NDA
203356

APPROVAL LETTER

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 20-356

FEB 2 1995

Miles Inc.
Pharmaceutical Division
Attention: Nancy Motola, Ph.D.
400 Morgan Lane
West Haven, CT 06516-4175

Dear Dr. Motola:

Please refer to your March 31, 1993 new drug application resubmitted on August 3, 1994 under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Nisocor (nisoldipine) Tablets, 10, 20, 30 and 40 mg.

We acknowledge receipt of your amendments and correspondence dated May 31, June 20 and 27, July 18 and 29 (two), September 8 and 16, October 19, November 8, 9, 17, 18 and 21, and December 16, 20 (two), 22 (three) and 28, 1994; and January 20, 23, and 27, 1995.

This new drug application provides for the use of Nisocor in the treatment of hypertension.

We have completed the review of this application including the submitted draft labeling and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the enclosed marked-up draft labeling. Accordingly, the application is approved effective on the date of this letter.

The final printed labeling (FPL) must be identical to the enclosed marked-up draft labeling. Marketing the product with FPL that is not identical to this draft labeling may render the product misbranded and an unapproved new drug.

The approved dissolution specifications are as follows:

3 hours
6 hours
12 hours

Please submit fifteen copies of the FPL as soon as it is available, in no case more than 30 days after it is printed. Please individually mount ten of the copies on heavy weight paper or similar material. For administrative purposes this submission should be designated "FINAL PRINTED LABELING" for approved NDA 20-356. Approval of this labeling by FDA is not required before it is used.

Should additional information relating to the safety and effectiveness of the drug become available, revision of that labeling may be required.

In addition, please submit three copies of the introductory promotional material that you propose to use for this product. All proposed materials should be submitted in draft or mock-up form, not final print. Please send one copy to the Division of Cardio-Renal Drug Products and two copies of both the promotional material and the package insert directly to:

Food and Drug Administration
Division of Drug Marketing, Advertising and Communications, HFD-240
5600 Fishers Lane
Rockville, Maryland 20857

Validation of the regulatory methods is ongoing. At the present time, it is the policy of the Office not to withhold approval because the methods are being validated. Nevertheless, we expect your continued cooperation to resolve any deficiencies that may occur.

Please submit one market package of the drug when it is available.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any questions, please contact:

Mr. David Roeder
Consumer Safety Officer
(301) 594-5300

Sincerely yours,

Robert Temple, M.D.
Director
Office of Drug Evaluation I
Center for Drug Evaluation and Research

Enclosure

cc:
Original NDA
HF-2/MedWatch (with labeling)
HFC-130/JAllen
HFD-2/MLumpkin
HFD-80 (with labeling)
HFD-100 (with labeling)
HFD-110
HFD-110/CSO
HFD-240 (with labeling)
HFD-638 (with labeling)
HFD-735/DBarash (with labeling)
HFD-110/DRoeder/12/28/94
sb/12/28/94;12/29/94
R/D: RWolters/12/28/94
SChen/12/28/94
GBuehler for NMorgenstern/12/29/94

APPROVAL

DRAFT OF
APPROVED
LABELING

NISOCOR

(nisoldipine)

Extended Release Tablets

For Oral Use

DESCRIPTION

NISOCOR (nisoldipine) is an extended release tablet dosage form of the dihydropyridine calcium channel blocker nisoldipine. Nisoldipine is 3,5-pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, methyl 2-methylpropyl ester, $C_{20}H_{24}N_2O_6$, and has the structural formula:

supply formula →

Nisoldipine is a yellow crystalline substance, practically insoluble in water but soluble in ethanol. It has a molecular weight of 388.4. NISOCOR tablets consist of an external coat and an internal core. Both coat and core contain nisoldipine, the coat as a slow release formulation and the core as a fast release formulation. NISOCOR tablets contain either 10, 20, 30, or 40 mg of nisoldipine for once-a-day oral administration.

Inert ingredients in the formulation are hydroxypropylcellulose, lactose, corn starch, crospovidone, microcrystalline cellulose, sodium lauryl sulfate, povidone and magnesium stearate. The inert ingredients in the film coating

are: hydroxypropylmethylcellulose, polyethylene glycol, ferric oxide, and titanium dioxide.

CLINICAL PHARMACOLOGY

Mechanism of Action

Nisoldipine is a member of the dihydropyridine class of calcium channel antagonists (calcium ion antagonists or slow channel blockers) that inhibit the transmembrane influx of calcium into vascular smooth muscle and cardiac muscle. It reversibly competes with other dihydropyridines for binding to the calcium channel. Because the contractile process of vascular smooth muscle is dependent upon the movement of extracellular calcium into the muscle through specific ion channels, inhibition of the calcium channel results in dilation of the arterioles. *In vitro* studies show that the effects of nisoldipine on contractile processes are selective, with greater potency on vascular smooth muscle than on cardiac muscle. Although, like other dihydropyridine calcium channel blockers, nisoldipine has negative inotropic effects *in vitro*, studies conducted in intact anesthetized animals have shown that the vasodilating effect occurs at doses lower than those that affect cardiac contractility.

The effect of nisoldipine on blood pressure is principally a consequence of a dose-related decrease of peripheral vascular resistance. While nisoldipine, like other dihydropyridines, exhibits a mild diuretic effect, most of the antihypertensive activity is attributed to its effect on peripheral vascular resistance.

Pharmacokinetics and Metabolism

Nisoldipine pharmacokinetics are independent of the dose in the range of 20 to 60 mg, with plasma concentrations proportional to dose. Nisoldipine accumulation, during multiple dosing, is predictable from a single dose.

Nisoldipine is relatively well absorbed into the systemic circulation with 87% the radiolabeled drug recovered in urine and feces. The absolute bioavailability of nisoldipine is about 5%. Nisoldipine's low bioavailability is due, in part, to pre-systemic metabolism in the gut wall and this metabolism decreases from the proximal to the distal parts of the intestine. Food with a high fat content has a pronounced effect on the release of nisoldipine from the coat-core formulation and results in a significant increase in peak concentration (C_{max}) by up to 300%. Total exposure, however, is decreased about 25%, presumably because more of the drug is released proximally. This effect appears to be specific for nisoldipine in the controlled release formulation, as a less pronounced food effect was seen with the immediate release tablet. Concomitant intake of a high fat meal with NISOCOR should be avoided.

Maximal plasma concentrations of nisoldipine are reached 6 to 12 hours after dosing. The terminal elimination half-life (reflecting post absorption clearance of nisoldipine) ranges from 7 to 12 hours. C_{max} and AUC increase by factors of approximately 1.3 and 1.5 respectively from first dose to steady state. After oral administration, the concentration of (+) nisoldipine, the active enantiomer, is about 6 times higher than the (-) inactive enantiomer. The

plasma protein binding of nisoldipine is very high, with less than 1% unbound over the plasma concentration range of 100 ng/mL to 10 mcg/mL. Nisoldipine is highly metabolized; 5 major urinary metabolites have been identified. Although 60 - 80% of an oral dose undergoes urinary excretion, only traces of unchanged nisoldipine are found in urine. The major biotransformation pathway appears to be the hydroxylation of the isobutyl ester. A hydroxylated derivative of the side chain, present in plasma at concentrations approximately equal to the parent compound, appears to be the only active metabolite, and has about 10% of the activity of the parent compound. Cytochrome P_{450} enzymes are believed to play a major role in the metabolism of nisoldipine. The particular isoenzyme system responsible for its metabolism has not been identified, but other dihydropyridines are metabolized by cytochrome P_{450} IIIA4. Nisoldipine should not be administered with grapefruit juice as this has been shown, in a study of 12 subjects, to interfere with nisoldipine metabolism, resulting in a mean increase in C_{max} of about 3-fold (ranging up to about 7-fold) and AUC of almost 2-fold (ranging up to about 5-fold). A similar phenomenon has been seen with several other dihydropyridine calcium channel blockers.

Special Populations:

Renal dysfunction: Because renal elimination is not an important pathway, bioavailability and pharmacokinetics of NISOCOR were not significantly different in patients with various degrees of renal impairment. Dosing adjustments in patients with mild to moderate renal impairment are not necessary.

Geriatric: Elderly patients have been found to have 2 to 3 fold higher plasma

concentrations (C_{max} and AUC) than young subjects. This should be reflected in more cautious dosing (See Dosage and Administration).

Hepatic Insufficiency: In patients with liver cirrhosis given 10 mg NISOCOR, plasma concentrations of the parent compound were 4 to 5 times higher than those in healthy young subjects. Lower starting and maintenance doses should be used in cirrhotic patients (See Dosage and Administration).

Gender and Race: The effect of gender o race on the pharmacokinetics of nisoldipine has not been investigated.

Disease States: Hypertension does not significantly alter the pharmacokinetics of nisoldipine.

Pharmacodynamics

Hemodynamic Effects

Administration of a single dose of nisoldipine leads to decreased systemic vascular resistance and blood pressure with a transient increase in heart rate. The change in heart rate is greater with immediate release nisoldipine preparations. The effect on blood pressure is directly related to the initial degree of elevation above normal. Chronic administration of nisoldipine results in a sustained decrease in vascular resistance and small increases in stroke index and left ventricular ejection fraction. A study of the immediate release formulation showed no effect of nisoldipine on the renin-angiotensin-aldosterone system or on plasma norepinephrine concentration in normals. Changes in blood pressure in hypertensive patients given NISOCOR were dose related over the range of 10 - 60 mg/day.

Nisoldipine does not appear to have significant negative inotropic activity in intact animals or humans, and did not lead to worsening of clinical heart

failure in three small studies of patients with asymptomatic and symptomatic left ventricular dysfunction. There is little information, however, in patients with severe congestive heart failure and all calcium channel blockers should be used with caution in any patient with heart failure.

Electropyhysiologic Effects

Nisoldipine has no clinically important chronotropic effects. Except for mild shortening of sinus cycle, SA conduction time and AH intervals, single oral doses up to 20 mg of immediate release nisoldipine did not significantly change other conduction parameters. Similar electrophysiologic effects were seen with single iv doses, which could be blunted in patients pre-treated with beta-blockers. Dose and plasma level related flattening or inversion of T-waves have been observed in a few small studies. Such reports were concentrated in patients receiving rapidly increased high doses in one study; the phenomenon has not been a cause of safety concern in large clinical trials.

Clinical Studies in Hypertension

The antihypertensive efficacy of NISOCOR was studied in 5 double-blind, placebo-controlled, randomized studies, in which over 600 patients were treated with NISOCOR as monotherapy and about 300 with placebo; 4 of the five studies compared 2 or 3 fixed doses while the fifth allowed titration from 10 - 40 mg. Once daily administration of NISOCOR produced sustained reductions in systolic and diastolic blood pressures over the 24 hour dosing interval in both supine and standing positions. The mean placebo-subtracted reductions in supine systolic and diastolic blood pressure at trough, 24 hours post-dose, in these studies, are shown below. Changes in standing blood

pressure were similar:

MEAN SUPINE TROUGH SYSTOLIC AND DIASTOLIC

BLOOD PRESSURE CHANGES (mm Hg)

NISOCOR Dose (mg/day)	10 mg	20 mg	30 mg	40 mg	60 mg	10-40mg titrated
Systolic:	8	11	11	14	15	15
Diastolic	3	5	7	7	10	8

In patients receiving atenolol, supine blood pressure reductions with NISOCOR at 20, 40 and 60 mg once daily were 12/6, 19/8 and 22/10 mm Hg, respectively. The sustained antihypertensive effect of NISOCOR was demonstrated by 24 hour blood pressure monitoring and examination of peak and trough effects. The trough/peak ratios ranged from 70 to 100% for diastolic and systolic blood pressure. The mean change in heart rate in these studies was less than one beat per minute. In 4 of the 5 studies, patients received initial doses of 20 - 30 mg NISOCOR without incident (excessive effects on blood pressure or heart rate). The fifth study started patients on lower doses of NISOCOR

Patient race and gender did not influence the blood pressure lowering effect of NISOCOR. Despite the higher plasma concentration of nisoldipine in the elderly, there was no consistent difference in their blood pressure response except that the 10 mg dose was somewhat more effective than in non-elderly patients. No postural effect on blood pressure was apparent and there was no

evidence of tolerance to the antihypertensive effect of NISOCOR in patients treated for up to one year.

INDICATIONS AND USAGE

NISOCOR is indicated for the treatment of hypertension. It may be used alone or in combination with other antihypertensive agents.

CONTRAINDICATIONS

NISOCOR is contraindicated in patients with known hypersensitivity to dihydropyridine calcium channel blockers.

WARNINGS

Increased angina and/or myocardial infarction in patients with coronary artery disease: Rarely, patients, particularly those with severe obstructive coronary artery disease, have developed increased frequency, duration and/or severity of angina, or acute myocardial infarction on starting calcium channel blocker therapy or at the time of dosage increase. The mechanism of this effect has not been established. In controlled studies of NISOCOR in patients with angina this was seen about 1.5% of the time in patients given nisoldipine, compared with 0.9% in patients given placebo.

PRECAUTIONS

General.

Hypotension: Because nisoldipine, like other vasodilators, decreases

peripheral vascular resistance, careful monitoring of blood pressure during the initial administration and titration of NISOCOR is recommended. Close observation is especially important for patients already taking medications that are known to lower blood pressure. Although in most patients the hypotensive effect of NISOCOR is modest and well tolerated, occasional patients have had excessive and poorly tolerated hypotension. These responses have usually occurred during initial titration or at the time of subsequent upward dosage adjustment.

Congestive Heart Failure: Although acute hemodynamic studies of nisoldipine in patients with NYHA Class II-IV heart failure have not demonstrated negative inotropic effects, safety of NISOCOR in patients with heart failure has not been established. Caution therefore should be exercised when using NISOCOR in patients with heart failure or compromised ventricular function, particularly in combination with a beta-blocker.

Patients with Hepatic Impairment: Because nisoldipine is extensively metabolized by the liver and, in patients with cirrhosis, it reaches blood concentrations about 5 times those in normals, NISOCOR should be administered cautiously in patients with severe hepatic dysfunction (See Dosage and Administration)

Information for Patients: NISOCOR is an extended release tablet and should be swallowed whole. Tablets should not be chewed, divided or crushed. NISOCOR should not be administered with a high fat meal. Grapefruit juice, which has been shown to increase significantly the bioavailability of nisoldipine and other dihydropyridine type calcium channel

blockers, should not be taken with NISOCOR

Laboratory Tests: NISOCOR is not known to interfere with the interpretation of laboratory tests.

Drug Interactions: A 30 to 45% increase in AUC and C_{max} of nisoldipine was observed with concomitant administration of cimetidine 400 mg twice daily. Ranitidine 150 mg twice daily did not interact significantly with nisoldipine (AUC was decreased by 15 - 20 %). No pharmacodynamic effects of either H_2 antihistamine were observed.

Pharmacokinetic interactions between nisoldipine and beta-blockers (atenolol, propranolol) were variable and not significant. Propranolol attenuated the heart rate increase following administration of immediate release nisoldipine. The blood pressure effect of NISOCOR tended to be greater in patients on atenolol than in patients on no other antihypertensive therapy.

Quinidine at 648 mg bid ~~increased~~ decreased the bioavailability (AUC) of nisoldipine by 26%, but not the peak concentration. The immediate release, but not the coat-core, formulation of nisoldipine increased plasma quinidine concentrations by about 20 %. This interaction was not accompanied by ECG changes and its clinical significance is not known.

No significant interactions were found between nisoldipine and warfarin or digoxin.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Dietary administration of nisoldipine to male and female rats for up to 24 months

(mean doses up to 82 and 111 mg/kg/day, 16 and 19 times the maximum recommended human dose (MRHD) on a mg/m^2 basis, respectively) and female mice for up to 21 months (mean doses of up to 217 mg/kg/day, 20 times the MRHD on a mg/m^2 basis) revealed no evidence of tumorigenic effect of nisoldipine. In male mice receiving a mean dose of 163 mg nisoldipine/kg/day (16 times the MRHD of 60 mg/day on a mg/m^2 basis), an increased frequency of stomach papilloma, but still within the historical range, was observed. No evidence of stomach neoplasia was observed at lower doses (up to 58 mg/kg/day). Nisoldipine ~~tested~~ was when tested negative in a battery of genotoxicity assays, including the Ames test and the CHO/HGRPT assay for mutagenicity ~~mutagenicity and clastogenicity tests~~. and the in vivo mouse micronucleus test and in vitro CHO cell test for clastogenicity. When administered to male and female rats at doses of up to 30 mg/kg/day (about 5 and 6 times the MRHD on a mg/m^2 basis respectively) nisoldipine had no effect on fertility.

Pregnancy Category C. Nisoldipine was neither teratogenic nor fetotoxic at doses that were not maternally toxic. Nisoldipine was fetotoxic but not teratogenic in rats and rabbits at doses resulting in maternal toxicity (reduced maternal body weight gain). In pregnant rats, increased fetal resorption (post-implantation loss) was observed at 100 mg/kg/day and decreased fetal weight was observed at both 30 and 100 mg/kg/day. These doses are, respectively, about 5 and 16 times the MRHD when compared on a ~~body surface area basis~~ mg/m^2 basis. In pregnant rabbits, decreased fetal and placental weights were observed at a dose of 30 mg/kg/day, about 10 times the MRHD when compared on a ~~body surface area~~ mg/m^2 basis. In a study in which pregnant monkeys (both treated and control) had high rates of abortion and mortality, the only surviving fetus from a group exposed to a maternal dose of 100 mg nisoldipine/kg/day (about 30 times the MRHD when compared on a ~~body~~ mg/m^2 basis

~~surface area basis~~) presented with forelimb and vertebral abnormalities not previously seen in control monkeys of the same strain. There are no adequate and well controlled studies in pregnant women. NISOCOR should be used in pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers: It is not known whether nisoldipine is excreted in human milk. Because many drugs are excreted in human milk, a decision should be made to discontinue nursing, or to discontinue NISOCOR, taking into account the importance of the drug to the mother.

ADVERSE EXPERIENCES

More than 6000 patients world-wide have received nisoldipine in clinical trials for the treatment of hypertension, either as the immediate release or the NISOCOR extended release formulation. Of about 1,500 patients who received NISOCOR in hypertension studies, about 55% were exposed for at least 2 months and about one third were exposed for over 6 months, the great majority at doses of 20 to 60 mg daily.

NISOCOR is generally well-tolerated. In the U.S. clinical trials of NISOCOR in hypertension, 10.9% of the 921 NISOCOR patients discontinued treatment due to adverse events compared with 2.9% of 280 placebo patients. The frequency of discontinuations due to adverse experiences was related to dose, with a 5.4% discontinuation rate at 10 mg daily and a 10.9% discontinuation rate at 60 mg daily.

The most frequently occurring adverse experiences with NISOCOR are those related to its vasodilator properties; these are generally mild and only

occasionally lead to patient withdrawal from treatment. The table below, from U.S. placebo-controlled parallel dose response trials of NISOCOR using doses from 10 - 60 mg once daily in patients with hypertension, lists all of the adverse events, regardless of the causal relationship to NISOCOR, for which the overall incidence on NISOCOR was both >1% and greater with NISOCOR than with placebo.

Adverse Event	Nisoldipine (%) (n=663)	Placebo (%) (n=280)
Peripheral Edema	22	10
Headache	22	15
Dizziness	5	4
Pharyngitis	5	4
~~Asthenia~~	4	4
Vasodilation	4	2
Sinusitis	3	2
Palpitation	3	1
Chest Pain	2	1
Nausea	2	1
Rash	2	1

not greater on Nisocor

Only peripheral edema and possibly dizziness appear to be dose related.

Adverse Event	Placebo	NISOCOR 10 mg	NISOCOR 20 mg	NISOCOR 30 mg	NISOCOR 40 mg	NISOCOR 60 mg
(Rates in %)	N=280	N=30	N=170	N=105	N=139	N=137

Peripheral Edema	10	7	15	20	27	29
Dizziness	4	7	3	3	4	10

The common adverse events occurred at about the same rate in men as in women, and at a similar rate in patients over age 65 as in those under that age, except ~~with the exception~~ that headache was much less common in older patients. Except for peripheral edema and vasodilation, which were more common in whites, adverse event rates were similar in blacks and whites.

The following adverse events occurred in ≤1 % of all patients treated for hypertension in U.S. and foreign clinical trials, or with unspecified incidence in other studies. Although a causal relationship of NISOCOR to these events cannot be established, they are listed to alert the physician to a possible relationship with NISOCOR treatment.

Body As A Whole: cellulitis, chills, facial edema, fever, flu syndrome, malaise,

Cardiovascular: atrial fibrillation, cerebrovascular accident, congestive heart failure, first degree AV block, hypertension, hypotension, jugular venous distension, migraine, myocardial infarction, postural hypotension, ventricular extrasystoles, supraventricular tachycardia, syncope, systolic ejection murmur, T wave abnormalities on ECG (flattening, inversion, nonspecific changes), venous insufficiency,

Digestive: abnormal liver function tests, anorexia, colitis, diarrhea, dry mouth, dyspepsia, dysphagia, flatulence, gastritis, gastrointestinal

hemorrhage, gingival hyperplasia, glossitis, hepatomegaly, increased appetite, melena, mouth ulceration,

Endocrine: diabetes mellitus, thyroiditis,

Hemic and Lymphatic: anemia, ecchymoses, leukopenia, petechiae,

Metabolic and Nutritional: gout, hypokalemia, increased serum creatine kinase, increased nonprotein nitrogen, weight gain, weight loss,

Musculoskeletal: arthralgia, arthritis, leg cramps, myalgia, myasthenia, myositis, tenosynovitis,

Nervous: abnormal dreams, abnormal thinking and confusion, amnesia, anxiety, ataxia, cerebral ischemia, decreased libido, depression, hypesthesia, hypertonia, insomnia, nervousness, paresthesia, somnolence, tremor, vertigo,

Respiratory: asthma, dyspnea, end inspiratory wheeze and fine rales, epistaxis, increased cough, laryngitis, pharyngitis, pleural effusion, rhinitis, sinusitis.

Skin and Appendages: acne, alopecia, dry skin, exfoliative dermatitis, fungal dermatitis, herpes simplex, herpes zoster, maculopapular rash, pruritus, pustular rash, skin discoloration, skin ulcer, sweating, urticaria,

Special senses: abnormal vision, amblyopia, blepharitis, conjunctivitis, ear pain, glaucoma, itchy eyes, keratoconjunctivitis, otitis media, retinal detachment, tinnitus, watery eyes, taste disturbance, temporary unilateral loss of vision, vitreous floater, watery eyes,

Urogenital: dysuria, hematuria, impotence, nocturia, urinary frequency, increased BUN and serum creatinine, vaginal hemorrhage, vaginitis.

experience with

In addition to NISOCOR, there is extensive experience with the immediate release formulation of nisoldipine. Adverse events were generally similar to

those seen with NISOCOR. Unusual events observed with immediate release nisoldipine but not observed with NISOCOR, were one case each of angioedema and photosensitivity. Spontaneous reports from postmarketing experience with the immediate release formulation of nisoldipine have not revealed any additional adverse events not identified in the above listings.

OVERDOSAGE

There is no experience with nisoldipine overdosage. Generally, overdosage with other dihydropyridines leading to pronounced hypotension calls for active cardiovascular support including monitoring of cardiovascular and respiratory function, elevation of extremities, judicious use of calcium infusion, pressor agents and fluids. Clearance of nisoldipine would be expected to be slowed in patients with impaired liver function. Since nisoldipine is highly protein bound, dialysis is not likely to be of any benefit; however, plasmapheresis may be beneficial.

DOSAGE AND ADMINISTRATION

The dosage of NISOCOR must be adjusted to each patient's needs. Therapy usually should be initiated with 20 mg orally once daily, then increased by 10 mg per week or longer intervals, to attain adequate control of blood pressure. Usual maintenance dosage is 20 to 40 mg once daily. Blood pressure response increases over the 10 - 60 mg daily dose range but adverse event rates also increase. Doses beyond 60 mg once daily are not recommended. NISOCOR has been used safely with diuretics, ACE inhibitors, and beta-

blocking agents.

Patients over age 65, or patients with impaired liver function are expected to develop higher plasma concentrations of nisoldipine. Their blood pressure should be monitored closely during any dosage adjustment. A starting dose not exceeding 10 mg daily is recommended in these patient groups.

NISOCOR tablets should be administered orally once daily. Administration with a high fat meal can lead to excessive peak drug concentration and should be avoided. Grapefruit products should be avoided before and after dosing. NISOCOR is an extended release dosage form and tablets should be swallowed whole, not bitten or divided.

HOW SUPPLIED

NISOCOR extended release tablets are supplied as 10 mg, 20 mg, 30 mg, and 40 mg round film coated tablets. The different strengths can be identified as follows:

Strength	Color	Markings
10 mg	Oyster	**891** on one side and **MILES 10** on the other side.
20 mg	Yellow Cream	**892** on one side and **MILES 20** on the other side.
30 mg	Mustard	**893** on one side and **MILES 30** on the other side.
40 mg	Burnt Orange	**894** on one side and **MILES 40** on the other side.

NISOCOR Tablets are supplied in:

	Strength	NDC Code
Bottles of 30	10 mg	0026-8911-30
	20 mg	0026-8921-30
	30 mg	0026-8931-30
	40 mg	0026-8941-30
Bottles of 100	10 mg	0026-8911-51
	20 mg	0026-8921-51
	30 mg	0026-8931-51
	40 mg	0026-8941-51
Unit Dose Packages of 100	10 mg	0026-8911-48
	20 mg	0026-8921-48
	30 mg	0026-8931-48
	40 mg	0026-8941-48

The tablets should be protected from light and moisture and stored below 86°F (30°C). Dispense in tight, light-resistant containers.

Distributed by:

Miles Inc.

Pharmaceutical Division

400 Morgan Lane

West Haven, CT 06516 USA

Made in Germany

Revised Labeling

Nisocor

891130 NDC [redacted]

[redacted]

(nisoldipine)
Extended Release Tablets
10 mg
100 Tablets Unit Dose

For institutional use only
RECOMMENDED STORAGE
[redacted]
Dosage: [redacted]

W 7.1 mm × H 106 mm × D 72.5 mm
[redacted]

[redacted]
Job # 18356
[redacted]
Date [redacted]

891130 NDC 0026-8911-46

NIS® CC

(nisoldipine)
Extended Release Tablets
10 mg
100 Tablets Unit Dose

NO SBA
WILL BE
PREPARED

CSO OVERVIEW

Reader

FEB 2 1995

CSO Application Overview

Application: NDA 20-356
Nisoldipine Coat Core Tablets

Sponsor: Miles Pharmaceuticals

NDA Receipt Date: April 1, 1993

NDA Resubmission Date: August 3, 1994

User Fee Goal Date: February 3, 1995

Date of Overview: November 28, 1994

Background

NDA 20-356 provides for the use of a sustained release (once-daily) formulation of nisoldipine in the treatment of hypertension. No formulation of nisoldipine is currently approved in the U.S.

A non-approval letter was issued on March 25, 1994, that listed deficiencies in the Chemistry, Pharmacology, and Clinical sections. The firm responded fully to this letter on August 3, 1994.

Review

Chemistry

Reviewer: Danute Cunningham

Reviews: 6/7/93 11/29/93 2/4/94 9/16/94

Ms. Cunningham's review of the application has been completed.

The trade name "Nisocor" has been approved by the Nomenclature Committee.

The facility inspection has been completed and was found to be satisfactory. We received a satisfactory response to our FUR on November 22, 1994.

The deficiencies outlined in the environmental assessment review were sent to the firm on October 27, 1994. The response from the firm has not been received yet.

Pharmacology

Reviewers: Xavier Joseph, D.V.M.
Sidney Stolzenberg, Ph.D.

Review: September 2, 1994

The reviewers comments have been incorporated into the draft labeling. The application went before the CAC, and the recommendations from that committee have also been incorporated into the labeling. The minutes of the CAC meeting have not been completed yet.

Biopharmaceutics

Reviewer: Patrick Marroum, Ph.D.

The Biopharm Day was held for this application, revisions have been made, and the draft is now under supervisory review.

Dr. Marroum has made a number of comments including extensive revisions of the labeling (see pp 13-16 of Dr. Marroum's review). The labeling recommendations have been incorporated into the draft package insert. He has also recommended that the dissolution specifications be revised as follows:

from:	3 hours	to:	3 hours
	6 hours		6 hours
	12 hours		12 hours

Statistical

Reviewer: Nancy Smith

Review (hypertension): 1/4/94

Dr. Smith has reviewed only the hypertension indication. There were no serious problems identified in the review.

Clinical

Reviewers: Shaw Chen, M.D., Ph.D. (Clinical Pharmacology): 2/16/94
Phil Dern, M.D. (Safety): 9/27/93
Cristobal Duarte, M.D. (hypertension): 8/4/93
Norman Stockbridge, M.D., Ph.D. 8/4/93

The reviewers recommend approval for the hypertension indication.

The final safety update is under review.

DSI Audits

Four of the seven requested DSI audits are completed. There have been no problems so far.

Labeling

Dr. Chen has provided a marked up copy of the package insert.

David Roeder
Consumer Safety Officer

dr/9-4-94/9-27-94/10-28-94/11-23-94/11-28-94

cc: NDA 20-356
HFD-110
HFD-111/DRoeder

MEDICAL OFFICER REVIEW

Reader

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

Date: 12/22/94

From: Shaw T. Chen, M.D., Ph.D., Medical Group Leader, HFD-110
Through: Director, Division of Cardiorenal Drug Products, HFD-110 Lipicky
To: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: NDA 20-356, Nisoldipine Core-Coat for Hypertension, Labeling

We did not get to see your memo in final prior to responding to your memo. There was one question that you asked that was not answered in our response of 12/17/94.

There were 2 "food studies" conducted by Miles. One of them was not the FDA "high fat" and studied only the 20 mg tablet (Study 666, it was more than average but not high) and in that study there was no evidence of "dose-dumping'. The other study was conducted using the FDA "high fat" meal and in that study there was dose dumping. However, in this study (D92-045-02), 30 and 40 mg tabs were used and the 20 mg dose was not repeated. The food effect on the C_{max} was average 3 fold, and 5 of the 28 subjects had 5-11 fold changes. Thus either the fat content in food is important or dose-dumping by food may be dose-related.

Miles did conduct a pharmacokinetic/pharmacodynamic study, a review of that study was done by Dr. Marroum, in which they found that the pharmacodynamic effects (lowering of blood pressure) was a function of the log of the plasma concentration. So ten-fold changes in plasma concentration make a sizable difference, three-fold changes do not make a big difference. The slope of the concentration-response curve goes over 2 order of magnitude from beginning of effect to definitely over the maximum effect.

Although Dr. Marroum's review criticized the analysis of the study, the qualitative statements above are not materially affected by the quantitative problems that Dr. Marroum found.

So, it seems to us that there is considerable latitude that can be given with respect whether nisoldipine **must** be taken fasting. We do not think it must be taken fasting. To be silent about fasting or fed in the Dosage and Administration section is reasonable but since there are more than 5-fold changes in C_{Max} in 18% of subjects, the Dosage and Administration should probably say "..., preferably in a fasted state (see Clinical Pharmacology)". In Clinical Pharmacology the 11-fold increase in C_{Max} should be stated to be an upper limit when a High fat meal is ingested.

or even other alternatives as we discussed in the 17 M of 12/22/94. SC

Shaw T. Chen, M.D.,Ph.D.

cc:
ORIG: NDA- 20-356
HFD-110
HFD-110/CSO
HFD-110/SChen/12/22/94

10.3 Display and Analysis of All Adverse Events (Continued)

except for two episodes of moderate headache, one occurring in the fed state and the other in the fasted state. Overall, there was a similarity in adverse events reported in the fed and fasted condition. Headache was the most common adverse event (42.9% fasted, 35.7% fed) followed by dizziness (3.6% fasted and fed) and flushing (3.6% fasted and fed). There was no suggestion of a dose relationship in the incidence of adverse events for the Nisoldipine CC 30 mg and 40 mg doses either in the fed or the fasted state.

The relationship of C_{max} values to the adverse events, was evaluated. Geometric mean C_{max} concentrations at the 30 mg dose were 1.9 ng/ml (range: 0.7-4.9) in the fasted state and 4.5 ng/ml (range: 1.7-13.3) in the fed state. Geometric mean C_{max} concentrations at the 40 mg dose were 2.7 ng/ml (range: 1.2-8.1) in the fasted state and 7.5 ng/ml (range: 2.1-26.7) in the fed state (Section 13.9.5.2). Inspite of the much higher mean C_{max} concentrations, in the fed state as compared to the fasted state, there were no notable differences in the incidence rates or intensities of the adverse events in the fed state as compared to the fasted state (Table 1, Section 13.9.6).

Even though the overall incidence of adverse events was similar between the fed and fasted states, it was of interest to define whether the subjects with especially high fed/fasted C_{max} ratios showed a higher propensity for adverse events in the fed state as compared to the fasted state. Adverse events in subjects with a fed/fasted C_{max} ratio of ≥ 5 are shown below:

SUBJECT #	DOSE (mg)	C_{max} (ng/ml)		FED/FASTED C_{max} RATIO	ADVERSE EVENTS*	
		FASTED	FED		FASTED	FED
2103	30				NONE	NONE
2104	30				NONE	NONE
2110	30				HEADACHE	HEADACHE
2202	40				PERIPHERAL EDEMA HEADACHE	DIZZINESS PERIPHERAL EDEMA PALPITATION
2204	40				HEADACHE	NONE

* All adverse events in the fasted and fed states were mild in intensity.

Kreeter

DEC 19 1994

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

Date: 12/15/94

From: Shaw T. Chen, M.D., Ph.D., Medical Group Leader, HFD-110.
Through: Director, Division of Cardiorenal Drug Products, HFD-110 Concurred
To: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: NDA 20-356, Nisoldipine Core-Coat for Hypertension, Approvability

This is in response to the comments and questions raised in your draft memo of 12/13/94 regarding some labeling issues for the above application

1. As stated in the Secondary Review (Dose Response), we also think that the effective dosage range is 20-60 mg/day and the recommended doses should include 60 mg. Usual maintenance doses in the labeling were changed to 20-40 mg in the Division's draft, which were concurred in your memo. We agree with your reasoning that dose titration should start at 20 mg.

3. We understand that the effects of food and grapefruit on the kinetics of nisoldipine CC are different. They were put together only in "Information for Patients", as they are both "food". The wording in your marked-up draft certainly described the problem much clearer.

4. A cleaned-up draft of package insert with your mark-ups has been prepared.

Shaw T. Chen, M.D.,Ph.D.

cc:
ORIG: NDA- 20-356
HFD-110
HFD-110/CSO
HFD-110/SChen/12/15/94

Reader

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

Date: 10/25/94

From: Shaw T. Chen, M.D., Ph.D., Medical Group Leader, HFD-110
Through: Director, Division of Cardiorenal Drug Products, HFD-110 NOV 21 1994
To: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: NDA 20-356, Nisoldipine Core-Coat for Hypertension, Approvability

OVERVIEW

This memorandum and the attached material constitute the Division's recommendation that NDA 20-356, Nisoldipine Core Coat (referred to as CC formulation) Tablets be approved for treatment of hypertension.

This package is being transmitted with a draft Summary Basis of Approval (SBA) prepared by the sponsor, which has not been edited by the Division but appears to be accurate in its contents to serve as one of the references for secondary/tertiary reviews of the application. In the draft SBA, any description or interpretation of the data different from that of this memo should be disregarded.

As one of the new team approaches, the primary medical review of the NDA were conducted in parallel by the following medical officers:

Clinical Pharmacology:	Dr. Chen
Hypertension -Efficacy:	Dr. Duarte
Hypertension -Safety:	Dr. Dem

Pharmacology sections of the application were also reviewed concurrently by two reviewers (Drs Joseph and Stolzenberg); a synoptic summary of all pharmacologic issues has been prepared by Dr. Joseph. As of the date of this memo, the chemistry, biopharmaceutical, pharmacological and statistical reviews have been completed. There are no major, unresolved preclinical issues which may affect the action recommended. Related labeling have been suitably edited.

Nisoldipine is a new calcium channel blocker of the dihydropyridine type and structurally related to nifedipine. It appears to be a less active inotrope than nifedipine *in vitro* but the two were not distinguishable in intact animals. There are no major efficacy or safety issues that should preclude the approvability of this drug for the hypertension.

The adverse experiences in the NDA have been amended with the First Safety Updates of 08/17/93. Selected major trials should be inspected before final approval of the application.

PRECLINICAL EVALUATIONS

Chemistry

There are no outstanding issues regarding the manufacturing and analytical controls. Final inspection will be scheduled.

Preclinical Pharmacology

Nisoldipine has been adequately characterized with respect to its preclinical pharmacokinetic and pharmacodynamic properties. There are no outstanding issues related to animal toxicity or carcinogenicity which may affect approvability of the drug.

Changes in proposed labeling, as recommended by the pharmacology reviewers, are summarized and commented below. They have been adopted with minor modification.

- Negative findings in carcinogenic studies should be qualified with the dosages studied, comparison with human dose should be based on both body weight and surface area calculations.
- Fetotoxicities in animals are suggestive, not conclusive. However, detailed description of the problematic monkey studies is not necessary. Again, basis of safety margin (toxic animal dose vs maximal human dose) should be specified (body weight and surface area).
- The pharmacology reviewers do not think malformation is increased in rabbits. Other recommendations related to fetotoxicity in rats/rabbits, sections of *Labor and Delivery*, and *Nursing Mothers* are all appropriate (Pharmacology Review, p 145).

CLINICAL PHARMACOLOGY

Pharmacokinetics/Pharmacodynamics

At the proposed dosages of 10-40 mgs, the **pharmacokinetic** profile of nisoldipine CC formulation supports a once-daily regimen. Compared with the immediate release (IR) form, availability of nisoldipine from the CC tablets was prolonged with lower C_{max} and higher AUC over 24 hrs. Bioavailability of nisoldipine CC was low for the unchanged drug but linear and dose-proportional over the range of 10-60 mg; it accumulates moderately after multiple oral dosing (7 days). While nisoldipine is extensively metabolized, the only active metabolite contributes about 10% of the pharmacologic effects.

The states of both hepatic and renal functions are potentially important for pharmacokinetics of the active drug, since nisoldipine is extensively metabolized and excretion of the metabolites is predominantly renal. Bioavailability of the parent drug was indeed increased by 4-5 fold in patients with hepatic failure, but changes in AUC and C_{max} due to various degree of renal impairment were only transient and diminished with multiple dosing. Plasma levels of nisoldipine were also higher in the elderly but dosage adjustment may not be required (see Efficacy -Hypertension). Nisoldipine metabolism probably involves P_{450} cytochrome system (as nifedipine), but no attempt to identify isozyme has been documented. Modest changes in bioavailability of CC nisoldipine were observed with

concomitant use of ranitidine (decreased 15-20%), cimetidine (increased 30-45%), quinidine (reduced by 25%), and propranolol ($t_{1/2}$ shorter by 20%). These interactions are probably of no significant clinical consequences, but labeling has been edited accordingly.

As noted in the Clinical Pharmacology and Biopharmaceutical Reviews, the problem of dose-dumping when nisoldipine CC is administered in a non-fasted state or with grapefruit juice (see biopharm review of Study 770) can not be ignored. Nisoldipine CC should not be administered concomitantly with meal or grapefruit juice, but instead after overnight fast and 1-2 hours before breakfast. Appropriate instructions to avoid dose administration in such settings have been included in the labeling.

Nisoldipine is a vasodilating antihypertensive with **pharmacodynamic** activities similar to other approved calcium channel blockers. Cardiovascular and hemodynamic effects of nisoldipine have been fairly well-established. Correlation between nisoldipine dose, plasma level and blood pressure reduction was good over the recommended dosage range.

Nisoldipine has no appreciable inotropic effects, but its clinical advantages over nifedipine has not been documented. Except for T-wave changes mostly at high doses (see Safety), nisoldipine had minimal electrophysiology activities. There is some evidence that iv nisoldipine improves coronary blood flow, but its anti-ischemic effect was not established in clinical pharmacology studies.

Nisoldipine did not affect regional blood flow in kidney or liver, and has no significant pharmacologic activities on non-cardiovascular systems.

Biopharmaceutics

Issues raised in the Biopharmaceutical Review are commented as follows.

The ratio of two enantiomers (and other metabolites) in special patient groups were not determined. Since no surprising clinical effects were observed in these patients which may required explanation, such data are not relevant for approval or prescription instruction.

Nisoldipine is metabolized by the P-450 enzyme system, but the specific iso-enzyme involved has not been identified. While metabolism of nisoldipine is not expected to be significantly different from that of nifedipine, the study should be done post approval, however.

Inconsistent C_{max} (by 2-folds) obtained after a 30 mg dose in two small pharmacology studies may be related to the variability in dissolution and need further clarification, as different blood pressure reductions from placebo were also noted in the efficacy trials (see below). Since no efficacy/safety problems were attributed to this variation, approval is not affected.

Assay validation was described in most of the studies, but missing in a few reports. It is reasonable to assume that same assay was used in all trials.

Variations in dose proportionality in two Phase II studies were small and of no clinical significance.

Pharmacokinetics and metabolism sections of the labeling have been edited to accommodate the recommendations of Biopharmaceutic Review.

CLINICAL : EFFICACY

Major Trials Supporting Approval

Nisoldipine has been evaluated as an antihypertensive treatment in 1,914 patients at dosages up to 80 mg/day. The efficacy data supporting approval were derived from the results of 7 double blind, parallel placebo controlled studies in 1,360 patients with hypertension, 886 of whom received nisoldipine. Long-term efficacy was supported by five open-label, 6-12 month follow-up of 554 patients (Studies X89-039, X90-019, X90-006, 675, 690).

The primary efficacy endpoint in each of these studies was the change in supine diastolic blood pressure (SDBP) from baseline at the end of dosing interval (trough effect) after 4-9 weeks of therapy. Data from five of the seven studies should be considered for major evidence of efficacy:

Study	Doses, mg/day	Duration		Remarks
D88-054	10, 20, 30	QD	4 wks	fixed dose
D89-026	10-40	QD	9 wks	dose titrated per response
D90-019	30, 60	QD	6 wks	fixed dose (after week 1)
D89-039	20, 40	QD	8 wks	fixed dose (after week 1)
D90-006	10, 20, 30	QD	6 wks	fixed dose (after week 1)

In the last three trials listed above, high doses were phased in after one week of low dose treatment. It should be noted that in Study D89-039, another group of 15 patients were randomized to receive nisoldipine CC 80 mg qd, but the arm was terminated due to safety concerns before collection of efficacy data. Nisoldipine was also compared with verapamil 240 mg (additional group of 78 patients) in this parallel placebo controlled study.

The remaining two controlled studies may provide instructions on how to use nisoldipine in a practical setting, but are not very useful as primary evidence for assessing efficacy of nisoldipine (vs placebo in general population who are not treated with other concurrent antihypertensive agents). Nisoldipine was evaluated in patients all receiving atenolol 50 mg qd as background therapy in one study (D89-029), and in the other (Study D90-029), lisinopril, hydrochlorothiazide (HCTZ) and placebo were compared in the presence of nisoldipine in all groups. Results of these studies will be commented in the Section of "Comparison/Combination with other Antihypertensives".

Overall Treatment Effects vs Placebo

The **primary efficacy** data in Table 1 on Page 6 demonstrate that nisoldipine, at 20-60 mg qd, is a consistently and significantly more effective antihypertensive agent than placebo with adequate duration of activity for once daily treatment. At this dose range, the placebo subtracted net decreases in SDBP at trough ranged from 3.6 to 9.9 mmHg after 4-9 weeks of therapy. Treatment effects were less consistent for the 10 mg dose, but was superior to placebo in the larger trial (D90-006) with a decent drop in SDBP. Similar results were obtained for supine systolic blood pressures (SSBP) (same Table) and standing blood pressures (excluding the smallest trial, D88-054, results not shown in this memo).

The **percentages of responders** (SDBP reduction of ≥ 10 mmHg at trough or to ≤ 90 mmHg) are also summarized in Table 2 on next page. In the major trials, the response rates for 20-60 mg/day were in the range of 17-45% more than that of placebo. Again, less patients (around 17% over placebo) responded to 10 mg dose.

With respect to blood pressure changes and response rate, there were no significant differences between various statistical analyses, i.e. per-protocol or intent-to-treat (final visit).

Dose Response

The **dose-response** relationship, at trough, has been examined within the range of 10-60 mg once daily (Tables 1 & 2, in the associated Figure 1, % response curve was shifted on the dose axis for clarification). While dose of 10 mg/day was not consistently better than placebo as monotherapy, it is appears that blood pressure reduction may increase further at doses above 60 mg (but not for % responders). Although there are evidence from small pilot studies of hypertensive patients that dose-response for 30-90 mg/day was rather flat (Study D90-022, see Clinical Pharmacology Review), the finding should be accepted with reservation because dosages were forced-escalated rapidly in that study. There was a concern, also in the same early phase study, of asymptomatic T-wave changes at high doses (see Safety below). However, when doses were increased slowly as in efficacy trials, less patients reported the same abnormality. Besides, such ECG changes were common in hypertensive patients and their clinical meaning are not yet clear. Thus, effective doses of nisoldipine CC range from 20 to 60 mg once daily, with a weak support for the nigh-end limit.

Correlation between blood nisoldipine level and blood pressure reduction has been demonstrated at trough in several clinical trials.

Time-Effect Relationship

While only once-daily regimen was used in clinical trials and no direct comparison with other dosing schedule was performed, appropriate **dosing interval** for nisoldipine was established in the following studies:

	Studies	
Peak/Trough Effect	D88-054, D90-019, D89-039, D90-006	10-60 mg/day, QD
24-hour BPs	D88-054, D90-019, D89-039, D90-006	10-60 mg/day, QD

For the doses studied, the placebo-subtracted **trough-to-peak** ratios appeared to be acceptable, ranging from 70 to 100% for SDBP and SSBP.

NISOLDIPINE: Hypertension Efficacy
Final visit, Placebo subtracted.

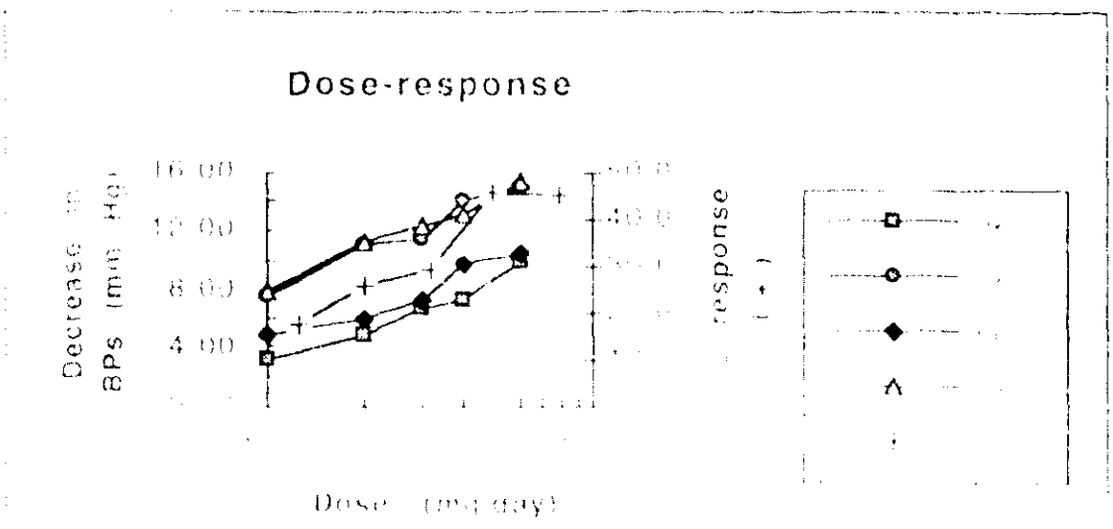
Bold these are significantly different from placebo

TABLE 1 Change in supine BPs from baseline at trough

Analysis	protocol	Last Visit Wks	mg/day: N/arm	regimen: 10	20	30	40	60	10-40 titrated
SDBP	D88-054	1-4	30	2.96	**3.81**	**4.63**			
ITT	D89-026	1-9	72						**8.35**
	D90-019	1-6	74			**6.66**		**9.91**	
	D89-039	1-8	76		**4.09**		**7.33**		
	D90-006	1-6	52	**3.54**	**6.85**	**8.00**			
	wt'd avg		SDBP	3.12	4.81	6.70	7.33	9.91	
SSBP	D88-054	1-4	30	5.31	**8.49**	**9.61**			
ITT	D89-026	1-9	72						**14.29**
	D90-019	1-6	74			**9.86**		**14.90**	
	D89-039	1-8	76		**7.28**		**13.96**		
	D90-006	1-6	52	**7.24**	**10.98**	**13.86**			
	wt'd avg		SSBP	7.55	10.98	11.44	13.96	14.90	

TABLE 2 Response Rate (Trough SDBP < 90 or drop by > 10)

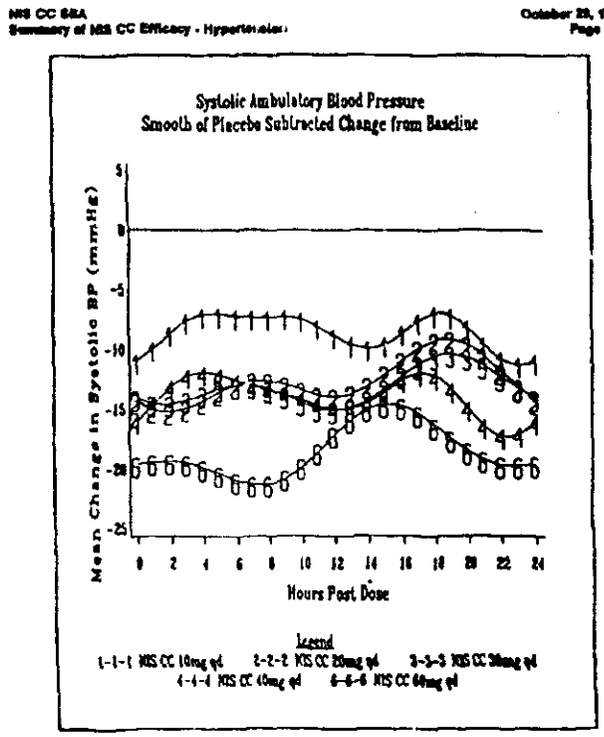
Analysis	protocol	Last Visit Wks	mg/day: N/arm	regimen: 10	20	30	40	60	10-40 titrated
ITT	D88-054	1-4	30	16.7	16.7	**26.9**			
	D89-026	1-9	72						**40.8**
	D90-019	1-6	74			**22.0**		**41.9**	
	D89-039	1-8	76		**9.5**		**15.8**		
	D90-006	1-6	52	17.3	**23.1**	**25.0**			
	wt'd avg		response	17.2	25.8	29.0	15.8	41.9	

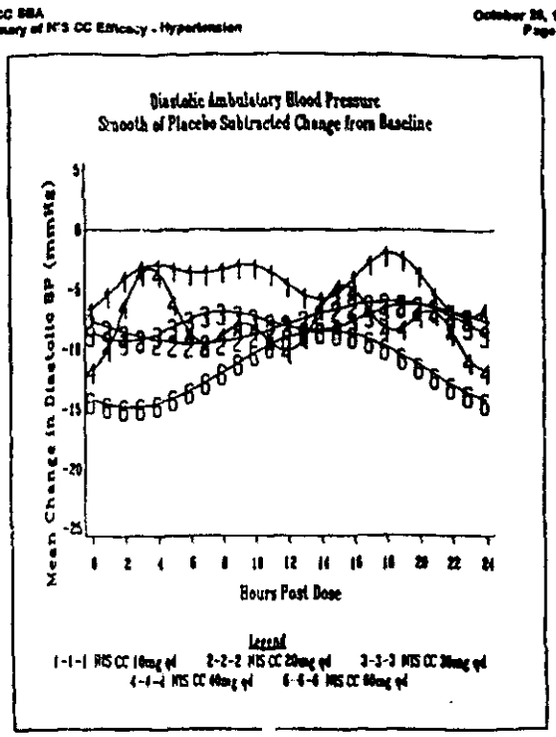


Total of 359 patients from the listed studies were pooled for analysis of **24 hours** ambulatory blood pressure change, the majority were white (65%) and male (60%). As shown by the 24-hour blood pressure curves, treatment effects of at least 5 mmHg reduction in SDBP over placebo were maintained during 24 hours for doses 20 mg and above (see Figure 2 below).

It is concluded that although other dosing schedules have not been evaluated, once-daily treatment with nisoldipine CC 20-60 mg per day appeared to be adequate to cover the dosing period.

Figure 2





Responses in Demographic Groups

In post hoc analyses (see draft SBA), nisoldipine appeared to be equally effective in **male/female**, with a slightly more pronounced dose-response relationship in the male patients. Despite increased bioavailability in the **elderly**, dose-response was less evident in such patients and there were no significant differences in blood pressure reduction between groups of age below and above 65 years. While blood pressure responses to nisoldipine were numerically greater in **black** than in white patients, such retrospective finding should not be described in the labeling or used in promotion. Not surprisingly, response to nisoldipine was greater in patients with higher **baseline blood pressure**, with a more significant dose-response relation.

Comparison/Combination with other Antihypertensives

Nisoldipine was compared or combined with the following antihypertensive agents in 3 controlled trials.

Comparison groups			Studies
nisoldipine+atenolol	vs	placebo+atenolol	D89-029
nisoldipine+lisinopril	vs	nisoldipine+placebo	D90-029
nisoldipine+HCTZ	vs	nisoldipine+placebo	D90-029

While results of the first study listed above indicated that concomitant atenolol did not affect the efficacy of nisoldipine CC, the second study suggested that some patients may have further response when a diuretic or ACE inhibitor is added. Up to one third of all patients received additional antihypertensive therapies in long-term, uncontrolled, follow-up studies. Overall, not much weight can be placed on these active controlled data for the efficacy claim.

Long-Term Efficacy

Long-term effectiveness of nisoldipine was evaluated in five open-label studies up to one year. Without a placebo control, reductions in supine blood pressures from baseline appeared to be sustained in more than 80% of 554 patients treated with nisoldipine for 6 months to one year

CLINICAL: SAFETY

Database

The database appeared to be adequate for analysis of the safety of nisoldipine, which includes cumulative experiences of nearly 4,200 hypertensive patients as of 10/29/93. Of 1,466 patients# (921 in US trials) who were treated with nisoldipine Coat-Care formulation, about 55% were exposed for at least 2 months (approx. 33% over 6 months) and a great majority were on doses of 20 to 60 mg.

The majority of comparative experience was based on the results of 6 randomized, double-blind, parallel group, placebo-controlled trials of 4-9 week duration (all U.S. double-blind controlled trials, see list in Efficacy Section)*, which included 678 patients on nisoldipine and 280 patients on placebo.

Data from the first 120-day Safety Update were not incorporated into the following summary, however, the numbers added were small and did not change the safety profile of the drug (see Reviews of Safety Update by Dr. Dern).

Comparative Experiences

There were no surprising findings in the safety profile of nisoldipine CC used in hypertensive patients. Overall frequency and rates of some specific adverse clinical experiences and abnormal laboratory findings were more common in nisoldipine than placebo treated patients, but none were serious or unexpectedly frequent.

The percentage of nisoldipine-treated patients reporting an **adverse event** in controlled trials (68%, N=678) was higher than that in the placebo group (53%, N=280). Among the adverse experiences, the following were more common for nisoldipine than placebo with incidence of $\geq 3\%$:

ADE	Nisoldipine(%) N=678	Placebo(%) N=280
peripheral edema	22	10
headache	22	15
dizziness	5	4
asthenia	4	4
vasodilatation	4	2
palpitation	3	1

From draft SBA. Different numbers of trials and patients exposed were given in Integrated Summary and Draft SBA, the later is probably more updated. Some calculations shown below were based on data from Integrated Summary of Safety.

* Foreign data also included some placebo-controlled safety experiences (D90-006). However, a great majority of the non-U.S. studies were not controlled and thus were not considered in comparative experiences. Nisoldipine was used in all treatment groups in one U.S. study (D90-029), but results of that study were not excluded from the comparative analysis.

As expected, the most commonly reported adverse events were related to nisoldipine's vasodilating effects. Most were mild and infrequently leading to withdraw.

While the overall frequency of adverse experiences was not affected significantly by patient **age, sex, race,** or **body weight** in the controlled trials, some minor differences in the incidence of a few adverse events may change the tolerability of nisoldipine in demographic subgroups. Headache appeared to be less common in the elderly, which would be surprising if the adverse event is pharmacokinetics-related (see Clinical Pharmacology). Peripheral edema was more frequent in female (as suspected with other dihydropyridine agent) and heavier patients (>185 lbs), but the sex difference was only seen in foreign studies, not in the U.S. controlled trials. Compared to blacks, incidences of headache and edema were slightly higher in whites.

The percentage of nisoldipine-treated patients **withdrawn due to adverse clinical experiences** was higher than that of placebo (7.8 vs 3.2%, U.S. controlled trials only), and dose-related (up from 5.4% at 10 mg to 10.9% at 60mg). The reasons for withdrawal were mostly related to nisoldipine's pharmacologic activities and within the scope of common adverse experiences:

Reasons for withdrawal	Nisoldipine(%) N=678	Placebo(%) N=280
headache	3.8	0.4
peripheral edema	2.9	0.4
vasodilatation	1.5	0.0
nausea	0.9	0.0
palpitation	0.9	0.0
dizziness	0.7	0.4

There were 2 **deaths** (car accident and metastatic prostate cancer) in nisoldipine-treated patients in controlled trials (non-U.S. studies only), compared with two deaths in the placebo groups. None were considered drug-related. Other **serious events** occurred with similar frequencies in nisoldipine (2.0%) and placebo group (1.5%). However, they are dose-related (increased from 0.2% at 20 mg to 4.5% at 60 mg) and half of these serious events led to withdrawal.

Abnormal **laboratory** findings in controlled trials were both rare and no different between nisoldipine and placebo groups. In U.S. controlled trials, incidences of such reports were in the range of 0-4% for hematology, 0-2% for hepatic functions, 0-1% for creatinine/BUN and 0-6% for lipid profile. While there were more reports of increased fasting blood glucose in nisoldipine than in placebo group from non-U.S. controlled trial, the phenomenon was not dose-related, not seen in the U.S., and cases of increases to above 140 mg/dl were not more frequent (than placebo).

Overall Exposures

In general, the overall safety experiences in all patients treated with nisoldipine in all clinical trials were not unexpectedly different from those described above for controlled trials.

Approximately 62% of all patients reported one or more **adverse events**, while the incidence was lower in European trials (43% vs 75% in U.S.). Prominent complaints were similar to those in controlled trials (e.g., 18% headache, 15% edema in U.S. Trial).

In all clinical trials, about 9% were **withdrawn** due to adverse experiences (10% in U.S. studies), not too different from that of comparative experiences. There was no additional death other than those noted above in the comparative experiences. Accumulative experiences of abnormal **laboratory** findings in all U.S. studies were also similar to that in the controlled trials.

Class Specific Safety Issues

As noted above, adverse experiences relatively specific to calcium channel blockers were also reported in nisoldipine-treated patients. They were not more severe or frequent than in other members of the class; however, the database may not be large enough for detecting some of the rare events.

Clinically significant **hypotension** and other related adverse experiences in nisoldipine treated patients were not common and rarely resulted in withdrawal. As described earlier in time-effect relationship, at doses that produced adequate trough blood pressure reduction, average peak response was not excessive. In all U.S. and non-U.S. trials, symptomatic hypotension occurred in about 0.2% and syncope was reported in 0.1% of patients. **Orthostatic** hypotension and related symptoms were slightly more common, reported in approx. 0.4% of patients on nisoldipine monotherapy, but very few were considered serious and required inervention. Overall, hypotensive reactions to nisoldipine treatment did not appear to be more frequent or severe than those with other calcium channel blockers. Appropriate warning related to hypotensive reaction is included in the draft labeling.

Like other dihydropyridines, nisoldipine has no significant effects on electrophysiology or **cardiac rhythms**. Tachycardia was reported in about 1% of all nisoldipine-treated patients, with a small mean changes in heart rate (< 1bpm, placebo-adjusted). It is most likely due to hypotensive reflex, rarely led to withdrawal, and occurred equally frequently in placebo groups. Some minor changes in ECG (QRS) were noted more frequently than that in placebo group, especially in patients receiving concomitant atenolol, but the magnitudes were of no clinical meaning. While dose (plasma level) and magnitude of BP reduction-related **T-wave flattening/inversions** were observed in a small phase II study (D90-022) with rapid dose escalation, such ECG finding was less clearly related to dose and not as frequent (similar to that in placebo groups) in a retrospective but blinded analysis of data from three efficacy trials. It is somewhat re-assuring that no angina or thallium test-documented ischemia were reported in any of the patients with T-wave changes in Study D90-022.

Limited experiences with concomitant use of nisoldipine and atenolol, lisinopril or HCTZ have not identified any unexpected safety or tolerability issue. Combination of nisoldipine with HCTZ or lisinopril may increase slightly the incidences of asymptomatic hypotension, tachycardia, palpitation and dizziness. Rebound hypertension after withdrawal has not been a problem with other dihydropyridines and was not significant in a small pharmacodynamic study for nisoldipine.

PEDIATRIC/GERIATRIC USE

There are no clinical trials assessing the efficacy or safety of nisoldipine in pediatric patients, either completed or in progress. The sponsor claimed that the drug has little potential for use in children and thus did not commit to any study in hypertensive children.

Efficacy and safety of nisoldipine as treatment for hypertension in the elderly (65 year and older) are not significantly different from that of general patient population.

DRAFT LABELING

The draft labeling submitted by the sponsor has been edited.

CONCLUSIONS

Nisoldipine appeared to be an effective and safe treatment for hypertension.

While there is little doubt that nisoldipine at 20-60 mg/day is an antihypertensive more effective than placebo, it is not certain if the entire useful dose range has been fully explored. Nisoldipine should be started at 10 mg once daily and titrated slowly (e.g. every few weeks) to 60 mg according to blood pressure response.

It is recommended that nisoldipine be approved with the edited draft labeling.

Shaw T. Chen, M.D.,Ph.D.

cc:
ORIG: NDA- 20-356
HFD-110
HFD-110/CSO
HFD-110/SChen/10/26/94

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MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

Date: 03/11/94

From: Shaw T. Chen, M.D., Ph.D., Medical Group Leader, HFD-110
Through: Director, Division of Cardiorenal Drug Products, HFD-110
To: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: NDA 20-356, Nisoldipine Core-Coat for Hypertension and Angina
Summary of Efficacy Data

INTRODUCTION

This memorandum will only summarize results from major controlled efficacy trials intended to support the above application, so preliminary decisions regarding approvability of the two indications can be made and deficiencies in efficacy data delineated. For the approvable claim, dose-response, safety and other labeling related issues are not covered here and will be described in a more comprehensive secondary review later.

As of the date of this memo, all primary medical reviews have been completed for both indications. While the hypertension claim is clearly approvable, as described below, the support

addition, Pharmacology Review is pending CAC deliberation on some animal tumorigenicity findings, which may be a potential approvability/labeling issue. Most importantly, several serious deficiencies in chemistry section of the NDA have been identified in the Chemistry Review, which may be ground for non-approval since they have not been corrected despite repeated request to do so by the Division.

This package is being transmitted with a draft Summary Basis of Approval (SBA) prepared by the sponsor, which has not been edited by the Division but appears to be accurate in its contents to serve as one of the references for secondary/tertiary reviews of the application. In the draft SBA, any description or interpretation of the data different from that of this memo should be disregarded.

HYPERTENSION

Major Trials Supporting Approval

Nisoldipine has been evaluated as an antihypertensive treatment in 1,914 patients at dosages up to 80 mg/day. The efficacy data supporting approval were derived from the results of 7 double-blind, parallel placebo controlled studies in 1,360 patients with hypertension, 886 of whom received nisoldipine. Long-term efficacy was supported by five open-label, 6-12 month follow-up of 554 patients (Studies X89-039, X90-019, X90-006, 675, 690).

The primary efficacy endpoint in each of these studies was the change in supine diastolic blood pressure (SDBP) from baseline at the end of dosing interval (trough effect) after 4-9 weeks of therapy. Data from five of the seven studies should be considered for major evidence of efficacy:

Study	Doses, mg/day		Duration	Remarks
D88-054	10, 20, 30	QD	4 wks	fixed dose
D89-026	10-40	QD	9 wks	dose titrated per response
D90-019	30, 60	QD	6 wks	fixed dose (after week 1)
D89-039	20, 40	QD	8 wks	fixed dose (after week 1)
D90-006	10, 20, 30	QD	6 wks	fixed dose (after week 1)

In the last three trials listed above, high doses were reached after one week of low dose administration. It should be noted that in Study D89-039, another group of 15 patients were randomized to receive nisoldipine CC 80 mg qd, but the arm was terminated due to safety concerns before collection of efficacy data. Nisoldipine was also compared with verapamil 240 mg (additional group of 78 patients) in this parallel placebo controlled study.

The remaining two controlled studies may provide instructions on how to use nisoldipine in a practical setting, but are not very useful as primary evidence for assessing efficacy of nisoldipine (vs placebo in general population who are not treated with other concurrent antihypertensive agents); in one (Study D89-029) nisoldipine was evaluated in patients all receiving atenolol 50 mg qd as background therapy and the other (Study D90-029) compared lisinopril, hydrochlorothiazide (HCTZ) and placebo in the presence of nisoldipine in all groups. Results of these studies will be commented in the Section of "Comparison/Combination with other Antihypertensives" in the final secondary review.

Overall Treatment Effects vs Placebo

The **primary efficacy** data in the table on Page 4 demonstrate that nisoldipine, at 20-60 mg qd, is a consistently and significantly more effective antihypertensive agent than placebo with adequate duration of activity for once daily treatment. At this dose range,

the placebo subtracted net decreases in SDBP at trough ranged from 3.6 to 9.9 mmHg after 4-9 weeks of therapy. Treatment effects were less consistent for the 10 mg dose, but was superior to placebo in the larger trial (D90-006) with decent drop in SDBP. Similar results were obtained for supine systolic blood pressures (SSBP) (same Table) and standing blood pressures (excluding the smallest trial, D88-054, results not shown in this memo).

The **percentages of responders** (SDBP reduction of $\geq$ 10 mmHg at trough or to $\leq$ 90 mmHg) are also summarized in the table on next page. In the major trials, the response rates for 20-60 mg/day were in the range of 17-45% more than that of placebo. Again, less patients (around 17% over placebo) responded to 10 mg dose.

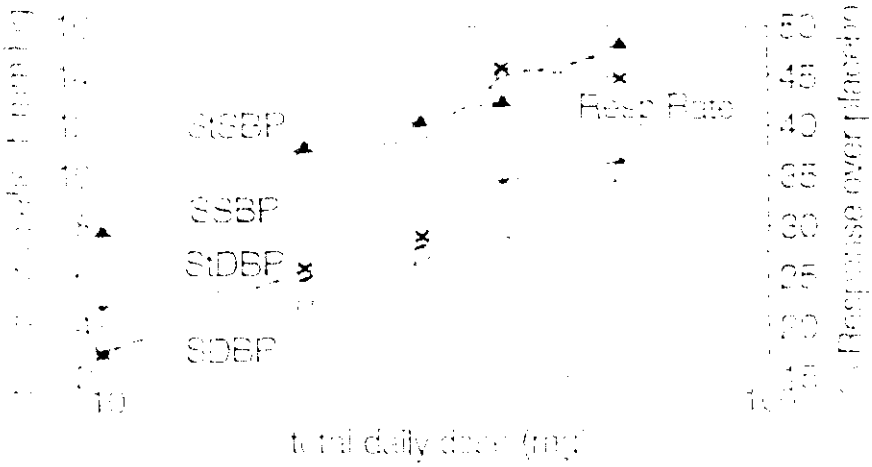
With respect to blood pressure changes and response rate, there were no significant differences between various statistical analyses, i.e. per-protocol or intent-to-treat (final visit).

Final visit. Placebo subtracted. These a significantly different from placebo

Change in supine BPs from baseline at trough

Analysis	regimen: Last Visit reg/day Wks	N/arm	10	20	30	40	60	10-40 titrated
SiDBP	D88-089 1-4	80	2.96	3.61	4.53			
ITT	D88-026 1-4	72						8.35
	D90-019 1-6	74			6.66		9.91	
	D89-039 1-8	76		4.03		7.33		
	D90-006 1-6	52	3.21	6.65	8.00			
	wtd avg		3.12	4.81	6.70	7.33	9.91	
SiSBP	D88-089 1-4	80	5.31	8.43	7.65			
ITT	D88-026 1-4	72						14.73
	D90-019 1-6	74			9.86		14.90	
	D89-039 1-8	76		7.33		13.96		
	D90-006 1-6	52	8.84	17.78	15.88			
	wtd avg		7.55	10.98	11.44	13.96	14.90	

Analysis	regimen: Last Visit reg/day Wks	N/arm	10	20	30	40	60	10-40 titrated
				16.7	25.8			
								40.9
					17.7		44.8	
				26.5		45.8		
				29.9	45.3			
				25.8	35.0	45.8	44.8	



Pages 5-9

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CONFIDENTIAL

COMMERCIAL

INFORMATION

CONCLUSIONS

Based on the efficacy data, nisoldipine appeared to be approvable for treatment of hypertension

Shaw T. Chen, M.D.,Ph.D.

cc:
ORIG: NDA- 20-356
HFD-110
HFD-110/CSO
HFD-110/Dern/Duarte/Stockbridge
HFD-110/SChen/03/11/94

13.1

NDA REVIEW
Clinical Pharmacology

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

FEB 16 1994

NDA:	20-356
Name of Drug:	Nisoldipine, Coat Core Tabs
Sponsor:	Miles
Indications:	Hypertension
Submitted:	03/31/93
Received:	04/05/93
Assigned:	04/09/93
Reviewed:	08/25/93
Review Completed:	02/14/94
Reviewer:	Shaw T. Chen, M.D.,Ph.D.

Overview of NDA (Clinical Pharmacology)

Nisoldipine is a calcium channel blocker of dihydropyridine derivative type being developed for the treatment of hypertension In this initial application, approvals of a sustained release formulation (coat-core) for both indications are requested. As a part of new parallel, team approach, this medical review covers only the areas related to clinical pharmacology.

The sections on clinical pharmacology (Section 8.* of NDA) contain data of 17 studies, involving 393 patients/subjects, on sustained released formulation (coat-core, referred to as CC in this memo). Of these, 183 participated in 6 U.S. studies. In addition, the submission also includes results of 47 studies on the immediate release preparation (IR), most of which were conducted in foreign countries. Except for three small studies (total 12 normal subjects, copies of publications only), full reports of all studies listed in Section 8.12 were submitted.

Additional pharmacokinetic and bioavailability data were presented in Section 6 of the NDA, which include 129 foreign studies on IR or other non-CC formulations not repeated in the clinical sections (Section 8, as noted above). These studies will be reviewed by the biopharmaceutical group of the Agency and not commented in this report.

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PHARMACOKINETICS

Formulation Design

The new coat-core formulation, which has a slowly-dissolving coat and an immediate release core, was designed based on the observation that nisoldipine is readily absorbed in the upper gastrointestinal tract but cleared by a first pass rapidly and a marked decrease in the rate of absorption but lower first pass metabolism in the colon.

Absorption/Disposition

The absorption of radiolabeled oral nisoldipine solution was rapid (T_{max} 0.42 hr) and extensive (87%). Despite efficient absorption, absolute bioavailability of the parent drug was only 8.4%, due to a high first pass effect (Study 400). Measured by iv infusion, nisoldipine has a volume of distribution about 2.3 to 3.4 L/kg (Study 330).

In **single dose** studies (Studies 102-106), oral doses of nisoldipine 6-20 µg/kg administered as IR capsules resulted in C_{max} of 2.7-19.3 µg/L within 30 minutes[1] after dosing. Nisoldipine was detectable (>1 µg/L) 4 hrs later only in the high dose groups (12 µg/kg and above). Compared with the IR formulation, nisoldipine administered in the **controlled release** (CR) forms had reduced C_{max}, greater AUC, prolonged mean residence time (MRT), duration of plasma concentration above 0.3 ng/ml and T_{max} (6 subjects, Study 632):

Formulation	C_{max} ng/ml	AUC_{norm} g.hr/L	MRT hrs	$T_{c>0.3}$ hrs	T_{max} hrs
CR (E 029)	0.86	57.2	21.2	23.9	12
IR	1.55	31.3	4.2	4.4	2
CR/IR	0.55	1.82	5.02	5.42	
(95% Confidence)	(0.34-0.90)	(1.30-2.56)	(3.48-7.23)	(3.48-8.45)	

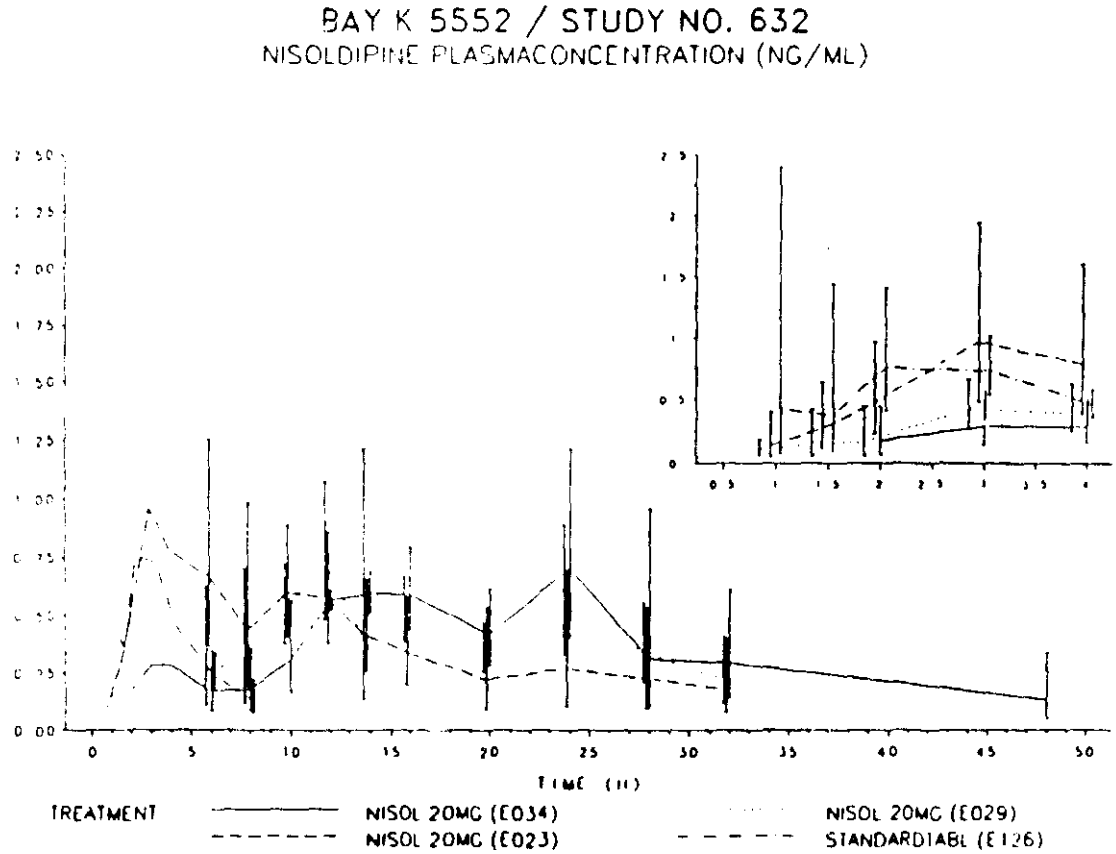
Time-courses of plasma concentrations after administration of the three CR formulations were compared with that of the IR dose in Figure 1. Based on these characteristics, the CR formulation E 029 was chosen from the three studied for further development. While the bioavailability was relatively higher than the IR form, absolute bioavailability of the CR formulation was still low (5.5%) in another study (Study 637).

Bioavailabilities (C_{max} and AUCs) of nisoldipine were dose-proportional for both the IR (at 2.5-20 mg, Studies 125, 339, D85-C24-01) and CC (at 10-60 mg) formulation (Study D91-035)[2].

[1] There may be greater variability in T_{max}, which was longer (mean 2 hrs) for IR nisoldipine in another study (632).

[2] The 10 mg dose may not be as linear as other doses, but the deviation is non-significant.

Figure 1



Although there is no evidence of accumulation with **multiple doses** of IR formulation (10 mg bid), bioavailability of CC nisoldipine increased moderately after 7 days of daily 20 mg dosing (see Table below, Study 645). At these doses, fluctuations of plasma nisoldipine levels were lower for the CC formulation (113% vs 434% for IR).

Formulation		C_{max} ng/ml	AUC_{norm} g*h/L	$T_{c>0.3}$ hrs	T_{max} hrs
CC	Day 1	0.84	40.3	14.9	11.1
	Day 7	1.09	58.9	28.4	9.2
IR	Day 1	2.18	40.8	10.8	2.4
	Day 7	1.95	40.3	11.6	2.3
CC/IR	Day 7	0.56	1.46	2.45	
(95% Confidence)		(0.47-0.66)	(1.27-1.69)	(1.95-3.08)	

While the AUC_{norm} remain similar regardless of fed or fasted state, C_{max} was increased by 38-48% when 20 mg of CC nisoldipine was administered together with or 1 hour after breakfast (Study 666). **Dose-dumping by food** of the CC formulation (administered within 5 minutes after completion of a meal) was even more pronounced with 30 mg (65-236% increase in C_{max}, 11-42% decrease in AUC) and 40 mg (92-292% increases in C_{max}, 7-39% decrease in AUC) doses in Study D92-045-02 (range given are 90% CI's). This food effect was both dose and formulation dependent, since C_{max} and AUC_{norm} were increased only modestly (31 and 28% respectively) by food for the IR formulation (20mg, Study 323), and may have clinical implications in patients usually older than those in the food studies (see Comments on Individual Studies below).

At the concentrations 20 times or higher than that observed in kinetic studies, nisoldipine and its enantiomers are >99% **protein bound**. Partitioning between plasma and blood or erythrocytes were moderate (0.7 blood to plasma, 0.3 erythrocyte to plasma). (Study 339, Ref. 3 (PB19611))

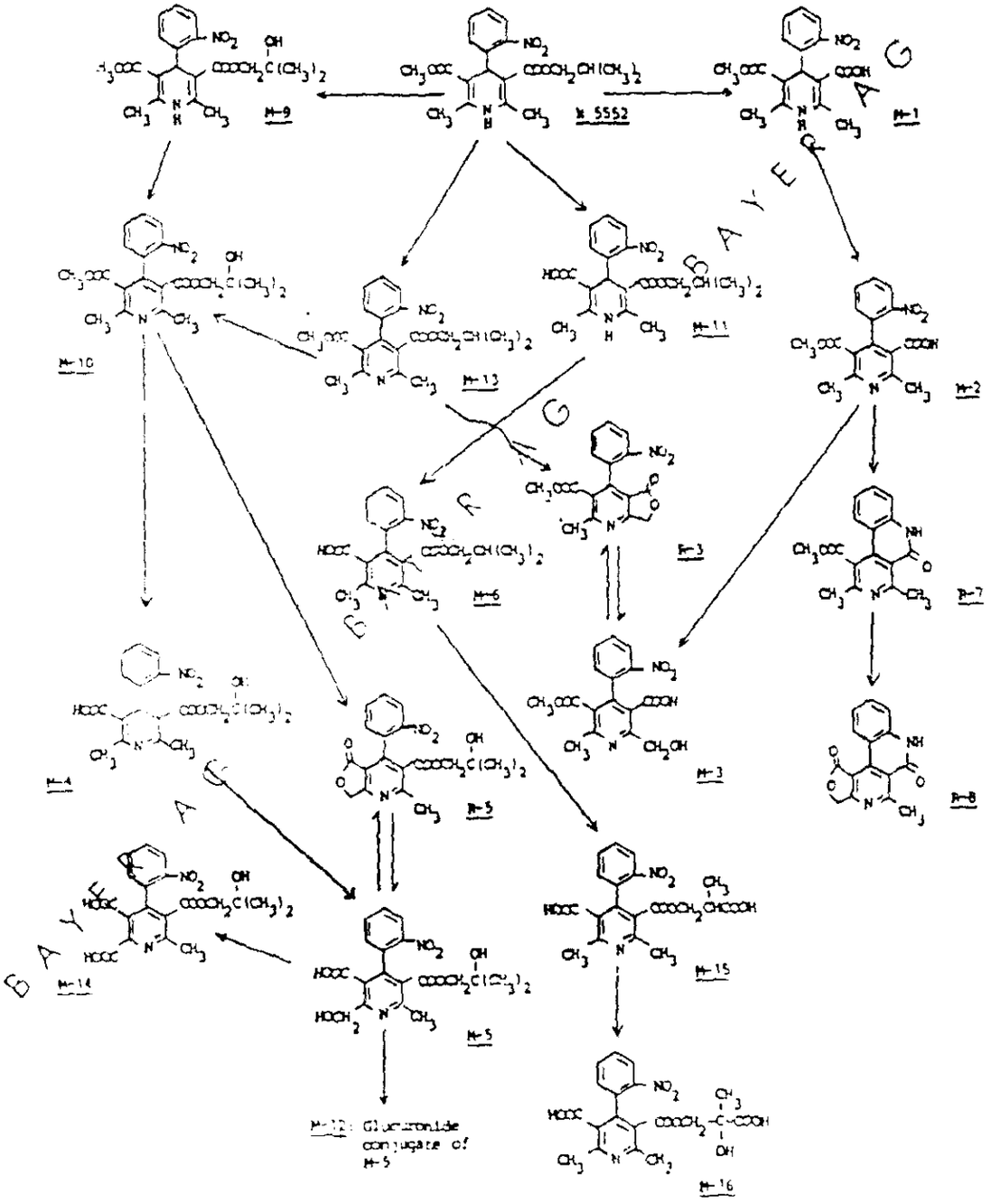
Metabolism

Following oral administration of nisoldipine solution, eleven metabolites, but no unchanged parent drug, was detected in the urine (Study 400, Reference 15 (PB 16626)). The proposed biotransformation pathways of nisoldipine in humans are described in Fig 2. In man, hydroxylation of the isobutyl ester appears to be the major product. Of the three metabolites detected in human plasma (M9, M10, M13), M-10 (Bay r 9590) is most abundant (approx 10-20 times of parent drug) and M-9 (Bay r 9425) is the only one with biological activity (about 1/10 of parent drug, Study P1010947). The latter is present at approximately the same[3], dose-proportional concentration as the parent drug (Studies 339, D85-024-01).

[3] In another study (No. 125), a metabolite was present at higher C_{max} and AUC (2-4 times of parent drug), which was later identified as sum of M-9 + M-10 due to nonspecific assay (12/20/93 Amendment)

Figure 2

PROPOSED METABOLIC PATHWAYS OF NISOLDIPINE



Excretion

Excretion of nisoldipine metabolites was predominantly renal (70% urine, 12% feces with oral dose), and varies little with route of administration (80% urine, 14% feces with iv dose) (Study 400). Terminal elimination half lives of iv nisoldipine, as measured in 4 healthy subjects in Study 330, were 11-12 hrs, with systemic clearance of 544-768 ml/hr*kg. Nisoldipine is probably not dialyzable (Study 311).

Pharmacokinetics of Enantiomers

The bioavailability of nisoldipine is dominated by the (+) enantiomer, when administered as racemic mixture with only the (+) enantiomer labeled with radioisotope (Ref 43), which is also the one with higher cardiovascular activity (in vitro studies). Since all clinical pharmacology studies and efficacy/safety trials were conducted using the racemic mixture, the kinetic parameters for the two enantiomers have no practical relevancy.

Pharmacokinetics in Disease States

Pharmacokinetics of nisoldipine CC formulation in **hypertensive patients** was examined in two double blind, parallel placebo controlled studies (D90-022 & D88-059). While the CC formulation was not compared with the IR form in any hypertensive groups, kinetics of nisoldipine in both normotensive and hypertensive elderly subjects were described in a third study (Study 712).

In study D90-022, 23 patients (5 placebo, 18 nisoldipine) were randomized and treated for 22 days with nisoldipine dosage increased every 4 days[4] from 30 to 60 mg and every 7 days from 90 to 120 mg. As summarized below, the kinetic parameters measured at the end of dosing periods were dose-proportional at 30-90 mg, non-linearity of 120 mg was dismissed for small number of patients (3). In this study, higher C_{max} of nisoldipine in hypertensive patients were reached at approximately the same T_{max} as that in normotensive subjects, but cross-study comparison is difficult to interpret.

Dose (mg qd)	C_{max} ng/ml	AUC_{0-24} ng.hr/ml	T_{max} hrs
30	4.79±0.68	74.28± 7.96	7.22±0.93
60	8.48±0.81	129.76±12.74	9.08±1.97
90	13.02±1.20	199.31±16.45	6.78±2.30
120	14.92±2.01	226.58±12.41	4.00±1.00

[4] Since the bioavailability of CC nisoldipine increased moderately from Day 1 to Day 7 in a study on normotensive subjects (Study 645, see above), 4 days may not be sufficient for reaching steady state for the two low doses in this study. Also, the ascending doses were not separated by washout period (see comments on individual study).

In the second study in hypertensive patients (D88-059), total of 69 patients were randomized in parallel to receive placebo, 5, 10, 20, or 30 mg CC nisoldipine for 7 days. Plasma nisoldipine concentrations were dose-proportional within the range of these doses both on Day 1 and Day 7 (with 40-70% increases from Day 1 to Day 7). The least square mean kinetic parameters at the end of 7 day dosing period are shown below:

Dose (mg qd)	C_{max} ng/ml	AUC_{0-24} ng.hr/ml	T_{max} hrs
5	0.65	8.39	9.21
10	1.02	16.17	4.79
20	2.13	28.24	3.65
30	2.79	40.34	3.73

At least in the elderly (>65), bioavailability of nisoldipine CC was not influenced by the elevated blood pressure in hypertensive patients (Study 712):

Day 7	Normotensive	Hypertensive
C_{max} (ng/ml)	2.61	2.59
AUC_{0-24} (ng*h/ml)	36.9	38.7

Bioavailability of CC nisoldipine in **angina patients** were also dose-proportional over the range of 20-60 mg (45 day treatment, long-term extension of Study D90-015). Data from 21 angina patients with mean age of 62 (range 43-77) resembled that of the elderly hypertensive patients (>65 year old, Study 712, see above):

Dose (mg qd)	No Patients	C_{max} ng/ml	AUC_{0-24} ng.hr/ml	T_{max} hrs
20	10	2.70	41.9	7.8
40	9	6.27	92.3	6.4
60	2	9.59	102.1	3.0

Despite the fact that metabolites of nisoldipine are eliminated predominantly by renal excretion, patients with various degrees of **renal impairment** (but not requiring dialysis) had similar pharmacokinetic parameters for the parent drug when given nisoldipine 20 mg in CC formulation for 7 days (Study D92-001).

Cr clearance	>90	61-90	30-60	<30
Day 8, Mean (ml/min/1.73m^2)				
C_{max} (ng/ml)	3.33	3.21	2.54	2.97
AUC_{0-24} (ng*h/ml)	40.0	50.3	38.4	43.8

However, pharmacokinetic effects of renal function on nisoldipine were slightly greater (although not significantly) with initial doses (Day 1)

Cr clearance	>90	61-90	30-60	<30
Day 1, Mean (ml/min/1.73m^2)				
C_{max} (ng/ml)	1.77	2.37	2.71	2.57
AUC_{0-24} (ng*h/ml)	25.3	32.8	36.1	32.1

Thus the accumulations of nisoldipine with multiple doses appeared to be blunted somewhat by the decrease in renal function (Day 8 vs Day1). As expected, elimination of some metabolites was more affected by renal function than the parent drug, but like nisoldipine, the differences were noted mostly on the first day and diminished over multiple dosing (Study D92-001). While there is less pharmacodynamic concern because the only active metabolite (Bay r 9425) was the least influenced by renal impairment, it not clear whether substantial increases (with the initial doses) of other more abundant metabolites by renal impairment has any long-term toxic effect. In this study the group with moderate renal impairment (Cr Cl 30-60 ml/min/1.73m^2) had higher mean age (63 vs 52-54 for other groups), but there is no clear trend suggesting that the conclusion was affected by such difference in age. Administered in the IR form, nisoldipine bioavailability was increased by about 40% when creatinine clearance decreased from >80 to <25 ml/min (Study 364). While nisoldipine was not detectable in the dialysate, thus probably not removed by hemodialysis, bioavailability of nisoldipine IR in patients on dialysis resembled that in subjects of normal renal function in the same study.

Hepatic failure increases bioavailability of nisoldipine administered as CC tablets. Compared with normal subjects, cirrhotic patients who received 10 mg nisoldipine CC had higher C_{max} and AUC_{0-24} (4-5 folds, Study D90-026). There appeared to be less effect of liver function on the bioavailability of nisoldipine in IR formulation, however, the studies were not controlled and the results were variable (Studies 294, 452).

Demographics Differences in Pharmacokinetics

While there is no significant difference with acute dosing (1 day), bioavailability of nisoldipine, administered as CC 20 mg daily for one week, was increased in the elderly normotensive subjects (65-84 years old), as compared with that in the younger subjects (Study 712):

Day 7	Young	Elderly
C_{max} (ng/ml)	1.41	2.61
AUC_{0-24} (ng*h/ml)	14.7	36.9

Bioavailability of IR nisoldipine was also higher (2-3 folds) in the elderly, but little accumulation was observed after one week dosing (Study 563).

Drug Interactions

The **effects of other drugs on nisoldipine pharmacokinetics** were evaluated in the following studies:

Immediate Release Formulation

Second Drug, Doses	Nisoldipine Doses	Interaction Observed	Study
Ranitidine, 300 mg qd X 3 days vs placebo	IR 20 mg one dose on Day 3	nisoldipine AUC increased 24%	385
Cimetidine, 400 mg one dose then 200 mg tid X 3 doses vs no treatment	oral & iv solution 10 mg po, 0.374 mg iv one dose each period	bioavailability of oral nisoldipine increased by 48%	399
Propranolol, 40 mg one dose vs placebo	IR 20 mg one dose	nisoldipine AUC, C_{max} increased by 30% & 57%	417

CC Formulation

Second Drug, Doses	Nisoldipine Doses	Interaction Observed	Study
Ranitidine, 150 mg bid X 6 days vs placebo	CC 20 mg one dose on Day 5	nisoldipine AUC, C_{max} decreased by 15-20%	738
Cimetidine, 400 mg bid X 6 days vs placebo	CC 20 mg one dose on Day 5	nisoldipine AUC, C_{max} increased by 30-45% t_{max} decreased by 4 hrs	738
Propranolol, 40 mg tid X 5 days vs no treatment	CC 20 mg qd X 5 days	nisoldipine AUC, C_{max} unchanged, $t_{1/2}$ decreased by propranolol (by 20%)	704
Quinidine, 648 mg bid x 2 doses vs no treatment	CC 20 mg qd x 1 dose	nisoldipine AUC reduced by 25%, C_{max} unchanged but at lower t_{max}	703

Bioequivalence of Various Formulations

Bioequivalence between clinical trial and market tablets and between various coat-core dosage forms have been determined in several studies. Details of the results are referred to the Biopharmaceutical Review.

Comments on Individual Pharmacokinetic Studies

In general, pharmacokinetic studies on nisoldipine, either CC or IR formulation, were well designed and properly conducted. Compared with the translated foreign reports, the U.S. studies (study numbers beginning with D) were better documented and probably more reliable. Minor deficiencies for a few studies and interpretation of the data different from that of sponsor have been pointed out, mostly as footnotes, in previous sections. Other than that, there are no study defects collectively serious enough to invalidate the conclusion on kinetic behavior of CC nisoldipine. In addition to the following comments, which are arranged below in the order of Study numbers, further detailed reviews on individual kinetic studies are referred to the Biopharmaceutical Review.

Studies 102-106

Based on a summary report (no detailed protocol), there is nothing remarkable in these placebo controlled, dose-escalating, kinetic studies using IR formulation in 12 normal subjects.

Studies 125, 339

These were placebo-controlled, double-blind, randomized, 3-4 sequence crossover studies on dose-proportionality of nisoldipine IR and metabolites in 12 normal subjects (6 actually treated in Study 125). While treatments were separated by at least one week in Study 339, they were given in three successive days in Study 125. Thus results of the latter study may be confounded by residual effect of preceding dose.

In vitro protein binding of nisoldipine at 20 ng/ml was also performed in Study 339, using each subject's pre-dose plasma.

Studies 294/452

These are two pharmacokinetic studies of IR nisoldipine and its metabolites in cirrhotic patients. The results were translated from foreign reports and no detailed protocols were submitted with the NDA. Subjects in Study 294 were hypertensive but the blood pressures were not described in Study 452. Neither was controlled with subjects of normal liver function.

Study 311

This is a kinetic and tolerability study in patients requiring regular hemodialysis. Seven patients were treated with nisoldipine 10 mg once daily, with dosage titrated up to 40 mg per day, for 3 months. The treatment effects on blood pressure and tolerability were not controlled.

Study 323

This is a food-pharmacokinetic study of IR nisoldipine 20 mg dose. Eight healthy, young male subjects were randomized to receive a single dose of the study drug either in a fasted (1.5 hrs pre-meal) or fed (20 minutes after start of meal) state and crossed over to the opposite food state two-weeks later. Small differences in heart rate response in fasted/fed states were noted (increased 10 bpm vs increase 15 bpm), but probably of no clinical significance.

Studies 330, 400

These were two uncontrolled bioavailability studies using oral/iv solution in small groups of healthy volunteers. A few subjects were excluded from data analysis due to radioisotope overdose in 2 of 12 subjects in Study 400 and leakage of infusion system in 2 of 6 subjects in Study 330. Washout interval was adequate for the crossover study (28 days, Study 400).

Study 364

This is also a kinetic study in renally impaired patients (Cr Clearance >80 ml/min, <25 ml/min or uremic on dialysis, 29 patients), but treated with single dose IR formulation only. The sponsor concluded that a 40% increase in nisoldipine bioavailability in renal failure was not a significant effect (see discussion above).

Study 400 (PB 14514, Biotransformation)

This was part of study 400 (see above for comments on bioavailability study) which described metabolite profile in human urine after oral and iv administration of radioisotope labeled nisoldipine. Eleven metabolites were identified, but no test of biologic activity was performed and plasma metabolite profile was not investigated.

Study 563

This is a pharmacokinetic study of IR nisoldipine in 21 normotensive subjects, 9 were of 20-28 years of age and 12 were older than 65. All were treated with IR nisoldipine 10 mg for 8 days.

Study 632

Three controlled release formulations (CR) were evaluated in this non-blind, randomized, crossover, single dose study in six healthy volunteers. The kinetics of CR formulation was compared with that of IR nisoldipine administered in the fourth period to all subjects, the wash-out between treatment periods was 6 days. Treatments were given in fasted states.

Study 637

Bioavailability of a controlled release formulation (CR E 029) selected from Study 632 was evaluated further, relative to an iv solution of nisoldipine, in this non-blind, crossover study in 12 normal subjects. Washout out between treatments was adequate (6 days) and study drugs were administered in fasted states.

Study 645

Steady-state pharmacokinetics of nisoldipine CC 20 mg was compared with that of IR formulation given as 10 mg bid in this non-blind crossover study. The study drugs were given in fasted state for one week and the treatments were separated by 7-day washout periods. Total of 18 male subjects were treated and included in the data analysis.

Study 666

This is an open-label study of food effect on pharmacokinetics of nisoldipine 20 mg CC tablets. Twelve young male subjects (24-33 years old) were randomized to receive the a single dose of the study drug in fasted (2 hrs pre-meal), together with (within 7 minutes after start of meal), or one hour after an American breakfast. All subjects also receive another dose together with a Continental dinner in a non-randomized fourth period. Treatment periods were separated by one week washouts. Moderate increase in C_{max} were observed when nisoldipine 20 mg CC was given in fed states, but no difference in clinical adverse events was noted.

Study 712

Bioavailabilities of nisoldipine CC in normotensive and hypertensive elderly (65 and older) patients were compared in this open-label, non-randomized study. In addition, influence of age on pharmacokinetic was also assessed in young and old normal subjects in the same study. Total of 58 subjects (46 young/elderly normal subjects, 12 hypertensive elderly) were treated with daily doses of 20 mg for 7 days.

Study D85-024-01

This is another dose-proportionality study using an ascending-dose, uncontrolled, non-crossover design. Single doses of nisoldipine IR 2.5-20 mg were administered at weekly intervals to twenty subjects. Kinetics of major metabolites in plasma were also described.

Study D88-059

This is a double-blind, placebo-controlled, multiple-dose, kinetic and tolerability study of nisoldipine CC in 69 hypertensive patients. After a 3-week placebo run-in, patients with SDBP of 95-115 mmHg were randomized to receive placebo, 5, 10, 20 or 30 mg nisoldipine CC in five parallel groups and treated for 7 days. The study design was better than that of higher dose range (D90-022, above) and dose proportionality in hypertensive patients was clear over the range of doses in this study. However, quite a few patients were excluded from the analyses for various reasons, which included 6 disqualified for low baseline SDBP (but included in the kinetic data), 10 without steady state blood samples and data of another 10 were considered invalid because these patients took nisoldipine instead of placebo on Day 0. One additional drop-out was due to blood drawing discomfort.

Study D90-015

Data from long-term extension of this angina efficacy trial were cited for bioavailability of nisoldipine CC formulation in patients with angina. See Medical Review on angina efficacy by Dr. Stockbridge for description of trial design and execution.

Study D90-022

This is a double blind, placebo-controlled multiple ascending dose, kinetic and tolerability study of nisoldipine CC in 23 hypertensive patients. Patients were randomized after a 3-week placebo run-in and the dosages were increased every 4 days for the two low doses (30 & 60 mg)

and every 7 days for higher doses (90 & 120 mg) without washout intervals (see above). The concern was that 4 day may not be adequate for reaching steady state and the carry over effects from previous lower dose can not be excluded. Thus the results of this study should be accepted with reservation and dose-proportionality is better supported by a parallel design (Study D88-059, but covered a lower dose range, see next).

Study D90-026

Pharmacokinetics of nisoldipine CC in patients with cirrhosis was evaluated and compared with that in normal subjects in this study. Sixteen, 8 in each group with well matched age, sex and weight, were treated with 10 mg daily for 7 days. Accumulation was observed in both groups.

Study D91-035

This was an open-label, four period crossover study on dose-proportionality of CC nisoldipine. Twenty-four healthy male subjects were randomized to one of four treatment sequences with single doses of 10-60 mgs. Treatments were separated by 7 day wash-outs and given in a fasted state.

Study D92-001

This is a unblinded pharmacokinetic study in patients with renal impairment, using the group with creatinine clearance of >90 ml/min/1.73m^2 as the control for renal function. In addition to the parent drug, kinetics of three major metabolites were also described. Of the 46 patients were treated with nisoldipine CC 20 mg once daily for 7 doses, 42 had valid kinetic data. While the overall recruitment had encountered some difficulty, two subjects were excluded because of "over-enrollment" in the mild renal impairment group. Two additional patients had indeterminate renal function and were excluded from the kinetic analysis. As noted in the above, age was not well matched in the four treatment groups, but no trend was discernible.

Study D92-045-02

This is the most important pharmacokinetic study on food effects, which described a significant dose-dumping phenomenon when nisoldipine CC was administered immediately (within 5 minutes) after a 20-minutes standard high-fat breakfast (see above for description of pharmacokinetic results). This was a randomized, open-label, two-way crossover study. Twenty-eight healthy, young (19-42 years), male subjects with near ideal weights were randomized to receive a single 30 or 40 mg CC tablet in a fasted (4 hour pre-meal) or fed state. After a one-week washout period, all subjects were crossed over at the same dose level to the opposite food state. Except for a higher heart rate in the fed state, the sponsor claimed that despite the marked change in kinetic behavior of nisoldipine CC, there were no pharmacodynamic consequences of dose-dumping by food. In 5 subjects with fed/fasted C_{max} ratio of ≥ 5, more complaints of headache were seen in the fasted state (3 vs 1) and one subjects reported dizziness under fed state only.

Study PB 16626

This was a study on biotransformation of nisoldipine in several animal species and in human. Metabolites detected in urine, but not in plasma, were identified. There were no qualitative differences in biotransformation of nisoldipine in rats, dogs, monkeys or man. Biological activities of metabolites were not described.

Study PB 19611

This is an *in vitro* human plasma protein binding and erythrocyte-plasma partitioning study of nisoldipine over the concentration range of 0.1 to 10 μg/ml. Results of protein-binding in this ^{14}C study were consistent with that of Study 339.

Study P101094.

Results of this foreign study (possibly animal) was cited in the NDA as the basis of the biological activities of various nisoldipine metabolites. However, there is no synopsis of the study anywhere in the application and despite repeated request by the Agency, the sponsor has had difficulty locating and submitting a full report as of the date of this memo.

Study (Reference 43)

This is a manuscript published by Frost et al in *Dose-Response Relationships of Drugs*, no original data were submitted.

Drug Interaction Studies

The results of studies on drug-interactions were separated into the following three groups:

Effect of other drugs on pharmacokinetics of nisoldipine.
Effect of other drugs on pharmacodynamics of nisoldipine.
Effect of nisoldipine on other drugs' pharmacokinetics/dynamics.

Only the first category was included in the Pharmacokinetic Sections, the remaining two will be discussed in the pharmacodynamic sections below. Limitations of individual studies, such as single/multiple doses, duration of treatment and use of control groups, have been described in tables summarizing the results.

Summary of Pharmacokinetic Issues

In summary, pharmacokinetic properties of nisoldipine administered as IR or CC formulations have been studied adequately and well-described in the submission. Bioavailability of nisoldipine CC was low due to high first pass effect and appeared to be linearly proportional to doses of 5-90 mg. Accumulation after 7 days of administration was modest and products of extensive metabolism were excreted renally. Relative to the young subjects, nisoldipine was slightly more bioavailable in the elderly after one week dosing. Bioavailability of nisoldipine was not significantly different in patients with hypertension, angina or renal impairment (except for the initial doses for the latest), but were markedly increased in hepatic failure subjects. Pharmacokinetics of nisoldipine was affected by concomitant administration of cimetidine, ranitidine and quinidine, other drug interactions may be possible through high degree of protein binding (> 99%).

While the kinetic data of CC nisoldipine support a longer dosing interval than the IR form, clinical effectiveness of once-daily dosing depends on the correlation with pharmacodynamic and efficacy findings. It should also be noted that the problem of dose-dumping when nisoldipine CC is administered in a non-fasted state can not be ignored and must be addressed appropriately in the labeling. This phenomenon is more pronounced than with the IR formulation and not exactly unexpected since similar problem has been observed in another approved drug formulated identically and developed by the same sponsor.

The differences in food effects between 20 mg (Study 666) and 30, 40 mg doses (Study D92-045-02) of nisoldipine CC may be due to variations in relative timings of drug administration and meal ingestion both in fasted and fed states. Both the absolute C_{max} and increase relative to fasted state were greatest when nisoldipine CC 30-40 mg was administered immediately after completion of a meal and compared to a prolonged fasted state (for additional 4 hrs post dose) (Study D92-045-02). While this sustained "fasted state" (4 hrs post dose) may not be a realistic simulation of large efficacy trials or practical settings, the dramatic increase in plasma nisoldipine concentration by food may result in excessive hypotension because nisoldipine plasma levels in efficacy trials resembled that of fasted state and there is a good kinetic-dynamic correlation (see below). Thus nisoldipine CC should not be administered concomitantly with meal, but instead after overnight fast and 1-2 hours before breakfast. Appropriate instruction to avoid dose administration in a fed state should be included in the labeling.

PHARMACODYNAMICS

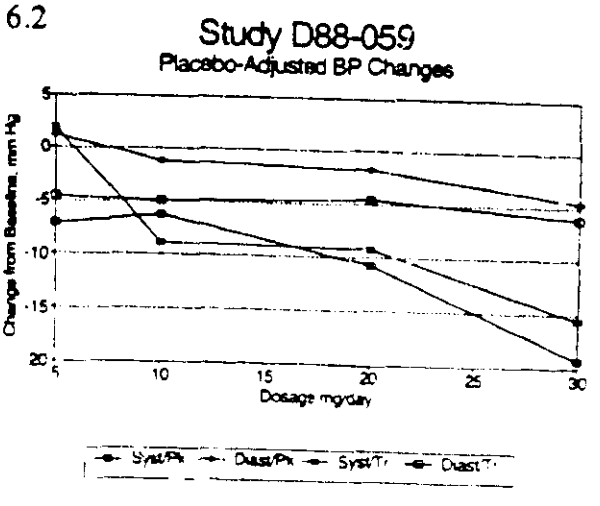
Pharmacodynamic data of nisoldipine are described in three groups in this review: **Principal cardiovascular effects**, **Non-cardiovascular pharmacological activities** and **Drug interactions**. Integrated summary of pharmacodynamic data as presented in the application and reviewed below were in general based on U.S. studies. Results of small scale foreign studies, mostly open and uncontrolled, were only commented briefly whenever appropriate. It should be noted that most dynamic data were obtained from studies using iv or IR oral form, only the effects on blood pressure and heart rate have been evaluated with the CC formulation. Issues related to tolerability of nisoldipine CC formulation in kinetic studies were also summarized at the end of this section.

Principal Cardiovascular Effects

In contrast with what the sponsor has stated in the NDA, **blood pressure and heart rate** changes were not small nor inconsistent in normal subjects treated with CC nisoldipine. Approximately 4-8 hours after a single dose of 10-60 mg, mean supine diastolic pressure decreased by a maximum of 5-7 mm Hg and mean heart rate increased by 6-11 bpm in a uncontrolled study (D91-035, Appendix 13.9.10). Similar but slightly greater effects (8-9 mm Hg) on SDBP were also seen with a single 60 mg dose in Study D90-020, before adjusted for a placebo response of about 4 mm Hg drop (Study Report Section 13.9.4). Mean Heart rate increases in the same study were 10-15 bpm (vs 2-4 bpm for placebo). The IR formulation produced more rapid but comparable degrees of heart rate and SDBP changes (Study 125, single dose). Relative to the normal subjects, the blood pressure responses of hypertensive patients were certainly more notable with multiple dosing at 5-40 mg/day (about 10 mm Hg drop in SDBP over placebo with IR form, Study 372). *Placebo-subtracted* blood pressure reductions in patients with mild to moderate hypertension were dose-related from 5 to 30 mg of CC nisoldipine (especially in systolic pressures, Study D88-059):

Dose	Changes in Systolic/Diastolic BPs, mm Hg, Day 7	
mg/d	8 hrs post dose	24 hrs post dose
5	- 7.1 / + 1.2	+ 1.9 / - 4.6
10	- 6.3 / - 1.2	- 8.8 / - 4.9
20	-10.7 / - 1.7	- 9.2 / - 4.6
30	-19.2 / - 4.6	-15.6 / - 6.2

On the right dose-response plot, 8 hrs data were used for peak effects. However, due to a large placebo response at 6-10 hours post dose in this study, the SDBP changes at 8 hrs did not appear to be the peak effects and greater antihypertensive activity was noted around 14 hours (-2.8 to -8.6 mm Hg over placebo, Study Report Section 13.9.4).



However, such dose-response relationship was not observed at higher dose range (30-90 mg/day) in another study of hypertensive patients (see Table on next page, D90-022)[5].

Study D90-022

Dose	Changes in Diastolic BPs, Placebo adjusted, mm Hg	
mg/d	8 hrs post dose	24 hrs post dose
30	- 9.4	- 6.4
60	- 9.2	- 6.7
90	- 9.1	- 6.6

Responses to 120 mg was distinctively higher (19.0 and 9.3 mm Hg at 8 and 24 hrs post dose) in the same study, but there were too few patients (3) received this maximum dose. As noted before, the dosages were forced-escalated in rapid sequence and not evaluated in parallel groups in this study, thus made the interpretation difficult.

Changes of heart rate in hypertensive patients due to nisoldipine appeared to be less consistent than that observed in normal subjects (Study 372, IR form). As noted in the NDA, heart rate increases were in the range of 5-10 bpm over baseline values for all nisoldipine groups in Study D90-022. However, it is not apparent whether the changes were caused by nisoldipine since the variation of heart rate in the placebo patients was in the same range and there is no clear trend in the difference between groups that suggests a treatment effect. Heart rate changes were not documented in Study D88-059.

With intravenous administration of nisoldipine at 3-13 µg/kg, changes in blood pressures (decreased by 11-16 mm Hg) and heart rates (increased by 10-30%) were dose-related and slightly more pronounced than that observed in the oral studies. Most of such studies were single-dose, uncontrolled, in small number of patients with underlying coronary artery disease (some were receiving concurrent beta blockers during measurements, Studies 344, 560, Ref 1-5)[6]. Directly compared with diltiazem in a published report (Ref 4)[6], nisoldipine (6 µg/kg) increased heart rate (by 14 bpm) but not diltiazem (500 µg/kg). Both drugs reduced peak systolic pressure by 24-28%.

[5] The sponsor claimed that blood pressure reductions not adjusted for placebo response were dose-related (see Summary of Clinical Pharmacology, Section 8.14), but admitted that the relationship did not exist when placebo responses were subtracted (see Study Report, Section 10.6).

[6] For reference cited, see List and Location of Publications, Section 8.14.3, NDA Vol 116. It should be noted that Ref 1-4 were reported by the same group of investigators.

Effects of nisoldipine on **other hemodynamic parameters** were summarized from 9 studies, three with full reports submitted with the NDA and 6 from published literatures (Ref 1-6, reprints only, no original data). Of these, hemodynamic effects were measured with intravenous administration of nisoldipine in 7 reports and with oral dosing in 2. In a double-blind, placebo controlled study (Study 372), nisoldipine IR titrated from 5 to 40 mg/day (bid) every 2 weeks were effective in blood pressure reduction for 36 hypertensive patients (see above) and reduced peripheral resistance as expected from a calcium channel blocker. Compared to placebo, cardiac index was increased either at rest or during exercise, but the effect of oral nisoldipine on LVEF was probably not meaningful. Changes in hemodynamic parameters as measured by radionuclide techniques are summarized below:

Changes/Baseline:	Resting		Exercise	
Parameters	Nisoldipine	Placebo	Nisoldipine	Placebo
Heart rate (bpm)	- 3.1/77	- 1.9/76	- 0.5/129	- 3.4/131
SBP (mm Hg)	- 24.1/172	- 8.5/171	- 20.4/219	- 13.3/217
DBP (mm Hg)	- 20.1/110	- 8.3/109	- 13.8/123	- 3.5/116
Total Peripheral Resistance (dynes/s/cm^5)	-302/1435	-132/1519	-165/953	- 32/940
Cardiac Index (L/min/m^2)	+ 0.31/4.1	+ 0.05/3.9	+ 0.37/7.4	- 0.01/7.4
Stroke Index (ml/m^2)	+ 5.6/53	+ 2.4/52	+ 2.7/58	+ 1.3/56
LVEF	+ 0.04/0.63	- 0.01/0.67	+ 0.02/0.75	+ 0.00/0.75
Double Product	- 23.9/134	- 9.2/130	- 27.4/283	- 24.7/288

A single oral dose of 10 or 20 mg IR nisoldipine decreased systemic vascular resistance significantly, but maintained same cardiac output, as measured invasively in 12 patients undergoing electrophysiology evaluations (Study 178, uncontrolled):

	Baseline	2 hrs post dose	
Heart rate	64	73	bpm
arterial pressures, Syst/Diast	130/76	120/69	mm Hg
Right Atrial Pressure	4	2	mm Hg
Pulmonary Arterial Pressures S/D	19/8	20/8	mm Hg
Pulm Cap Wedge Pressure	8	7	mm Hg
Cardiac Index	3.75	3.71	L/min/m^2
Systemic Resistance	1307	1038	dynes/s/cm^5
Stroke Index	50	53	ml/m^2

In another single oral dose study (Ref 6)[6], nisoldipine 10 mg attenuated both the drop in exercise LVEF in patients with coronary disease (-10% after vs -18% before treatment) and the decrease in LVEF during cold pressor test in patients with signs of ischemia but normal coronary artery .

Acute effects of intravenous nisoldipine on invasive hemodynamic factors are fairly consistent in Study 560 and several published reports (Ref 1-5)[6]. As noted above, most of these studies were single-dose (3-13 μg/kg)[7], uncontrolled, in small number of patients with coronary artery disease (some were on concurrent beta blockers during measurements). Similar to that observed with oral administration, total systemic vascular resistance was decreased (by about 30-35%) with iv nisoldipine in all studies described (Ref 1-3)[6]. Nisoldipine iv also decreased intra-aortic and left ventricular systolic pressure (by 15-30%), but not LV end diastolic pressure in these studies. Nisoldipine did not appear to have negative inotropic activities. Ejection fraction and stroke volume were increased by 16 and 21-24%, respectively, and cardiac out was up 26-36%.

The hemodynamic effects of nisoldipine on **coronary blood flow** have been examined in several uncontrolled studies using intravenous nisoldipine. In patients with coronary artery disease, nisoldipine administered intravenously increased coronary blood flow, but only in normal vessels or in area with collateral supplies (by 38-52%, 6 patients on background therapy of atenolol, Study 344). It also dilated the coronary arteries for up to 15 minutes after a 0.5 or 1.0 mg dose infused over 4 minutes, which was not plasma level related, however (Study 560). In several published reports, nisoldipine increased coronary blood flow by 17-50% (Ref 1-4)[6], thus reduced the calculated coronary vascular resistance by 40-50%. Myocardial oxygen consumption changes (decreased by 4-8%) were not significant in these studies. Similar to nicardipine, nisoldipine reduced myocardial lactate production in patients evaluated for angina (Ref 5)[6].

In patients with stable angina, nisoldipine IR given as a single oral dose of 20 mg increase exercise tolerance by 200 watts-min, as compared with 10 watts-min for placebo (Study 126). Nisoldipine also reduced ST segment change more than that by placebo at the maximum stress (0.8 mm vs 0.1 mm) in the same study. Exercise duration was increased in a published report (Ref 6)[6], but the study was not controlled and no data on ST change were described. These **anti-ischemic effects**, however, were not observed in another single oral dose study of similar design (Study 643/649).

In an **electrophysiology** study using oral nisoldipine (baseline controlled, Study 178), sinus cycle length and AH intervals were shortened by 12% and 9%, respectively, about 120 minutes after a 10 or 20 mg single dose. Other ECG changes (QTc, QRS, HV, corrected sinus recovery time, effective ventricular and atrial refractory periods) were not significant. In another similar study using the same IR oral doses (Study 135), there were no significant changes in intra-cardiac conduction times and the effects of nisoldipine on sinus node were also mild (except for sinus node recovery time and SA conduction time, which were decreased by 11-15%, all other changes were less than 10%). However, electrophysiological measurements may not be performed at time of maximum effect (within 20-45 minutes of dosing) in the latter study.

[7] In [illegible] measurements were performed after a 4.5 μg/kg bolus followed by a constant infusion of [illegible]

With intravenous administration at single dose of 1.5 μg/kg, similar shortening of sinus period were noted at 15-40 minutes post dose, without other changes in refractory periods (Study 144). The effect of iv nisoldipine on sinus cycle length was blunted some what (to 7%) if the patients were pre-treated with beta-blockers (Study 257).

Some ECG changes, usually seen as T-wave flattening or inversion, appeared to associated with nisoldipine in several tolerability studies. They occurred most frequently (65% of 17 treated patients vs none of placebo) in Study D90-022, a 3-week double blind, placebo-controlled, ascending dose study in hypertensive patients. The incidence is probably dose and plasma concentration related, since in this study, it increased from 22% to 80% with doses up from 30 mg to 120 mg, observed at peak but not trough drug level in some patients, and reversible when study drug was discontinued. Also, patients who reported the ECG changes had significantly higher C_{max} than those who did not (Study Report Section 10.8.4). These reactions were correlated with blood pressure drop and hemodynamic effects have been proposed as the mechanism in literatures for this and other calcium blockers. Higher rate of T-wave ECG changes in Study D90-022 was attributed to rapid and forced dose escalation. This issue has been discussed in more details by Dr. Dern in his Medical Review.

Based on one double-blind, crossover study comparing **neurohormonal effects** of nisoldipine IR 10 mg, nifedipine 20 mg, and placebo (Study 199), nisoldipine has no significant effects on the renin-angiotensin-aldosterone system or noradrenaline concentration in normal volunteers.

Nisoldipine IR at 20 mg daily increased **regional blood flows** in liver and kidney, but the effect was transient and no different from that of placebo by Day 4 (Ref 7)[8]. Similar time course of effects was noted for some renal function parameters (GFR and sodium excretion) in the same study. Nisoldipine, administered intravenously, increased blood flow in forearm more than nitrendipine or nifedipine (Ref 8)[8].

Non-cardiovascular Pharmacological Activities

Nisoldipine IR given at 10 mg bid for 4 weeks has no significant effect on **thyroid** function tests or **prolactin** level in young and healthy subjects (placebo controlled, Study 670). Similar to verapamil (160 mg), nisoldipine inhibit **platelet aggregation** after 4 days of treatment with 20 mg daily. However, nisoldipine plasma concentration at such dose did not have the same anti-platelet effect *in vitro* and unlike verapamil, nisoldipine did not displace yohimbine from specific platelet binding sites (Ref 9)[8]. The sponsor claimed that nisoldipine, at 5-20 mg daily for 4 weeks, induced favorable changes (increased HDL and apoprotein A) in plasma **lipoproteins** in a group of 15 hypertensive patients (Ref 10)[8]. The study, however, had no concurrent controls. In a double-blind, placebo-controlled 3-week trial in normal subjects (Study 479), there were no significant differences between placebo and nisoldipine (10 mg bid) in **psychomotor** performance tests.

[8] For reference cited, see List and Location of Publications, Section 8.15.3, NDA Vol 116. Reference # 10 cited in this review correspond to Publications 1-4 of the list on Page 08.15.0000016, Section 8.15.3.

Drug Interactions

Pharmacodynamic interaction studies were conducted for nisoldipine and the following drugs:

a. Effect of Other Drugs on Nisoldipine Pharmacodynamics

Immediate Release Formulation

Second Drug, Doses	Nisoldipine Doses	Interaction Observed	Study
Ranitidine, 300 mg qd X 3 days vs placebo	IR 20 mg one dose Day 3	no differences in hemodynamics	385
Cimetidine, 400 mg one dose then 200 mg tid X 3 doses vs no treatment	oral & iv solution 10 mg po, 0.374 mg iv one dose each period	cimetidine had no additional hemodynamic effects[9]	399
Propranolol, 40 mg one dose vs placebo	IR 20 mg one dose	propranolol attenuates heart rate increase by nisoldipine	417

CC Formulation

Second Drug, Doses	Nisoldipine Doses	Interaction Observed	Study
Propranolol, 40 mg tid X 5 days vs no treatment	CC 20 mg qd X 5 days	no significant changes in hemodynamics	704

b. Effect of Nisoldipine on Other Drugs

Second Drug, Doses	Nisoldipine Doses	Interaction Observed	Study
Quinidine, 500 mg bid X 5 doses	IR 10 mg bid X 7 days vs placebo	quinidine AUC increased 17-26%, no ECG changes	384
Quinidine, 648 mg bid x 2 doses	CC 20 mg qd x 1 dose vs no treatment	no significant changes in quinidine kinetics	703
Warfarin, "steady state"	IR 10 mg bid X 21 days vs placebo	no change on warfarin level or anti-coagulation effect	349

[9] model dependent, see comments on individual studies

Propranolol, 40 mg one dose	IR 20 mg one dose vs placebo	propranolol AUC, C_{max} increased by 43% & 68% no effect on beta blockade	417
Propranolol, 80 mg bid X 7 days	IR 10 mg bid for 7 days vs placebo	propranolol AUC, C_{max} unchanged, higher heart rate w/ nisoldipine	Ref 57
Propranolol, 160 mg qd X 2 weeks	IR 20 mg qd for 2nd week vs placebo	propranolol AUC, C_{max} increased by 30% & 50%, further BP reduction and higher heart rate by nisoldipine	382[6]
Propranolol, 40 mg tid X 5 days	CC 20 mg qd X 5 days vs no treatment	propranolol AUC, C_{max} decreased by 14-15%, $t_{1/2}$ increased by 25%, no effects on hemodynamics	704
Atenolol, 100 mg qd X 2 weeks	IR 20 mg qd for 2nd week vs placebo	atenolol C_{max} increased by 20% further BP reduction and higher heart rate by nisoldipine	382[10]
Digoxin, 0.6 mg qd X 2 days then 0.3 mg qd X 20 days	IR 10 mg bid Days 9-22 (no control)	digoxin plasma level increased 7% (95%CI 3-20%)[11] no dynamic interaction	413
Digoxin, 0.25 mg bid X 7 days (pre-treated 15 days)	IR 10 mg bid X 7 days vs placebo	digoxin plasma level increased 15% ($p<0.05$) no dynamic interaction[13]	Ref 58[12]

[1] see comments on individual studies for design problems

[2] the sponsor has claim no kinetic interaction in this study, but the data are consistent with the results of Ref 58.

[3] heart failure patients

[4] the authors also noted interaction in pre-ejection periods, 139±11 ms with placebo vs 129±11 ms with nisoldipine

Tolerability Findings

Tolerability findings in pharmacologic studies are described as follows, which are included as part of dynamic characterization and not to be relied on heavily for safety assessment. For complete assessment of adverse experiences and safety profile of nisoldipine, reference is made to the reviews on efficacy/safety trials by Drs. Dern and Stockbridge.

As noted in the above sections on pharmacokinetics, studies referred to as "dose-tolerability" in the NDA were of short term (mostly 7 days) and conducted in small number of subjects. In general, nisoldipine administered in CC formulation was well-tolerated in healthy volunteers (Studies D91-035, D90-020), hypertensive patients (D88-059, D90-022) and in subjects with hepatic (D90-026) or renal impairment (D92-001). Adverse experiences reported frequently in these small studies were roughly dose-related and not unexpected from those observed for other calcium channel antagonists, which included dizziness, edema, flushing, headache, nausea, postural hypotension and tachycardia. Somnolence was a frequent complaint in patients with hepatic dysfunction and overall frequency of adverse events was higher in the renally impaired patients. Abnormal ECG with T-wave changes (see above in Pharmacodynamics, Electrophysiology) was noted in at least three studies, one in normal subjects (D90-020), one in hypertensive patients (D90-022) and one in patients with renal impairment (D92-001). However, it was not clear whether the ECG changes were correlated with any clinically intolerable signs and symptoms. Similar ECG changes had not been as prominent in Phase III efficacy/Safety trials (see Dr Dern's Review).

Comments on Individual Pharmacodynamic Studies

While most pharmacodynamic studies of nisoldipine were of reasonable design and execution, some dynamic activities were not controlled and such results should be accepted with reservation. Again, the U.S. studies were better documented and probably more reliable than the translated foreign reports. Minor deficiencies and interpretation different from that of NDA have been noted in the discussion above. The following comments are arranged in the order of Study numbers for reference.

Studies 101-107, 109, 110, 115, and 116

These were all small tolerability studies in 4-6 normal subjects each. The adverse effects observed were similar to that of U.S. studies.

Study 125

In this single dose, placebo controlled crossover study, decreases in SDBP and increases in heart rate were dose-related in 6 normal subjects. However, there may not be adequate separation between doses to rule out carry-over effect (see Comments on Individual Pharmacokinetic Studies for study design)

Study 126

Effect of a single oral dose (20 mg) nisoldipine on exercise tolerance was evaluated in this placebo-controlled, double blind study in 12 patients with stable angina. While nisoldipine improved exercise capacity and ST-segment changes, single dose data are basically of little use for these endpoints.

Study 135

This a electrophysiologic study of single oral dose of nisoldipine (10-20 mg). No significant changes in intra-cardiac conduction were found, but the study was not controlled and the measurements may have been performed too soon after dosing (see description of data above).

Studies 144, 257

These were uncontrolled electrophysiology studies of similar design and same dosage (1.5 μg/kg iv). In Study 257, patients were pretreated with atenolol 75 mg/day or pindolol 15 mg/day for 3 days.

Study 178

This is an uncontrolled hemodynamic and electrophysiologic study of nisoldipine in 12 patients undergoing arrhythmia evaluation. The patients were randomized to receive a single dose of nisoldipine IR 10 or 20 mgs orally. Invasive hemodynamic and electrophysiologic data were collected before and 120 minutes after dosing. Electrophysiology data were not cited by the sponsor in the pharmacodynamic summary (see description of results above).

Study 199

In this double blind, single dose, crossover study, nisoldipine IR 10 mg was compared with nifedipine 20 mg and placebo in 9 young and healthy subjects. Effects on blood pressure, heart rate and neurohormonal system were measured. Separation of treatment periods was adequate (one week).

Study 201

This is a small study in 12 Japanese healthy subjects. Nisoldipine was well-tolerated at single doses of 2.5-20 mg. No kinetic data were available.

Study 344

This is an open label, uncontrolled study of iv nisoldipine (3 µg/kg/3 min) on regional myocardial blood flow in 6 patients with coronary heart disease. Background therapy with atenolol 100 mg/day was continued for all patients.

Study 372

This is a double blind, placebo-controlled, 8-week study in 72 hypertensive patients. After a 3 week washout, the dosage was forced-titrated from 5 mg qd to 20 mg bid every two weeks. Ergometric exercise was performed by every patient but hemodynamic measurements were done in only randomly selected half of the patients. Treatment groups were well-matched.

Study 382

This is a double blind, placebo controlled drug-interaction study to assess the effects of adding nisoldipine to established beta blocker (atenolol or propranolol) therapies in normotensive subjects. Eight young and healthy subjects were randomized to one of the following two treatment sequences (a or b, reproduced from NDA Vol 148, Page 08 17 00130291.

a	Weeks	0-------1-------2-------3-------4-------5
	beta blocker (b) or placebo (p)	bbbbbbbbbbbb-------pppppppbbbbbb
	nisoldipine (n) or placebo (q)	---------nnnnnn----------qqqqqq
b	Weeks	0-------1-------2-------3-------4-------5
	beta blocker (b) or placebo (p)	ppppppbbbbbb--------bbbbbbbbbbbb
	nisoldipine (n) or placebo (q)	---------qqqqqq----------nnnnnn

In the above scheme, the third week (between the end of Week 2 and beginning of Week 4) was a washout. With either sequence, nisoldipine (n) was administered after one week of beta blocker (b) therapy but the matching placebo (q) was given after a week of beta blocker matching placebo (p), although they were both concomitant with beta blockers during Week 2 or 5. Thus the nisoldipine treatment was not well controlled.

Study 399

This is another drug-interaction study to evaluate the effects of pretreatment with cimetidine on the pharmacokinetics and pharmacodynamics of nisoldipine. Eight normal subjects were given a single dose of nisoldipine as a 10 mg oral solution or 0.374 mg iv infusion over 40 minutes (two crossover periods separated by 5 days), without (no placebo) and with cimetidine treatment (400 mg x 1 followed by 200 mg tid the next day of measurement). Using a sigmoidal E_{max} model, it was calculated that the hemodynamic changes due to cimetidine pre-treatment can be attributed to a 48% increase in nisoldipine bioavailability, and the sponsor concluded that cimetidine has no additional dynamic interaction with nisoldipine.

Study 479

This a double blind, parallel placebo controlled, 3 week study to evaluate the effect of nisoldipine (10 mg IR bid) on psychomotor functions in 30 normal healthy subjects.

Study 560

Changes in diameters of coronary arteries before and after iv nisoldipine (0.5 or 1.0 mg over 4 minutes) treatment were measured by angiography in 26 patients with coronary heart disease in this uncontrolled study. Plasma drug levels correlated with other hemodynamic activities but not vasodilating effects.

Study 648/649

This is another single oral dose study on the anti-ischemic effect of nisoldipine, but unlike Study 126 (see above), a CC tablet (20 mg) was tested. Despite a rigorous design (double blind, parallel placebo controlled, multi-center), nisoldipine had no effect on several angina endpoints measured. But again, not much has been shown with a single dose study.

Study 670

This study was double blind, placebo controlled in young and healthy subjects. Effects of nisoldipine IR treatment at 10 mg bid for 4 weeks on thyroid function and prolactin were assessed.

Study D88-059

See Comments on Individual Pharmacokinetic Studies for study design and execution.

Study D90-020

This is a double blind, parallel placebo controlled, two single-dose crossover study designed to demonstrate bioequivalence of 3x20mg and 2x30mg nisoldipine CC tablets. Blood pressure and heart rate effects, as well as tolerability data, were also collected.

Study D90-022

See Comments on Individual Pharmacokinetic Studies for study design and execution.

Study D91-035

See Comments on Individual Pharmacokinetic Studies for study design and execution.

Ref 1-4

As noted above, these four studies were published by the same group of investigators, thus not to be considered as four independent reports. Invasive hemodynamics, both systemic and coronary, of nisoldipine were measured in patients with coronary heart disease. Except for Ref 4, which compared nisoldipine (6 µg/kg) with diltiazem (500 µg/kg), none of the other studies were controlled. For these studies, only publication reprints were submitted (no original data).

Ref 5

Effects of nisoldipine on myocardial metabolism were compared with that of nicardipine in this paper published by M F. Rousseau et al. Thirty-two patients with angina pectoris were treated with nisoldipine 0.06-0.12 µg/kg infused intravenously over 10 minutes. Measurements were performed at basal state and during a cold pressor test.

Ref 6

This is a hemodynamic study of oral nisoldipine (10 mg IR single dose) on left ventricular function, using radionuclide angiographic techniques. Changes in left ventricular function were measured during exercise in 20 patients with chronic stable angina and coronary disease and responses to a cold pressor test was evaluated in additional 12 patients with ischemic pain and abnormal exercise test but normal coronary arteries. The study was not controlled. Reprint of publication only, no original data were presented in the NDA.

Ref 7, 8 (References 1, 2 of Section 8.15.3)

These are published reports of studies on regional blood flow (liver and kidney, Ref 7, forearm, Ref 8). Nisoldipine was given orally (20 mg/day x 4 days) in the former and intravenously (6 µg/kg) in the latter. The investigation was conducted in young, normal subjects in both studies. Reprints only, no original data reviewed.

Ref 9 (References 3 of Section 8.15.3)

Effects of nisoldipine (20 mg/day for 4 days) on platelet aggregation was compared with that of verapamil (160 mg/day for 4 days) in this published report. Reprint only, no original data.

Ref 10 (References 4 of Section 8.15.3)

Without a concurrent control, the claimed favorable effects of nisoldipine on lipoprotein profile can not be taken seriously and will not be described in the labeling. Also reprint of publication only, no original data.

Drug Interaction Studies

As noted above in the kinetic sections, drug-interactions were separated into the following three groups:

Effect of other drugs on pharmacokinetics of nisoldipine.
Effect of other drugs on pharmacodynamics of nisoldipine.
Effect of nisoldipine on other drugs' pharmacokinetics/dynamics.

The first category has been described in the Pharmacokinetic Sections. For the remaining two, limitations of individual studies, such as single/multiple doses, duration of treatment and use of control groups, have been described in tables summarizing the results. Studies 382 and 399 have been commented above in this section.

Summary of Pharmacodynamic Issues

Antihypertensive activity of nisoldipine has been demonstrated for both IR and CC formulations. Dosages ranging from 5 to 120 mg have been studied in at least 6 studies, however, dose-response relationship in these short-term studies on CC formulation was not consistent. While the placebo adjusted blood pressure reductions were proportional to doses of 5-30 mg/day, the response was flat over higher doses of 30-90 mg/day in a less well-designed study (see comments above). Systemic vascular resistance was consistently decreased by nisoldipine, but heart rate changes appeared to be mild in the pharmacodynamic studies.

Nisoldipine did not appear to have significant negative inotropic activities and except for a modest decrease in sinus cycle length, had no appreciable chronotropic effects either. However, the changes in T wave as observed in a few pharmacologic studies need to be re-examined in large efficacy/safety trials. Nisoldipine may increase coronary blood flow in patients with coronary artery disease but other measurements of anti-ischemic effect were not consistent.

There were no significant pharmacodynamic interactions between nisoldipine and ranitidine, cimetidine, or propranolol (with CC formulation of nisoldipine). Nisoldipine may increase bioavailability of quinidine, propranolol, atenolol and digoxin, but the extends were variable and of unclear dynamic consequences or clinical meaning.

Based on a somewhat limited experience, nisoldipine had no adverse pharmacologic effects on neurohormonal system, regional blood flow, thyroid and prolactin activity, lipoprotein profile or psychomotor functions. Nisoldipine may inhibit platelet aggregation and its clinical implication should be reviewed in the safety data. Nisoldipine CC appeared to be well-tolerated in the small scale clinical pharmacology studies, some of which were conducted in normotensive subjects. Adverse events were commonly seen in other calcium channel blockers.

PHARMACOKINETICS/DYNAMICS CORRELATIONS

As noted in the Summary of Pharmacodynamic Issues, placebo-adjusted blood pressure changes were dose-related for 5-30 mg (one week study n CC formulation, Study D88-059), correlation with plasma drug concentration and total bioavailability was also good:

Dose	C_{max}	AUC_{0-24}	Changes in Systolic/Diastolic BPs, mm Hg, Day 7	
mg/d	ng/ml	ng.hr/ml	8 hrs post dose	24 hrs post dose
5	0.65	8.39	- 7.1 / + 1.2	+ 1.9 / - 4.6
10	1.02	16.17	- 6.3 / - 1.2	- 8.8 / - 4.9
20	2.13	28.24	-10.7 / - 1.7	- 9.2 / - 4.6
30	2.79	40.34	-19.2 / - 4.6	-15.6 / - 6.2

Fit by linear regression was reasonable and suggested that plasma nisoldipine concentration of 2 ng/ml was required for a 5 mm Hg drop in diastolic BP over placebo. Such relationship was less clear at higher doses (30-90 mg/day, Study D90-022). When blood pressure responses and plasma drug concentrations were fitted with linear regression which included placebo data for plasma level of zero, estimated slopes were significant or nearly so for 30 mg and 60 mg. However, as noted before, placebo-corrected blood pressure reductions were not dose-related and the dosages were forced titrated rapidly in sequence in this study.

In four large hypertension efficacy trials (D88-054, D89-029, D89-039 and D90-019), which covered doses from 10 to 60 mgs/day, overall correlation between systolic/diastolic blood pressure reductions and plasma drug concentration was good for each study pooled over all dosages, and for 30-60 mg doses pooled over all four studies (Table on next page). The estimated slopes from linear regression analysis indicated that trough supine diastolic blood pressure decreased by 1.57 mm Hg per 1 ng/ml (overall pooled analysis), or 1.67-2.38 mm Hg per ng/ml for the three monotherapy studies.

In two Phase III angina trials (D88-060 and D90-015), placebo-subtracted changes in exercise durations from baseline were related to dose/plasma concentration as follows:

Dose	C_{max}	C_{min}	Changes in seconds, p for correlation with drug levels			
mg/d	ng/ml	ng/ml	Peak	p	Trough	p
10	-	0.79	-		1	0.037
20(D88-060)	-	1.25	-		20	0.905
20(D90-015)	2.10	1.57	29	0.012	34	0.184
30	-	2.16	-		32	0.054
40	3.70	2.56	6	0.108	7	0.017
60	5.70	4.07	34	0 577	37	0.777

Of these, the estimated slopes were significant or nearly so for 10, 30, 40 mgs at trough, and 20 mg at peak. Pooled over all dosages, the correlation was significant in Study D88-060 (10-30 mg, trough) only. Overall correlations between dose, plasma level and exercise tolerance in angina patients were poor.

Correlation of trough nisoldipine level and blood pressure responses:
(From NDA Section 8.13.8.2, Vol 116)

NISOLDIPINE/EFFICACY POOL — TABLE
US CC HTN — CORRELATION COEFFICIENTS OF TROUGH BLOOD LEVELS WITH TROUGH BLOOD PRESSURE
FOR ALL PATIENTS VALID FOR EFFICACY ANALYSIS

PROTOCOL	NIS CC 00 DRUG GROUP	N	SUPINE SYSTOLIC BP R	SUPINE SYSTOLIC BP P	SUPINE DIASTOLIC BP SLOPE	SUPINE DIASTOLIC BP R	SUPINE DIASTOLIC BP P	STANDING SYSTOLIC BP R	STANDING SYSTOLIC BP P	STANDING DIASTOLIC BP R	STANDING DIASTOLIC BP P
D88-054	ALL	87	-0.17853	0.0980	-2.11	-0.30895	0.0036	-0.11794	0.2766	-0.29230	0.0060
D89-029	ALL	158	-0.14702	0.0616	-1.03	-0.30298	0.0001	-0.22569	0.0044	-0.39461	0.0001
D89-039	ALL	114	-0.38121	0.0001	-2.38	-0.40175	0.0001	-0.37050	0.0001	-0.50104	0.0001
D90-019	ALL	119	-0.30830	0.0006	-1.67	-0.44582	0.0001	-0.29118	0.0013	-0.46786	0.0001
D88-054	10MG	30	0.01600	0.9331	---	-0.34391	0.0628	0.11973	0.5286	-0.23418	0.2129
D88-054	20MG	30	-0.47472	0.0080	---	-0.39234	0.0320	-0.39317	0.0316	-0.36920	0.0447
D88-054	30MG	27	-0.22195	0.2658	---	-0.16068	0.4233	-0.21617	0.2780	-0.34713	0.0761
D89-029	20MG	66	0.15689	0.2482	---	0.14361	-0.2910	0.12065	-0.3757	0.07007	0.6079
D89-029	40MG	49	-0.15621	0.2838	---	-0.32064	0.0247	-0.14882	0.3075	-0.53553	0.0001
D89-029	60MG	53	-0.09154	0.5145	---	-0.31901	0.0199	-0.17371	0.2135	-0.22792	0.1007
D89-039	20MG	59	-0.15072	0.2545	---	-0.26441	0.0430	-0.19165	0.1459	-0.28609	0.0280
D89-039	40MG	55	-0.36456	0.0062	---	-0.35004	0.0088	-0.41101	0.0018	-0.45768	0.0004
D90-019	30MG	66	-0.27969	0.0279	---	-0.32130	0.0085	-0.35486	0.0035	-0.42230	0.0004
D90-019	60MG	53	-0.27936	0.0428	---	-0.44821	0.0008	-0.22532	0.1048	-0.44383	0.0009
ALL	10MG	30	0.01600	0.9331	---	-0.34391	0.0628	0.11973	0.5286	-0.23418	0.2129
ALL	20MG	145	-0.05009	0.5496	---	-0.09741	0.2438	-0.15634	0.0604	-0.20408	0.0138
ALL	30MG	93	-0.26392	0.0106	---	-0.28517	0.0056	-0.31687	0.0020	-0.39962	0.0001
ALL	40MG	104	-0.22674	0.0195	---	-0.32601	0.0001	-0.25083	0.0102	-0.47381	0.0001
ALL	60MG	106	-0.17762	0.0685	---	-0.39735	0.0001	-0.19492	0.0453	-0.35785	0.0002
ALL	ALL	478	-0.26588	0.0001	-1.57	-0.39170	0.0001	-0.28519	0.0001	-0.45456	0.0001

CONCLUSIONS

It appears that pharmacokinetic properties of nisoldipine has been well-described for both the immediate release (IR) and the control release (CC) formulations. Variations in bioavailability of nisoldipine in patients of different concurrent diseases and demographic characteristics have been examined, which should be addressed in relevant sections of labeling, especially the issues of dose-dumping by food. Once daily use of nisoldipine CC tablets for hypertension is supported by the kinetic data.

While the pharmacodynamic profile of nisoldipine was studied mostly using intravenous and the IR formulations, it has been demonstrated that nisoldipine is a vasodilating antihypertensive with minor electrophysiologic effects and insignificant inotropic activities. There is no reason to expect significantly different behavior for the CC tablets. For major cardiovascular effects of nisoldipine, there is good correlation with dose (5-30 mg/day) and plasma drug concentration.

It is concluded that clinical pharmacology of nisoldipine CC has been adequately characterized for the patients to be treated that instructions on its clinical use for hypertension can be written for the labeling.

Shaw T. Chen, M.D.,Ph.D.

cc:
ORIG: NDA- 20-356
HFD-110
HFD-110/CSO
HFD-426/PMarroum
HFD-110/PDern, CDuarte, NStockbridge
HFD-110/SChen/02/15/94

D.Rueter

DIVISION OF CARDIO-RENAL DRUG PRODUCTS

MEDICAL OFFICER'S REVIEW OF NDA AUG 4 1993

NDA Number : 20-356

Name of Drug : Nisoldipine (NIS CC)

Drug Category : Calcium Channel Blocker

Indication : Hypertension

Sponsor : Miles Inc Pharmaceutical Division

Date of Submission : March 31, 1993

Date Received : April 1, 1993

Date Review Completed : July 30, 1993

Reviewer : Cristobal G. Duarte, MD

Background. NIS CC is an extended release tablet dosage form of the dihydropyridine calcium channel blocker Nisoldipine The sponsor has submitted a NDA for approval of Nisoldipine for the treatments of hypertension This review will be concerned only with the efficacy in the treatment of hypertension.

As pivotal protocols in support of the effectiveness of Nisoldipine in the control of hypertension the sponsor is submitting the following studies : D90-006, D90-019, D89-026, D89-029, and D89-039.

Protocol D89-026

Title of Study : " A Pilot Dose-Titration Study of the Safety and Efficacy of Nisoldipine Coat-Core 10 mg, 20 mg, 30 mg and 40 mg versus Placebo in Patients with Mild to Moderate Hypertension ".

Investigators : Ginsberg D, Flamenbaum W, Canzanello V, Townsend R, Winer N,Schnaper H.

Places of Study. Harleysville, Englewood Cliffs, Winston-Salem, Galveston, Kansas City, Birmingham/USA

Objectives. The objectives of this study were :

1. To determine whether Nisoldipine given once daily lowers the blood pressure significantly more than placebo.

2. To determine the efficacy and safety of Nisoldipine when titrated from 10 mg to 40 mg qd.

Inclusion Criteria. Ambulatory patients, male and female, 21 years of age or older, with a history of essential hypertension were eligible for the study. Hypertension was defined as mean supine diastolic blood pressure of 95-115 mmHg.

Exclusion Criteria. Patients with the following conditions were excluded from the study :

1. Labile hypertension.
2. Recent myocardial infarction
3. Patients with cerebrovascular accident or signs suggesting impending MI or CVA, heart failure, angina pectoris, intermittent claudication, major arrhythmia or cardiac conduction disturbances.
4. Insulin-dependent diabetes mellitus, failure of a major organ system, impaired renal function (serum creatinine >2 mg/dl), severe infection, malignancy or psychosis.
5. Patients likely to have impaired drug absorption such as with chronic diarrhea, ulcerative colitis, regional enteritis, diverticulitis, partial or complete gastrectomy or small bowel resection.
6. Women of childbearing potential, alcohol or drug abusers, history of allergy to dihydropyridines.
7. Excluded concomitant medications were : antihypertensive drugs, cimetidine, monoamino oxidase inhibitors, sedatives, tranquilizers, tricyclic antidepressants, neuroleptic drugs, anorectics and decongestants.

Qualification for Randomization. Patients with mild or moderate hypertension discontinued previous antihypertensive treatment and were given a single-blind placebo once daily (regimen A) during a three to four-week qualifying run-in period. There was an optional extension of one week if the blood pressure was not in the qualifying range. Those patients with mean supine diastolic pressure ≥ 95 mmHg to ≤ 114 mmHg after three to four weeks of placebo were randomized and were given regimen B (Nisoldipine or placebo).

Drug-Regimen Protocol. At week 0 qualified subjects were given either Nisoldipine 10 mg qd or placebo qd (regimen B) for 2 weeks. On subsequent visits 5 through 7 (scheduled every two weeks) the once daily dose of Nisoldipine was titrated in a stepwise fashion to 20 mg (regimen C), 30 mg (regimen D), or 40 mg (2X20 mg) (regimen E) if mean trough supine diastolic pressure for that visit was ≥ 85 mmHg. Patients randomized to placebo underwent corresponding dummy titration. Two patients were randomized to Nisoldipine for each patient that was randomized to placebo.

Patients took two tablets before 11 am through the study but did not take the medication on the morning of clinical visits until trough blood pressure has been measured. Patients took the medication fasting or with food.

Patients were seen either weekly or biweekly in the morning throughout the study. At each visit supine and standing blood pressures were measured 24 hours ± 30 minutes after the last dose.

The duration of the double-blind phase was 9 weeks.

Expulsion. A subject was to be dropped from the study if the mean supine diastolic blood pressure was greater than 114 mmHg at any visit or if they had significant physical or laboratory abnormalities or a significant concurrent illness.

They also were to be withdrawn for blatant non-compliance, for missing visits or significant adverse experiences.

Assessment. Patients were seen in the morning at weekly or biweekly intervals. A history was taken at the first visit. Complete physical examination and 12-lead electrocardiogram were done at the first visit, at baseline (after 3 to 4 weeks on placebo) and at the last visit (after 9 weeks of double blind drug). Brief physical examinations were done at all other visits. A chest X-ray was done at the first visit unless a report was available within the previous 6 months.

Blood was drawn for the following laboratory tests at the first visit, after 3 to 4 weeks of single-blind placebo, and after 9 weeks of double-blind drug : CBC, differential, and platelet count, serum glucose, uric acid, calcium, phosphate, sodium, potassium, chloride, bicarbonate, creatinine, BUN, total protein, albumin, cholesterol, triglycerides, CPK, SGOT, LDH, alkaline phosphatase and total bilirubin, Complete urinalysis including microscopic and casts.

The primary endpoint of the study was a change in trough diastolic blood pressure (measured 24 hours after dosing) from baseline (mean of diastolic blood pressure after 3 or 4 weeks of single-blind placebo) to endpoint (the last valid visit on double-blind drug for each valid patient) in the Nisoldipine group compared to the placebo group.

Secondary endpoints were supine systolic blood pressure at trough and standing blood pressure at trough.

Statistical Analysis. The primary efficacy analysis was based on change from baseline in trough supine diastolic blood pressure at endpoint. No analysis based on level of titration achieved was done. Responders were considered those who achieved efficacy results according to the following criteria : blood pressure 90 mmHg or less, at least a 10 mmHg fall in blood pressure from baseline, either of the above and both of the above.

All tests were two-sided and based on the least square means estimated by the model.

Data from previous hypertension studies had suggested that the standard deviation of change from baseline in trough supine diastolic blood pressure at endpoint would be 7.5 mmHg. In order to detect a 5 mmHg difference from placebo in an alpha = 0.05, two tailed tests of

significance, and in order to obtain as much data as possible on the Nisoldipine 40 mg qd, it was decided to randomized 72 patients to Nisoldipine and 36 to placebo. Based on this information, the study, as designed, had 80 % power to detect a significant difference of at least 5 mmHg.

Subjects Studied. Of 166 patients enrolled, 43 were disqualified for randomization. The reasons for which patients did not qualify for randomization id given in the following table :

Mean Diastolic blood pressure at visit 4 did not qualify for randomization (95 mmHg to 114 mmHg)	26
Supine diastolic blood pressure >114 mmHg-	
At any time	4
Non compliance	1
Illness not due to medication	3
Other	9
Total	43

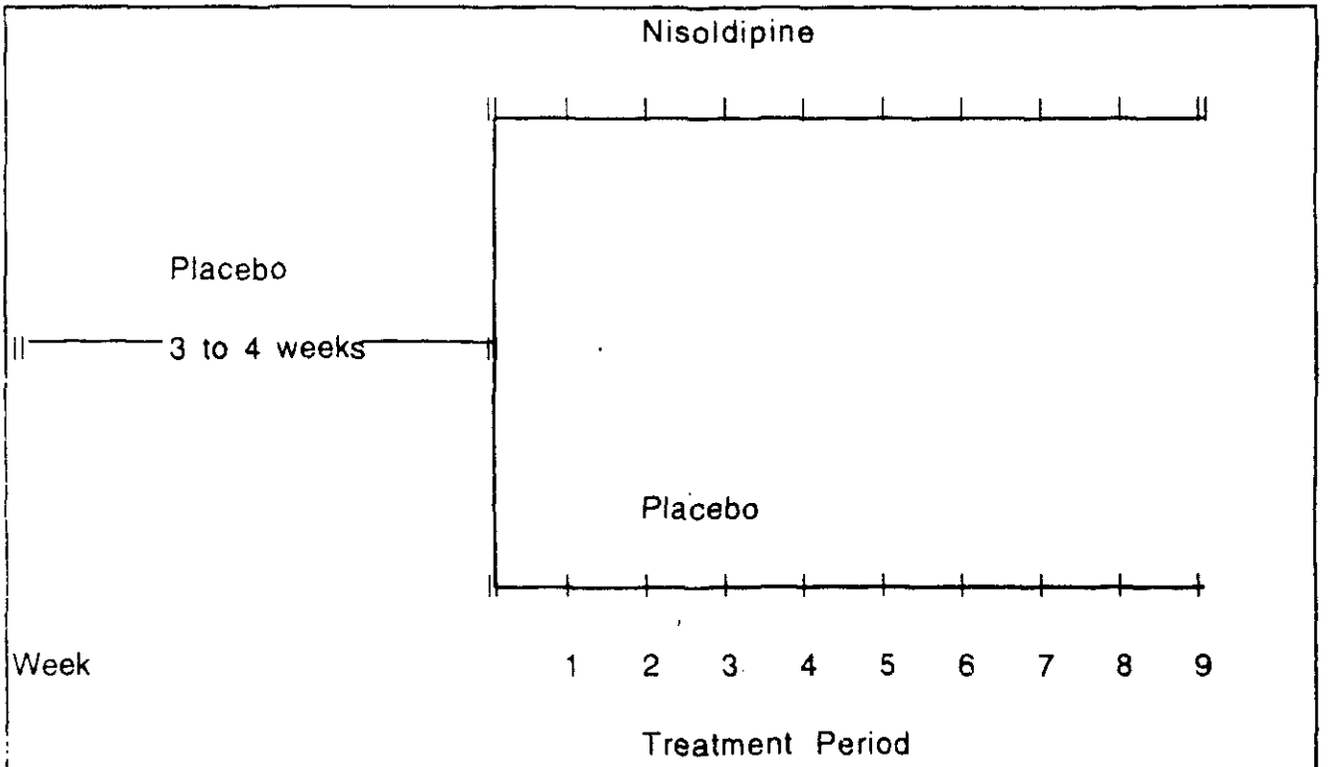
The demography and baseline characteristics of the patients valid for analysis of efficacy is given in the following table :

		Nisoldipine (n=79)	Placebo (n=38)
Sex	Male	46 (58 %)	22 (58 %)
	Female	33	16
Race	Caucasian	53 (67 %)	21 (55 %)
	Black	25	16
	Other	1	1
Age (years)	Mean	53	57
Years of hypertension	Mean	10	14
Baseline blood pressure	Supine	153/100	160/101
	Standing	149/100	156/102

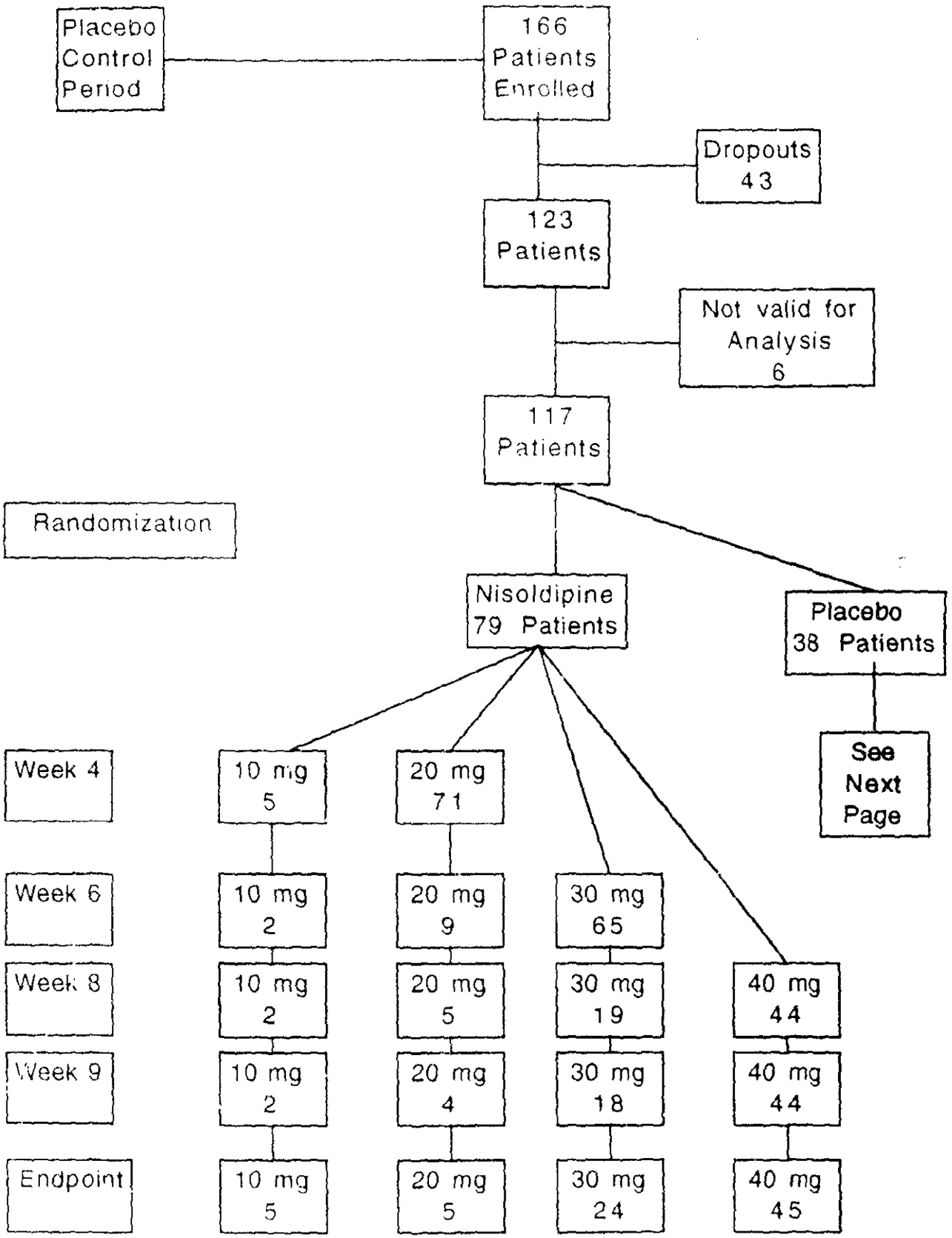
The reasons for discontinuation of double-blind therapy are given in the following table :

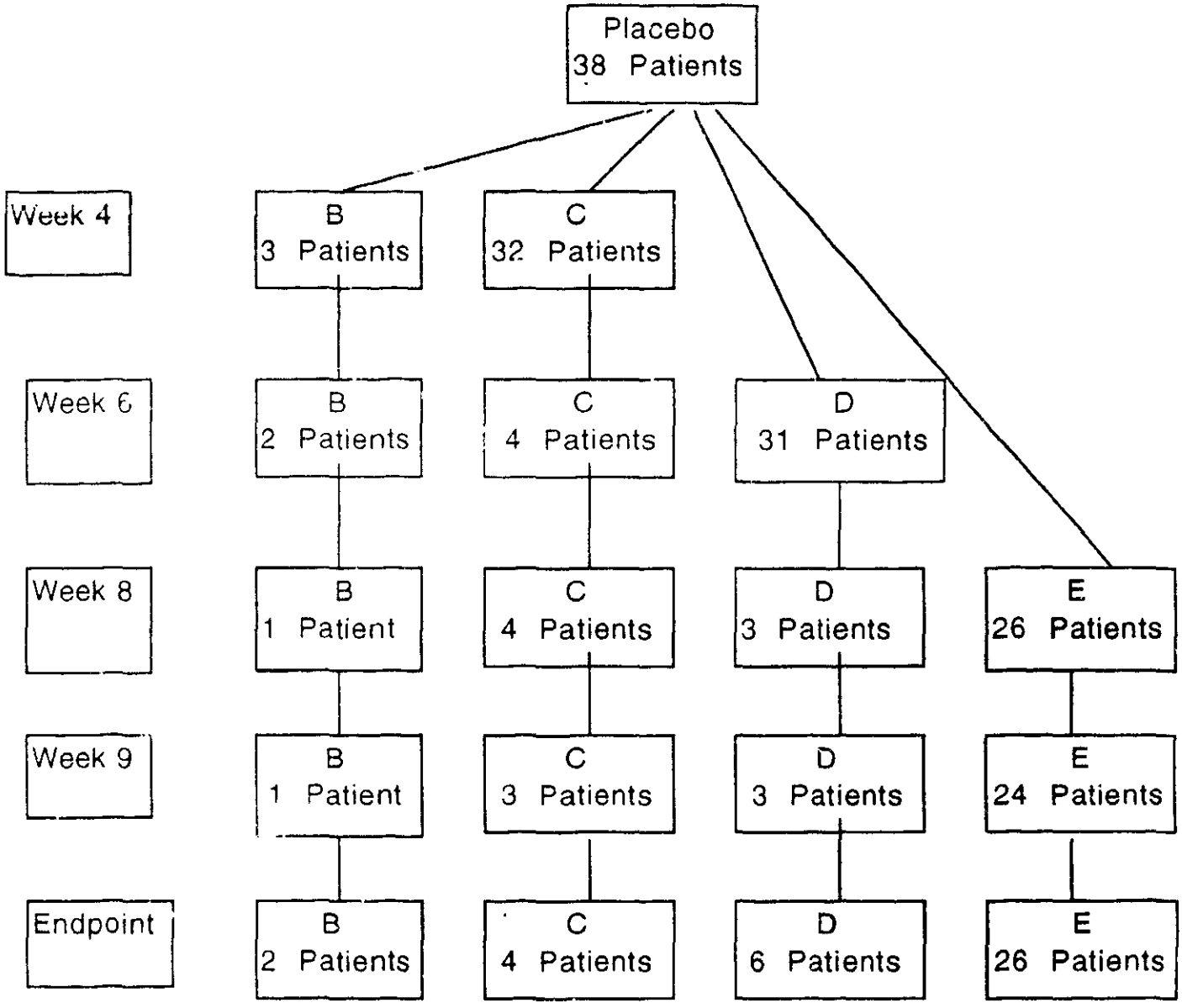
Reason	Nisoldipine n=83	Placebo n=40
Lack of Efficay	0	5
Adverse Event	6	0
Abnormal Laboratory Value	0	1
Lost to Follow-up	3	0
Other	2	9

The protocol that was followed is represented schematically in the following graph



The randomization of patients is given in the following two pages


Placebo Control Period
166 Patients Enrolled
Dropouts 43
123 Patients
Not valid for Analysis 6
117 Patients
Randomization
Nisoldipine 79 Patients
Placebo 38 Patients
See Next Page
Week 4
Week 6
Week 8
Week 9
Endpoint
10 mg 5
20 mg 71
10 mg 2
20 mg 9
30 mg 65
10 mg 2
20 mg 5
30 mg 19
40 mg 44
10 mg 2
20 mg 4
30 mg 18
40 mg 44
10 mg 5
20 mg 5
30 mg 24
40 mg 45


Placebo
38 Patients
Week 4
B
3 Patients
C
32 Patients
Week 6
B
2 Patients
C
4 Patients
D
31 Patients
Week 8
B
1 Patient
C
4 Patients
D
3 Patients
E
26 Patients
Week 9
B
1 Patient
C
3 Patients
D
3 Patients
E
24 Patients
Endpoint
B
2 Patients
C
4 Patients
D
6 Patients
E
26 Patients

Efficacy. Doses of Nisoldipine were titrated from regimen B (10 mg QD) to regimen E (40 mg QD) in 10 mg steps. The following table shows the actual number of patients that were given each dose level and had valid visits at each week for the population of patients valid for efficacy :

Week of Therapy

			2	4	6	8	9	End-point
Group	Reg.	Dose	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)
NIS	B	10 mg QD	79 (100)	5(7)	2 (3)	2 (3)	2 (3)	5 (6)
	C	20 mg QD		71 (93)	9 (12)	5 (7)	4 (6)	5 (6)
	D	30 mg QD			65 (86)	19 (27)	18 (27)	24 (30)
	E	40 mg QD				44 (63)	44 (65)	45 (57)
PLA	B		38 (100)	3 (9)	2 (5)	1 (3)	1 (3)	2 (5)
	C			32 (91)	4 (11)	4 (12)	3 (10)	4 (11)
	D				31 (84)	3 (9)	3 (10)	6 (16)
	E					26 (77)	24 (77)	26 (68)

The following table shows trough supine diastolic blood pressure response at different weeks of treatment and at endpoint for both groups for the set of all valid patients.

Trough Supine Diastolic Blood pressure
Mean Change (mmHg) by visit

	Week 2	Week 4	Week 6	Week 8	Week 9	End-point
Nisoldipine						
(n)	(79)	(76)	(76)	(70)	(68)	(79)
Mean Change	-5.7*	-6.5	-10.1*	-10.6*	-10.0*	-9.5*
Placebo						
(n)	38	35	37	34	31	
Mean Change	-3.0	-4.9	-3.4	-4.4	-3.4	-1.2

* Significantly different from placebo

In the following table, changes from baseline at endpoint by treatment regimen at endpoint for all valid patients is demonstrated :

	Nisoldipine			
	Reg B (n=5)	Reg C (n=5)	Reg D (n=24)	Reg E (n=45)
Supine				
Systolic	-8.3	-23.1	-10.9	-16.7
Diastolic	-5.9	-12.4	-9.4	-9.8
Standing				
Systolic	-5.3	-13.5	-10.3	-15.3
Diastolic	-7.2	-9.9	-6.6	-8.6

	Placebo			
	Reg B (n=2)	Reg C (n=4)	Reg D (n=6)	Reg E (n=26)
Supine				
Systolic	-19.7	-8.0	+20.4	-2.2
Diastolic	+ 1.7	-10.0	+6.9	-1.4
Standing				
Systolic	-9.7	-1.3	+ [illegible]	-0.1
Diastolic	-0.7	-7.5	+5.0	-1.6

The responder rates are given in the following table :

Responder Rates
Based on Trough Supine Diastolic Blood Pressure at Endpoint

	Nisoldipine	Placebo
BP<90 mmHg at endpoint	49 %	18 %
At least a 10 mmHg fall at Endpoint	54 %	13 %
Either of the Above	62 %	21 %
Both of the Above	42 %	11 %

The change from baseline in trough blood pressure by treatment for all patients valid for analysis of efficacy is given in the following table :

Supine Diastolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg.B	Reg. B or C	Reg. BC or D	Reg.BCD or E	Reg BCDorE	
Nisoldipine n	79	76	76	70	68	79
Baseline Mean LS	100.36	100.36	100.36	100.14	100.36	100.36
LS Mean Change	-5.65*p	-6.52*	-10.06*p	-10.58*p	-9.98*p	-9.51*p
SE of Change	0.68	0.75	0.88	0.84	0.80	0.89

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg B	Reg B or C	Reg BC or D	Reg BCD or E	Reg BCD or E	
Placebo n	38	35	37	34	31	38
Baseline LSMean	101.45	101.31	101.20	100.52	100.79	101.45
LS Mean Change	-3.04*	-4.89*	-3.39*	-4.42*	-3.36*	-1.16
SE of Change	0.99	1.10	1.26	1.21	1.20	1.29
p Values						
Drug	0.0323	0.2234	0.0001	0.0001	0.0001	0.0001
Drug-Center	0,1582	0.2604	1.381	0.0539	0.0472	0.0332

P Significantly different from placebo
* Significant Change from baseline

Values for supine systolic blood pressure are given in the following tables:

Supine Systolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg B	Reg B or C	Reg BC or D	Reg BDC or E	Reg BCD or E	
Nisoldipine n	79	76	76	70	68	79
Baseline LS Mean	153.11P	153.39	153.41	153,35	153.42	153,11p
LS Mean Change	-8.67*	-10.123*p	-15.04*p	-14.10*p	-15.62*p	-14.73*p
SE of Change	1.33	1.61	1.61	1.61	1.64	1.89
Placebo n	38	35	37	34	31	38
Baseline LS Mean	159.59	158.99	158.23	157.78	159.71	159.59
LS Mean Change	-4.96*	-2.39	-1.90	-4.91*	-5.51*	-0.00
SE of Change	1.92	2.38	2.33	2.31	2.43	2.72

P-Values	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug	0.1140	0.0074	0.0001	0.0015	0.0008	0.0001
Drug Center	0.6059	0.0481	0.0416	0.1372	0.1224	0.0726

p Significantly different from placebo
* Significant change from baseline

Standing Diastolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg.B	Reg.B or C	Reg. BC or D	Reg BDC or E	Reg BCD	or e
Nisoldipine n	79	76	76	70	68	79
Baseline LSMean	100.40	100.31	100.31	100.21	100.27	100.40
LS Mean Change	-5.03*p	-6.24*p	-8.62*p	-10.18*p	-8.29#p	-7.86*p
SE of Change	0.75	0.75	0.85	0.83	0.98	1.00
Placebo n	38	35	37	34	31	38
Baseline SL Mean	102.15	101.85	101.85	101.22	100.89	102.15
LS Mean Change	-2.05	-1.99	-1.87	-4.27*	-3.65*	-1.18
SE of Change	1.08	1.11	1.22	1.20	1.45	1.44
P Values						
Drug	0.0253	0.0021	0.0001	0.0001	0.0095	0.0002
Drug-Center	0.2060	0.0052	0.6446	0.5733	0.4304	0.3160

p Significantly different from placebo
* Significant change from baseline

Standing Systolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg B	Reg B or C	Reg BC or D	Visit BCD or E	Visit BCD or E	
Nisoldipine n	79	76	76	70	68	79
Baseline LS Mean	149.22p	149.54	149.56	149.41	149.57	149.22p
LS Mean Change	-8.37*	-10.78*p	-14.18*p	-14.84*p	-13.76*p	-13.*p
SE of Change	1.34	1.49	1.78	1.73	1.66	1.77
Placebo n	38	35	37	34	31	38
Baseline LS Mean	156.22	155.79	155.30	154.22	155.46	156.22
LS Mean Change	1.94	2.20	2.55	2.49	2.48	2.57
P values						
Drug	0.0832	0.0005	0.0001	0.0002	0.0002	0.0001
Drug-Center	0.1162	0.0979	0.1092	0.2475	0.0099	0.0330

P Significantly different from placebo * Significant change from baseline

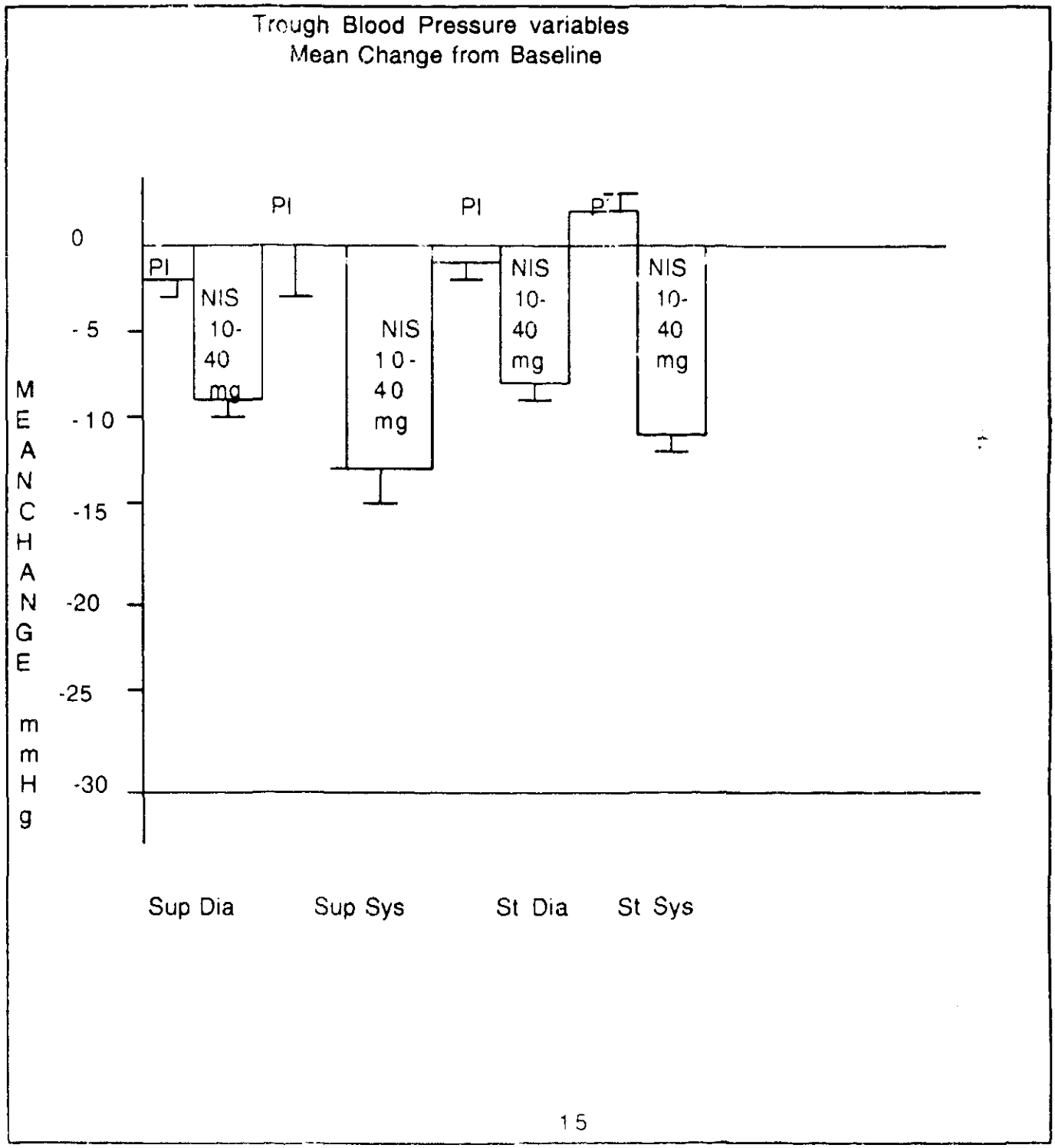
The effect of Nisoldipine on trough supine and standing systolic and diastolic blood pressure at study endpoint in all Nisoldipine treated patients is shown in the following table :

Change from Baseline to Endpoint in Trough Blood Pressures
Mean and SEM in mmHg

	Placebo n=38	Fisoldipine n-79
Supine Diastolic Blood Pressure	-1.16±1.29	-0.51±0.89*
Supine Systolic Blood Pressure	-.000±2.72	-14.73±1.89*
Standing Diastolic Blood Pressure	-1.18±1.44	-7.86±1.00*
Standing Systolic Blood Pressure	+0.89±2.57	-13.00±1.77*

* Significantly different from placebo. $p<0.05$

The change from baseline to endpoint in the primary efficacy blood pressure parameter supine diastolic blood pressure, as well as the 3 secondary blood pressure parameters is shown for placebo and all Nisoldipine doses in the figure below :



Conclusion. This was a titration study in which doses of Nisoldipine 10 mg, 20 mg, 30 mg, 40 mg and placebo were evaluated. In the course of the study most patient were moved to the higher doses in order to decrease the blood pressure and very few patients remained in the lower doses (see flow sheets pages 7, 8, and 9). Therefore a dose-range study could not be carried and only a global evaluation was possible. Such assessment demonstrated that Nisoldipine was very effective in lowering the blood pressure (pages 10-15).

Protocol D89-029

Title of Study : " Double-Blind Randomized Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine 20, 40 and 60 mg (2X30 mg) Core-Coat Tablets vs Placebo in Combination with Atenolol 50 mg in Hypertensive Patients ".

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Objectives . The objectives of this study were to determine the dose response and safety of 20 mg, 40 mg, and 60 mg Nisoldipine tablets as compared to placebo when administered once daily as additive treatment for hypertensive patients not controlled on once daily Atenolol 50 mg.

Inclusion Criteria. Ambulatory patients, male or female, of age 21 or older, with a history of essential hypertension were eligible for enrollment in the placebo run-in period.

Exclusion Criteria. Criteria foe exclusion were : labile hypertension, renal failure (plasma creatinine > 2.0 mg/dl), significant liver disease, insulin-dependent diabetes mellitus, history or presence of bronchial asthma, obstructive pulmonary disease, significant peripheral vascular disease, recent (3 months) myocardial infraction, cerebrovascular accident, or clinical signs suggesting impending myocardial infarction or cerebrovascular disease. Also excluded were patients with heart failure, major arrhythmias, conduction disturbances greater than first degree block, sinus bradycardia, failure of a major organ system, malignancy, psychosis, impaired absorption (such as chronic diarrhea), pregnancy,

women with childbearing potential, abuse of alcohol or drugs, allergy to dihydroperidines or beta blockers and participation in an investigational drug study within the past 30 days.

Qualifications for Randomization. Patients discontinued all previous antihypertensive medication and were given a single-blind placebo once daily in a 2-week qualifying period (Regimen A). Patients with a mean SUDBP 100-119 mmHg at the end of the placebo run-in period were given 1 capsule containing 50 mg Atenolol and 2 placebo tablets under single-blind conditions for 4 weeks (Regimen B). Patients with mean SUDBP 95-114 mmHg after 4 weeks of single-blind Atenolol were randomly assigned to 1 to 4 treatment groups and given double-blind drug.

Drug-Regimen Protocol (Regimen C). Patients who qualified for randomization received Atenolol 50 mg + Nisoldipine (20 mg, 40 mg, or 60 mg) or Atenolol 50 mg + placebo for 6 weeks.

Drugs for the double-blind period (Regimen C) contained encapsulated Atenolol 50 mg with one of the following :

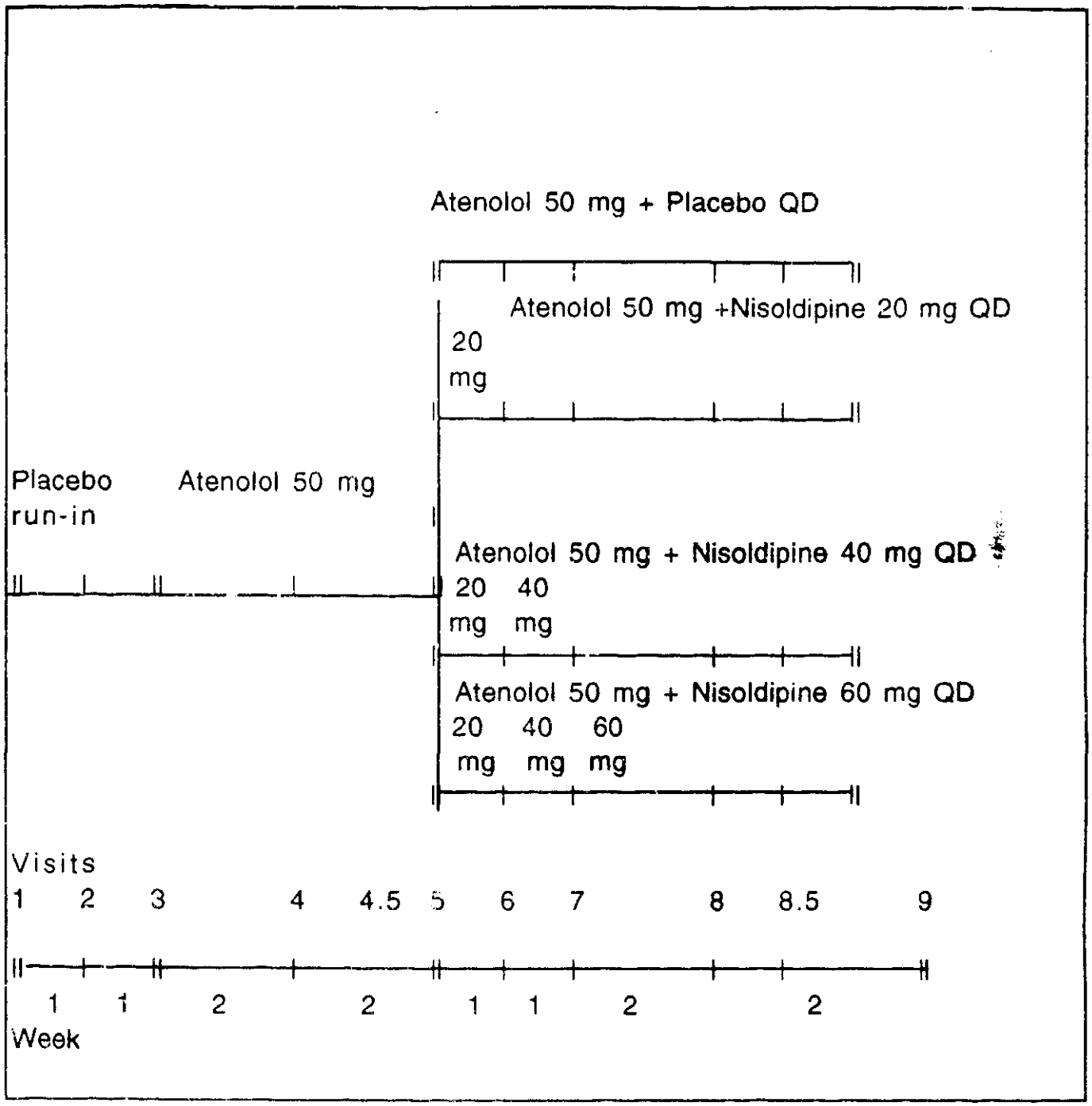
One Nisoldipine 20 mg tablet and
one placebo tablet once daily for 6 weeks

One Nisoldipine 20 mg tablet and
One placebo tablet once daily for 1 week
Forced titrated to
One Nisoldipine 40 mg tablet and
One placebo tablet once daily for 5 weeks

One Nisoldipine 20 mg tablet and
One placebo tablet once daily for 1 week
Forced titrated to
One Nisoldipine 40 mg tablet and
One placebo tablet once daily for 1 week
Forced titrated to
Two Nisoldipine 30 mg tablets once daily for 4 weeks

Two placebo tablets once daily for 6 weeks

The study design is illustrated in the following graph :



Removal of patients from Study or Analysis. Patients could leave the study at any time if they so wished. Patients could be discontinued if they had significant physical or laboratory abnormalities, or if they had significant concurrent illness or deterioration of their condition. Patients could also be withdrawn if they were blatantly non-compliant. Patients with significant adverse events and those patients with elevations in SUDBP > 114 mmHg were also discontinued from the study.

Statistical Methods. All statistical tests were two-tailed and were conducted at a significant level of 0.05. Pairwise comparisons and within-group changes were tested via the least square means estimated by the model.

Results. . Demographic Characteristics. The demographic characteristics are given in the following table :

	Atenolol+ Nis 20 mg n=61	Atenolol+ Nis 40 mg n=59	Atenolol+ Nis 60 mg n=59	Atenolol+ Placebo n=59
Mean Age (years)	52	54	56	54
Mean wt (lbs)	201	198	198	195
Baseline BP (mmHg)				
Supine	159/101	159/101	162/101	156/110
Standing	154/102	157/103	156/103	152/101
% Male	79	73	70	64
% Caucasian	61	53	58	53
% Diabetic	8	9	14	12
% Mild Hypertensive	60	56	51	59
% Moderate Hypertensives	40	44	49	41

The sponsor states that there were no statistically significant differences between the groups for any of the characteristics examined.

Assessment. Patients were seen in the morning at weekly and biweekly intervals. A history complete physical examination and 12-lead electrocardiogram were taken in the firs visit, at baseline (after 4 weeks of Atenolol) and at the last visit on double-blind drug. Electrocardiograms

were included in visits 7 and 8. Twenty-four hour ambulatory electrocar-cardiograms were taken at some centers. on weeks 4.5 after 3 weeks of single Atenolol and at 8.5 weks of double-blind therapy. Chest X-ray were taken after 2 weeks on placebo.

Laboratory tests performed in the course of the study included blood hematology, serum electrolytes, battery of liver function tests, and urinalysis.

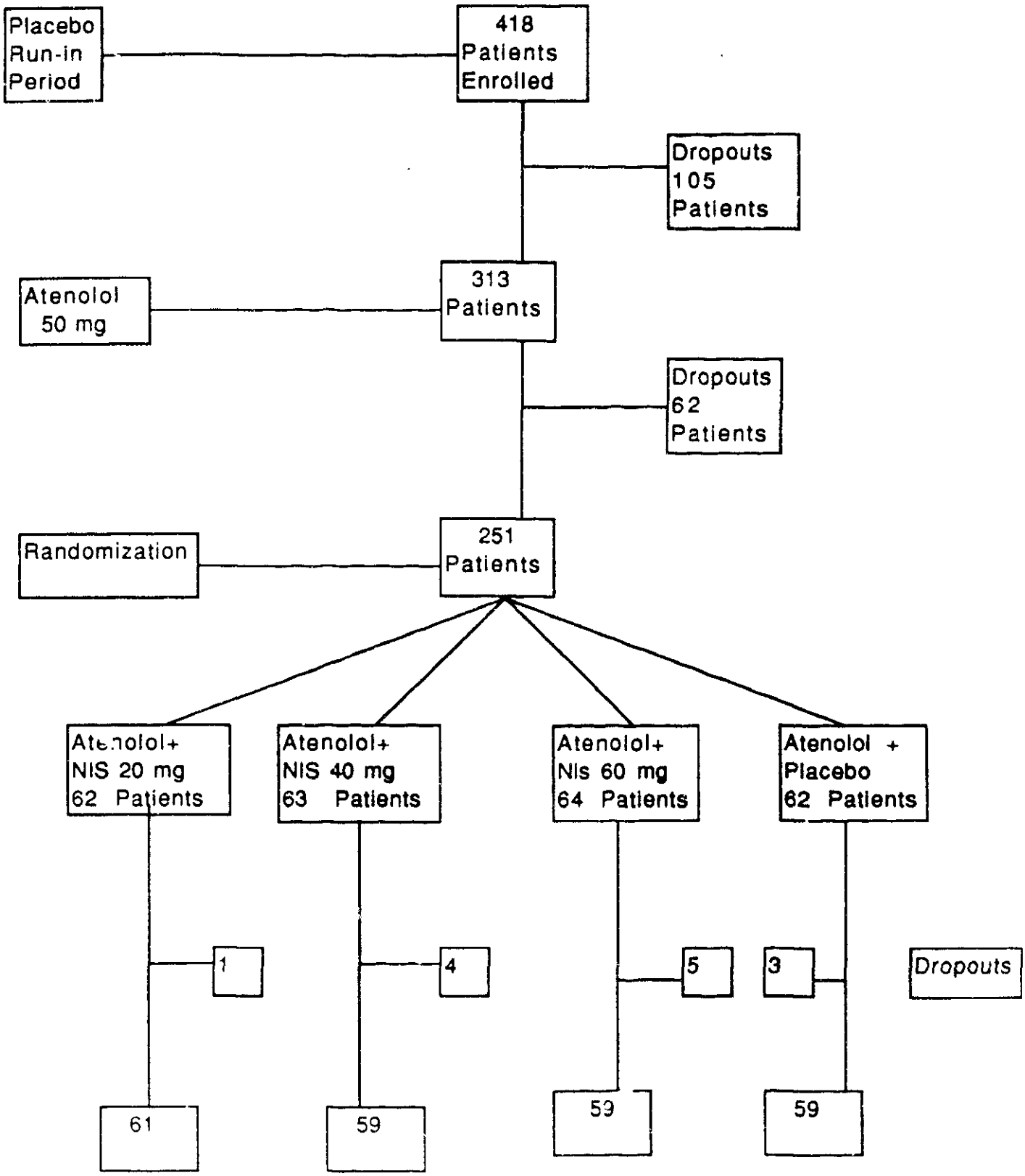
At the end of the single blind Atenolol phase and at the end of the double-blind phase blood was taken at trough for Nisoldipine assay.

At each visit vital signs were taken.

Criteria for Effectiveness. The change from baseline in SUDBP was the primary efficacy variable in this study. The primary time point was the endpoint which was defined as the last double-blind visit for all valid patients. A valid patient was one who had at least 3 weeks of double-blind drug. This criterion was later amended before braking the random code to 19 days. The overall treatment efficacy was determined by the change from baseline in trough SUDBP at endpoint between the average of the three Atenolol-Nisoldipine groups and the Atenolol-Placebo group. Secondary efficacy parameters were supine systolic blood pressure chang[illegible]t trough, standing blood pressure changes at trough, and amb[illegible]y blood pressure trough/peak ratios.

An average decrease in diastolic blood pressure of at least 5 mmHg more than placebo was considered to be clinically meaningful. The actual power for the study was >95 %.

The disposition of the patients is given in the following flow-sheet :


Placebo Run-in Period
418 Patients Enrolled
Dropouts 105 Patients
Atenolol 50 mg
313 Patients
Dropouts 62 Patients
Randomization
251 Patients
Atenolol+ NIS 20 mg 62 Patients
Atenolol+ NIS 40 mg 63 Patients
Atenolol+ Nis 60 mg 64 Patients
Atenolol + Placebo 62 Patients
1
4
5
3
Dropouts
61
59
59
59

The reasons because the patients were withdrawn from the placebo run-in period are given in the following Table :

Mean SUDBP at visit 3 <100 mmHg or > 119 mmHg	=58
Mean SUDBP >119 mmHg during placebo run-in period	= 6
Adverse event during placebo run-in period	=10
Other illness	= 3
Abnormal laboratory value	= 4
Abnormal electrocardiogram	= 2
Noncompliance	= 3
Investigator discretion	= 5
Consent withdrawn	= 9
Lost to follow-up	= 5
Total	105

Patients withdrawn during the single Atenolol period and therefore not randomized :

Mean SUDBP at visit 5 <95 mmHg or >114 mmHg	=37
Mean SUDBP > 114 mmHg on 2 consecutive visits after placebo run-in	= 4
Adverse Event	= 4
Other illness	= 2
Abnormal electrocardiogram	= 1
Noncompliance	= 1
Investigator discretion	= 4
Consent withdrawn	= 4
Lost to follow-up	= 2
Enrolled after enrollment date	= 3
Total	6[illegible]

The reasons for invalidity for patients that were withdrawn during the treatment period are given in the following table :

Drug Group	Number of Patients	Reasons for Invalidity
Atenolol + NIS 20 mg	1	Less than 19 days on double-blind drug
Atenolol + NIS 40 mg	4	Less than 19 days on double-blind drug
Atenolol + NIS 60 mg	4	Less than 19 days on double-blind drug
	1	Visit 5 diastolic BP not between 95 and 114 mmHg
Atenolol + Placebo	2	Less than 19 days on double-blind drug
	1	Visit 5 diastolic BP not between 95 and 114 mmHg
Total	13	

Effectiveness. The change from baseline in trough blood pressure by treatment for all patients valid for analysisof efficacy are given in the following tables

Supine Diastolic

Drug Group	Visit 6 Week 1	Visit 7 Week 2	Visit 8 Week 4	Visit 9 Week 6	Endpoint
ATN+NIS 20 mg					
N	58	61	61	61	61
Baseline BP	100.56	100.56	100.56	100.57	100.56
Mean Change	-8.53*C	-9.80*C	-9.10*AB	-10.09*	-10.09*
SE	0.85	0.78	C 0.89	ABC 0.88	ABC 0.88
ATN+NiS 40 mg					
N	57	58	58	57	58
Baseline BP	100.75	100.75	100.75	100.77	100.75
Mean Change	-8.52*C	-11.26*C	-12.87*C	-12.75*C	-12.69*C
SE	0.86	0.81	0.92	0.92	0.91
ATN+NIS 60 mg					
N	56	59	58	57	59
Baseline BP	101.12	101.11	101.00	101.27	101.11
Mean Change	-9.72*C	-12.15*C	-12.82*C	-14.36*C	-14.24*C
SE	0.87	0.80	0.92	0.91	0.90
ATN+PL					
N	58	59	59	59	59
Baseline BP	99.93	99.93	99.93	99.93	99.93
Mean Change	-4.00*	-4.31*	-4.61*	-4.29*	-4.28*
SE	0.85	0.80	0.91	0.90	0.90

Drug Group	Visit 6 Week 1	Visit 7 Week 2	Visit 8 Week 4	Visit 9 Week 6	Endpoint
ATN+NIS 20 mg					
N	61	61	61	61	61
Baseline BP Mean	158.71	158.70	158.70	158.71	158.70
Change	-10.82°C	-12.96° ABC	-13.30° BC	-13.14° ABC	-13.15° ABC
SE					
ATN+NIS 40 mg					
N	58	58	58	57	58
Baseline BP Mean	158.78	158.77	158.77	158.97	158.77
Change	-12.93°C	-19.04°C	-19.03°C	-20.09°C	-19.83°C
SE	2.01	2.11	2.29	2.05	2.05
ATN+NIS 60 mg					
N	58	59	58	57	59
Baseline BP Mean	161.46	161.58	161.70	162.01	161.58
Change	-12.29°C	-20.38°C	-21.00°C	-23.43°C	-23.09°C
SE	2.00	2.08	2.28	2.03	2.02
ATN+Pla					
N	59	59	59	59	59
Baseline BP Mean	156.30	156.29	156.29	156.28	156.29
Change	-3.78	-2.51	-0.02	-0.87	-0.85
SE	1.99	2.09	2.26	2.01	2.02

Standing Diastolic

ATN+NIS 20 mg					
N	61	61	61	61	61
Baseline BP	102.15	102.16	102.16	102.16	102.16
Mean Change	-7.50*C	-8.46*C	-7.90* ABC	-8.93* ABC	-8.93* ABC
SE	0.87	1.00	0.89	0.89	0.89
ATN+NIS 40 mg					
N	58	58	58	57	58
Baseline BP	103.42C	103.43C	103.43C	103.45C	103.43C
Mean Change	-8.55*C	-12.23*C	-12.85*C	-15.00*C	-14.93*C
SE	0.89	1.02	0.92	0.93	0.91
ATN+NIS 60 mg					
N	58	59	58	57	59
Baseline BP	102.97	102.94	102.97	103.18	102.94
Mean Change	-8.55*C	-12.23*C	-12.85*C	-15.00*C	-14.93*C
SE	0.89	1.02	0.91	0.91	0.91
ATN+Pla					
N	59	59	59	59	59
Baseline BP	101.13	101.13	101.13	101.12	101.13
Mean Change	-3.29*	-4.24*	-3.19*	-1.96*	-1.95*
SE	0.89	1.02	0.91	0.91	0.91

Standing Systolic

Drug Group	Visit 6 Week 1	Visit 7 Week2	Visit 8 Week4	Visit 9 Week 6	Endpoint
ATN+NIS 20 mg					
N	61	61	61	61	61
Baseline BP	152.33	152.23	152.23	152.20	154.37
Mean Change	-10.69*C	-11.17* ABC	-11.20* ABC	-10.97* ABC	-10.97* ABC
SE	2.00	2.10	2.17	2.04	2.00
ATN+NIS 40 mg					
N	58	58	58	57	58
Baseline BP	156.89	156,87	156.87	156.85	156.87
Mean Change	-13.25*C	-17.66*C	-21.52*C	-22.35*	-22.38*C
SE	2.10	2.18	2.25	2.13	2.11
ATN+NIS 60 mg					
N	58	58	58	57	59
Baseline BP	156.17	156.30	156.33	156.73	156.30
Mean Change	-11.16*C	-19.76*C	-20.50*C	-22.36*C	-22.10*C
SE	2.08	2.15	2.24	2.12	2.08
ATN+Pla					
N	59	59	59	59	59
Baseline BP	152.23	152.23	152.23	152.20	152.23
Mean Change	-3.17	-1.42	-0.90	1.88	1.89
SE	2.07	2.15	2.22	2.08	2.09

P-values

	Visit 6 Week1	Visit 7 Week2	Visit 8 Week 4	Visit 9 Week 6	Endpoint
Drug* Center	0.0203	0.6905	0.1363	0.1813	0.1478
NIS vs PLA	0.0001	0.0001	0.0001	0.0001	0.0001
20 mg vs PLA	0.0002	0.0001	0.0004	0.0001	0.0001
40 mg vs Pla	0.0003	0.0001	0.0001	0.0001	0.0001
60 mg vs Pla	0.0001	0.0001	0.0001	0.0001	0.0001

A: Significantly different from ATN+NIS 40 mg QD
B. Significantly different from ATN+NIS 60 mg
C. Significantly different fron ATN+Pla
* Significant change from baseline

The effect of Nisoldipine on trough SUDBP during the course of the double-blind treatment is shown in the following table :

Placebo Substracted Change in SUDBP
Mean in mmHg

NIS Dose	Week 1	Week 2	Week 4	Week 6
20 mg	-4.53*	-5.49*	-4.49*	-5.80*
40 mg	-4.52*	-6.95*	-8.26*	-8.46*
60 mg	-5.72*	-7.84	-8.21*	-10.07*

* Denotes values when Nisoldipine blood pressure responses are significantly different from placebo, <0.05

The changes from baseline to endpoint in trough blood pressure, men and SEM in mmHg are given in the following table :

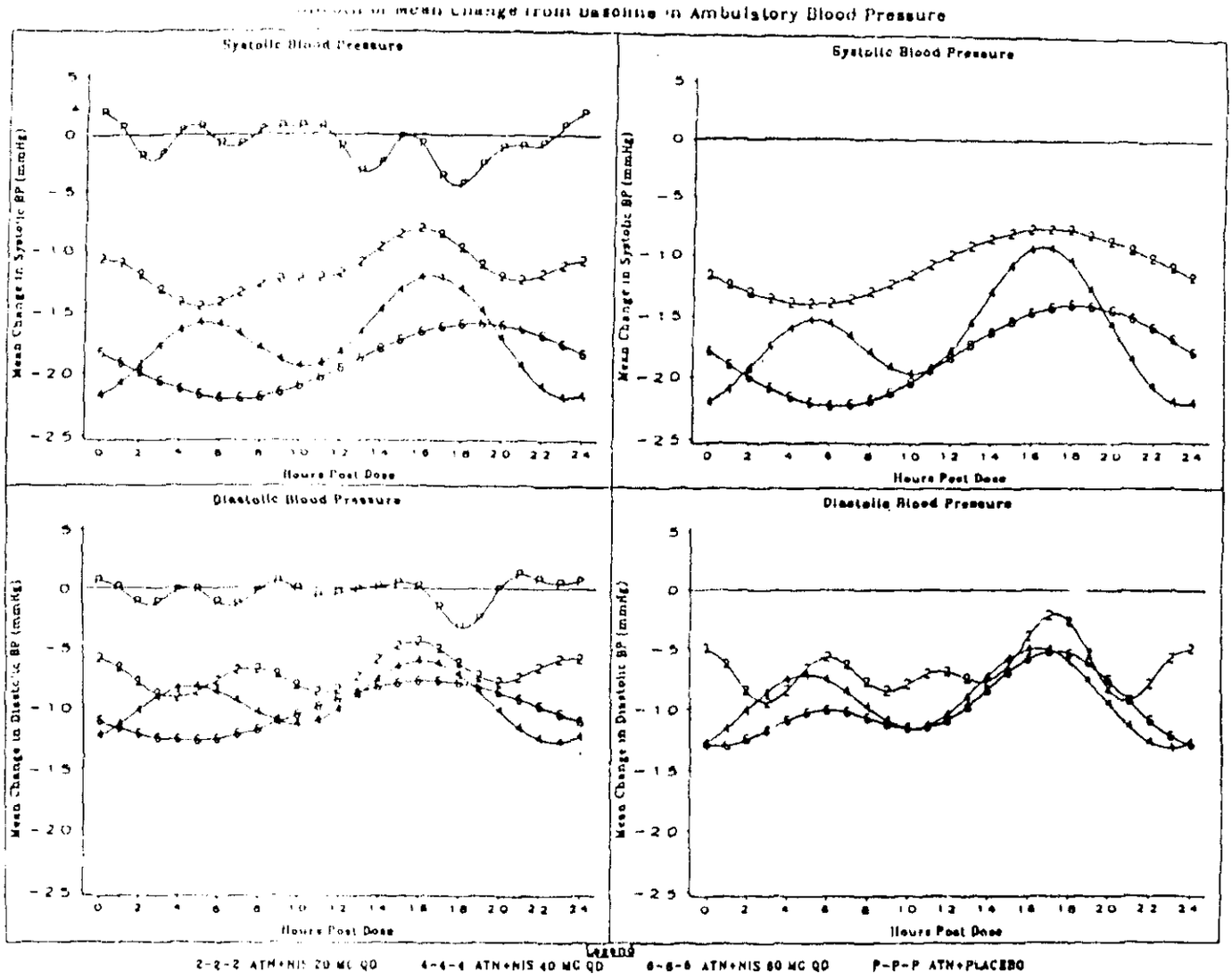
	ATN+PLA n=59	ATN + NIS 20 mg n=61	ATN + NIS 40 mg n=58	ATN + NIS 60 mg n=59
SUDBP	-4.28±0.90	-10.08±0.9[B]	-12.69±0.9*	-14.24±0.9*
SUSBP	-0.85±2	-13.15±2[AB]	-19.83±2*	-23.1±2*
STDBP	-1.95±0.91	-8.93±0.9[AB]	-13±0.92*	15±0.91*
STSBP	+1.89±2.09	-11±2.03*	-22.38±2.1*	-22.10±2.1*

A denotes values NIS 20 mg significantly different from placebo,p<0.05
B denotes values NIS 20 mg significantly different from Nisoldipine 60 mg<0.05.

	ATN+PL:A	ATN+NIS 20 mg	ATN+NIS 40 mg	ATN+NIS 60 mg
Trough response mmHg	% Patients	% Patients	% Patients	% patients
SUDBP ≤90	32.2	55.7*	67.8*	66.1*
Fall in SUDBP ≥10	23.7	50.8*	67.8*	74.6*
SUDBP≤90 or Fall in SUDBP ≥10	39	63.9*	74.6*	78
SUDBP ≤90 and fall in SUDBP ≥ 10	16.9	42.6*	61*	62.7*

* p <0.01 vs placebo

At 8 centers ambulatory blood pressure monitoring (ABPM) was done after 3 weeks on single blind Atenolol and after 5 weeks of double-blind therapy. Smoothed and unsmoothed means for the ambulatory data are shown in the graphs in the following two pages :



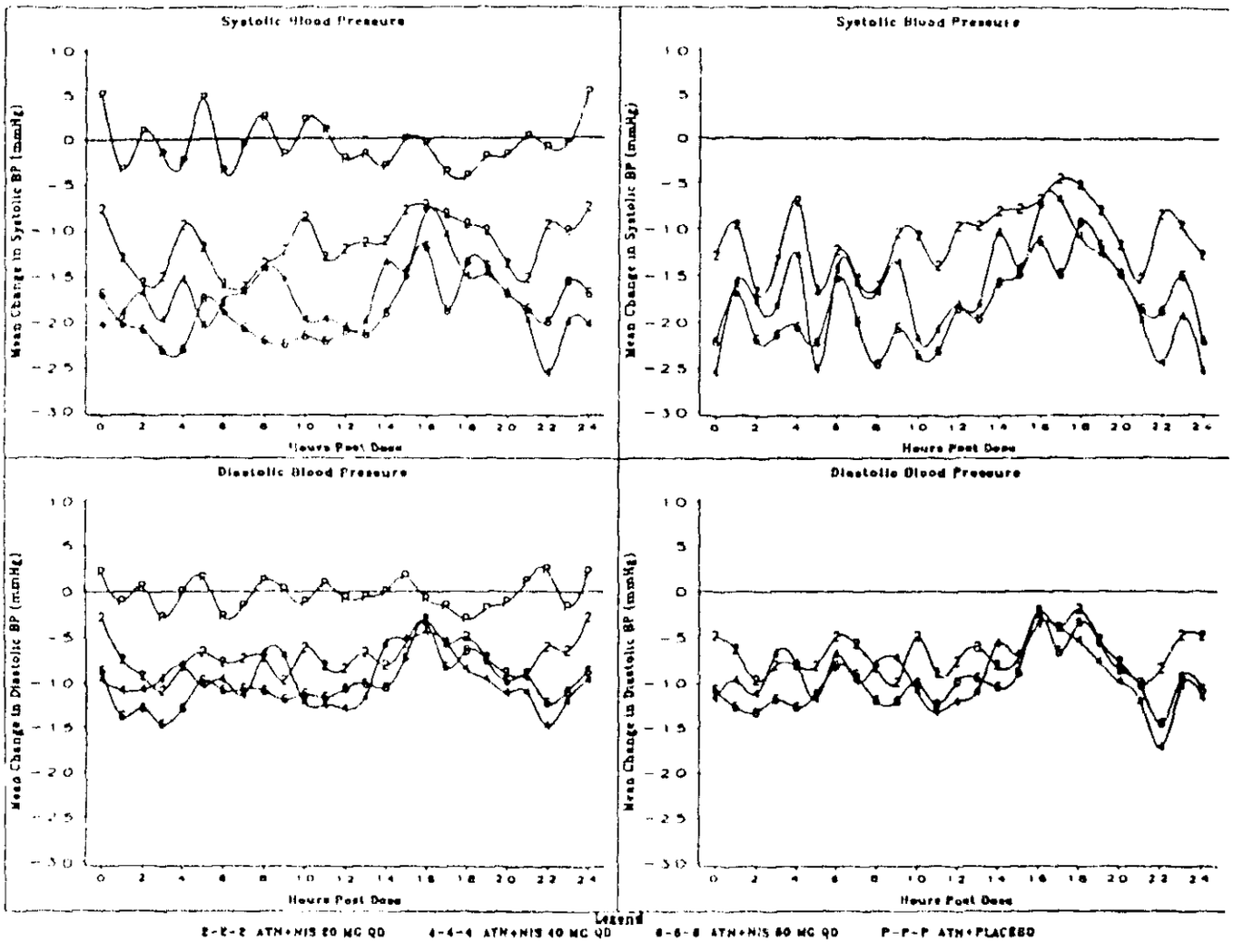
2-2-2 ATN+NIS 20 mg 4-4-4 ATN+NIS40 mg 6-6-6 ATN+NIS 60 mg
P-P-P ATN+Placebo

D00-029

Figure 10

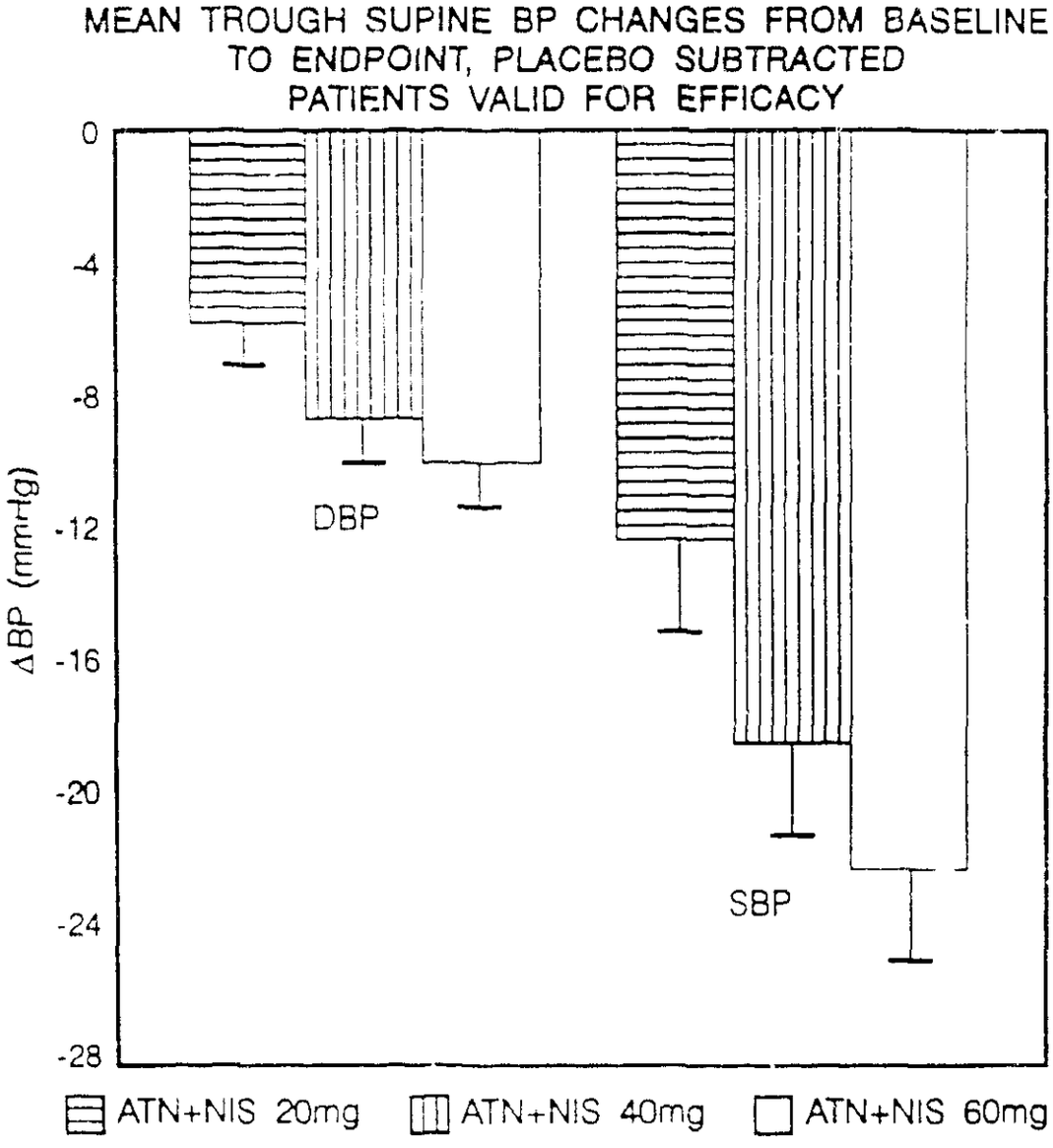
8JUL9

Mean Change from Baseline in Ambulatory Blood Pressure



Symbols as in previous graph

The placebo-substracted trough SUDBPs are showed in the following graph:



The trough and peak ambulatory blood pressure changes and trough to peak ratios for patients valid for efficacy analysis are given in the following table :

Smoothed Data - Difference from Placebo Group

		Trough mmHg	Peak mmHg	Hours to Peak	Trough to Peak Ratio
Variable	Drug				
Diastolic	ATN+NIS 20 mg QD	-5.0	-9.4	3	53%
	ATN+NIS 40 mg QD	-12.8	-13.1	23	97%
	ATN+NIS 60 mg QD	-12.9	-13	1	99%
Systolic (at corresponding diastolic peak)	ATN+NIS 20 mg QD	-11.7	-13.6	3	86%
	ATN+NIS 40 mg QD	-22.0	-22.0	23	100%
	ATN+NIS 60 mg	-17.9	-19.0	1	94%
Systolic (actual)	ATN+NIS 20 mg QD	-11.7	-14.0	5	83%
	ATN+NIS 40 mg QD	-22.0	-22.0	24	100%
	ATN +NIS 60 mg QD	-17.9	-22.3	6	80%

Pharmacodynamic Results. Trough plasma samples were drawn at visits 5 and 9. Visit 9 samples for all patients were analyzed for Nisoldipine. The results for patients whose treatment regimens did include Nisoldipine are shown in the following table :

	n	Mean Trough Concentration (ng/ml)	Range of Trough Concentrations (ng/ml)
AT+NIS 20 mg	57	1.6	0-7.41
AT+NIS 40 mg	55	2.5	0-12.0
AT+NIS 60 mg	59	3.3	0-10.40

Conclusions. In this protocol Atenolol was used as a positive control and studies were performed with Atenolol in combination with placebo and with NIS in the concentrations of 20 mg, 40 mg and 60 mg. Atenolo with placebo had not significant effect in blood pressure but in combination with NIS demonstrated significant hypotensive effects in relation with the concentration of NIS. Threfore the possibility of drug interaction should be considered. Measurements of NIS in plasma were performed and they increased as would be expected with increasing concentrations of NIS and in relation to their effects on blood blood pressure but unfortunately concentrations of Atenolol in plasma were not measured. In other studies the sponsor did not find clinically relevant drug interaction between Nisoldipine, and the beta blocker Propanol (study 704, PB#21521, Volume 142, pp. 08-17-0010374). However some studies in the literature do not agree with this conclusion.

Elliott et al studying the interactions between Nisoldipine and Atenolol and Propanolol found that Nisoldipine, in single and multiple doses, significantly increased the peak plasma concentration of Propanolol and Atenolol. There was no evidence that either beta blocker influenced the pharmacokinetics of Nisoldipine. (The interactions between Nisoldipine and two beta-adrenoceptor antagonists : atenolol and propanolol. H.L.Elliott et al. Brit J Clin Pharmacol 1991;32(6):379-85).

Levine et al demonstrated pharacodynamic and pharmacokinetic interactions between Nisoldipine and Propanolol (MAH Levine et al. Pharmacokinetic and pharmacodynamic interactions between Nisoldipine and Propanolol. Clin Pharmacol Ther 1988; 43:39-48).

These contradictions could have been clarified had the sponsor included in this protocol a true placebo group and groups of Nisoldipine without Atenolol. Plasma levels of Atenolol should have been also measured.

Protocol D90-019

Title of Study : " A Double-Blind Randomized Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine Coat-Core Tablets 30 mg, 60 mg (2X30) and 90 mg (3X30) vs Placebo in Hypertensive Patients ".

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Objectives. The objectives of this study were :

1. To determine the antihypertensive efficacy and safety of NIS tablets in doses of 30 mg. 60 mg and 90 mg daily in patients with mild to moderate hypertension.

2. To assess the time and magnitude of peak blood pressure response and the ratio of trough to peak antihypertensive effect by 24-hour ambulatory blood pressure monitoring.

Inclusion Criteria. Ambulatory patients, male and female, 21 to 75 years of age, with a history of mild to moderate essential hypertension were eligible for the study.

Exclusion Criteria. Patients with the following conditions were excluded from the study : labile hypertension, renovascular or other secondary forms of hypertension, patients whose SUDBP after 3 and 4 weeks of placebo run-in were not ≥100 or ≤ 114 mmHg, previous myocardial infarction or cerebrovascular accident, heart failure, frequent arrhythmias, conduction disturbances, angina pectoris, use of other antihypertensive drugs, and many other drugs.

Also excluded were patients with failure of a major organ system, liver, kidney disease, malignancy or psychosis, patients with previous history of gastrointestinal disease which could result in incomplete absorption of the drug, women with childbearing potential, patients with alcohol or drug abuse, or allergy to dihydropyridines

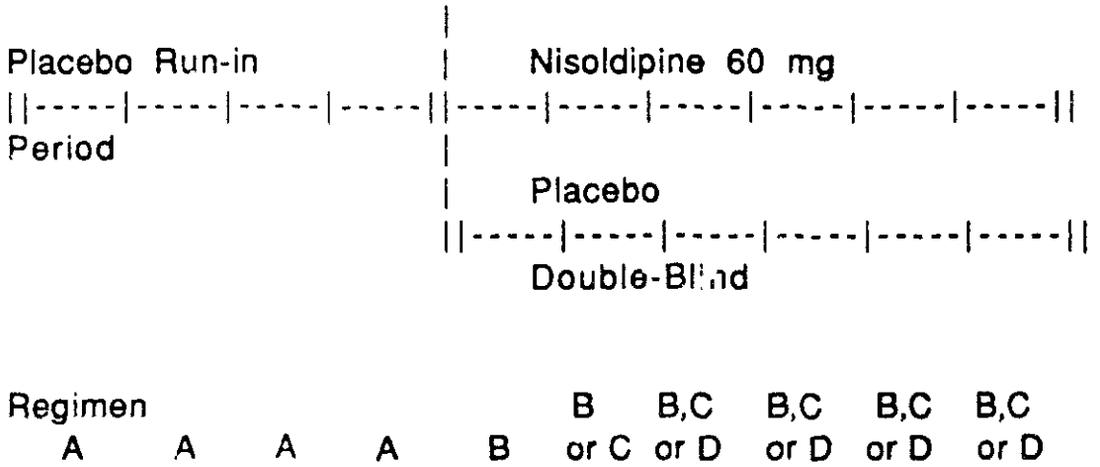
Study Design. . The study consisted of a single-blind placebo run-in period and a treatment period.

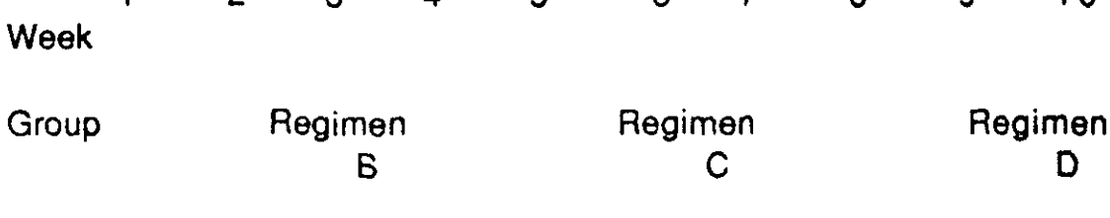
Placebo Run-in Period. During this period of 4 weeks duration pacients discontinued all previous antihypertensive medication and were given a single-blind placebo which consisted of 3 tablets once a day in the morning for a 4-week qualifying run-in period. Then patients with confirmed hypertension, with a trough SUDBP ≥100 to ≤ 114 mmHg after 3 and again after 4 weeks of placebo and whose SUDBP at these 2 visits were within 7 mmHg of each other were admitted into the treatment period.

Treatment Period. After four weeks of single-blind placebo patients with confirmed hypertension were randomized to one of three treatment groups: Placebo, Nisoldipine 30 mg or Nisoldipine 60 mg. Patients randomized to placebo received placebo for the remainder of the study. Placebo randomized to Nisoldipine 30 mg received the same dose for the remainder

of the study. Patients randomized to Nisoldipine 60 mag were given Nisoldipine 30 mg for one or two weeks and the dose was titrated to Nisoldipine 60 mg (2X30) for the final 4 to 5 weeks of the double-blind treatment. A group of patients was to be titrated to 90 mg (3X30) but this arm was discontinued before any patients were randomized because a concurrent high-dose forced-titration clinical pharmacological study showed evidence of symptomatic T wave flattening/or inversion predominantly at doses above Nisoldipine 60 mg.

The study design is shown schematically in the following graph :



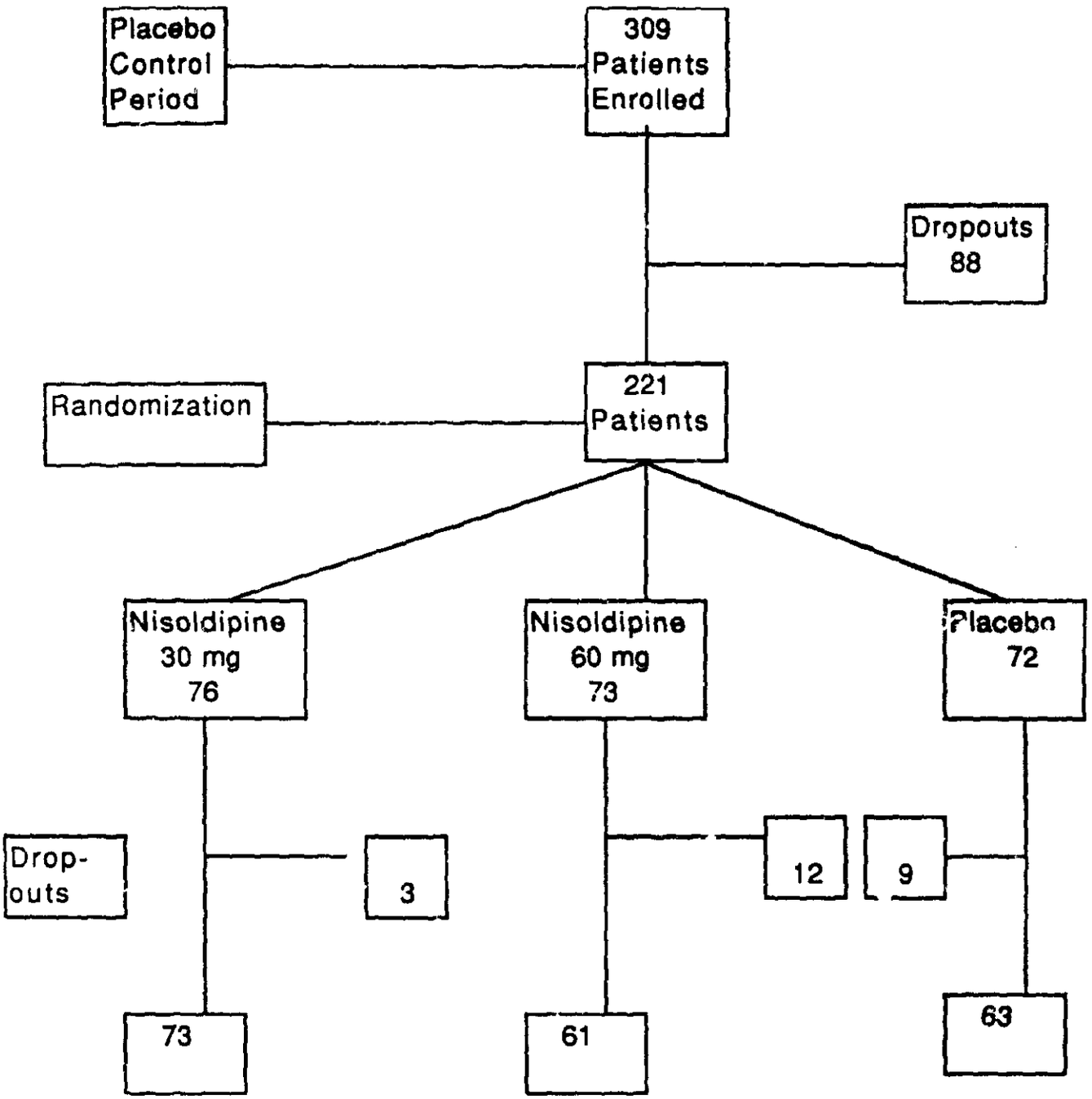


Group	Regimen B	Regimen C	Regimen D
NIS 30 mg	30 mg	30 mg	30 mg
NIS 60 mg	30 mg	60 mg	60 mg
Placebo	Placebo	Placebo	Placebo

Demography. The demography and baseline characteristics in patients valid for analysis of efficacy is given in the following table :

	NIS 30 mg N=76	NIS 60 mg N=66	Placebo N=71
Mean Age (years)	52	52	52
Mean wt (Lbs)	197	197	202
Baseline BP Supine Standing	 157/104 153/104	 158/105 154/104	 155/104 151/103
History of Hypertension (years)	11	9	10
% Male	61	56	52
% Caucasian	58	58	72
% History of Diabetes	9	11	13
% History of Hyperlipidemia	13	12	8
% History of MI	0	2	1
% Mild Hypertensives	62	58	70
% Moderate Hypertensives	38	42	30

The distribution and randomization of patients is seen in the following graph



The listing of patients who did not qualify for randomization is given in the following table :

Mean supine diastolic blood pressure at visit 4 and at visit 5 did not qualify	45
Adverse events	7
Low diastolic blood pressures	6
Patient request	6
Blood pressure too high	5
Elevated serum transaminase levels	5
Childbearing potential	2
Elevated serum lipids	2
Family considerations	2
Illness not due to study medication	2
Intercurrent medical considerations	2
Administrative problems	1
Change in supine diastolic blood pressure > 7 mmHg from visit 4 to visit 5	1
Low hemoglobin/hematocrit	1
Protocol violation	1
Total	88

The reasons for discontinuation of double-blind therapy population in all randomized patients are given in the following table :

Reason	Nisoldipine		Placebo
	30 mg N=76	60 mg N=73	N=72
Adverse Event	1	11	3
Lack of efficacy	1	0	2
Physician dissatisfied with treatment	0	1	2
Patient dissatisfied with treatment	0	0	2
Lost to follow-up	1	0	0

The listing of dropouts due to adverse events for patients valid for safety analysis is given in the following table :

Drug Group	Adverse Experience Causing Patient to Drop	Day of Onset	Dose/ Duration (Days)	Intensity/ Relation-ship to Drug
Placebo	EKG abnormality	-1	Pla/1	Mild/ Remote
	Cardiac arrest	24	Pla/1	Severe/ Remote
	Frontal headaches	28	Pla/>3	Severe/ Possible
Nisoldipine	Edema lower extremity	24	30 mg/ >7	Moderate/ Probable
	Severe headache	3	30 mg/ >11	Severe/ Possible
	Headache	-1	Pla/5	Severe/ Possible
	Severe headache	0	30 mg/7	Severe/Pos
	2+ ankle edema, bilateral	24	60 mg/ 10	Moderate/ Probable
	3+ pretibial edema, bilateral	24	60 mg/ 10	Severe/ Probable
	Trace edema, bilateral	24	60 mg/ 10	Mild/ Probable
	Atypical chest paln	9	60 mg/5	Mod/Poss
	Headache	12	60 mg/ >1	Severe/ Possible
	3 + ankle edema	33	60 mg/ >3	Severe/ Possible
	Headache,vomiting	7	60 mg/ 1	Severe/ Possible
	Headache	12	60 mg/>1	Sev/Rem
	Confusion,	0	30 mg/>1	Sev/Rem
	Nausea,	0	30 mg/3	Mild/Prob
	Headache	6	30 mg/2	Mod/ Probable

Efficacy

Actual Dosage and Duration of Treatment. Patients were to receive Nisoldipine 30 mg, 60 mg or Placebo over a 6 week double-blind treatment period. Upward titration from Nisoldipine 30 mg to Nisoldipine 60 mg was required in the Nisoldipine 60 mg group after one or two weeks of double-blind medication if trough SUDBP was greater than or equal to 80 mmHg. Placebo and Nisoldipine 30 mg underwent sham titration. The following table shows the number of patients that were given each dose level and had valid visits at each week for the population of patients valid for efficacy :

Double-Blind Visit			6	7	8	9	10	
Week of Double-Blind Therapy			1	2	3	4	6	Endpoint
Group	Regimen	Dose	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Nis 30 mg	B (30 mg qd)		75 (100)	10 (13)	4 (5)	3 (4)	3 (4)	3 (4)
	C (30 mg QD)			66 (87)	71 (95)	71 (96)	70 (96)	73 (96)
NIS 60 mg	B (30 mg QD)		65 (100)	7 (11)	5 (8)	5 (8)	4 (6)	5 (8)
	C (60 mg QD)			59 (89)	59 (92)	59 (92)	58 (94)	61 (92)
Placebo	B (Placebo)		71 (100)	6 (8)	2 (3)	2 (3)	2 (3)	2 (3)
	C (Placebo)			65 (92)	65 (97)	64 (92)	62 (97)	69 (97)

Analysis of Effectiveness. Two hundred thirteen of the 221 enrolled patients had at least one valid blood pressure measurement after randomization and were included in the primary efficacy analysis (endpoint) :76 were randomized to the Nisoldipine 30 mg group, 66 were randomized to the Nisoldipine 60 mg group, and 71 were randomized to the placebo group.

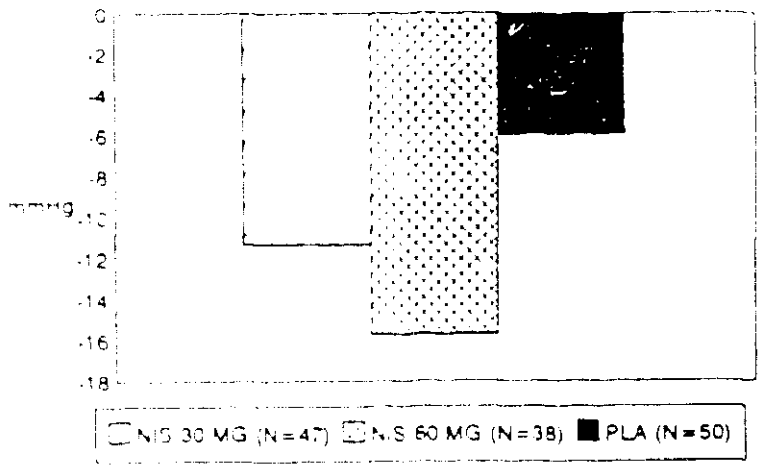
Trough raw means of blood pressure at each visit as well as at endpoint are given in the following table :

	Supine			Standing		
	NIS 30 mg	NIS 60 mg	Placebo	NIS 30 mg	NIS 60 mg	Placebo
Base-line (N)	158/ 104 (76)	158/ 105 (66)	155/ 104 (71)	154/ 104 (76)	155/ 104 (66)	152/ 103 (71)
Week 1 (N)	148/95 (75)	146/94 (65)	152/98 (71)	144/96 (75)	142/94 (65)	149/ 100 (71)
Week 2	147/93 (76)	141/90 (66)	152/98 (71)	143/95 (76)	139/91 (66)	149/99 (71)
Week 3 (N)	144/92 (75)	139/89 (64)	149/97 (67)	140/93 (75)	136/90 (64)	148/98 (67)
Week 4 (N)	142/92 (75)	139/87 (64)	152/98 (66)	140./93 (75)	135/87 (64)	150/99 (66)
Week 6 (N)	145/92 (73)	140/89 (62)	152/98 (64)	140/94 (73)	135/90 (62)	149/ 100 (64)
End-point (N)	146/93 (76)	141/90 (66)	154/99 (71)	141/94 (76)	137/91 (66)	150/ 101 (71)

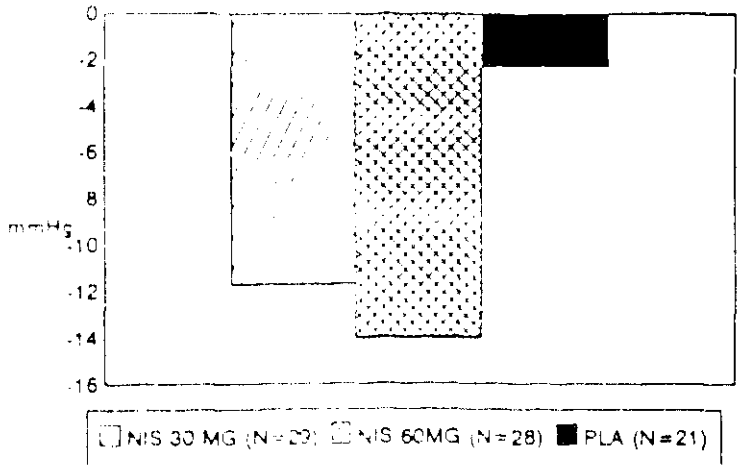
Mean changes (mmHg) in SUDBP at endpoint for patients wit mild (baseline SUDBP ≥ 100 to ≤ 104 mmHg)and moderate (baseline SUDBP ≥ 105 to ≤ 114 mmHg) hypertension are shown in the followings table and figure:

	Nisoldipine 30 mg		Nisoldipine 60 mg		Placebo	
	(N)	Change	(N)	Change	(N)	Change
Mild	47	-11.3	38	-15.7	50	-6.0
Moderate	29	-11.7	28	-14.0	21	-2.3

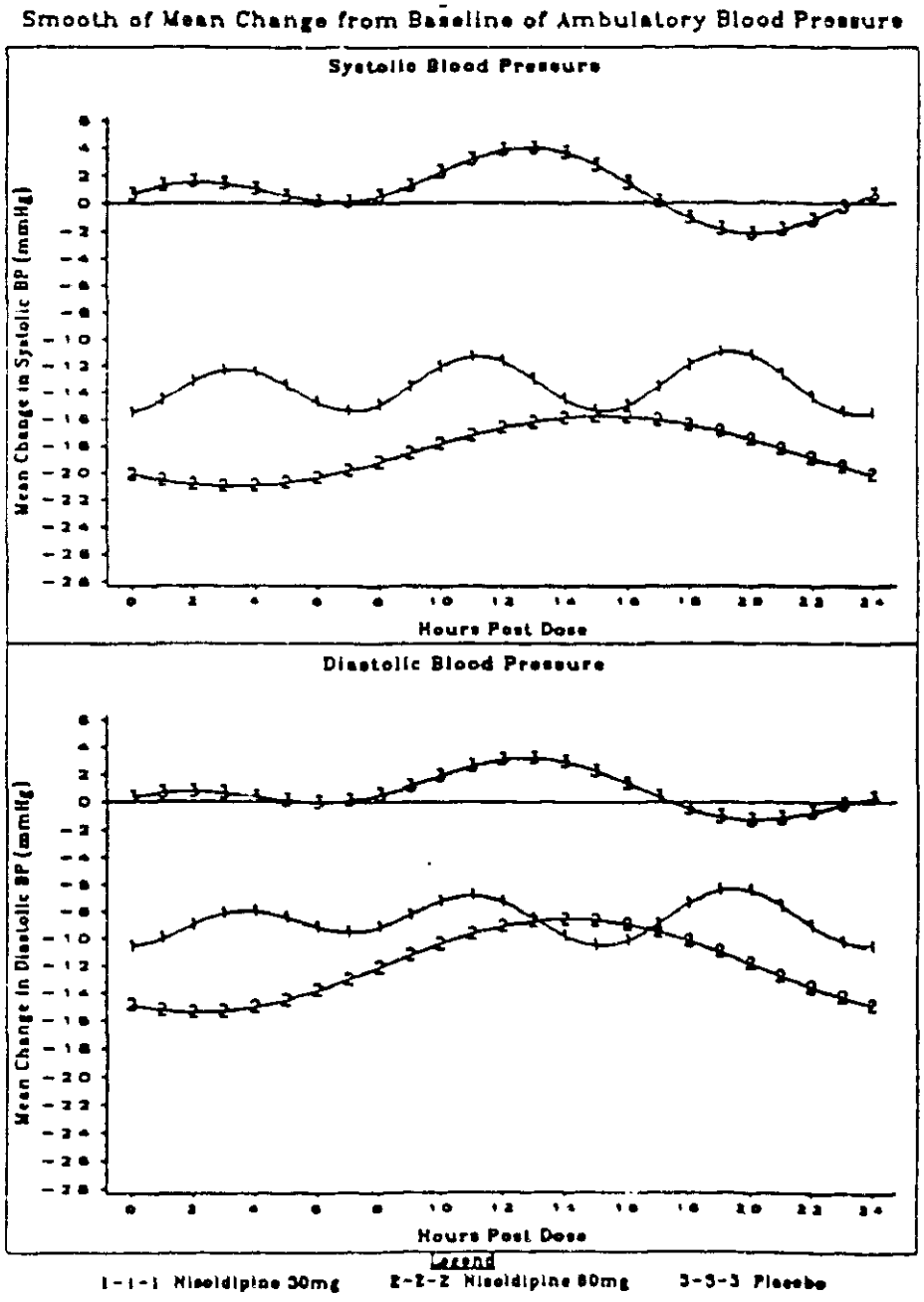
MEAN CHANGE FROM BASELINE (mmHg)
FOR SUPINE DIASTOLIC BP AT ENDPOINT
PATIENTS WITH MILD HYPERTENSION



MEAN CHANGE FROM BASELINE (mmHg)
FOR SUPINE DIASTOLIC BP AT ENDPOINT
PATIENTS WITH MODERATE HYPERTENSION



The 24-hour ambulatory blood pressure profile of mean systolic and diastolic blood pressure responses for the three treatment groups are shown in the following graph :



The ambulatory blood pressure falls and trough to peak ratios change from placebo for patients valid for efficacy analysis are given in the following table :

	N	Peak Hour	Peak value * (mmHg)	Trough* (mmHg)	Trough to Peak ratio
Diastolic BP					
NIS 30 mg	39	13	12.13	9.52	78 %
NIS 60 mg	29	4	15.24	14,22	93 %
Systolic BP (at corresponding time of diastolic peak)					
NIS 30 mg	39	13	17.46	14.52	83 %
NIS 60 mg	29	4	23.13	17.66	76 %
Systolic Blood Pressure (actual)					
NIS 30 mg	39	13	17.46	14.52	83 %
NIS 60 mg	29	5	23.71	17.66	75 %

*Change from placebo, baseline corrected

The mean changes from baseline in diastolic blood pressure over the 24-hour period of ambulatory blood pressure were -8.6 mmHg for Nisoldipine 30 mg, -12.0 mmHg for Nisoldipine 60 mg and +0.7 mmHg for placebo.

Pharmacokinetics Results. Trough blood samples were drawn at all 16 centers at visits 5 an 10. Seven centers also drew blood samples at visit 10.1 at 2 and 12 hours post-dosing. Samples were assayed for Nisoldipine blood levels. Results are presented in the following table :

	N	Mean Concentration (SD) ng/ml	Change in SUBP (Systolic/Diastolic) in mmHg
NIS 30 mg :			
Trough	68	1.5 (1.3)	-12/-11
2 hours post dosing	27	2.3 (1.9)	-17/-15
12 hours post dosing	27	2.1 (1.2)	-19/-17
NIS 60 mg:			
Trough	55	3.2 (2.8)	-17/-16
2 hours post dosing	27	6.0 (5.2)	-20/-19
12 hours post dosing	26	4.9 (2.8)	-21/-22

There was a statistically significant correlation between plasma concentration and change from baseline to endpoint in supine diastolic blood pressure at trough. The greater the correlation, the greater was the decrease in supine diastolic blood pressure. Twenty percent of the variability in the observed change in supine diastolic blood pressure was explained by plasma concentration.

Assessment. The results of this study indicate that Nisoldipine, at the dose of 30 mg and 60 mg daily once daily, is effective in reducing systolic and diastolic blood pressure at trough in patients with mild to moderate hypertension. The reductions in systolic and diastolic blood pressure were greater than 50 percent of peak effect at trough. Furthermore, ambulatory measurements of blood pressure for 24-hours demonstrated that reductions in blood pressure in Nisoldipine-treated patient was maintained through the hours of observation. The effect was more effective with the 60 mg of Nisoldipine than with the 30 mg dose and in the latter more effective than placebo. Pharmacokinetic studies demonstrated that effect on diastolic blood pressure was proportional to the concentration of Nisoldipine in blood.

Side effects were significantly increased by drug administration as compared to control and were greater with the 60 mg Nisoldipine dose than with the 30 mg. Adverse events are to be discussed by another reviewer.

Protocol D89-039

Title of Study : " Comparative Double-Blind Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine 20 mg. 40 mg, 80 mg Coat Core (CC) Tablets vs a Twice Daily Dose of Verapamil SR 240 mg caplets vs Placebo in Hypertensive Patients ".

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Objectives. The objective of this study was to determine the efficacy and safety of once daily doses of Nisoldipine 20 mh, 40 mg and 80 mg to a twice daily dose of Verapamil 240 mg and to Placebo in patients with mild to moderate hypertension.

Inclusion and Exclusion Criteria. Ambulatory male and female patients, 21 years of age or older, with history of mild to moderate hypertension, were eligible for enrollment in this study.

Patients with the following conditions were excluded from this study : recent myocardial infarction or cerebral vascular accident ; herat failure, major arrhythmias, conduction distrurbances, angina pectoris, sinus bradycardia or severe left ventricular dysfunction ; patients with impaired absorption of the drug ; females pregnant or with childbearing potential ; patients with failure of a major organ system such as liver, renal disease, malignancy or psychosis ; alcohol abuse or drug intake ; allergy to dihydropyridines, verapamil or other antagonists ; also excluded were patients who participated in another investigational drug study within the previous 30 days.

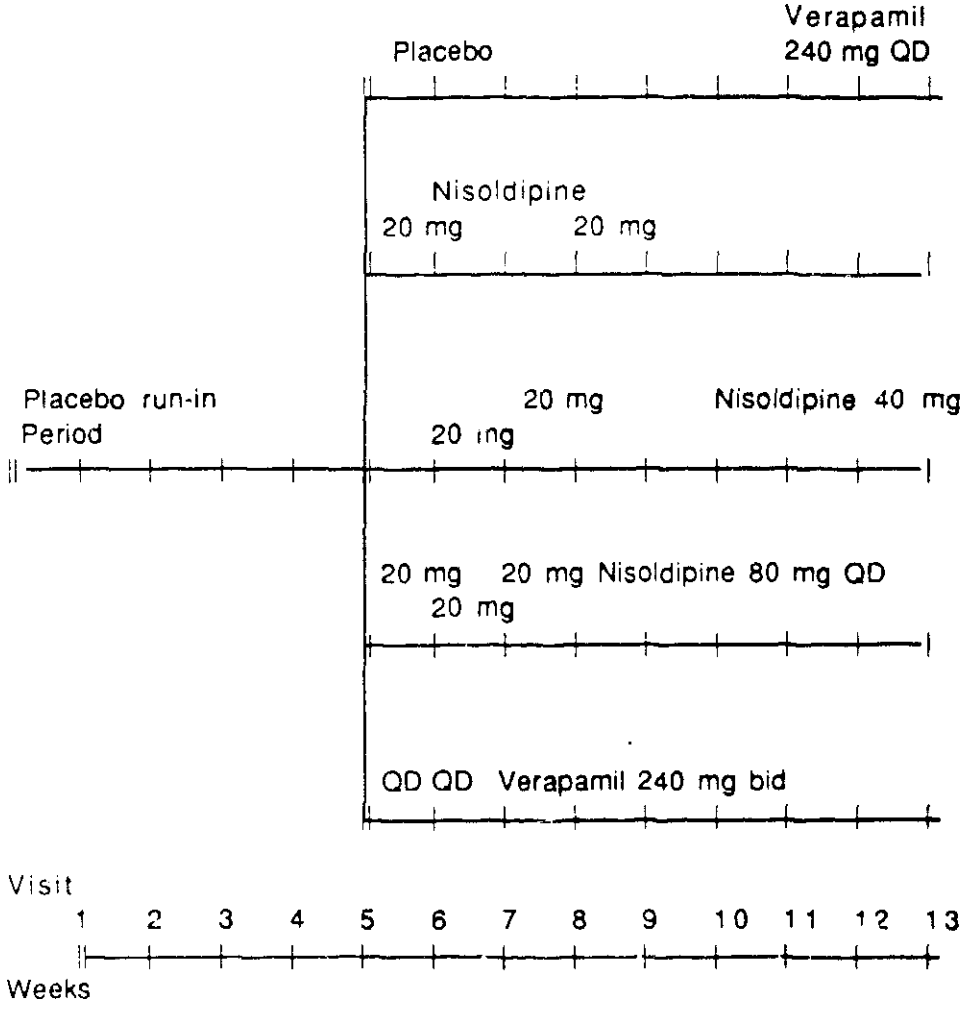
Study Design. The study consisted of a single-blind run-in period and a treatment period.

Single-Blind Run-In Period. Patients were given two placebo tablets and one placebo capsule in the morning and anothe placebo-capsule in the evening each day during a 4-week single-blind-run-in period. Drug for the single-blind placebo run-in period was labeled as Regimen A.

Qualification for Randomization. Patients whose mean SUDBP (the average of 3 readings over a five minute period in the supine position) were 95-114 mmHg after 3 and after 4 weeks on placebo and whose SUDBP after 3 and 4 weeks on placebo were within 7 mmHg of each other were eligible for randomization.

Double-Blind Treatment Period. After the placebo run-in period, a forced titration was designed as follows : Regimen B : Nisoldipine 20 mg, Verapamil 240 mg qd or Placebo which patients took for one week; Regimen C : Nisoldipine 20 mg, Nisoldipine 40 mg, Verapamil 240 mg twice daily or Placebo which patients took for one week ; Regimen D : Nisoldipine 20 mg, Nisoldipine 40 mg, Nisoldipine 80 mg (2 X 40), Verapamil 240 mg twice daily or Placebo which patients took for 6 weeks. After 8 weeks of double-blind drug, patients given Nisoldipine or Verapamil continued on the same drug regimen while patients given Placebo were switched to Verapamil 240 mg qd for the remaining of the 4 weeks of study.

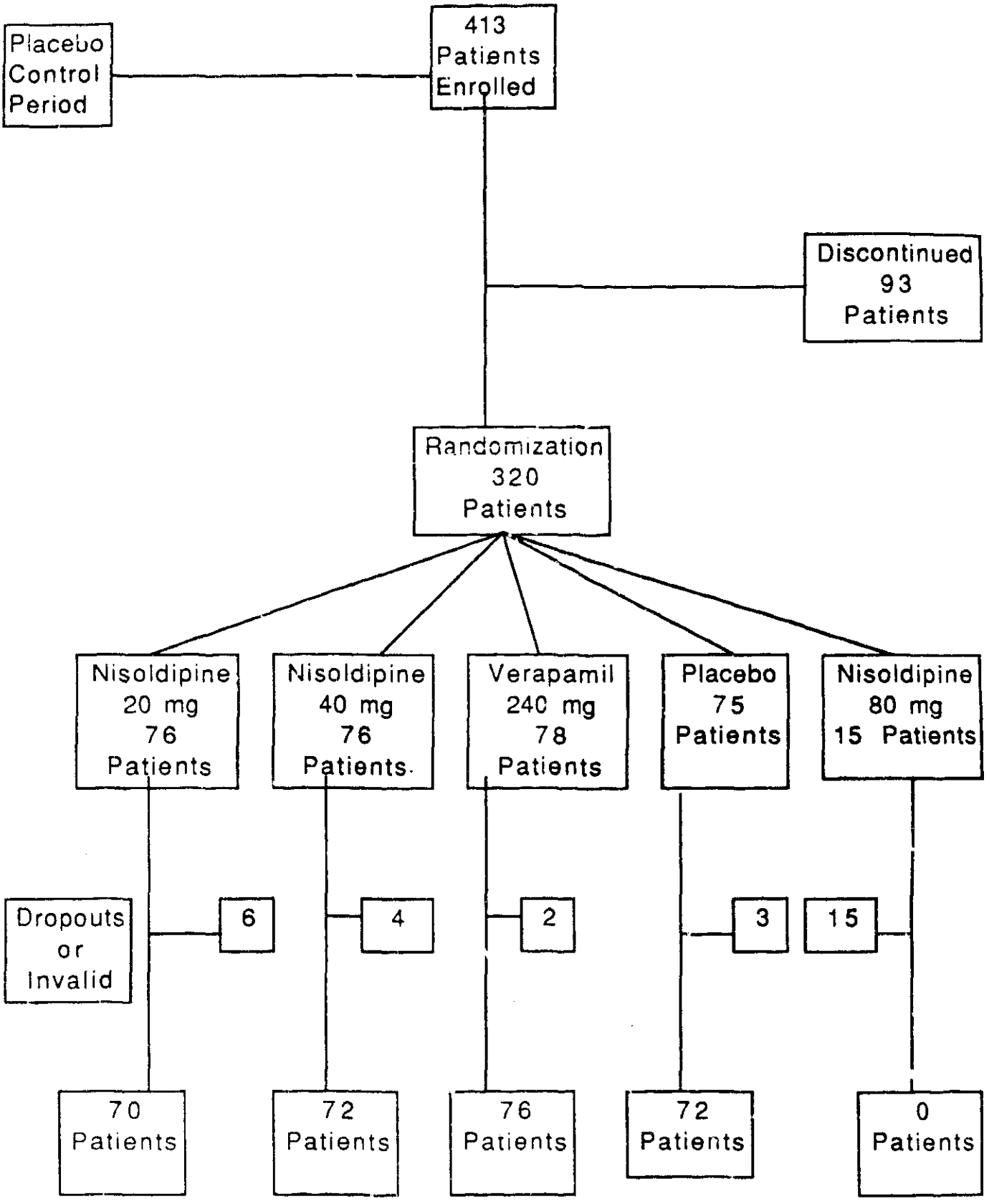
The stuoy design is demonstrated schematically in the following graph :



Demographics. The demographic characteristics are shown in the following table :

	Nisoldipine 20 mg n=70	Nisoldipine 40 mg n=72	Verapamil n=76	Placebo n=72
Mean age (years)	53	54	52	55
Mean wt (lbs)	202	197	198	196
Baseline BP (mmHg)				
Supine	153/100	155/100	151/100	154/100
Standing	151/101	151/101	148/101	151/100
Male	57 %	61 %	55 %	67 %
Black	31 %	24 %	28 %	21 %
History of Diabetes	9 %	1 %	11 %	8 %
History of Hyperlipidemia	3 %	1 %	3 %	10 %
History of MI	3 %	1 %	0 %	1 %
Hypertensives				
Mild	84 %	86 %	83 %	89 %
Moderate	16 %	14 %	17 %	11 %

The distribution of patients and randomization are given in the following graph:



The reasons that disqualified enrolled patients for andomization are given in the fo'lowing table :

Mean Supine Diastolic Blood Pressure at visit 4 or visit 5 did not qualify for randomization (95 mmHg to 11 mmHg)	47
Adverse events	13
Patient chose to withdraw	9
Other illness/Surgery/Screening abnormality	5
Blood pressure too high off medications for patient's safety	5
Lost to follow-up	4
Elevated transaminases at screening	3
Noncompliance	3
Called to military service	2
Blood pressure too low after in-clinic	1
Inadequate quality control during ambulatory blood pressure	1
Total	93

The number of dropouts during the treatment period and the reasons for elimination from the study are given in the following table :

Nisoldipine 20 mg. N=76

Event	Days on Drug
Palpitations, depression, headache, emesis	2
Headache, shortness of breath, fatigue	3
Headache, flashing, head congestion	4
Headache, flushing, palpitations	6
Peripheral edema	12
Headache, nausea	12
Peripheral edema	24
Peripheral edema	73
Pleural effusion	77
Myocardial infarction	89
Noncompliance	7
Chose to withdraw	54

Nisoldipine 40 mg. N=76

Event	Days on Drug
Headache, rash	1
Headache, nausea	2
Headache, nausea	2
Peripheral edema	10
Peripheral edema	12
Myocardial infarction	13
Headache, tremor, flushing, palpitations hypesthesia, asthenia	14
Peripheral edema	16
Peripheral edema	22
Peripheral edema	40
CVA	41
Chose to withdraw	16
Chose to withdraw	32

Nisoldipine 80 mg. N=15

Headache, flushing, palpitations, chest pain	1
Flushing, palpitation	14
Deep T wave inversion	15
Discontinued	3
Discontinued	4
Discontinued	6
Discontinued	12
Discontinued	13
Discontinued	14
Discontinued	17
Discontinued	20
Discontinued	20
Discontinued	24
Discontinued	28
Discontinued	31

Verapamil 240 mg. N=78

Event	Days on Drug
Headache, dizziness, tachycardia, leg pain, tinnitus	0
Peripheral edema	17
Hypotension	22
Headache, chills, peripheral edema	36
Cholecystitis	7

Placebo. N=75

CVA	6
Fatigue, edema	14
Peripheral edema	32
Lack of efficacy	5
Lack of efficacy	48
Lost to follow-up	21
Lost to follow-up	69
Chose to withdraw	47
Chose to withdraw	62
DBP> 114 mmHg	27
Retinal disorder	55

Efficacy

Criteria for Effectiveness. The change from baseline to endpoint in trough SUDBP (blood pressure measured 24 hours after the previous day's morning dose and 12 hours after the previous day's evening dose) in the Nisoldipine 40 mg group compared to the placebo group was the primary criterion used to determine the effectiveness of the drug.The comparison of Nisoldipine 20 mg to Placebo was of secondary importance.

Secondary efficacy parameters included standing diastolic blood pressure and both standing and supine systolic blood pressure. In addition in eight centers ambulatory blood pressure changes (the difference between measurements made over the 24 hours after 3 weeks of placebo run-in and the 24 hours after 7 weeks of double-blind therapy) were compared among

groups. The 12-hour in-clinic monitoring data were also compared among groups. The peak effect and the time to peak effect were calculated for both the ambulatory and 12-hour in-clinic monitoring. In addition the trough to peak ratio was calculated for ambulatory blood pressure. Plasma samples were drawn at baseline (visit 5) and at visit 11 for analysis of Nisoldipine plasma concentrations.

Statistical Methods. All statistical methods were two-tailed and were conducted at a significance level of 0.05. Pairwise comparisons and within group changes were tested via the least squares means estimated by the model.

Analysis of Effectiveness. The mean blood pressure changes at endpoint (mmHg) for patients valid for efficacy analysis are given in the following table :

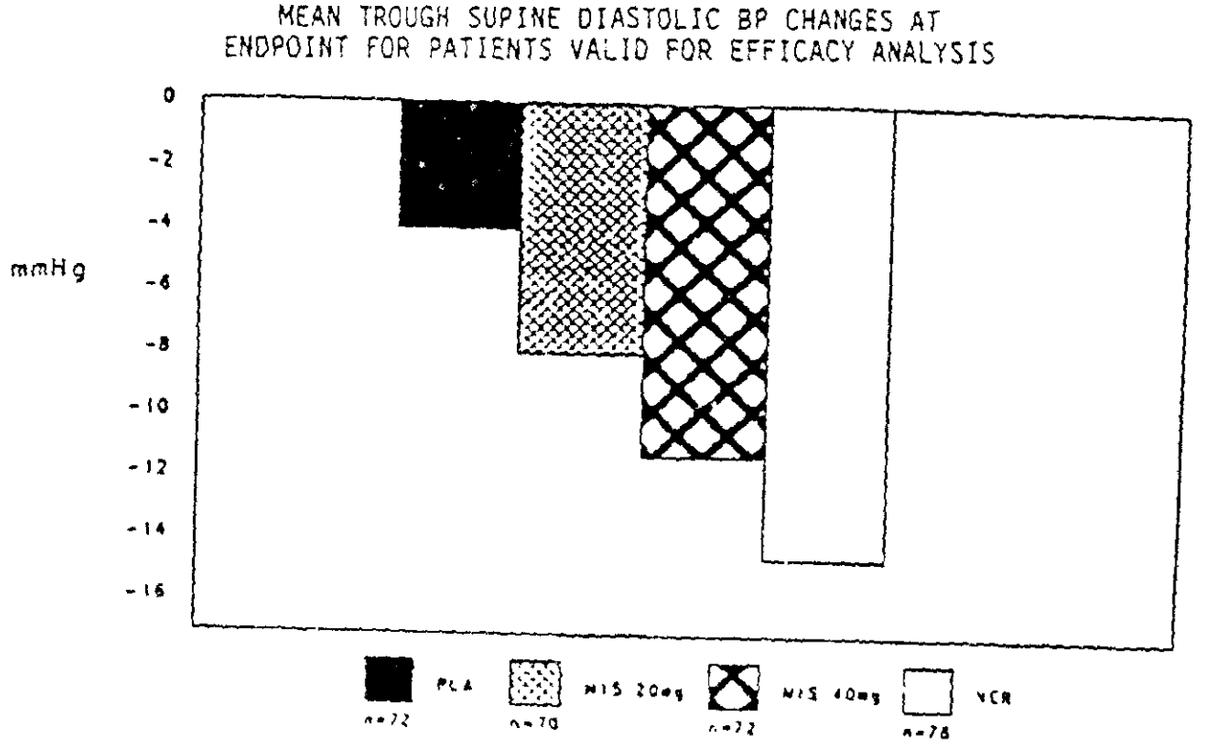
	Nisoldipine 20 mg N=70	Nisoldipine 40 mg N=72	Verapamil N=76	Placebo N=72
Supine				
Diastolic	-8.1 ABP	-11.4 BP	-14.7 P	-4.0
Systolic	-9.6 ABP	-16.2 P	-16.0 P	-2.2
Standing				
Diastolic	-7.1 ABP	-11.8 BP	-13.9 P	-2.0
Systolic	-11.6 BP	-15.4 P	-16.4 P	-2.4

A Significantly different from Nisoldipine 40 mg
B Significantly different from Verapamil
P Significantly different from Placebo

Mean changes (mmHg) in SUDBP at endpoint for patients with mild (baseline SUDBP 95-104 mmHg) and moderate (Baseline SUDBP 105-114 mmHg) are shown in the following table :

	Nisoldipine 20 mg		Nisoldipine 40 mg		Verapamil		Placebo	
	n	Change	n	Change	n	Change	n	Change
Mild	59	-8.4	62	-11.3	63	-14.1	64	-4.1
Moderate	11	-6.5	10	-13.0	13	-18.0	8	-3.5

The effect on SUDBP at endpoint in the Nisoldipine (24 hours after dose), Verapamil group (12 hours after dose) and Placebo group is shown in the figure below :



The results by visit for SUDBP are shown in the following graph :

	Week 1	Week 2	Week 3	Week 4	Week 6	Week 8
NIS 20 mg						
n	69	70	66	65	68	68
Mean Change	-7.8	-7.2	-8.6	-7.5	-7.9	-8.0
NIS 40 mg						
n	72	72	66	65	65	63
Mean Change	-7.4	-9.8	-9.9	-11.2	-11.0	-11.8
Ver						
n	76	75	73	69	73	71
Mean Change	-5.9	-11.0	-12.8	-13.1	-12.6	-14.6
Placebo						
n	72	72	68	67	69	67
Mean Change	-4.0	-4.7	-5.1	-4.4	-5.7	-4.1

During the secon phase of the double-blind period, the differences between the active drugs decreased, while the Placebo group experienced the expected further decrease in blood pressure after switching to Verapamil. The changes from baseline in trough SUDBP at the two visits in thus phase are presented below :

	Week 10	Week 12
NIS 20 mg	-8.8	-10.1
NIS 40 mg	-12.2	-10.3
Verapamil	-13.1	-11.5
Placebo	-7.3	-7.0

Various demographic variables were examined including sex, weight, age, smoking status, race and baseline blood pressure. Of these only age exhibited a marked difference in blood pressure response. Mean changes from baseline in supine blood pressures for each drug group for patients at least 60 years old vs patients younger than 60 years old are provided in the table below :

	NIS 20 mg		NIS 40 mg		Verapamil		Placebo	
	n	Mean	n	Mean	n	Mean	n	Mean
Diastolic								
Age ≥ 60	28	-10.4	26	-13.6	24	-16.2	23	-4.0
Age < 60	42	- 6.6	46	-10.4	52	-14.1	49	-4.0
Systolic								
Age ≥ 60	28	-14.0	26	-21.2	24	-19.3	23	-2.3
Age < 60	42	-6.8	46	-13.6	52	-14.5	49	-2.2

Responders rate based on trough SUDBP are presented in the following table :

	NIS 20 mg N=70	NIS 40 mg N=69	Verapamil N=76	Placebo N=72
DBP ≤ 90 mmHg	35 (50 %)	50 (69%)	62 (82 %)	19 (26 %)
DBP decrease ≥ 10 mmHg	28 (40 %)	47 (65 %)	59 (78 %)	10 (14 %)

In clinic monitoring was done for 12 hours and 24-hour ambulatory blood pressure monitoring for 24 hours.

The in clinic monitoring, that covered only half of the dosing interval yielded the following results :

Dose	Mean Change Range mmHg/	Hour
Nisoldipine 20 mg	-9.8	12
	-13.5	8
Nisoldipine 40 mg	-10.8	12
	-15.5	4
Verapamil	-11.8	
	-15.1	
Placebo	-2.5	
	-5.5	

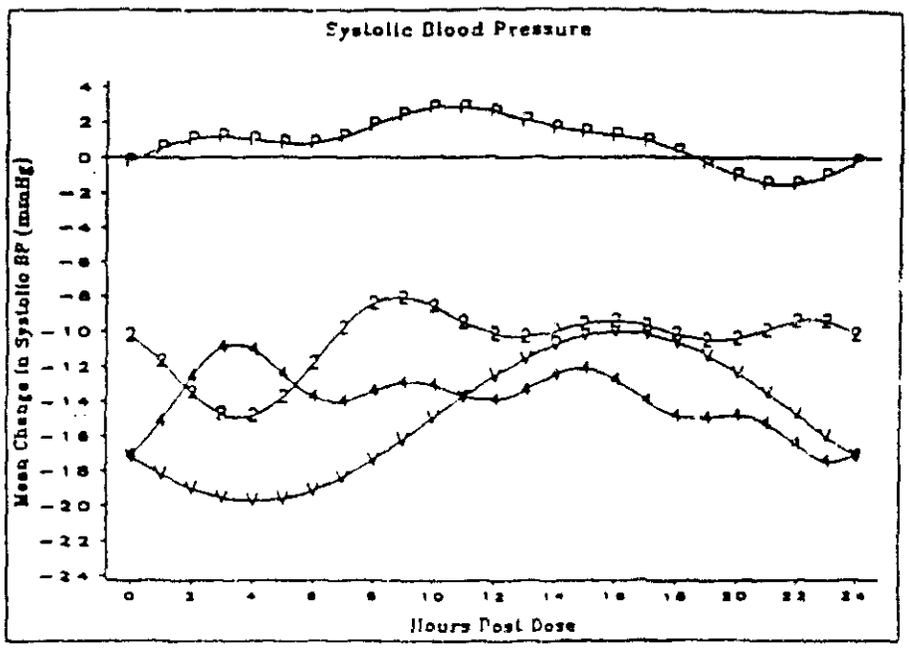
On ambulatory blood pressure monitoring response after 7 weeks of therapy was observed for 24 hours after Nisoldipine 40 mg therapy, 4 hours after Nisoldipine 20 mg therapy, and 4 hours after the morning dose of Verapamil. with blood pressure changes (systolic/diastolic)of -17.5/ -10.7 mmHg, -15.1/10.2 mmHg, and -19.7/-14.3 mmHg respectively. The mean 24-hour systolic and diastolic blood pressure changes during ambulatory blood pressure monitoring were :

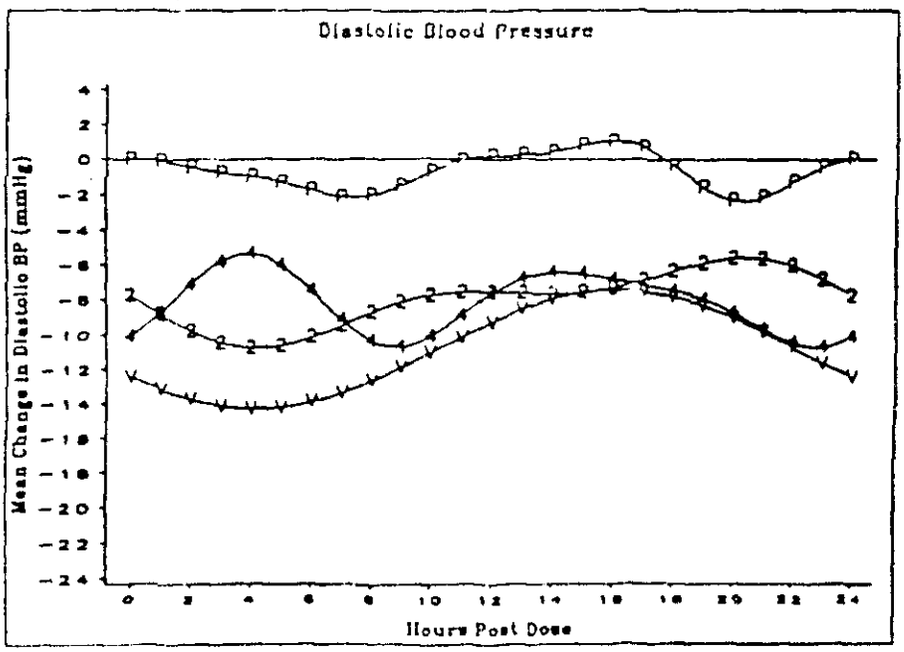
Nisoldipine 40 mg	-13.6/-8.0
Nisoldipine 20 mg	-11.1/-7.9
Verapamil	-14.8/10.8

Based on smoothed ambulatory blood pressure data, the trough/peal ratios for the treatment groups are summarized in the following table :

	Trough mmHg	Peak mmHg	Trough to peak ratio
Diastolic BP			
NIS 20 mg	-S.7	-9.7	69 %
NIS 40 mg	-11.7	-11.7	100 %
Verapamil	-11.1	-12.9	86 %
Systolic BP			
NIS 20 mg	-9.9	-15	66 %
NIS 40 mg	-14.3	-14.3	100 %
Verapamil	-15.9	-20.9	78 %

The unsmoothed change from baseline ambulatory data in systolic and diastolic blood pressure are shown below

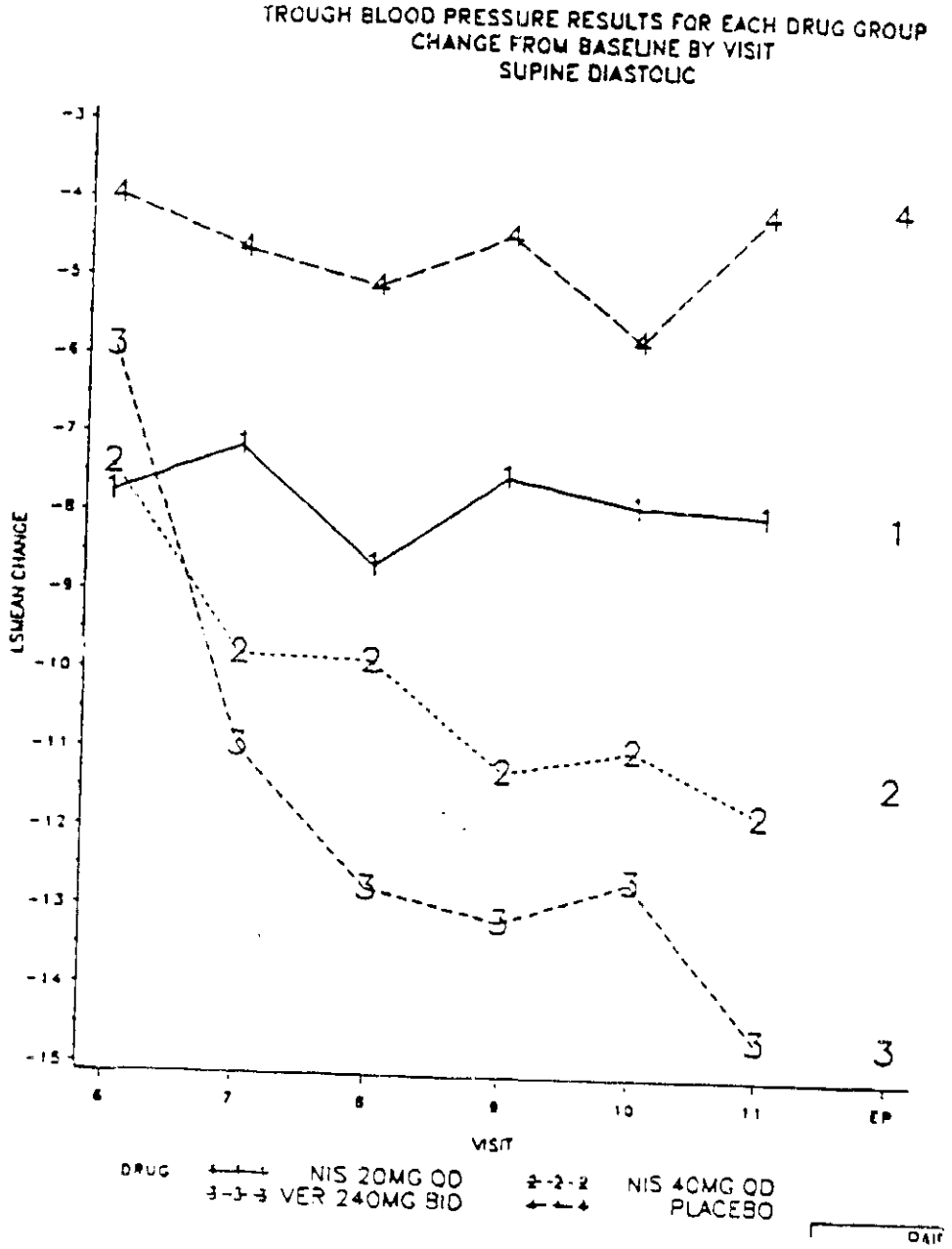




Legend

2-2-2 Nis 20mg 4-4-4 Nis 40mg V-V-V Verapamil P-P-P Placebo

The trough blood pressure results for each drug group change from baseline by visist supine diastolic is given in the following graph :



Pharmacokinetic Results. Trough blood samples were drawn at visits 5 and 11. Visit 11 samples were analyzed for Nisoldipine and results are summarized below :

	n	Range of Concentrations (ng/ml)	Mean Concentrations (ng/ml)
NIS 20 mg	66	0-3.19	1.0
NIS 40 mg	61	0-6.83	2.2
NIS 80 mg	3	0-5.24	2.3

Assessment. The study was initially designed to determine the effectiveness of Nisoldipine at doses of 20, 40, 80 mg, Verapamil and placebo. The 80 mg dose of Nisoldipine was dropped when in another study of a high-dose forced-titration study of Nisoldipine 120 mg daily showed asymptomatic T waves flattening and/or inversion on electrocardiogram predominantly at doses above 60 mg daily.

The 20 and 40 concentrations of Nisoldipine demonstrated to be more effective in lowering the blood pressure than placebo, and the 40 mg more effective than the 20 mg. Aiso the effectiveness was greater in subjects older than 60 years especially in lowering the systolic blood pressure. Verapamil bid was more effective in lowering blood pressure than any of the concentrations of Nisoldipine.

Peak and trough values were determined by ambulatory blood pressure monitoring and the antihypertensive effect was well sustained at 24 hours after dose administration in all concentrations of Nisoldipine evaluated in this study.

By pharmacokinetic studies the concentration of Nisoldipine in blood was determined and was found to be more elevated after the 40 mg administrations of Nisoldipine than after the 20 mg ncentration. There was no major difference between the 40 mg and 80 mg dose of Nisoldipine.

Protocol D90-006

Title of Study : " South-African Multicentre Study to Investigate the Anti-Hypertensive Effect of Three Single Oral Daily Doses of Nisoldipine Administered as a Long Acting "Coat-Core" Tablet Formulation."

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Objectives. The objectives of this study were :

1. To compare the anti-hypertensive efficacy and safety of three daily doses of Nisoldipine coat-core formulation, namely 10 mg, 20 mg and 30 mg with placebo.

2. To study a dose-response relationship for Nisoldipine coat-core.

3. To assess the consistency of anti-hypertensive response over 6 weeks.

Additional objectives were :

1. To describe the blood pressure profile of the last day of therapy by continuous automated ambulatory blood pressure monitoring in a group of patients, and hence :

2. To quantify the trough/peak blood pressure relationship for this therapy.

Inclusion Criteria. Patients with newly diagnosed mild to moderate hypertension were eligible to enter the study. In addition patients with mild to moderate hypertension being treated who, in the opinion of the investigator, were no significantly placed at risk by withdrawal of previous anti-hypertensive medication during the 4-week placebo run-in period could also be enrolled in the study.

Exclusion Criteria. Patients were not eligible if they had labile hypertension, clinical evidence of major arrhythmias, angina pectoris, conductio disturbances or heart failure, or recent or impending myocardial infarction, or a cerebral vascular accident in the previous 3 months, history of allergy to dihydropyridines, type 1 diabetes mellitus, impaired renal function, liver disease, elevated transaminases, treatment with antihypertensives or any other drug that may affect the blood pressure or may interact with the effects of calcium antagonists.

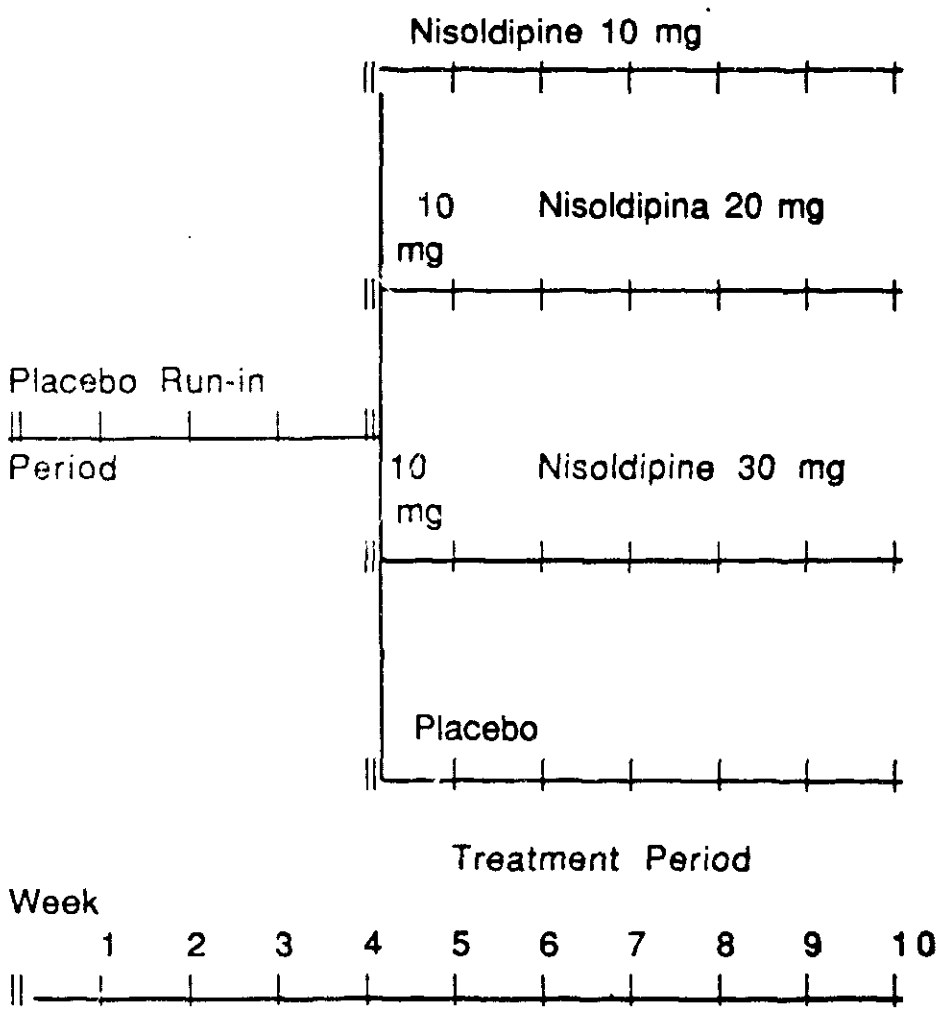
Study Design. This was a 10 week, multi-centre, randomized, placebo controlled, parallel group comparison of Nisoldipine coat-core 10 mg, 20 mg, 30 mg versus placebo. The study consisted of two periods : a single-blind placebo run-in period and a double-blind, randomized, placebo-controlled, group comparison (treatment period.

Placebo run-in Period. During this period of 4 weeks duration all antihypertensive medication was discontinued and one placebo tablet was given to be taken in the morning before breakfast. Patients whose SUDBP was ≥ 95 mmHg and ≤ 114 mmHg at visits 2 and 3 were eligible for enrollment in the active treatment phase.

Treatment Period. Eligible patients were randomized to one of four arms : placebo, 10 mg Nisoldipine, 20 mg Nisoldipine and 30 mg Nisoldipine.

Patients randomized to placebo or 10 mg Nisoldpine were to receive their treatment for 6 weeks. Patients in the two higher dose groups (Nisoldipine 20 mg or Nisoldipine 30 mg) were to receive 10 mg for the first week following by 5 weeks of their randomized treatment in order to avoid rapid exposure to the higher doses.

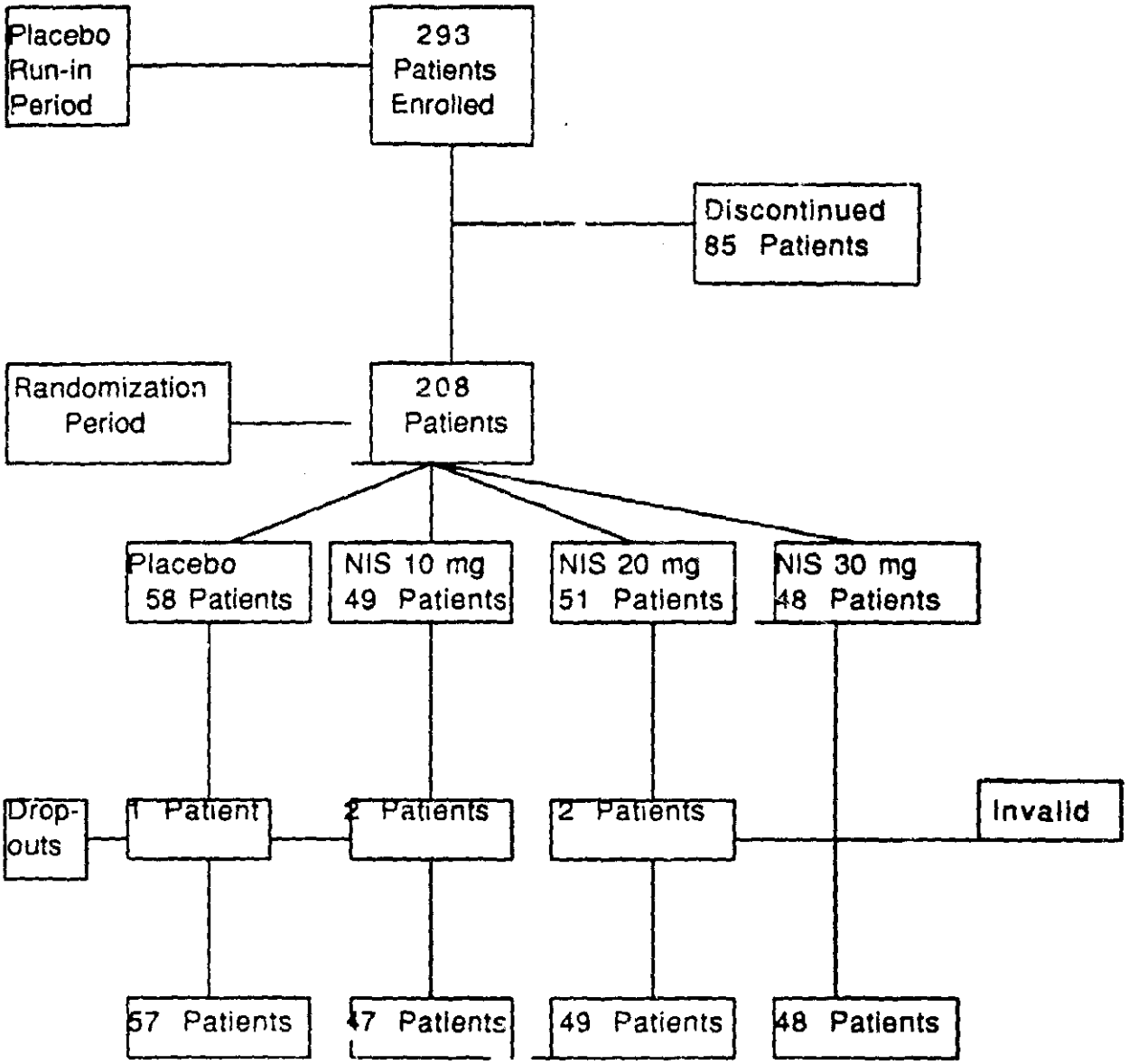
The study design is demonstrated schematically in the following graph :



The demographic information is given in the following table :

		Placebo n=58	NIS 10 mg n=49	NIS 20 mg n=51	NIS 30 mg n=48
Sex (p=0.78)	Male Female	27 (47 %) 31 (53 %)	24 (49 %) 25 (51 %)	20 (39 %) 31 (61 %0	21 (44 %) 27 (56 %)
Race (p=0.98)	Caucasian Black Asian Other	30 (52 %) 27 (29 %) 6 (20 %) 5 (9 %)	24 (53 %) 16 (33 %) 2 (4 %) 5 (10 %)	25 (49 %) 18 (35 %) 6 (12 %0 2 (4 %)	26 (54 %) 14 (29 %) 5 (11 %) 3 (6 %)
Age (years) Mean	Mean Mean=0.2	53	50	55	50
Weight (kg)	Mean (p=0.69)	80.8	77.3	79.7	80.3
Baseline Means BP Supine Systolic Diastolic Standing Systolic Diastolic	 (p=0.17) (p=0.65) (p=0.69) (p=0.09	 163.8 103.5 160.5 105.1	 161.2 104.7 159.7 107.9	 167.2 104.8 163.8 107.4	 164.3 104.4 161.7 107.4
Mild Hypert. n Baseline SDBP Moder. Hypert. N Baseline SDBP		 33 99.7 25 108.5	 25 99.3 24 110.2	 25 99.2 26 110.1	 27 100.1 21 110.1

The distribution and randomization of patients is illustrated in the following graph :



The reasons for patients who did not enter the double-blind treatment period is given in the following table :

Reason	Number of Patients
Supine diastolic blood pressure < 95 mmHg	58
Supine diastolic blood pressure > 114 mmHg	13
Unwilling to continue	5
Patient had raised serum calcium levels	1
Uncontrolled non-insulin dependent diabetes mellitus	1
Raised liver enzymes	3
Left ventricular failure	1
Right ventricular failure when taken off diuretic	1
Major arrhythmias	2
Total	85

Invalid Results and Drop-outs During the Treatment Period. Three patients dropped-out during tne treatment period. One patient in the placebo group died after experiencing cerebral hemorrhage 33 days after entering the double-blind treatment period. One patients in the Nisoldipine 10 mg experienced severe tinnitus 30 days after entering the double-blind treatment period. Another patient in the 10 mg Nisoldipine group had a severe headache and dropped 17 days after entering the double-blind treatment period.

Efficacy.

Criteria for Efficacy. The primary variable for assessing efficacy was the trough 24-hour supine diastolic blood pressure (SUDBP), and especifically the change in suDBP from baseline to endpoint (visit 6, week 6 or the last valid visit). The change from baseline in each of tne three Nisoldipine treatment groups was compared to the Placebo group. Secondary efficacy variables were supine systolic BP and standing diastolic and systolic blood pressure.

Statistical Analysis. Two types of analysis were followed. The first and primary analysis was the standard endpoint analysis, also referred as the main efficacy analysis. The second was the intent-to treat analysis (ITT).

All patients adherent to the protocol with a valid treatment duration of at least 2 weeks on double-blind treatment were included in the main efficacy analysis. These patients completed at least a two-week double-blind treatment period during which they were compliant. and after which the blood pressure was taken between 22.5 h and 25.5 h after the last tablet intake. Patients who discontinued treatment because of lack of efficacy or adverse events were also included. Only 2 patients who received double-blind treatment were considered invalid for the main efficacy

analysis. They were included in the intent to treat analysis. In one patient the baseline measurements were lost
and in another patient only 10 tablets instead of 20 tablets were dispensed.

The results of change from baseline at endpoint in trough blood pressure in all patients valid for the main efficacy analysis (n=206) are given in the following table :

	Placebo n=58	NIS 10 mg n=49	NIS 20 mg n=51	NIS 30 mg n=48
Supine DBP				
Baseline	103.5	104.7	104.8	104.4
Endpoint	101.1	99.3	95.7	94.3
Difference (NIS-Placebo)		-3.2	-6.7	-8.0
Supine SBP				
Baseline	163.8	167.2	167.2	164.3
Endpoint	163.3	149.8	149.8	148.8
Difference (NIS-Placebo)		-8.9	-17.8	-15.9
Standing DBP				
Baseline	105.1	107.9	107.4	107.4
Endpoint	104.6	101.1	98.1	96.9
Difference (NIS-Placebo)		-6.9	-9.2	-10.6
Standing SBP				
Baseline	160.5	159.7	163.8	161.7
Endpoint	160.5	150.6	147.5	145.7
Difference (NIS-Placebo)		-9.5	-15.9	-16.2

The results on SDBP are demonstrated in the following graph :

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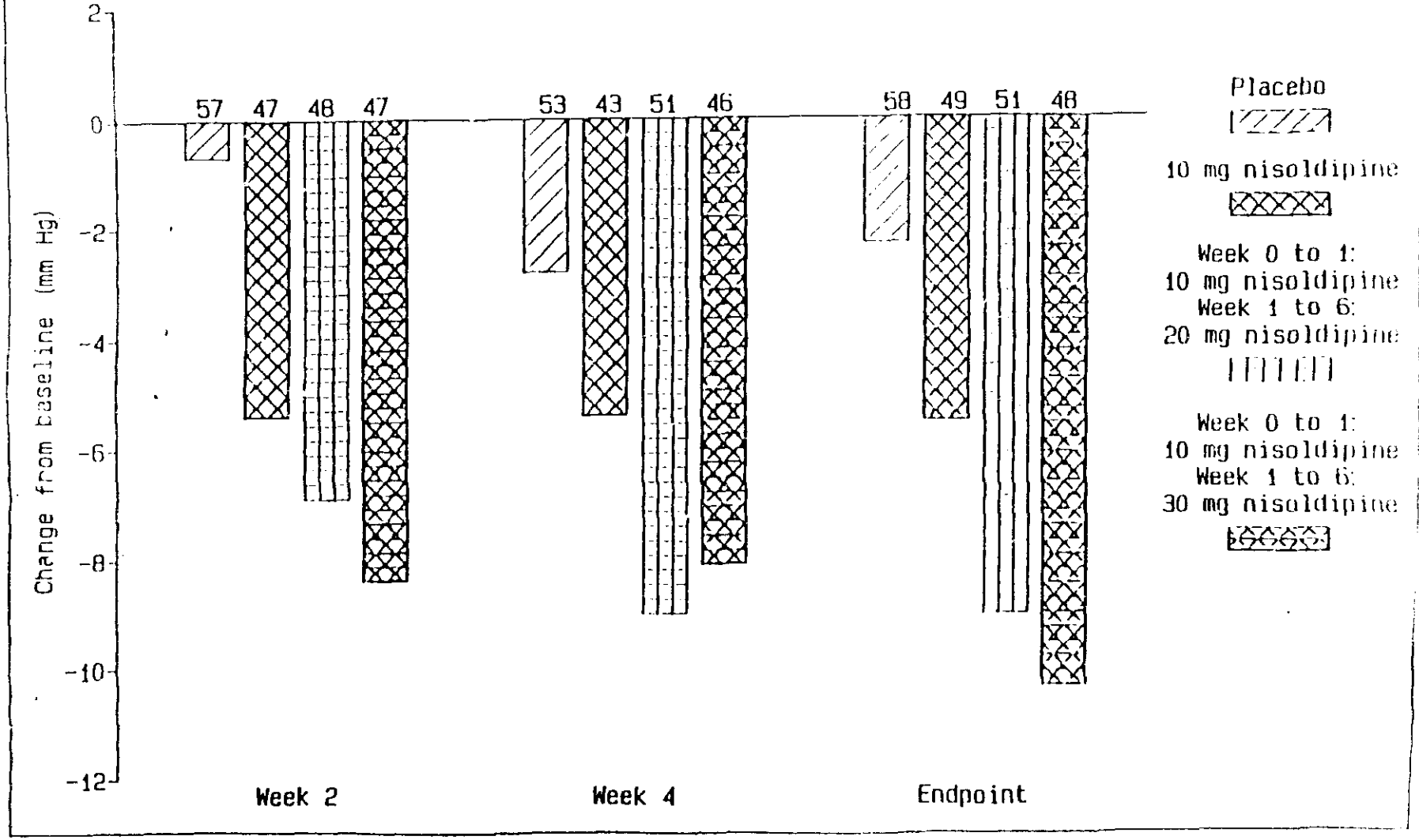
Supine diastolic blood pressure (Average of three measurements)

Change from baseline: Least squares means (n as indicated)

[For standard endpoint analysis; all centres]



The mean change from baseline in supine diastolic pressure (mmHg) for each treatment group after stratification for age is shown in the following table : (Main efficacy analysis = 206).

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg	Overall Least Square Means
Age < 45 years	-6.5 (12)*	-4.1 (14)	-10.6 (11)	-8.9 (15)	-7.5
Age≥ 45 and < 65 years	-1.4 (38)	-6.9 (28)	-8.3 (31)	-10.7 (27)	-6.8
Age ≥ 65 years	-1.3 (8)	-2.0 (7)	-10.1 (9)	-10.7 (6)	-6.0

* The number of patients used for calculating the mean values are given in brackets.

Results from ANOVA

Age effect : p=0.62
Treatment Effect : p=0.0001
Treatment by age interaction effect : p=0.12.

These results indicate that there is no association between age and the diastolic blood pressure response.

The mean change from baseline in supine diastolic blood pressure (mmHg) for each treatment group after statification for race is given in the following table : (Main efficacy analysis = 206).

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg	Overall Least Square Means
Caucasian	-1.2 (30)*	-4.6 (26)	-7.5 (25)	-9.2 (26)	-5.6
Black	-2.3 (17)	-5.9 (16)	-10.3 (18)	-10.1 (14)	-7.2
Other	-6.3 (11)	-7.0 (7)	-11.5 (8)	-13.1 (8)	-9.5

Analysis of Response and Normalization Rates. Responders were defined as patients who had SDBP of less than or equal to 90 mmHg or patients who had a drop in SDBP of at least 10 mmHg at endpoint. A patient's blood pressure was said to be normalized when satisfied these two conditions, namely, a drop in supine DBP to 90 mmHg or below, and a drop of at least 10 mmHg.

The following table shows the response rates for each treatment group, odds ratio and relative efficacy of each Nisoldipine treatment relative to Placebo from the main efficacy analysis of the last visit:

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg
Total number of Patients	58	49	51	48
Responders	10	17	24	30
Response Rate	17 %	35 %	47 %	63 %
Odds Ratio (OR) NIS relative to Placebo		2.4	4.6	8.8
95 % CI for OR		1.0 ; 5..5	1.8 ; 12	3.6 ; 22

Relative Efficacy (RE) NIS relative to Placebo 95 % CI for RE		2.0 1.0 ; 3.9	2.6 1.5 ; 4.8	3.7 2.1 ; 6.2

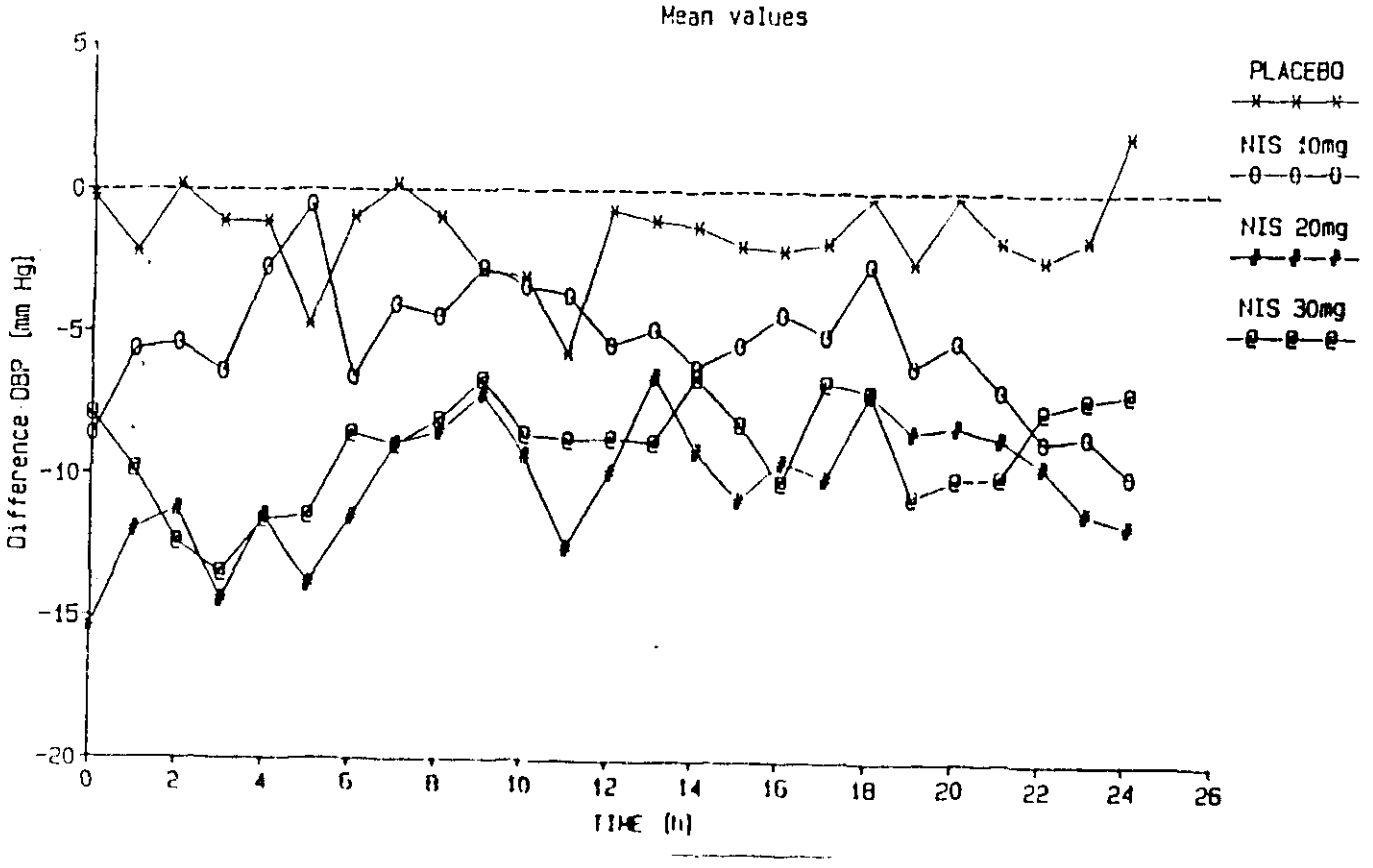
These results can be interpreted as indicating that the response rate for placebo was 17 %, Nisoldipine 10 mg 35 %, Nisoldipine 20 mg 47 % and Nisoldipine 30 mg 63 %. A relative efficacy of 2.6 of Nisoldipine 20 mg vs Placebo means that a positive treatment response is 2.6 times more likely to occur under Nisoldipine 20 mg than placebo. The confidence interval of 1.5 to 4.8 indicates that the true relative efficacy is likely (95% confidence limits) to be at least 1.5 and at most 4.8.

The following table shows the normalization rates for each treatment group, odds ratio, and relative efficacy of each Nisoldipine treatment relative to Placebo from the main efficacy analysis of the last visit :

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg
Total Number of patients	58	49	51	48
Number of Patients	5	5	13	13
Normalization Rate	8.6 %	10 %	25 %	27 %
Odds Ratio (OR) NIS relative to Placebo 95% CI for OR		1.2 0.31 ; 4.8	4.3 1.4 ; 1.3	4.3 1.4 ; 13
Relative Efficacy (RE) NIS relative to Placebo 95 % CI for RE		1.2 0.37 ; 3.9	3.1 1.3 ; 7.3	3.3 1.3 ; 8.1

These results can be interpreted in the same manner as described for the response rates.

Analysis of Ambulatory Blood Pressure Monitoring. Of the 165 patients who entered the ambulatory blood pressure monitoring phase of the study 137 patients were evaluable. The means across patients (change from baseline in diastolic blood pressure) are graphically presented in the following figure



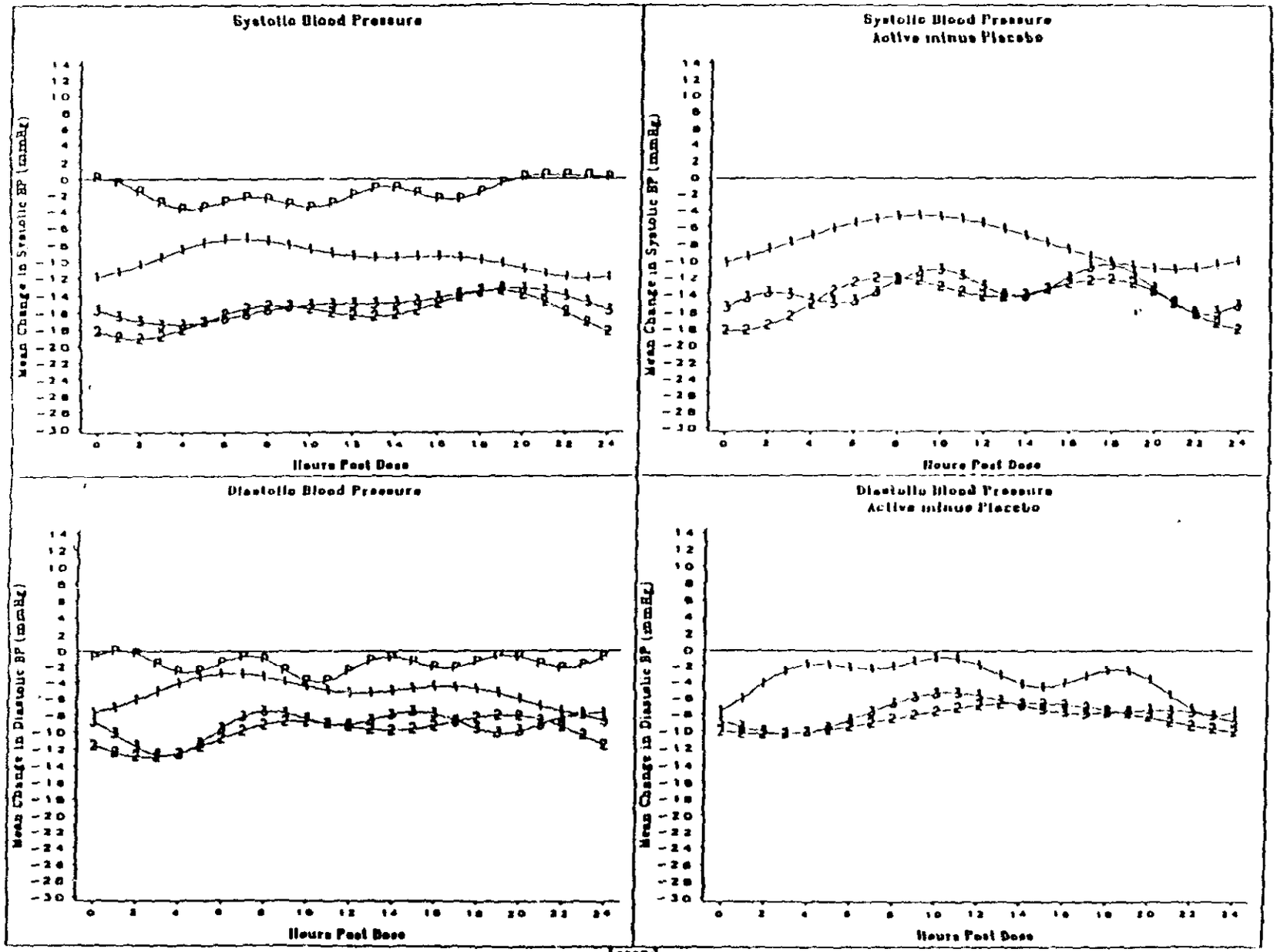
Various clinically meaningful variables could be calculated from the hourly mean diastolic blood pressure profiles. The following table shows results of trough/peak ratios calculated from hourly means of ambulatory monitoring data :

	Trough (mmHg)	Peak (mmHg)	Hour of Peak	Trough to peak Ratio
Diastolic BP				
NIS 10 mg.	-11.95	-11.95	24	100 %
NIS 20 mg	-13.70	-13.70	24	100 %
NIS 30 mg	-9.03	-12.57	2	72 %
Systolic BP *				
NIS 10 mg	-15.70	-15.70	24	100 %
NIS 20 mg	-20.72	-20.72	24	100 %
NIS 30 mg	-18.31	-18.31	24	100%
Systolic BP#				
NIS 10 mg	-15.70	-15.70	24	100 %
NIS 20 mg	-20.72	-20.72	24	100%
NIS 30 mg	-18.31	-10.64	2	172%

* Using timepoint of systolic peak.
# Using timepoint of diastolic peak

The results indicate that there was a good dose-response pattern in both systolic and diastolic blood pressure falls from baseline for placebo Nisoldipine 10 and 20 mg while the fall of Nisoldipine 30 mg was very similar to that in the 20 mg group. The effect of the 3 Nisoldipine group was maintained over the entire dosing period. This is also in evidence by observing the following graph of hourly means in a smoothed curve :

Smooth of Mean ange from Baseline of Ambulatory Blood P sure



Assessment. This study demonstrated that Nisoldipine, at concentrations of 10 mg, 20 mg and 30 mg, was more effective than placebo in lowering the blood pressure, but this effect was not potentiated when the dose was increased from 20 to 30 mg. Ambulatory blood pressure measurements were done which demonstrated that the effectiveness of Nisoldipine extended throughout the 24 hours after administration , trough values frequently being equal to peak values.

It is interesting that in this study this calcium channel blocker demonstrated to have a greater effectiveness in blacks, a patients population usually more refractory to antihypertensive treatment, than in caucasians.

In reference to age, this study concluded that Nisoldipine was more effective in individuals 65 years of age or older. (table page 73). This finding is consistent with those of protocol D89-039 in which Nisoldipine was more effective in this age range especially in lowering systolic blood pressure. (table page 60).

Protocol D88-054

Title of Study : " Comparative Double-Blind Pilot Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine 10, 20, 30 mg Core-Coat Tablets vs Placebo in Hypertensive Patients ".

Principal Investigators and Sites of Investigation :

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Objectives. The objectives of this study were :

1. To test whether Nisoldipine core-coat given 10 mg, 20 mg, 30 mg once daily lowers the blood pressure significantly more than placebo at the end of 24-hour dosing interval (trough).

2. To record blood pressure and pulse rates for four hours after the first dose of double-blind drug to monitor patient response to acute administration of the drug.

3. To determine peak response and calculate ratios of trough to peak effect by 24-hour ambulatory blood pressure monitoring.

Inclusion and Exclusion Criteria. Male or female patients, 21 to 70 years of age, with a history of mild to moderate essential hypertension and a mean supine diastolic blood pressure of 95 to 114 mmHg after three and four weeks of placebo were eligible for the study.

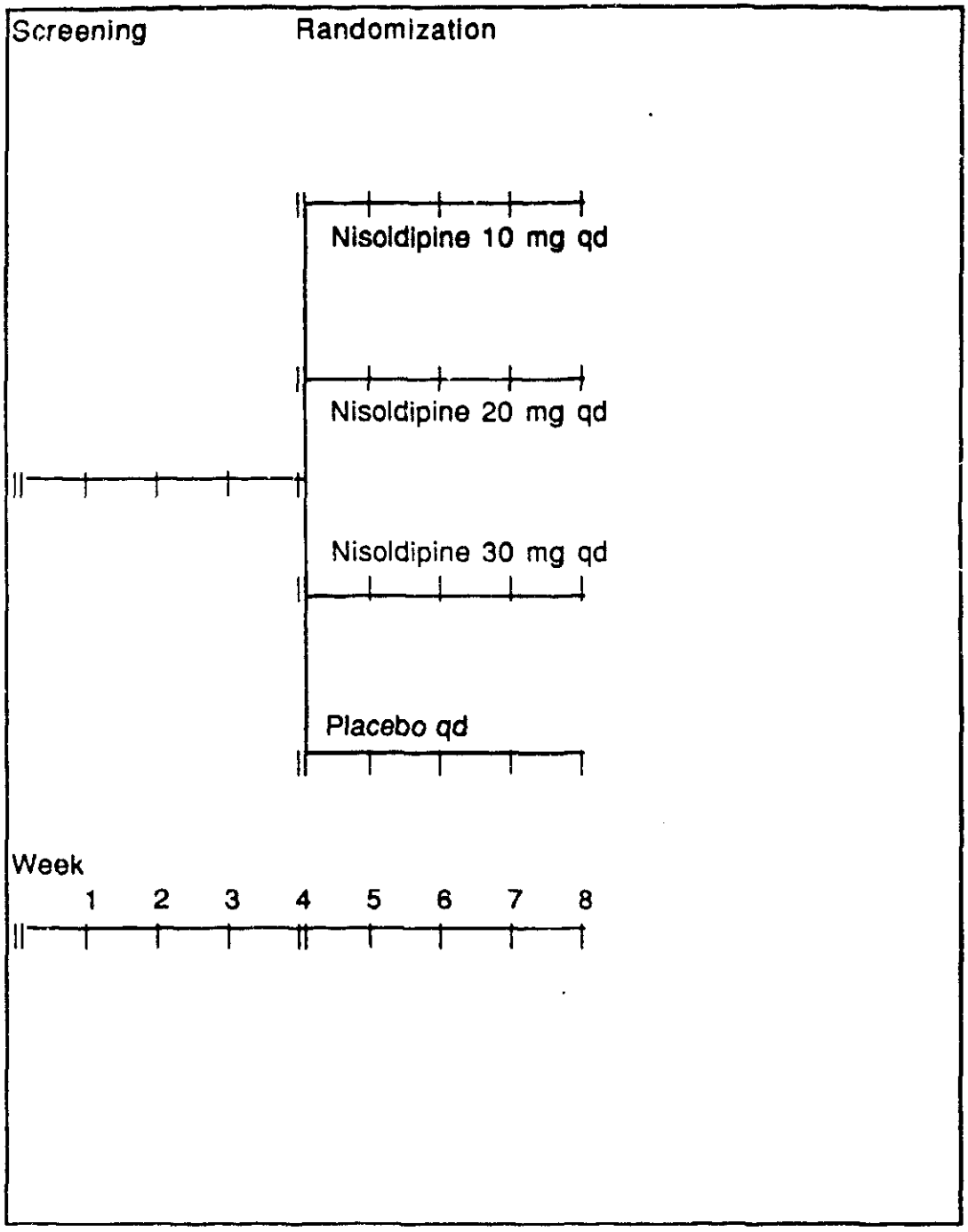
Excluded from the study were patients with labile hypertension, a change in supine diastolic blood pressure greater than 7 mmHg between the last 2 placebo run-in visits, impaired renal or liver function, recent or impending myocardial infarction, or cerebral vascular accident, angina pectoris or intermittent claudication, heart failure, major arrhythmias, conduction disturbance, failure of a major organ system, severe infection, malignancy, psychosis, chronic diarrhea, ulcerative colitis, regional enteritis, diverticulitis, partial or complete gastrectomy or small bowel resection, history of allergy to dihydropiridines, pregnant women or those with childbearing potential and patients known to abuse alcohol or drugs.

Study Design. This was a randomized, double-blind, parallel group, placebo controlled study of eight weeks duration consisting of a screening period and a randomization treatment period.

Screening Period. During this period of 4 weeks duration patients discontinued all previous antihypertensive medication and were given a single-blind placebo once daily. Those patients with a mean supine diastolic pressure ≥ 95 mmHg to ≤ 114 mmHg after three to four weeks of placebo and within 7 mmHg at both visits were transferred to the treatment period.

Randomization Period. Patients were randomized to receive either Nisoldipine 10 mg qd, Nisoldipine 20 mg qd, Nisoldipine 30 mg qd or Placebo qd for four weeks.

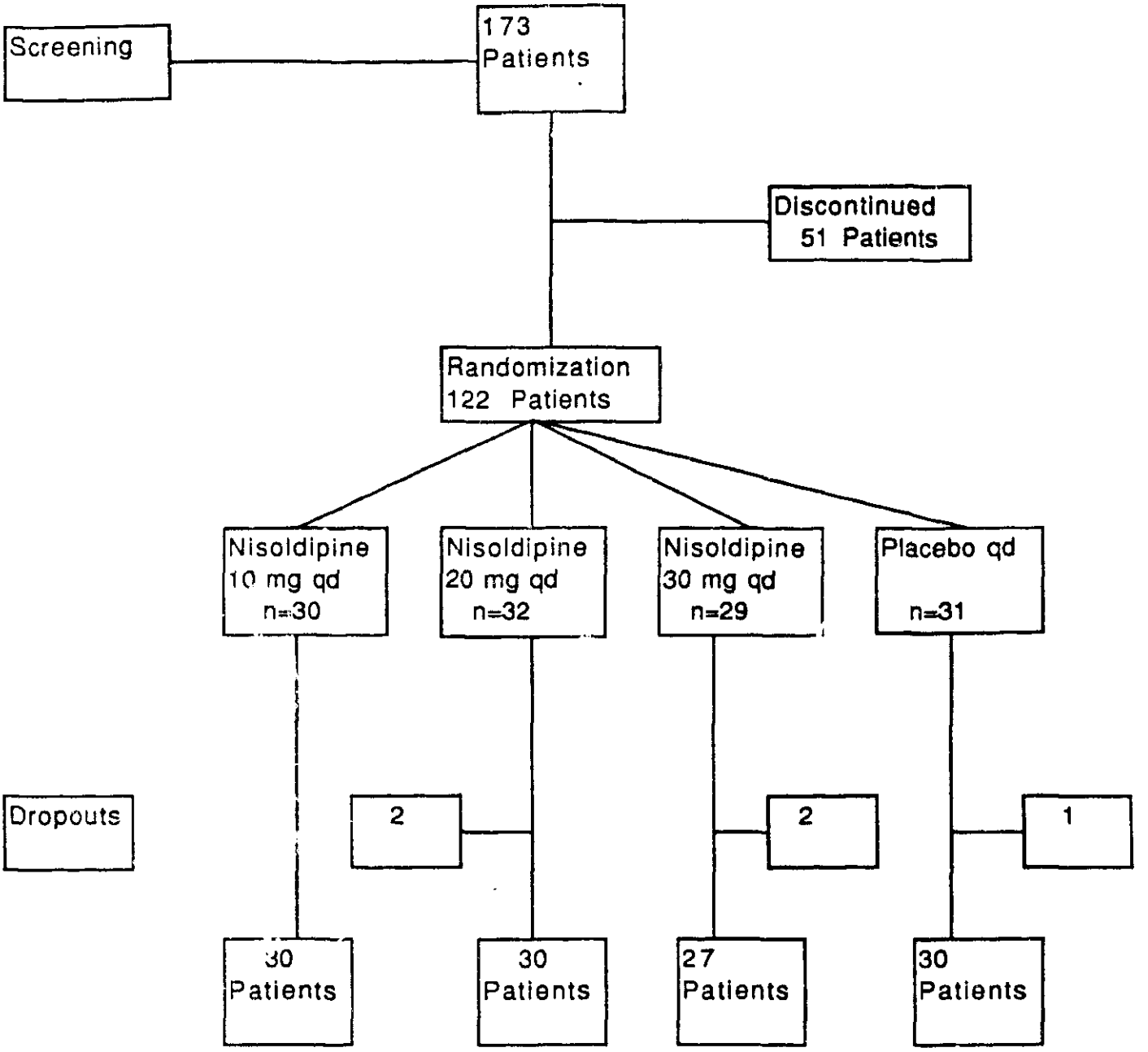
The study design is demonstrated schematically in the following graph :


Screening
Randomization
Nisoldipine 10 mg qd
Nisoldipine 20 mg qd
Nisoldipine 30 mg qd
Placebo qd
Week
1
2
3
4
5
6
7
8

Demography. The demography and baseline characterisitics are given in the following table :

		Nisoldipine 10 mg n=30	Nisoldipine 20 mg n=30	Nisoldipine 30 mg n=29	Placebo n=30
Sex	Male Female	20 (67 %) 10	19 (63 %) 11	19 (66 %) 10	21 (70 %) 9
Race	Caucasian Black Hispanic	23 (77 %) 4 3	23 (77 %) 7 0	20 (69 %) 9 0	23 (77 %) 7 0
Age (years)		56	53	52	51
Weight (lbs)		186	200	207	190
Baseline Blood Pressure mmHg	Supine Standing	146/99 144/100	147/99 144/100	145/99 144/100	148/100 145/101

The distribution of patients and randomization are given in the following graph :


Screening
173 Patients
Discontinued 51 Patients
Randomization 122 Patients
Nisoldipine 10 mg qd n=30
Nisoldipine 20 mg qd n=32
Nisoldipine 30 mg qd n=29
Placebo qd n=31
Dropouts
2
2
1
30 Patients
30 Patients
27 Patients
30 Patients

The reasons that disqualified enrolled patients for randomization are given in the following table :

Reasons for disqualification	Patients
Supine diastolic blood pressure < 95 mmHg	21
< 7 mmHg difference in supine diastolic blood pressure (visits 4 and 5)	3
Supine diastolic blood pressure >114 mmHg	4
Unable to make scheduled visits	3
Illness not due to study medication	4
Lost to follow-up	2
Abnormal laboratory values	3
Non-compliance	2
Systolic blood pressure above acceptable limit	1
High blood pressure readings during ambulatory monitoring	1
Chest pain at visit 1	1
Chose to withdraw	1
Total	46

The reasons for dropping out during the double-blind randomization period are given in the following table :

Drug Group	Final visit	Days on Drug	Reasons for dropping-out- Severity Drug Relationship
Placebo	7.0	11	Dizziness-Moderate Probable
Nisoldipine 20 mg	6.0	5	Intolerance to all-night visits
	5.5	5	Shortness of breath-Cough Mild-Probable
Nisoldipine 30 mg	8.0	23?	Noncompliance
	6.0	7	Flushing-Severe-Probable

Efficacy

Criteria for Effectiveness. The change in trough supine diastolic blood pressure from baseline to endpoint compared among the groups was the primary efficacy criterion. Endpoint was defined as the last valid visit on the double-blind drug for each valid patient. Patients were to be included in this efficacy analysis if trough blood pressure data was available after at least 7 days on double-blind drug.

Statistical Methods. All tests of significance were performed as two-tailed tests with alpha + 0.05 unless stated otherwise.

Analysis of Efficacy. The mean blood pressures by visit for all patients valid for analysis of efficacy are given in the following table :

Week Post Randomization — Supine Blood Pressure

	Nisoldipine 10 mg Sys/Dia (n)	Nisoldipine 20 mg Sys/Dia (n)	Nisoldipine 30 mg Sys/Dia (n)	Placebo Sys/Dia (n)
Week-1	143/99 (30)	145/99 (30)	144/99 (29)	145/99 (30)
Week 0	145/99 (30)	146/98 (30)	144/98 (29)	147/100 (30)
Week 1	136/91 (30)	136/90 (30)	136/91 (29)	141/95 (30)
Week 2	140/91 (30)	139/92 (29)	133/90 (28)	140/94 (30)
Week 3	138/91 (30)	136/91 (30)	134/89 (28)	142/95 (29)

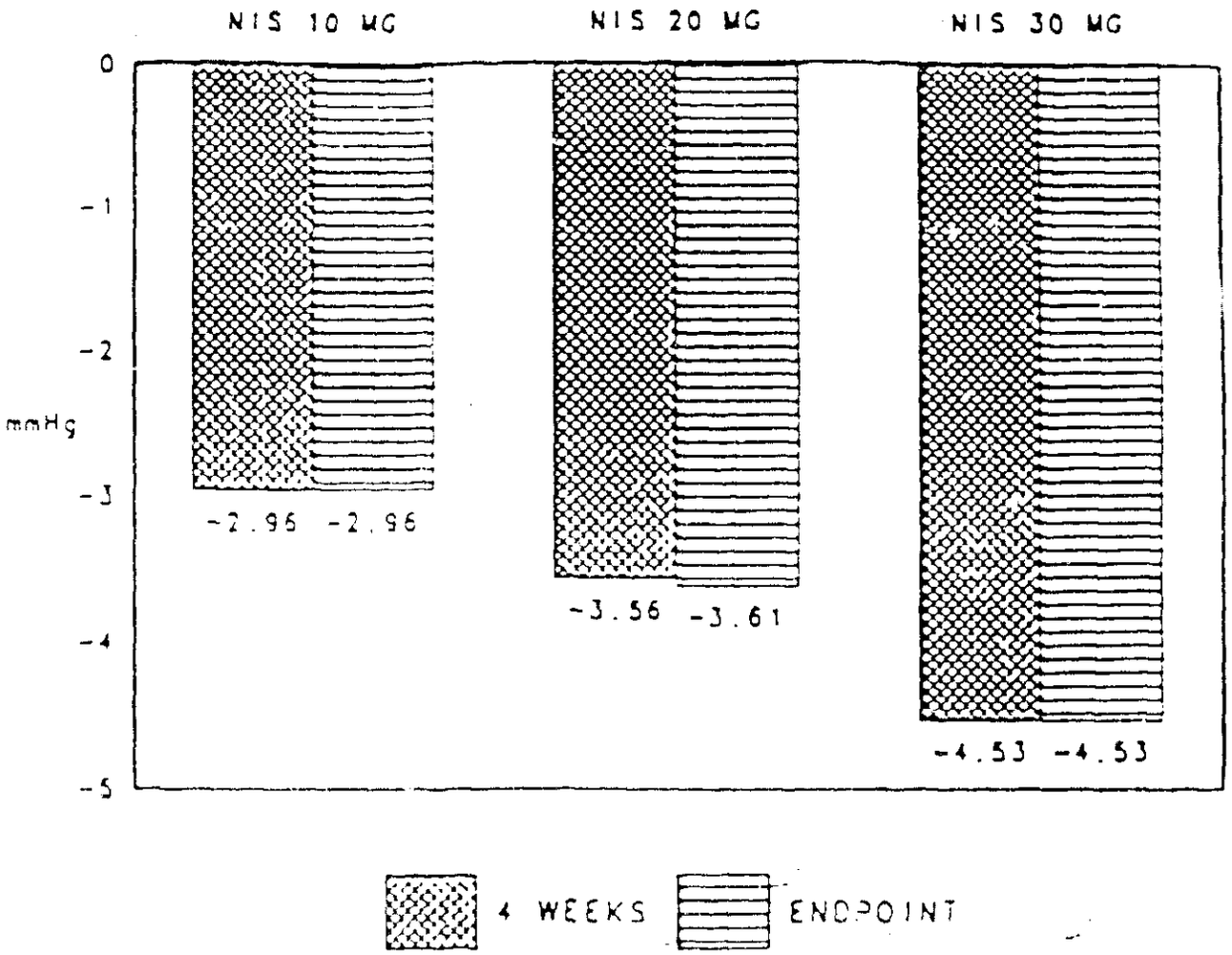
Week 4	137/90 (30)	134/90 (30)	133/89 (27)	144/94 (30)
Endpoint	137/90 (30)	134/90 (30)	133/89 (30)	144/94 (30)

In the following table, the results of the analysis at endpoint are summarized :

	Nisoldipine 10 mg n=30	Nisoldipine 20 mg n=30	Nisoldipine 30 mg n=29	Placebo n=30
Supine				
Systolic	8.4	11.5*	10.7*	3.0
Diastolic	8.3	8.9*	9.9*	5.3
Standing				
Systolic	8.3	11.8*	10.7*	3.4
Diastolic	6.2	7.3	7.0	5.1

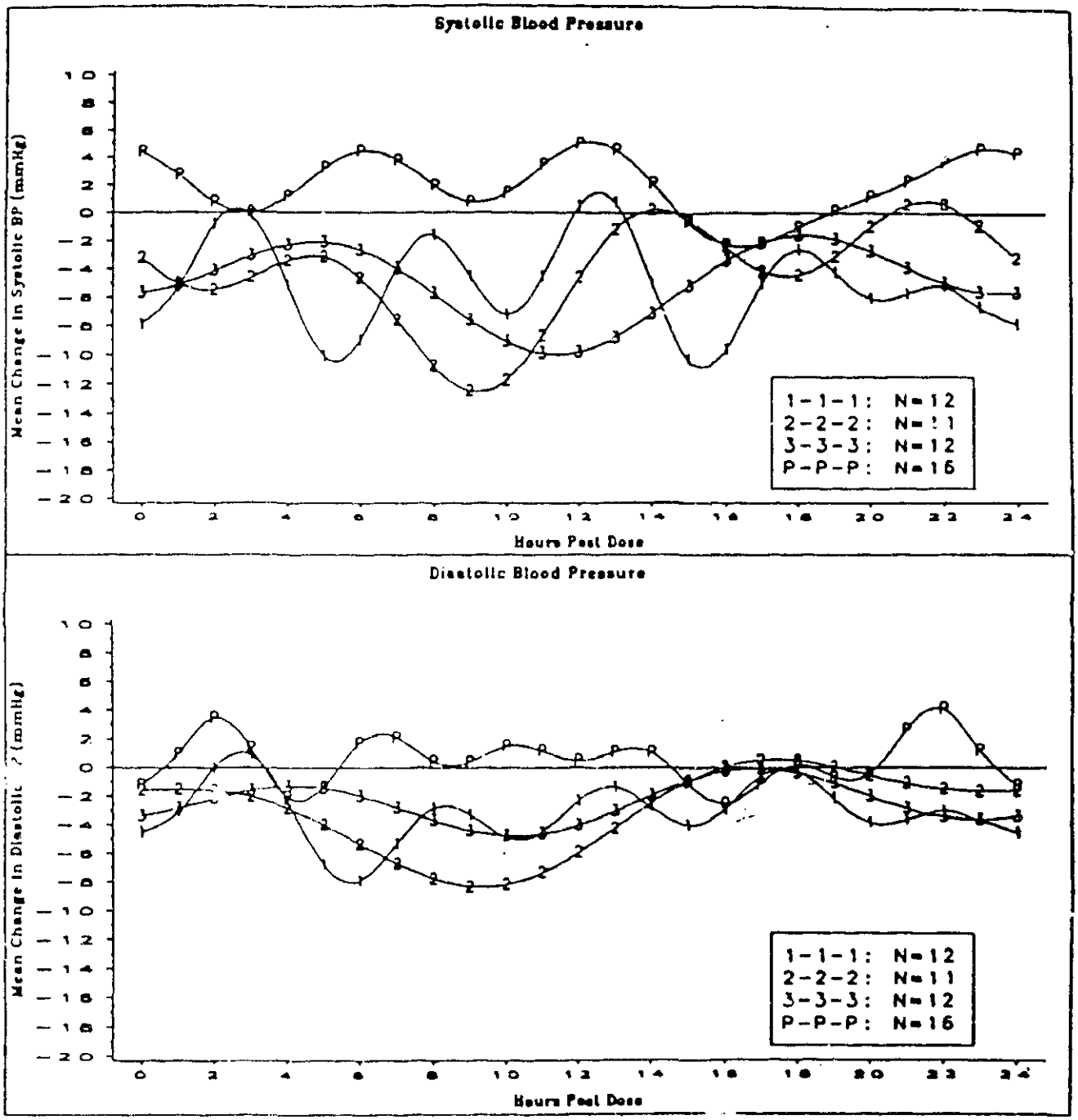
*Significant difference from the placebo group $p<0.05$

The change in trough supine diastolic blood pressure at 4 weeks and endpoint, placebo subtracted, are shown in the following graph :



Ambulatory monitoring and supine in-clinic blood pressures were smoothed and results are demonstrated in the following graph :

SMOOTH OF MEAN CHANGE FROM BASELINE OF AMBULATORY BLOOD PRESSURE



Legend

1-1-1 NIS 10mg
2-2-2 NIS 20mg
3-3-3 NIS 30mg
P-P-P Placebo

The Trough/Peak ratios from smoothed ambulatory monitoring data for valid patients are given in the following table :

	Nisoldipine 10 mg (n=12)	Nisoldipine 20 mg (n=11)	Nisoldipine 30 mg (n=12)
Diastolic	7 %	35 %	68 %
Systolic	92 %	43 %	108 %
Peak hour Post-dose	6	9	8
Nisoldipine levels at trough ng/ml	0.82	1.04	1.49

Assessment. This is a small pilot studied carried in a relatively small number of subjects consisting mostly of middle-age caucasian male obese patients. Although the results on blood pressure with the 10 mg dose of Nisoldipine was not significantly different from placebo the 20 and 30 mg doses were but the effect of both did not seem to be very different from each other.

Other Studies. Other studies were performed in which Nisoldipine was administered to patients with renal disease, to cirrhotic, elderly and young people. The effect of food on drug absorption was also investigated. The effects of combination with other antihypertensive agents was studied in long term extension studies.

Study in Cirrhosis. Protocol M.M.R.R. # 1118

Title of Study. "The Effect of Cirrhosis on the Steady-State Pharmacokinetics of Nisoldipine Coat-Core Sustained-Release Tablets ".

This was a single center, non-randomized, non-blinded, comparison of single dose and steady-state pharmacokinetics of Nisoldipine coat-core tablets in cirrhotic and healthy subjects.

Sixteen subjects participated in the study : 8 cirrhotic and 8 healthy subjects. There were 4 males and 4 females in each group. In stage 1 a

single 10 mg dose of Nisoldipine was administered and in stage 11 10 mg of Nisoldipine was administered qd for 7 days.

Results. Administration of Nisoldipine to patients with cirrhosis resulted in a 3 to 4-fold increase in peak plasma concentration and $AUC_{(024)}$. Nisoldipine had little effect on blood pressure in either group.

Assessment. These results are indicative of possibility that the dose of Nisoldipine may need to be adjusted in patients with cirrhosis.

Study in Renal Disease. Report 5837 (R).

Title of Study. "Influence of Renal Function on the Pharmacokinetics of Nisoldipine CC Tablets After Single and Multiple Dosing ".

This was a multicenter, non-blinded, non-randomized, comparative study among 4 groups to compare the effects of renal function on the pharmacokinetics of Nisoldipine CC after a single dose as well as after achievement of a steady state.

A total of 40 patients were enrolled in 3 centers. The following groups of patients were enrolled :

1. Control. Nine subjects with creatinine clearance > 90 ml/min/1.73 m^2

2. Mild Renal Failure. Twenty subjects with creatinine clearance 61 ≤ 90 ml/min/1.73 m^2

3. Moderate Renal Failure. Nine subjects with creatinine clearance 30 to ≤ 60 ml/min/1,73 m^2

4. Severe Renal Failure. Seven subjects with creatinine clearance < 30 ml/min/1.73 m^2.

Results. Although there was not a statistically significant difference in the Nisoldipine AUC_{norm} between the groups with impaired renal function and the normal control, in the former an increase in plasma Nisoldipine of approximately 2-fold could not be excluded.

Assessment. An increase in plasma levels of Nisoldipine in patients with impaired renal function may require the adjustment of the dose. There were only modest effects on blood pressure across all groups.

The Factor Age . Report 5857 (P).

Title of Study : "A Study to Determine the Single Dose and Steady-State Pharmacokinetic Profile of Nisoldipine Coat-Core (CC) Tablet 20 mg in Elderly and Young Volunteers and in Elderly Hypertensive ".

This was an open, multiple-dose, non-randomized study. Nisoldipine CC was administered at the dose of 20 mg qd for 7 days. Plasma samples were collected and blood pressure and heart rate were measured.

The following groups of patients were studied :

Young Volunteers. Twenty healthy young volunteers, 18 to 23 years of age, completed the study.

Elderly Volunteers. Twenty healthy elderly volunteers, 65 to 84 years of age, completed the study.

Hypertensive Elderly. Eleven hypertensive patients, 66 to 77 years of age, completed the study.

Results. The plasma concentrations of Nisoldipine were higher in elderly volunteers and hypertensive patients than in young volunteers. After multiple dose administration the supine diastolic blood pressure remained essentially unchanged in normal young healthy volunteers but a moderate decrease in elderly healthy volunteers and a significant decrease in elderly hypertensive patients was observed.

The Effect of Diet. Study Number D92-045-02.

Title of Study : " The Effect of Food on the Pharmacokinetics of 30 mg and 40 mg Nisoldipine CC Tablets in Healthy Male Volunteers ".

This study was an open-label, randomized, two-way cross over evaluation of the effect of food on the pharmacokinetics of 30 and 40 mg

Nisoldipine. Subjects were randomized to receive a single 30 mg or 40 mg dose of Nisoldipine either in a fasted or a fed state. After one week washout period there was a crossover to the opposite state.

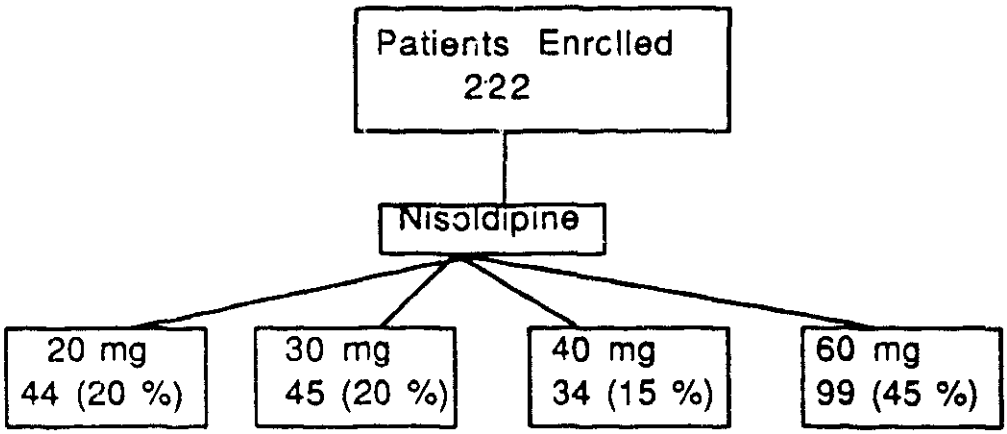
Twenty-eight healthy male subjects between the ages of 18 and 45 years completed the study. There were no significant effects on mean sitting diastolic blood pressures in the fed or fasted states at the 30 or 40 mg. doses.

Long Term Extension Studies. Drug Combination. Protocols X89-039 and X90-019.

These were long term extension studies of the 6-month efficacy studies and safety of Nisoldipine CC in the treatment of mild to moderate hypertension. Patients completing studies D89-039 and D90-019 were given the option of immediately entering an open-label extension protocol.

Patients were initially given Nisoldipine CC 20 mg or 30 mg tablets once a day as initial therapy. Then the dose of Nisoldipine was to be increased sequentially every one or two weeks as tolerated, to 40 mg qd, 60 mg qd and 80 mg qd. or 60 mg qd and 90 mg qd until SUDBP was ≤ 90 mmHg. However the maximum dose of Nisoldipine was in fact limited to 60 mg qd before any patient enrolled. Atenolol 50 mg to 100 mg qd and/or Hydrochlorothiazide 24 to 50 mg qd could be added at the investigator's discretion at any time. Thus tablets used were Nisoldipine CC 20, 40, 2X30 mg for monotherapy with the addition of Atenolol 50 and 100 mg and.or Hydrochlorothiazide 25 and 50 mg for combination therapy.

The distribution of patients is shown in the following graph :



With Atenolol
20 Patients (9 %)
1 Patient 25 mg
15 Patients 50 mg
4 Patients 100 mg

With Hydrochlorothiazide
78 Patients (35 %)
44 Patients 25 mg
34 Patients 50 mg

The results are summarized below :

	Supine		Standing	
	SBP mmHg	DBP mmHg	SBP mmHg	DBP mmHg
Baseline	154.0	101.1	149.7	100.3
Endpoint	135.7	86.0	132,4	86.5
Mean Dif	-18.3	-15.2	-17.3	-13.6

Assessment. These were open-label uncontrolled studies in which results were all pooled together and therefore they should not be valid for evaluation of combined therapy.

Total Assessment of Efficacy

Peak Drug Effect on Blood Pressure. The effect of Nisoldipine on blood pressure at the approximate time of peak drug plasma concentration (i.e. the maximal response between 6-10 hours post-dose) in the supine and standing position is shown below for the systolic and diastolic blood pressure.

	Placebo Substracted Change in Peak Blood Pressure Dose Nisoldipine				
	10 mg	20 mg	30 mg	40 mg	60 mg
Study			SUDBP		
D88-054	-11.6	-9.5	-14.1	NA	NA
D89-039	NA	-8.0	NA	-8.3	NA
D90-019	NA	NA	-6.3	NA	-10.6
			SUSBP		
D88-054	-8.6	-7.6	-12.8	NA	NA
D89-039	NA	-15.2	NA	-15.3	NA
D90-019	NA	NA	-13.0	NA	-11.1
			STDBP		
D88-054	-9.3	-7.8	-11.5	NA	NA
D89-039	NA	-7.6	NA	-8.5	NA
D90-019	NA	NA	-6.6	NA	-13.4
			STSBP		
D88-054	-4.7	-11.6	-11.0	NA	NA
D89-039	NA	-14.4	NA	-17.6	NA
D90-019	NA	NA	-15.5	NA	-19.1

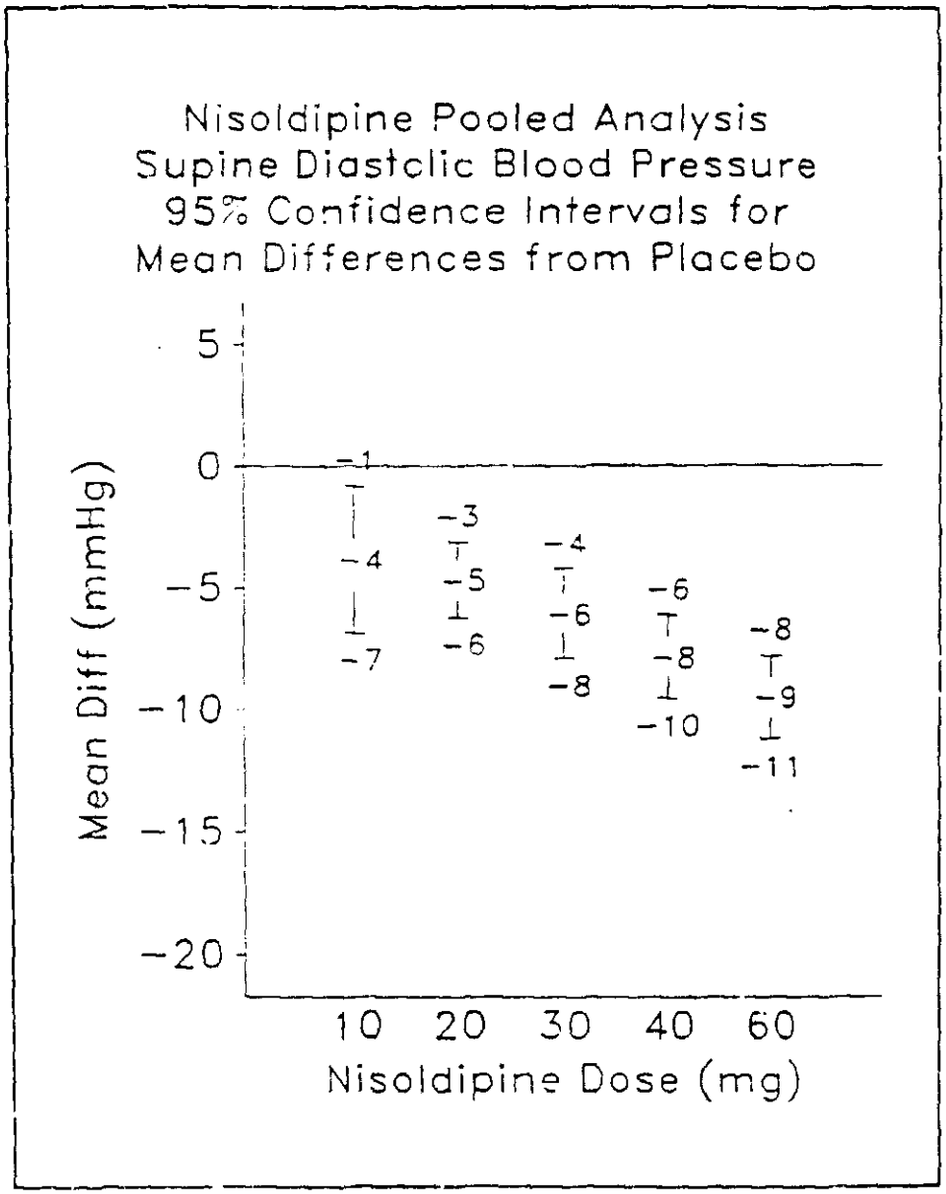
Twenty Four Hour Mean BP Reduction. Ambulatory blood pressure was used in a majority of the clinical trials of Nisoldipine in hypertension. In addition to characterizing the temporal profile of its effect on blood pressure, these data provide an estimate of the time-average reduction in blood pressure for each dosage of the drug. The pooled results of several studies are shown in the following table :

Nisoldipine Dosage (mg)	24 Hour AVG BP Reduction, Mean ± SEM Systolic	Diastolic
Placebo	-0.7±8.7	-0.9±6.3
10	-8.4±11.8	-4.6±7.5
20	-12.7±11.5	-8.4±7.1
30	-12.7±10.8	-7.9±6.9
40	-13.6±12.1	-8.0±6.8
60	-18.4±9.9	-12.0±7.2

The change in trough blood pressure from baseline to endpoint (**Mean±SEM** in mmHg) is given in the following table :

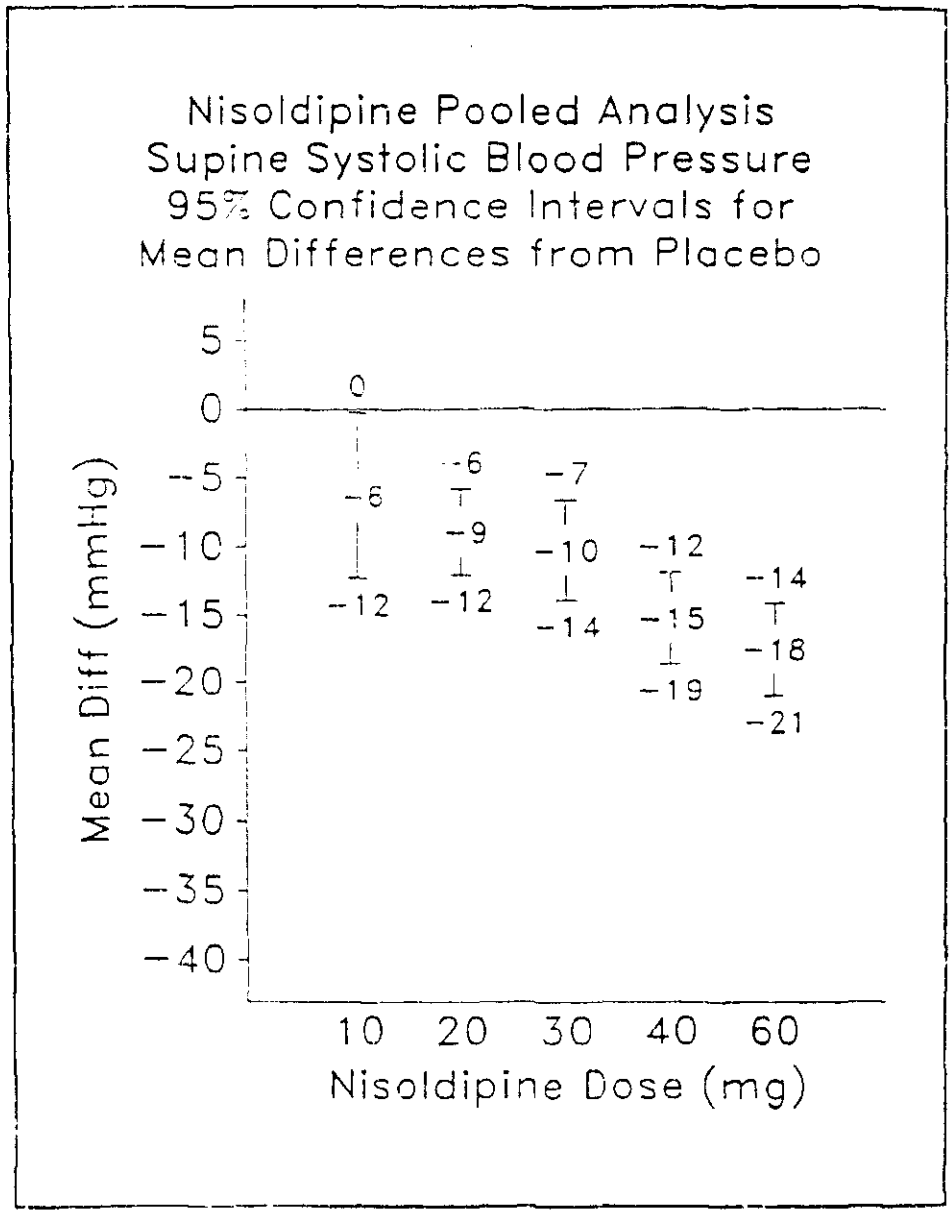
Pooled Dosage	Placebo N=232	Nisoldipine 10 mg N=30	20 mg N=161	30 mg N=105	40 mg N=131	60 mg N=125
SUDBP	-4±0.5	-8.4±1.4	-9.2±0.6	-10.6±0.8	-12.4±0.7	-14.0±0.7
SUSBP	-2.0±0.5	-8.3±2.9	-10.9±1.2	-12.2±1.6	-17.2±1.4	-19.5±1.4
STDBP	-2.7±0.5	-7.0±1.5	-7.9±0.7	-9.0±0.8	-12.6±0.8	-13.6±0.8
STSBP	-1.5±1.0	-8.2±3.0	-11.4±1.3	-12.9±1.6	-18.7±1.5	-19.2±1.5

In the following graph, pooled results of placebo subtracted values for trough SUDBP reduction by dose are demonstrated :



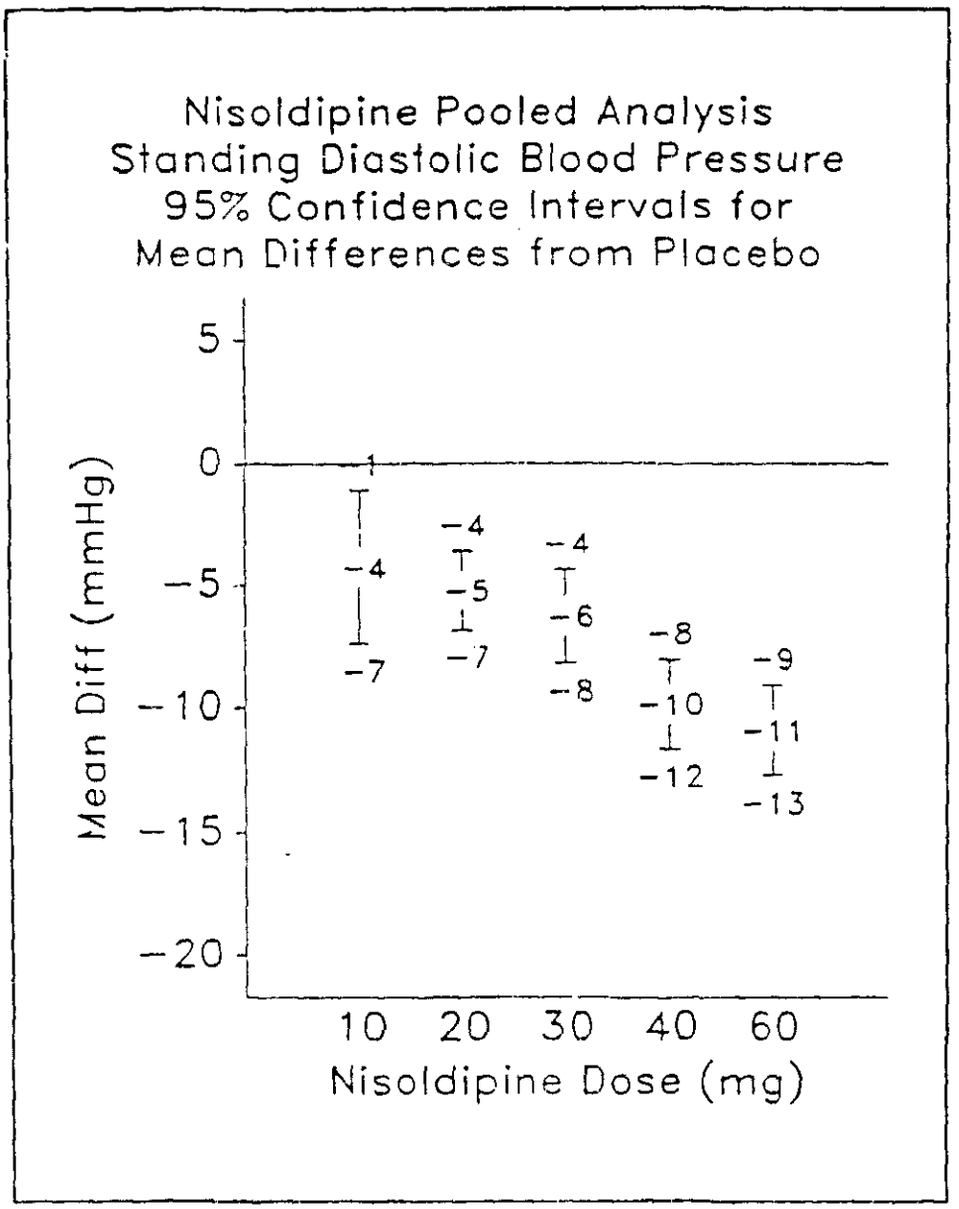
A linear relationship of blood pressure reduction by Nisoldipine in dosages between 10 and 60 mg is apparent without evidence of a plateau.

Similar results for SUSBP are shown in the following figure :



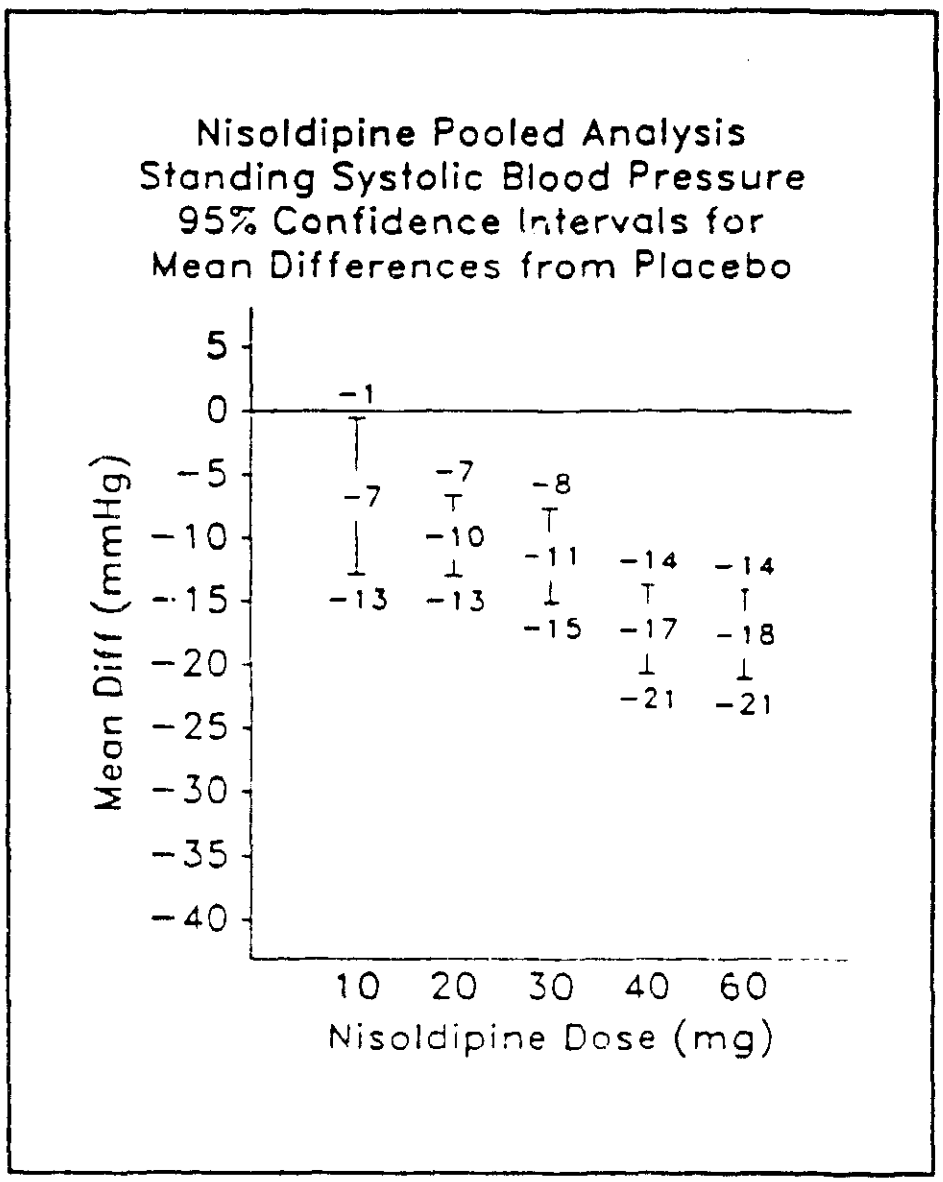
A linear relationship is not as evident as in previous graph but the maximum effect was achieved with 60 mg Nisoldipine dose

Similar results for STDBP are shown in the following figure :



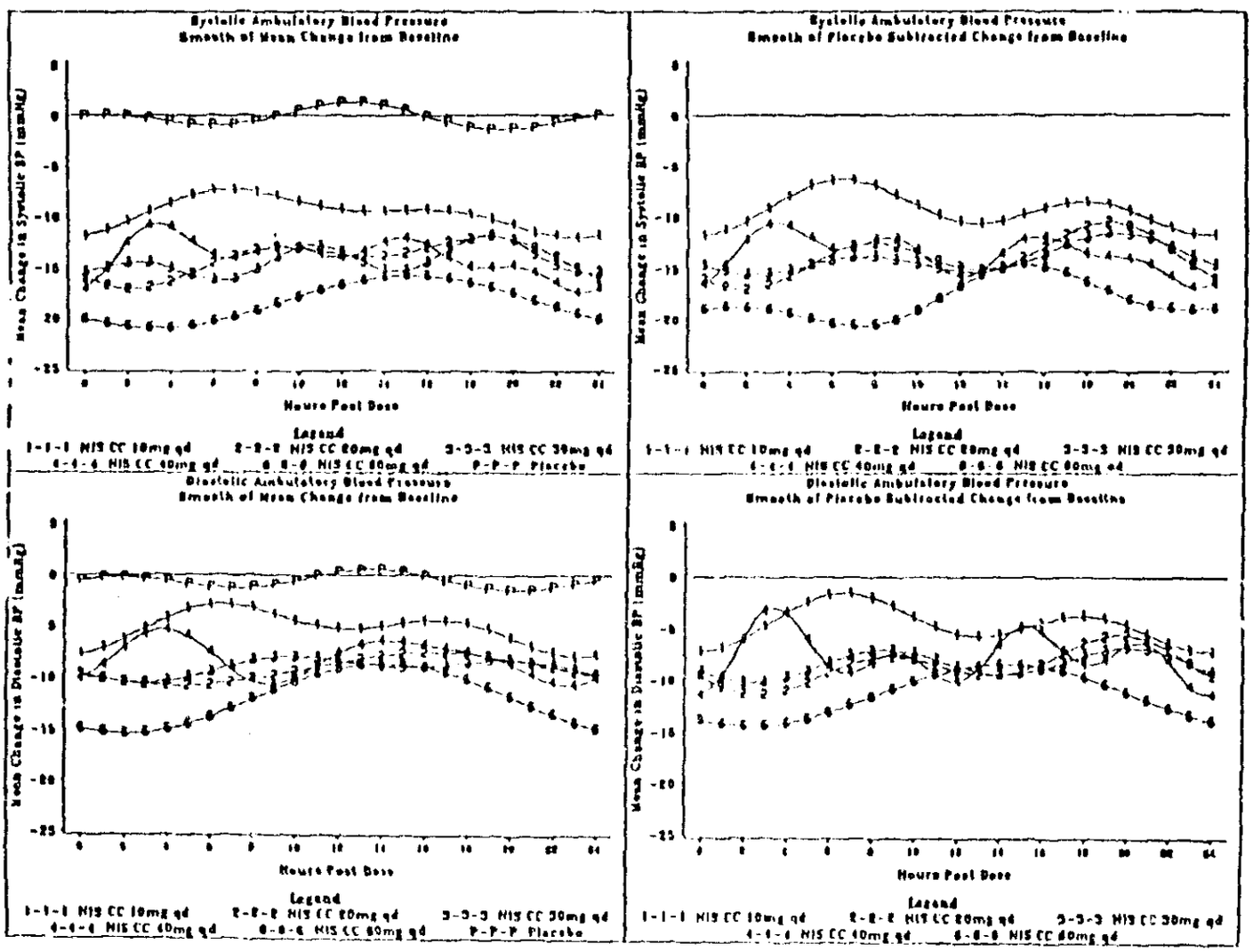
In this case the relationship of blood pressure reduction to dosage is roughly sigmoidal with an apparent plateau at 60 mg.

Similar results for STSBP are shown in the following figure :



The relationship of blood pressure reduction to dosage is sigmoidal with an apparent plateau at 40 mg

A pooled analysis of 24 hour ambulatory blood pressure monitoring is demonstrated in the following 4 graphs :



Through the 24-hour recording there seems to considerable overlapping especially among the higher doses but at trough there is evidence of blood pressure reduction that seems to be dose related.

The effects on diastolic blood pressure at peak and trough and the trough/peak ratios according to dose are given in the following table :

Dosage	Trough/Peak Ratio Diastolic Blood Pressure
10 mg	73 %
20 mg	75 %
30 mg	93 %
40 mg	100 %
60 mg	97 %

Time Course Effect of Nisoldipine. The therapeutic effect of Nisoldipine was achieved early in the course of treatment (approximately 2 weeks) and gradual incremental gain is evident for another 2-4 weeks.

The mean changes in sitting blood pressure from baseline after first dose is given in the following table :

Dose (mg)	N	8 Post-dose Systolic/ Diastolic	24 post-dose Systolic/ Diastolic
Placebo	10	-4.9/-1.9	3.8/-2.2
5	11	-10.4/-4.2	0.3/2.3
10	13	-6.7/-7.1	-0.7/-4.5
20	12	-11.3/-7.8	-5.8/-1.9
30	7	-15.4/-9.6	-13.3/-1.9

Pharmacokinetic and Blood Pressure Results. The mean sitting blood pressure change (mmHg) from baseline at peak and pharmacokinetic parameters (Mean ±SD) at steady state at each dose level is given below :

Dose (mg)	N	8h Post-Dose Sys/Dia	24h Post-Dose Sys/Dia	AUC (0-24h) (ng.h/ ml)	C_{max} (ng/ml)	T_{max} (h)
Placebo	10	-2.5/ -5.9	3.8/ 0.3			
5	11	-9.6/ -4.7	1.9/ -4.3	9.1±5.0	0.7±0.3	9.2±3.0
10	13	-8.9/ -7.1	-5.0/ -4.6	16.2± 3.0	1.1±0.3	6.3±4.8
20	12	-13.2/ -7.6	-5.4/ -4.3	29.4± 11.8	2.3±0.9	4.0±2.4
30	7	-21.7/ -10.5	-11.8/ -5.9	43.2± 23.1	2.9±1.1	5.4±5.0

The mean supine blood pressure change (mmHg) from baseline and pharmacokinetic parameters (mean±SD) at steady state for each dose level is given in the following table :

Dose (mg)	N	8h Post Dose	24 h Post Dose	AUC (0-24h) (ng.h/ ml)	C_{max} (ng/ml)	T_{max} (h)
30	18	-16.4/ -8.4	-14.0/ -10.2	74.28± 7.96	4.79± 0.68	7.22± 0.93
60	18	20.8/ 13.2	16.8/ 15.0	129.76± 12.74	8.48± 0.81	9.08± 1.97
90	9	-22.1± 12.1	-23.0± 13.4	199.31± 16.45	13.02± 1.20	6.78± 2.30
120	3	-30.7/ 25.0	-44.3/ -19.0	226.58± 12.41	14.92± 2.01	4.00± 1.00

To bring up more clearly the relationship between plasma Nisoldipine concentrations and blood pressure decrease, supine diastolic blood pressure changes from baseline at peak (8 h) and trough (24 h) were related to plasma Nisoldipine concentration at this time points using a simple linear regression. Placebo patients were used in this analysis with a plasma Nisoldipine level of Zero. The results for 30 and 60 mg are summarized in the table below :

Timepoint	Nisoldipine Mean Plasma Conc. (ng/ml)	Mean Change in SUDBP (mmHg)	Estimated Slope	Estimated Slope (P-Value)
Day 4, 30 mg (N=18)				
8 hours	3.5	-8.4	-2.55	0.0118
24 hours	2.6	-10.2	-1.42	(0.0689)
Day 8, 60 mg (N=17-18)				
8 hours	6.2	-13.2	-1.14	0.0507
24 hours	5.2	-15.0	-1.39	(0.0027)

Blood Pressure Rebound Upon Withdrawal. Blood pressure rebound was determined 24, 48 and 72 hours after cessation of Nisoldipine 60 mg qd in patients who had reached steady state. There was no evidence of for an exaggerated rebound effect on blood pressure after discontinuance of Nisoldipine at this high dose.

Maintenance of Blood Pressure Reduction in Long Term Studies. There was no evidence of tolerance to the antihypertensive effect of Nisoldipine over 6 months to 1 year of therapy.

Demographic Subgroups. Gender. Trough SUDBP changes from baseline to endpoint for male and female patients are given in the following table :

	Female	Male
Dosage	Nisoldipine-Placebo	Nisoldipine-Placebo
10 mg	-8.85	-2.27
20 mg	-3.21	-5.87
30 mg	-8.47	-6.1
40 mg	-7.82	-8.43
60 mg	-10.31	-10.79

Although dose-response profiles are somewhat erratic the overall effects are similar for men and women.

Race. A comparable analysis of efficacy for race related to dose is demonstrated in the following table :

	White	Black
Dosage	Nisoldipine-Placebo	Nisoldipine-Placebo
10 mg	-3.59	-4.37
20 mg	-4.54	-6.39
30 mg	-7.51	-8.82
40 mg	-6.69	-11.61
60 mg	-11.51	-11.1

Black patients responded with a greater decline in trough SUDBP than did white patients.

Age. In the following table the dose response for patients divided by age less than 65 years and equal or greater than 65 years is demonstrated.

	<65	≥65
Dosage	Nisoldipine - Placebo	Nisoldipine - Placebo
10 mg	-3.69	-6.2
20 mg	-4.98	-5.15
30 mg	-7.31	-5.48
40 mg	-8.21	-8.09
60 mg	-11.08	-8.14

The elderly demonstrated a greater low-dose response and a lesser high-dose response.

Quartile of Baseline Blood Pressure. For Nisoldipine as well as for many other antihypertensive drugs, a higher baseline blood pressure is associated with larger decline on medication. In the table below a dose response according to baseline SUDBP by quartile is demonstrated :

	Q1	Q2
Dosage	Nisoldipine-Placebo	Nisoldipine-Placebo
10 mg	-4.38	-5.97
20 mg	-4.23	-8.18
30 mg	-2.27	-11.49
40 mg	-6.36	-13.81
60 mg	-9.66	-14.16

The relationship of Nisoldipine dosage and decline in blood pressure is least evident in the first quartile and strongest in fourth quartile.

Combination Antihypertensive Therapy. Addition to a background of a beta blocker. One the pivotal studies (D89-029) evaluated the combination of Nisoldipine CC and a beta blocker.To patients who were already receiving Atenolol Nisoldipine was added. The sponsor claims the efficacy of Nisoldipine under these conditions. However there seems to be a drug interaction between these drugs that the sponsor has not recognized (see p. 35 this review).

Long Term Extension Trials. Based on open-label controlled trials and uncontrolled studies of one year duration the sponsor claims that meaningful responses were elicited by the combination of Nisoldipine with diuretics and or/ a beta blocker.

Recommendations. Nisoldipine should be approved as monotherapy for hypertension. The recommended dosage should be 10 mg to 40 mg.

Although the sponsor states that there is no drug interaction between Nisoldipine and beta blockers there are publications stating that such interaction exists (1, 2). This should be stated in the package insert.

Consideration should be given to advising that the dosage may need to be adjusted in patients with renal failure.

There were not well controlled studies of the combination of Nisoldipine with diuretics or other antihypertenive agents. Therefore the claim of efficacy with other drug combination is not well substantiated.

Cristobal G. Duarte M

Cristobal G. Duarte, MD - HFD-110

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HFD-110/CGD/30Jul93

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D. Roeder

SEP 27 1993

NDA20356 P1

DIVISION OF CARDIO- RENAL DRUG PRODUCTS
MEDICAL OFFICER'S NDA REVIEW

NDA: 20 356

DRUG: Nisoldipine Core- coat (BAY k 552)

SPONSOR: Miles Inc (Pharmaceutical Division)

DATE SUBMISSION: 3 March, 1993

DATE REVIEW: 20 August 1993

REVIEWER: Philip L. Dern M.D. P.L. Dern

RESUME:

This review deals entirely with safety aspects of the above submission; not efficacy. The primary approach is via examination of individual pools of data based on similar studies and provided by the Sponsor in this submission.

I. Hypertension

A. Exposure

Although the number of cases treated world-wide with nisoldipine exceeds 6000, according to the Sponsor, relatively few of these, N= 1292, were given nisoldipine core- coat (NIS cc) as shown below in completed studies:

PATIENTS EXPOSED TO NISOLDIPINE IN EACH CATEGORY

FORMULATION	NDA STATUS	INDICATION										TOTAL
		ANGINA		HYPERTENSION		HEART FAILURE		CLIN. PHARM.		OTHER		
		NON-US	US	NON-US	US	NON-US	US	NON-US	US	NON-US	US	
COAT-CORE	INCLUDED	624	474	518	776	142		210	183			2925
	EXCLUDED											0
	TOTAL	624	474	518	776	142	0	210	183	0	0	2925
IMMEDIATE RELEASE	INCLUDED	3472	521	2371	10	414	14	755	83	131		7771
	EXCLUDED	411		334		185		421		136		1487
	TOTAL	3883	521	2705	10	599	14	1176	83	267	0	9258
OTHER	INCLUDED	81				103		575				898
	EXCLUDED					31		126				157
	TOTAL	81	0	0	0	134		701	0	0	0	1055
TOTAL	INCLUDED	4104	995	2889	786	659	14	1540	266	131	0	11382
	EXCLUDED	411	0	334	0	216	0	547	0	136	0	1644
	TOTAL	4515	995	3223	786	875	14	2087	266	267	0	13025

STUDIES IN EACH CATEGORY

FORMULATION	NDA STATUS	INDICATION										TOTAL
		ANGINA		HYPERTENSION		HEART FAILURE		CLIN. PHARM.		OTHER		
		NON-US	US	NON-US	US	NON-US	US	NON-US	US	NON-US	US	
COAT-CORE	INCLUDED	4	3	4	7	21		11	8			37
	EXCLUDED											0
	TOTAL	4	3	4	7	21	0	11	8	0	0	37
IMMEDIATE RELEASE	INCLUDED	11	16	87	1	33	2	67	3	8		328
	EXCLUDED	36		21		14		38		7		116
	TOTAL	147	16	108	1	47	2	105	3	15	0	444
OTHER	INCLUDED	11				9		55				85
	EXCLUDED					3		13				16
	TOTAL	11	0	0	0	12	0	68	0	0	0	91
TOTAL	INCLUDED	116	19	91	8	44	2	133	9	8	0	430
	EXCLUDED	36	0	21	0	17	0	51	0	7	0	132
	TOTAL	152	19	112	8	61	2	184	9	15	0	562

The following table provides the total duration of treatment by total daily dose of longest duration for the US NIS CC (total controlled and uncontrolled) cases. The second table provides duration of treatment in the non- US studies.

TABLE 3
TOTAL DURATION OF TREATMENT WITH NISOLDIPINE
BY TOTAL DAILY DOSE OF LONGEST DURATION

POOL OF US CC HYPERTENSION
TOTAL CONTROLLED + TOTAL UNCONTROLLED

	ALL	DURATION									
		1-7 DAYS		8-30 DAYS		31 DAYS-60 DAYS		61 DAYS-180 DAYS		181 DAYS-360 DAYS	
	N	N	%	N	%	N	%	N	%	N	%
DRUG											
NIS CC 10MG QD	27			33	88 2	2	5 4	2	5 4		
NIS CC 20MG QD	203	8	4 4	37	18 2	67	33 0	64	31 5	26	12 8
NIS CC 30MG QD	130	1	0 7	22	22 2	48	24 8	36	27 0	28	20 3
NIS CC 40MG QD	188	3	1 6	17	6 8	65	34 6	91	48 4	16	8 5
NIS CC 60MG QD	189	6	3 0	111	5 5	94	49 2	23	11 6	61	30 7
NIS CC 90MG QD	11	4	36 4	6	54 5	1	8 1				
ALL	776	23	3 0	132	17 0	281	36 2	208	26 8	131	16 8

2. DURATION BY DOSE TABLE 10:22 TUESDAY, SEPTEMBER 22, 1992
NISOLDIPINE (BAY K 5552) - DATA POOL / NON US-STUDIES
TOTAL DURATION OF TREATMENT WITH NISOLDIPINE BY TOTAL DAILY DOSE OF LONGEST DURATION

POOL 80- CC - HYPERTENSION - TOTAL STUDIES

INSTITUTE OF BIOMETRY

DRUG	ALL	DURATION															
		NOT RECORDED		1 DAY		2-7 DAYS		8-30 DAYS		31-60 DAYS		61-180 DAYS		181-360 DAYS		> 360 DAYS	
	N	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
>5- <=10 MG	114	1	0.9	2	1.8	1	0.9	6	5.3	49	43.0	5	4.4	17	14.9	33	28.9
>10- <=20 MG	192	1	0.5					5	2.6	56	29.2	6	3.1	39	20.3	85	44.3
30 MG	106	1	0.9							47	44.3	1	0.9	22	20.8	35	33.0
40 MG	184									2	1.9	7	6.7	64	60.8	11	10.6
ALL IN POOL	516	3	0.6	2	0.4	1	0.2	11	2.1	154	29.8	19	3.7	142	31.4	164	31.8

In the non- US studies N= 329 received NIS CC for more than 6 months; N= 164 for more than 1 year. In the US studies the figure for greater than 6 months was N= 131 but, apparently, none were treated longer than 1 year.

Demographic characteristics in this safety evaluation will be related primarily to adverse effects and other safety- related features.

B.Safety

1. Deaths

No deaths occurred in the US NIS CC hypertension studies other than for a single subject receiving placebo. He was aged 68 years, collapsed, and failed to respond to resuscitation efforts. In the non-US NIS CC hypertension studies a placebo case died of cerebral hemorrhage; N= 2 NIS CC cases died, one due to an accident, another due to cerebral metastases from prostatic cancer.

2. Serious ADE

The next table shows the number and percentage of subject in the US NIS CC studies by dose for both cases with serious ADE, which display dose response, and for those withdrawing due to serious ADE, in whom dose response is probably present. The dose response for % of patients with ADE versus dose varys from 0.7% (10mg) to 9.1%(80mg).

Number (%) of Patients with Serious Adverse Events and Withdrawals from Study Participation because of Those Events by Dose of NIS CC							
DOSE OF NIS CC (n)	10mg (151)	20mg (395)	30mg (244)	40mg (292)	60mg (199)	80mg (11)	Total (1292)
NO. OF PATIENTS WITH SERIOUS AEs (% OF PTS ON DOSE)	1 (0.7)	8 (0.2)	2 (0.8)	5 (1.7)	9 (4.5)	1 (9.1)	26 (2.0)
NO. OF PATIENTS WHO WITHDREW BECAUSE OF SERIOUS AE (% OF PTS WITH SAE)	0	3 (38)	0	4 (80)	5 (55)	1 (100)	13 (50)

3. Discontinuations due to ADE

Of the N= 1292 patients (combined US+ non- US NIS CC), N= 25 (2.0%) ADE reports were received. Of these cases N= 13 were withdrawn because of these events. N= 17 of the 25 reports occurred during the double- blind phase of trials.

These N= 13 cases, among others, have narrative comments in Table 15a Pool 6 Vol 521. Each of the narratives on these cases was examined by the Reviewer. The final diagnoses were angina, MI, cellulitis of legs, possible MI, CVA, possible MI, infection, flu, pleural effusion, cholelithiasis, CVA, chest pain, pituitary tumor and berry aneurysm, elevated liver enzymes, edema and erythema plus petechiae, pain in legs with elevated CPK, chest pain.

Of the non- US NIS CC completed studies N= 35 cases on NIS withdrew due to ADEs. A listing, Vol 523 Table 15, provides reasons for discontinuation in N= 11 cases: These include tinnitus, non-response, pheochromocytoma, headache plus edema, atrial fibrillation, impotence, edema(2), vertigo, lack of efficacy, non- compliance. In the remaining cases a cause for discontinuation was not given although co-start terms for side effects were. There were a variable number of such terms for different patients. Most were manifestations of vasodilatation. No withdrawals for laboratory abnormalities were listed.

In the N= 6 placebo- controlled trials (US and non- US) with NIS CC 55/828 (6.6%) discontinued. Another N= 68 cases (11.5%) discontinued from among N= 590 patients on long- term uncontrolled studies. Since all but one of the 6 trial was a US study, the Sponsor focused on them. The following table shows that a dose response exists, except at 30 mg, for withdrawal due to ADE in the US placebo- controlled trials. The Sponsor does not believe dose response is evident, but this reviewer's logistic regression (below) shows a slope coeff t of 3.5 std errors.

Number and Percent of Patients in US Placebo-Controlled Studies Withdrawing Due to Adverse Experiences, by Dose of NIS CC							
	PLA (n=280)	10mg (n=37)	20mg (n=180)	30mg (n=125)	40mg (n=184)	60mg (n=137)	80mg (n=15)
N (%)	9 (3.21)	2 (5.4)	13 (7.2)	5 (4.0)	15 (8.2)	15 (10.9)	3 (20.0)

Table 15a Vol 521, not attached, provides a complete listing of reasons for withdrawal in this group. What is striking is that many subjects have multiple reasons for withdrawal. When more than one reason is listed, no single one is given most weight in this table. There was a suggestion, based on inspection of the table, that peripheral edema and rash might be associated but the number of cases is not more than a few.

The following table shows the most frequently reported ADE in subjects withdrawing due to ADE in the controlled US studies.

Incidence (%) of Most Frequently Reported Adverse Events in Patients Withdrawn Due to Adverse Events in U.S. Placebo-Controlled Studies		
ADVERSE EVENTS	PLA (n=280)	ALL NIS CC (n=678)
Any Body System	3.2	7.8
Headache	0.4	3.8
Peripheral Edema	0.4	2.9
Vasodilatation	0	1.5
Nausea	0	0.9
Palpitation	0	0.9
Dizziness	0.4	0.7

The Sponsor reports that the ratio of the number of ADE to the number of patients discontinuing was greater at lower doses than at higher ones.

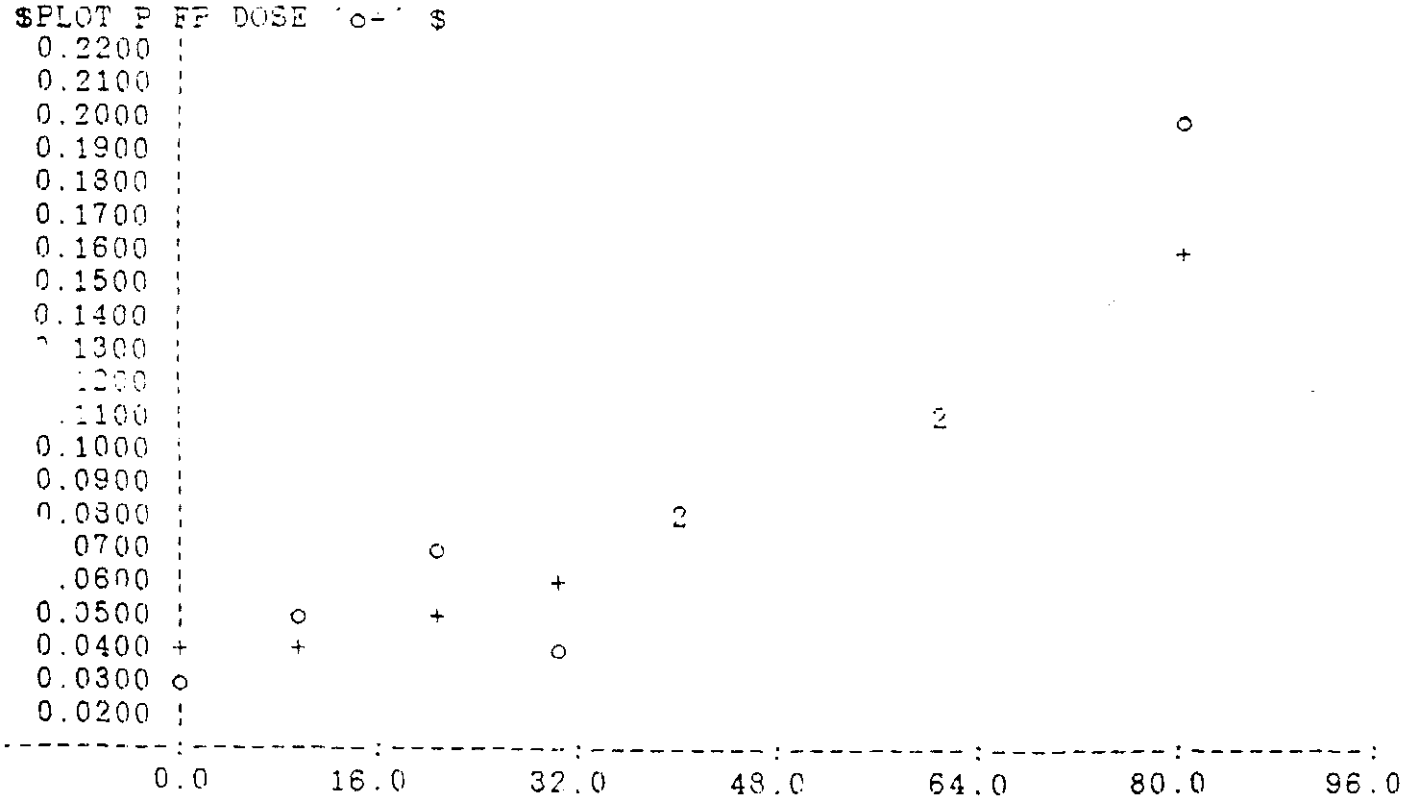
LOGISTIC REGRESSION OF WITHDRAWAL PROPORTION ON DOSE
Y AXIS: PROPORTION WITHDRAWN DUE TO ADE
X AXIS: DOSE MIS MG/DAY

Method: Iteratively- reweighted least squares (GLIM)
(analysis by reviewer)

o = OBSERVED

+ = PREDICTED

US PLACEBO- CONTROLLED HYPERTENSION TRIALS



The Sponsor suggests that multiple, but mild, events could be occurring at low doses with more severe ones, though fewer, at high doses. No analysis of this hypothesis is provided.

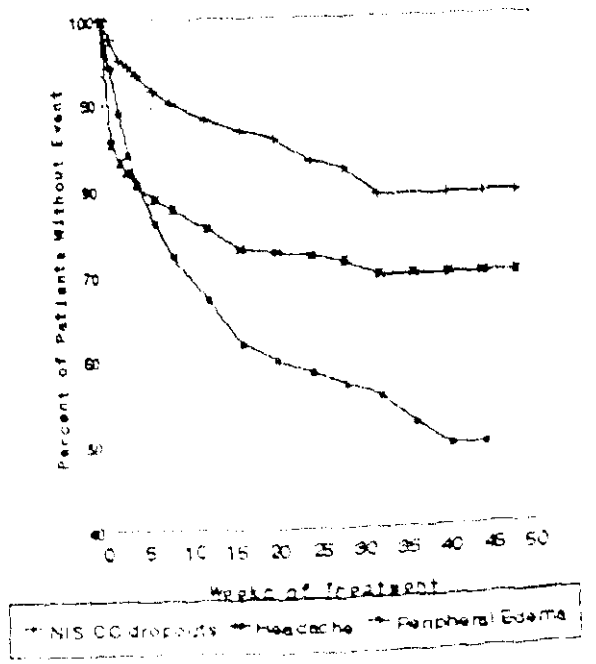
The following table shows cases withdrawing due to ADE from US uncontrolled studies. Patients are assigned the dose they were on for the longest time. There is a counterintuitive inverse association of withdrawal and dose. One possibility is that subjects on higher doses had the opportunity to have withdrawn on lower ones during the process of upward dose adjustment in these long- term studies.

Number and Percent of Patients in U.S. Uncontrolled Studies Withdrawing Due to Adverse Experiences, by Dose of NIS CC				
	20mg (n=55)	30mg (n=46)	40mg (n=34)	60mg (n=89)
N (%)	12 (21.8)	9 (19.6)	6 (17.6)	8 (9.0)

The most frequent ADE in patients withdrawn during the US uncontrolled studies, shown below, are ordered somewhat differently than in the short- term studies. In particular, peripheral edema is more frequent in the long- term trials probably because of a time-dependence for withdrawal such that headache occurs earlier than edema. This is shown in the following table and in a Kaplan-Meier plot.

Incidence (%) of Most Frequently Reported Adverse Events in Patients Withdrawn Due to Adverse Events in U.S. Uncontrolled Studies	
ADVERSE EVENTS	ALL NIS CC (n=224)
Any Body System	15.6
Peripheral Edema	12.1
Headache	7.6
Rhinitis	4.0
Asthenia	3.6
Dizziness	3.1
Chest Pain	2.7
Vasodilatation	2.7

Kaplan-Meier analysis for dropouts due to adverse events and for incidence of headache and peripheral edema



The Sponsor states events causing discontinuation are more severe or higher doses, say 60 mg, than on lower ones. In the absence of a specific analysis taking account of the correct denominators, this may be questioned.

4. Most frequent ADE

The following table lists the most frequent ADE regardless of whether they were associated with withdrawal. Data from US and non- US NIS CC cases are given. The prominance of symptoms related to vasodilation is again seen. The relatively greater incidence of edema in the pooled controlled + uncontrolled studies is also shown for both US and non- US data. In addition, the rates in the non- US studies are overall less than in the US ones.

Incidence Rate (%) of Adverse Experiences (≥ 3%) in Patients Treated in U.S. and Non-U.S. Studies						
Study Location	Studies conducted in the U.S.			Studies conducted outside the U.S.		
Type of Studies	Placebo-Controlled		Controlled + Uncontrolled	Placebo-Controlled		Controlled + Uncontrolled
Adverse Events	PLA (n = 280)	NIS CC (n = 678)	NIS CC (n = 776)	PLA (n = 58)	NIS CC (n = 150)	NIS CC (n = 516)
Headache	15	22	23	21	23	18
Peripheral Edema	10	22	29	7	12	15
Dizziness	4	5	7	9	4	5
Asthenia	4	4	6	0	1	3
Vasodilatation	2	4	5	0	3	5
Palpitation	1	3	3	3	4	3

5. ADE by Demographic features

ADE by demographic sub- groups is examined in the US placebo- controlled trials. The incidence is given below of ADE selected by the Sponsor for "common observance" with the type of compound used. These are mostly those with high incidence. There is no aggregation by sex though one might have expected this, say, for edema in females.

Breakdown by Gender of the Incidence (%) of Selected* Adverse Events† in US Placebo-Controlled Studies				
ADVERSE EVENT	NIS CC		PLACEBO	
	Male (n=424)	Female (n=254)	Male (n=172)	Female (n=108)
Any Body System	67	69	50	58
Headache	20	24	14	18
Peripheral Edema	22	21	8	14
Dizziness	5	5	2	6
Asthenia	4	4	4	3
Vasodilatation	4	4	1	4
Palpitation	3	4	1	1

* The above events were selected because of their common observance with dihydropyridine therapy

† The US data includes both adverse events and intercurrent illnesses

In the non- US placebo- controlled trials there was an increase in edema in females treated with NIS CC but data for the placebo group is not provided. The incidence of edema in treated females is about the same as in the US placebo group, above. The more frequent ADEs are shown below by race in all placebo- controlled studies. Rates tend to be higher for headache and edema in Caucasians.

Breakdown by Gender of the Incidence (%) of Selected* Adverse Events† in Non-US Placebo-Controlled Studies		
ADVERSE EVENT	NIS CC	
	Male (n=65)	Female (n=85)
Any Body System	33.8	38.8
Headache	23.1	22.4
Peripheral Edema	7.7	15.3
Dizziness	3.1	4.7
Vasodilatation	< 3	4.7
Palpitation	3.1	4.7

* The above events were selected because of their common observance with dihydropyridine therapy

ADE by age are shown below for placebo- controlled studies. Headache is more frequent in younger subjects, interestingly, though edema may be less frequent than in older cases. These trends are present in both US and non- US studies.

Breakdown by Age of the Incidence (%) of Selected Adverse Events† in US and Non-US Placebo-Controlled Studies				
ADVERSE EVENT	US Studies		Non-US Studies	
	≤ 65 Years (n=601)	> 65 Years (n=77)	≤ 65 Years (n=131)	> 65 Years (n=19)
Any Body System	59	57	37	37
Headache	24	5	24	11
Peripheral Edema	21	27	12	16
Dizziness	5	5	4	5
Asthenia	4	0	< 3	5
Vasodilatation	4	4	4	< 3
Palpitation	3	1	5	< 3

incidence ≥ 3% is

† The US data includes both adverse events and intercurrent illnesses

Peripheral edema was more frequent in heavier subjects than in the lighter ones shown below both in US and non - US studies.

The table below shows selected adverse events with incidence rate ≥ 3% broken down by median weight in the US and non-US placebo-controlled studies.

Breakdown by Weight of the Incidence (%) of Selected Adverse Events[1] in US and Non-US Placebo-Controlled Studies				
	US Studies		Non-US Studies	
ADVERSE EVENT	≤ 185 lbs (n = 2801)	> 185 lbs (n = 3981)	≤ median weight (n = 81)	> median weight (n = 691)
Any Body System	64	70	33	39
Headache	20	23	24	22
Peripheral Edema	18	28	10	15
Dizziness	5	5	4	4
Asthenia	4	4	< 3	< 3
Vasodilatation	5	4	< 3	6
Palpitation	3	3	< 3	6

[1] The US data includes both adverse events and intercurrent illnesses

A table, not attached, shows that when ADE are stratified by baseline BP below and above 108 mmHg diastolic, that cases with lower BP tended to have more vasodilatation. The respective rates, 5/537 and 1/141. These are not very impressive.

6. Hemodynamic safety

A. Hypotension

ADE suggestive of hypotension, syncope and "hypotension" were sought. Asymptomatic hypotension was determined by "first dose effect", by trough/peak ratios from in- clinic and 24hr ambulatory BP readings, and by examining supine and standing BP plots. No cases (Sponsor) of syncope in the US NIS CC trials on NIS. N= 6 patients in the US placebo- controlled studies had either "hypotension" or "postural hypotension" on NIS CC. The next table shows data from US placebo- controlled trials.(INSERT tp15 v309)
Note that only a few of the cases show orthostatic hypotension in casual blood pressures. It seems worth while to point out that "dizziness" occurred in about 7% of all the US NIS CC studies and that it is possible that a number of cases had this symptom due to hypotension. Clinical experience shows that "dizziness" is not often distinguished from light- headedness without vertigo due to inadequate questioning of patients.

A first- dose effect was examined in two studies without showing adverse symptomatology but with BP reductions. The following table shows the results of in- clinic BP monitoring. Dose related peak effects are seen.

Mean Pre-dose and Peak Supine and Standing Blood Pressure Changes During In-Clinic Monitoring Periods in US Placebo-Controlled Studies							
		Mean Change in Supine Blood Pressures (mmHg)		Time to peak (hr)	Mean Change in Standing Blood Pressures (mmHg)		Time to peak (hr)
Drug Group	n	Pre-dose	Peak		Pre-dose	Peak	
STUDY 088-054 (24-hour period, BPs every hour)							
Placebo	8	-0.9/-3.4	-5.0/-8.3	3	-2.3/-3.5	-5.3/-7.0	22
NIS CC 10mg	7	-11.5/-10.3	-12.6/-11.1	8	-17.4/-8.7	-9.7/-10.5	9
NIS CC 20mg	7	-6.0/-6.3	-7.1/-12.0	14	-3.6/-4.5	-7.1/-10.5	11
NIS CC 30mg	5	-6.8/-12.0	-18.0/-15.5	12	-5.9/-7.4	-14.9/-15.2	11
STUDY 090-039 (12 hour period, BPs every two hours)							
Placebo	35	-4.9/-5.0	-3.4/-3.5	9	5.7/-3.5	-1.5/-4.3	8
NIS CC 20mg	37	-11.7/-10.8	-16.7/-13.5	8	-14.3/-8.3	-15.2/-12.5	8
NIS CC 40mg	35	-18.0/-12.3	-19.1/-15.5	4	-19.2/-12.1	-22.9/-15.9	4
VER SR	40	-14.4/-14.8	-17.4/-15.1	2	-19.2/-14.9	-21.0/-15.8	8
STUDY 090-019 (12 hour period, BPs every hour)							
Placebo	27	-1.9/-7.2	-5.0/-8.1	2	-7.1/-5.0	-4.8/-7.5	4
NIS CC 30mg	32	-13.3/-13.5	-19.0/-14.4	8	-14.5/-12.8	-15.9/-15.8	5
NIS CC 60mg	29	-15.2/-12.8	-15.9/-13.1	9	-18.1/-15.2	-21.1/-20.3	7

Symptomatic Hypotensive Adverse Events
Occurring in US Placebo-Controlled Studies

Study Number	Patient No. / Drug & Dose	Investigator Term	Days on Drug	Duration (days)	Intensity	Baseline BPs (supine & standing)	BPs (day and blood pressures)
			SYNCOPE				
D89-039	16020 / PLA	Vaso-vagal Attack	43	1	Moderate	165/107 168/105	day 43 142/99 143/90
D90-019	1012 / PLA	Feeling Faint at Times	30	>13	Mild	149/107 141/107	day 28 155/113 171/113
			HYPOTENSION				
D89-039	13012 / NIS 20mg	Hypotension Post-Dose	62	1	Mild	136/95 135/97	day 62 129/82 108/70
D89-039	16015 / VER SR 240mg bid	Hypotension	23	>5	Severe	129/100 115/91	day 23 114/93 98/74
			POSTURAL HYPOTENSION				
D89-039	1005 / NIS 20mg	Dizziness upon standing	30	12	Mild	129/95 130/99	day 41 136/91 135/95
D90-019	16004 / NIS 30mg	Dizziness (Postural upon standing up)	10	9	Mild	165/105 162/106	day 14 151/99 146/98
D89-039	1008 / NIS 40mg	Postural Hypotension	16	1	Mild	155/98 141/93	day 16 139/89 129/85
D89-039	12007 / NIS 40mg	Postural Hypotension	25	1	Mild	157/97 150/97	day 25 146/89 136/88
D90-019	16007 / NIS 60mg	Postural Dizziness	28	>6	Mild	170/105 166/101	day 33 145/89 147/90
D89-039	4001 / [illegible]	Dizziness upon standing	3	9	Mild	181/105 146/90	day 6 153/98 150/93 day 14 153/94 98/67

N= 5 ambulatory monitoring studies were done. The distribution of doses by study is provided. The second table shows the trough/peak ratios from these studies.

Number of Patients Undergoing 24-Hour ABPM by Dose of NIS CC						
Study & (location)	PLA	10mg	20mg	30mg	40mg	60mg
D88-054 (US)	16	12	11	12		
D89-029* (US)	31		32		32	30
D89-039 (US)	26		24		24	
D90-006 (non-US)	33	33	32	37		
D90-019 (US)	31			39		29

* This study was conducted on the background of atenolol 50mg qd

Trough/peak ratios from the 24-hr ambulatory data are provided below. Note that for systolic BP two methods are used depending on whether peak systolic is a) determined at the time of peak diastolic BP or, b) whether the true peak is used. These ratios are consistant with peaks that are not substantially below the trough values. Nota, however, that if in a given subject the trough readings are quite low that a small, further drop at peak might be hypotensive. For that reason trough/peak ratios may not be very good means of exploring for BP reductions for safety purposes.

TABULATION OF TROUGH-TO-PEAK RATIOS ANALYZED DURING 24-HOUR AMBULATORY BLOOD PRESSURE MONITORING (BASELINE- AND PLACEBO-SUBTRACTED RESULTS)						
	DOSE OF NIS CC					VER SR
PARAMETER	10mg	20mg	30mg	40mg	60mg	240mg bid
Diastolic BP Trough/Peak Ratio (%)	73	75	93	100	97	86
Systolic BP* Trough/Peak Ratio (%)	75	83	114	100	101	78
Systolic BP† Trough/Peak Ratio (%)	75	85	93	100	89	78

* Peak values correspond to the time of peak diastolic effect
† Peak values are the actual maximum systolic effect

The table below shows the percentage of patients having either a change of 20 mm from baseline or a BP below 100mm Hg. There appears to be a fairly consistant percentage of cases with a fall

in systolic BP regardless of dose except for low numbers in the small sample on 40 mgm NIS. Note that a substantial number of placebo cases also show this degree of fall. Subtraction of the placebo values gives about 4- 8% of cases with reduction below 100 mm systolic. Thus supine reductions of note occurred in some subjects.

TABULATION OF SAFETY PARAMETERS ANALYZED FROM 24-HOUR AMBULATORY BLOOD PRESSURE MONITORING (BASELINE-SUBTRACTED RESULTS)

SAFETY PARAMETER	DOSE OF NIS CC					PLA	VER SR
	10mg (n=45)	20mg (n=87)	30mg (n=88)	40mg (n=24)	60mg (n=29)	(n=106)	240mg bid (n=29)
Diastolic BP Change > 20mmHg from Baseline for at least 1 Hour (% of patients)	82	85	78	79	96	62	96
Systolic BP < 100mmHg for at least 1 Hour % of patients)	24	25	22	4	20	16	24

The last method of assessing hypotension was to compare supine and standing blood pressures at trough. The correlation was near 1.0 and consistant with little orthostatic hypotension.

B. Reflex tachycardia

One of the effects of a vasodilator is reflex tachycardia. The Sponsor examined this by dose response of pulse rate; by frequency of tachycardia as an ADE; and by ECG HR.

In the US monotherapy studies the mean placebo- subtracted change in HR varied from -1.53/min to +0.48 over the dose range of 10 to 60mgm NIS CC. The change in HR for the combined doses was 0.52. Note that these are not specified as standing readings.

In the US placebo- controlled studies, tachycardia was an ADE in 1% of NIS CC patients. One patient in these studies withdrew for supraventricular tachycardia.Three patients in the US uncontrolled studies had tachycardia contributing to withdrawal. The Sponsor analyzed the transition from normal or low heart rate to high values in the US controlled trials and found this to have occurred in 1.4% of NIS CC patients and in 0.9% of placebo cases. Again, none of these readings are specified as taken standing, a position that might have exaggerated pulse change.

C. Rebound hypertension

A placebo- controlled study D90-022 in hypertensive patients treated for as long as 21 days sought evidence for rebound blood pressure elevations by a 72- hr follow-up after discontinuation of NIS CC. Examination of the Sponsor's table showed that the group means for diastolic BP show no evidence of rebound. However, the systolic BP values are higher at 72 hrs than at baseline except for the highest dose level, 120mg NIS. The systolic BP mean for the placebo group is also elevated at 72 hrs so that it is difficult to ascribe the increase in systolic BP to "rebound". It is more likely that loss of both the placebo and therapeutic effects are involved.

7. Clinical Laboratory Tests

A. US NIS CC placebo- controlled studies

1. Incidence rates of "high" lab abnormalities

The Sponsor provides Table 17a (not attached), in which the rates for "high" abnormalities are given by dose level from 0 mgm (PLAC) to 80mg. Sample sizes are very small for the lowest active dose, 10mgm and the highest, 80mgm. Examination of these rates show no evidence for dose response nor is it likely they would given the exceedingly small rates for the data pooled over doses. In particular, for items with overall higher rates including blood glucose, no dose response trend is seen. BUN has a 3% rate at 40mg NIS, 2% at 60mg, and 0% for placebo. No such trend is seen for creatinine. No trend is seen for increase with dose of serum calcium, alkaline phosphatase, or SGPT is seen.

Rates by dose/body weight are also provided but do not show trends of interest except, possibly, for alkaline phosphatase, which has a rate of 5% at the highest dose/weight level, >.55- <1.2 mgm/kg, versus a placebo rate of 2% and rates in lower active dose/weight levels of 1%.

For "low" values the hematologic values for all US NIS CC studies showed 2% of NIS patients with neutrophils below 1700/microl and 1% in the placebo group. The rate was also 2% in the pooled controlled and uncontrolled studies. No patients with platelets below 100,000/microl are shown. Thus addition of cases with long- term followup did not increase the rate of low values for these two tests.

Hematopoietic Parameter Abnormalities from Studies Conducted in the US

BLOOD CELL LINE	Placebo-Controlled		Controlled + Uncontrolled
RBC	PLA	NIS CC	NIS CC
	n=250	n=588	n=680
% PATIENTS LOW ABNORMAL	3	3	5
WBC	PLA	NIS CC	NIS CC
	n=232	n=553	n=636
% PATIENTS LOW ABNORMAL	5	4	5
% PT WITH NEUTROPHILS < 1700/μL	1	2	2
PLATELETS	PLA	NIS CC	NIS CC
	n=267	n=655	n=751
% PATIENTS LOW ABNORMAL	0	0	0
% PT WITH PLATELET < 100,000/μL	0	0	0

This submission contains a tabulation of mean difference from baseline for both NIS CC (N= 650) and placebo (N= 280) for US placebo- controlled subjects. A tabulation on the following page contains selected laboratory tests from the larger tables. The larger table, Table 21 Vol 522, also has standard deviations. The Reviewer calculated the mean difference +/- 2 SE limits for NIS cc and for placebo for hematocrit, platelets, %neutrophils, glucose, BUN, alkaline phosphatase, and SGPT. All of the 2SE limits for the NIS group overlapped those for the PLAC group for these particular tests so that the treatment groups are not likely to differ by this method.

Examination of 10 lowest or highest values of selected laboratory tests in all US studies(controlled and uncontrolled for individual subjects showed, among the lowest 10, N= 3 instances in which falls in hematocit occurred. None were associated with the lowest 10 values for total wbc or %neutrophils. Respective baseline and lowest values were 34,29; 36,30; and 36,33. The baseline values tended towards being low. The subject with the lowest hematocrit,29, was on many medicines at baseline including Naproxin, insulin, enalapril,labetalol, and glyburide. During the

Renal Function Parameter Abnormalities from Studies Conducted in the US			
RENAL FUNCTION TEST	Placebo-Controlled		Controlled + Uncontrolled
CREATININE	PLA	NIS CC	NIS CC
	n = 271	n = 646	n = 739
% PATIENTS HIGH ABNORMAL	0	0	1
BUN	PLA	NIS CC	NIS CC
	n = 269	n = 645	n = 738
% PATIENTS HIGH ABNORMAL	0	1	1

Hepatic Parameter Abnormalities from Studies Conducted in the US			
LIVER FUNCTION TEST	Placebo-Controlled		Controlled + Uncontrolled
SGOT	PLA	NIS CC	NIS CC
	n = 248	n = 603	n = 692
% PATIENTS HIGH ABNORMAL	3	2	3
% PT >3X NORMAL	0	0	0
SGPT	PLA	NIS CC	NIS CC
	n = 224	n = 562	n = 647
% PATIENTS HIGH ABNORMAL	3	2	4
% PT >3X NORMAL	0	0	0
ALKALINE PHOSPHATASE	PLA	NIS CC	NIS CC
	n = 262	n = 623	n = 713
% PATIENTS HIGH ABNORMAL	2	2	4
% PT >1.25X NORMAL	0	0	0
LDH	PLA	NIS CC	NIS CC
	n = 257	n = 628	n = 717
% PATIENTS HIGH ABNORMAL	3	2	4

All cases of high blood glucose on treatment were high at baseline, usually above 200mg%.

N=7 instances of mildly elevated BUN on treatment were found. Only one of these (value =27) was associated with an increased serum creatinine(1.4,2.0).
Serum calcium increased in association with treatment in N=3 cases baseline, on treatment:9.8,10.5; 10.0,10.5;9.1,10.4) but alkaline phosphatase was not in the highest N=10 for any of these. The last of the above N=3 cases had a low phosphate (2.3,1.9).

Although a number of cases had elevation of alkaline phosphatase during treatment, all were high at baseline. One instance of notable change (112,248), but with a subsequent fall to 150, despite some increase at baseline was more closely examined. The patient was a 64- year-old female with diabetes mellitus, hyperlipidemia, and edema. During a long-term extension trial she was on concomittant medications including atenolol, niacin, and glyburide. A slow rise in alkaline phosphatase over 1 and 1/2 years occurred with 3 high readings.

N=3 instances of elevation of SGPT with concomittant elevation of SGOT were noted (baseline, on treatment: 25,384; 39,209;35,88). The first of these also had elevated total bilirubin (0.6,3.3). This patient was a 63-yr-old male with a history of elevated transaminases on ACE inhibitors.During treatment he received Lovastatin. Despite continued treatment with NIS the final day SGPT and SGOT were well within normal limits though the previous two values, both obtained within a one-week period, were elevated. An additional N=2 instances of elevation of SGOT occur ed in the absence of enough rises in the other enzymes to reach the level of the highest 10 values.

increases in serum bilirubin occurred in N=4 cases. One of these had enzyme elevations described just above. Total CPK was elevated during treatment in N=2 patients. In one the MM and BB fractions were normal.

In the N=516 (depending on test) non- US controlled plus uncontrolled studies hematocrits were low in N= 3 cases but in N=2 they tended to increase subsequently. Total leucocytes were low in N=4 cases. In two of these the baseline value was also low. None of these cases were among the 10 lowest %granulocyte values. N=8 low platelet counts occurred in N= 8 cases. In N= 2 of these the baseline values were normal Except for N=2 cases the on- Rx values were not very low, and though below the assigned normal range, were all above 100,000. In the one of N=2 cases with platelets below 100,000 total wbc was low both at baseline and on treatment. In the other the wbc count was not among the N=10 lowest.

In N= 10 cases elevation of SGOT occurred but baseline values were elevated in these. In two cases use of country- specific normal values might have reduced baseline values to normal. N=7 cases with elevated alkaline phosphatase values occurred. In N= 5 of these the baseline values were also elevated. These elevations ere not associated with values for SGOT in the highest 10. N=6 cases of elevated serum bilirubin were found; in N=4 baselines were high. None were associated with SGOT values in the highest 10.

Serum creatinine was not elevated above normal in any of the values in the highest 10.

8. ECG

A. Background

Because of the finding of t-wave changes in study D90-022 (hypertension), the Sponsor examined that study and three phase III NIS CC trials for such alterations. The Sponsor has provided background information from the medical literature. T wave inversion or flattening occur during rapid reduction of BP with vasodlators. These reductions in BP do not appear to be associated with wall motion abnormalities on 2D echocardiography. Long- term treatment with minoxidil has been carried out with improvement in the initial t-wave changes.

B. The phase II trial D90-022

This was a trial in N= 26 patients with mild to moderate hypertension, mean age about 60 years. This was a forced titration trial 30- 120mg NIS CC with the first two doses given for 4 days each; the next two for 7days each. N=8 subjects were randomized to NIS CC and N=5 developed t- wave flattening on the ECG. The N=120mg dose level was discontinued due to poor tolerance (severe peripheral edema, ECG changes).A second group, N= 10, was randomized to NIS. N=6 of these cases developed t wave flattening and/orinversion with occurrence equal, N=2 cases, at each of the doses, 30,60, and 90 mg. No angina occurred. Thallium scans were reported as negative in N=5 of the first cohort.

The percent with T-wave changes and dose were, respectively, 0%(PLAC); 22%(30mg); 39%(60mg); 64%(90mg); 80%(120mg). The respective sample sizes were 5,18,18,11,5. n.b. The excess over the N=23 that were randomized must represent titration steps.

Stratifying results into cases with normal and abnormal ECG and into 6 and 24 hrs after dosing, showed significant differences between BP falls at 6hrs between the two ECG groups. Differences at 24 hrs (trough) were not significant.

Stratifying normal and abnormal ECGs by AUC and Cmax showed that the abnomal ECG group had larger pharmacokinetic parameters.

The Sponsor relates the ECG changes to the forced- titration design and, by analogy, to the literature reports of T- alterations in rapidly- induced hypotension.

C. Other Phase III trials

ECGs from trials D89-029,039,and D90-019 coded blindly and read by a cardiologist. These ECGs were usually taken at trough. Peak ECGs were obtained in one study. Dosage was up to 80mg NIS CC (N=494 in the pooled studies) or placebo. Mean age was about 55. One study had background atenolol.

In these titration studies the dose assigned to an ECG was, for one analysis, that to which a patient was randomized, not to, say, a lower dose they might have transited from. Another analysis assigned the dose as that on which the event occurred. Two analyses were done; one ignoring baseline ECG events and another eliminating cases with these. It seems likely that more than one event per person could occur since rates were calculated from the "total number of events" divided by the number of cases at risk for the first type of analysis. In the second analysis all subjects were at risk. One analysis was done for peak ECG responses.

The table below shows some evidence of dose response except for the small sample at 80mg.

STUDY D89-039						
ECG abnormality	PLA	ALL NIS CC	NIS CC 20 mg	NIS CC 40 mg	NIS CC 80 mg	VER SR
T flattening	5/70 (7%)	10/155 (6%)	3/71 (4%)	7/69 (10%)	0/15 (0%)	4/71 (6%)
T inversion	3/73 (4%)	11/157 (7%)	4/72 (6%)	6/71 (8%)	1/14 (7%)	3/72 (4%)
either	8/74 (11%)	20/166 (12%)	7/75 (9%)	12/76 (16%)	1/15 (7%)	7/76 (9%)

Similar results were obtained when 'all patients' (regardless of baseline findings) were studied and when the number of ECG tracings was used as the denominator. n.b. there tends to be a dose response in each of the above results except at 80mg.

Results for ECGs taken at peak were unrevealing. The sample sizes were far too small, about N=5 per active dose group.

Study DS029. This is the study with background atenelol in addition to NIS CC.The doses of NIS were 0,20,40, and 60mg.

The respective rates of T flattening or inversion at these doses were 18%,7%,20%13% so there was not much evidence of dose response.

For all cases, regardless of baseline status, there was a significant difference among doses for T-flattening (PLAC 21%; 20 21%; 40mg 41%; 60mg 27%).

For events per number of ECGs results were weaker.

Study D90-019. The rates for T-wave flattening or inversion by dose were PLAC 13%; 30mg 9%; 60mg 5%. Therefore no dose response is seen

Rates using all patients or number of ECGs in the denominator were not more useful.

ST- segment elevation or depression: Rates for these were very low in each of the three studies (1-2%). Comparing the incidence in the PLAC and pooled NIS CC groups by ST depression and by elevation showed that placebo and active dose rates were each no more than 2%.

There is little evidence from these three studies, taken together, of a dose response for the primary ECG T-wave changes of interest. However, the findings in the Phase II trial with forced titration do show a trend for dose response as well as effects of BP reduction and plasma NIS concentrations on T abnormalities. Thus the findings in the Phase III trials may just be at the opposite end of a spectrum of effects.

Conclusions for Hypertension Safety (NIS CC):

In placebo- controlled trials there is a dose- related incidence of ADE over the range 0 to 80 mgm of NIS CC. The most frequent ADE are those related to the vasodilatory action of the drug - headache and edema. The occurrence of these two ADE is time- dependent with headache occurring relatively early versus edema.

Marked symptomatic hypotension was not prominant with NIS CC although "dizziness" occurred and may have been a manifestation of hypotension. Asymptomatic hypotension in trough readings was not frequent though supine systolic BP values in the region of 100mm Hg were not infrequent in 24- hr ambulatory BP readings. Rebound hypertension was not present overall during monitored withdrawal. ECG T- wave abnormalities, inversion and flattening, occurred during forced titration in a phase II study but in studies in which dosage was increased slowly was not prominant. There was an association of these ECG changes to the degree of BP reduction and to drug blood levels in the forced titration study ECG S-T alteration was infrequent.

Clinical laboratory abnormalities: In the US, placebo- controlled (shorter- term) studies, evaluations by overall rates of abnormality, transition from normal, and overall rates by dose were not very revealing. present. Examination of the N= 10 highest or lowest values, as appropriate, in all US studies, controlled or uncontrolled (long-term), showed a few instances of falls in hematocrit, total wbc count, and %neutrophils. Several instances of increased transaminases, one with increased bilirubin were found. An instance of substantial elevation of alkaline phosphatase occurred. Serum calcium increased in three cases without increases in alkaline phosphatase.

In the non- US controlled and uncontrolled trials (approximately N= 516 NIS cases) several instances of decreases in hematocrit or wbc occurred. Platelet counts fell in some cases but not below 100,000. A number of cases had increases in SGOT but from elevated baseline levels. Serum creatinine was not elevated in the highest ten values.

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III. Congestive heart failure (NIS CC)

Safety information for this indication is based on two, non- US, placebo- controlled trials. Approval is not being sought for this indication though the safety information is useful since patients with hypertension may develop CHF.

A. Duration of exposure/ demography

Of N= 142 total CHF cases N= 68 were exposed to NIS CC for at least 1 month. Not quite half were exposed for 1- 2 months. Almost all cases, 90%, were male and about 3/4 were younger than 66 yrs of age. Most were NYHA class 1- 2. About half the cases in the placebo and active Rx groups were receiving beta blockers.

B. The following table shows ADE occurring in at least 1% of subjects. Oddly, headache is less frequent in the NIS CC group. Notably, dyspnea is also less frequent.

NIS CC IN PATIENTS WITH CONGESTIVE HEART FAILURE
TREATMENT EMERGENT ADVERSE EVENTS WITH INCIDENCE ≥ 1%

ADVERSE EVENTS	NIS CC n=142 %	PLACEBO n=71 %	PLACEBO SUBTRACTED
Chest pain	8.5	9.9	0
Dyspnea	7.7	12.7	0
Dyspepsia	6.3	5.6	0.7
Dizziness	5.6	7.0	0
Angina Pectoris	4.2	19.7	0
Palpitation	4.2	5.6	0
Vasodilatation	2.8	4.2	0
Headache	2.8	11.3	0
Peripheral Edema	2.8	4.2	0
Hypotension	2.1	4.2	0
Asthenia	1.4	4.2	0
Pain	1.4	0.0	1.4
Increased Cough	1.4	1.4	0

C. Discontinuations associated with ADE

N= 3 cases discontinued placebo treatment in association with ADE (angina, CHF, and ventricular fibrillation) and N= 2 NIS CC cases stopped (CHF, angina). Total discontinuations, regardless of assigned cause, were N=3 in each Rx group. No deaths were reported.

D. Biochemical tests

The submission contains tabulations including one for the percent of cases with "low" abnormalities. The sample sizes for NIS CC and PLAC are about N= 70 for the wbc and platelet data. N= 1 instance of low platelet count per each Rx group is recorded. No instances of low wbc counts are listed. SGOT and SGPT were more frequently elevated in the placebo group. Alkaline phosphatase levels are not listed. No elevations of serum creatinine are listed in either treatment group.

Cases with the ten lowest and ten highest lab values were examined by the Reviewer. One cases had a fall in hematocrit from 40 to 35.2. The lowest total wbc count was 3.9 and one instance occurred in each of the Rx groups. The lowest platelet counts were 75,000 (PLAC), 120,000 (NIS CC). The highest SGOT and SGPT on NIS CC were each 57 units.

Comment: In considering the above CHF safety data, note that the experience is limited to NYHA Class I and II cases.

IV. Congestive heart failure (CC IR)

A. Non- US studies, controlled plus uncontrolled

1. Exposure/ demography

Data was provided on N= 314 patients. Duration of exposure for about half the cases was 1- 2 months and for about 15% of cases was 6- 12 months. Most cases were NYHA Class II or III. Nearly half the patients were on concomittant diuretic therapy.

2. Patient completion status

Of NIS IR cases 60% (189/314) and 50% (29/58) of placebo cases completed therapy. Completion status was not available for 29% of NIS IR and 40% of placebo cases. There were N=2 and N= 1 deaths in those respective treatment groups.

a. Narratives of deaths in NIS IR cases

Case 1. This patient had pain in the right arm during the day prior to death during sleep. Death occurred on day 15 of NIS IR 20 mgm/daily.

Case 2. After N=12 days of NIS IR 20mgm/day the patient developed dypsnea at rest and was dropped from the study and put on an ACE inhibitor. Death occurred 35days later due to "protracted pump failure".

3. Adverse Events

Adverse events (ADE) occurring in at least 3% of cases of the NIS IR group included headache(6.1%); "vasodilatation"(5.4%); peripheral edema (3.8%); and "dizziness"(4.4%). Of N= 14 cases discontinuing in association with ADE relatively few were apparently due to increasing congestive heart failure or development of angina -perhaps one of each. Tachycardia occurred in N= 4 cases. In one case edema, angina, and decreased exercise tolerance all were listed as occurring on day 14. Non-US and US trial results often differ in this submission.

4. Biochemical tests

The Sponsor pooled the results of IR and solution data to study biochemical test results. Sample

sizes were very small. For example, in the placebo- controlled studies evaluation of elevation of SGOT could be made in only N= 17 NIS cases and N= 12 placebo ones (11.8 and 8.3%, respectively). Alkaline phosphatase was elevated in 2/17 (11.8%) of NIS cases and 0/13 (0.0%) of PLAC ones. No cases in either treatment group had "elevated" serum creatinines or decreased total wbc count. One NIS case had a decreased platelet count.

V. Clinical pharmacology studies (safety)

The Sponsor has gathered information from NIS CC (US, non- US) ; NI IR (US); NI IR + other formulations (non- US). Information in the last of these three groups is quite incomplete since safety information was often not recorded. In N=8 ongoing, non- US NIS CC trials in N= 135 subjects there were reports of two dropouts due to edema, both from a trial in subjects with hypertension and renal failure.

A. Exposure/demography

1. NIS CC US Studies

N= 183 cases received NIS CC; N=25, placebo. N= 65 healthy volunteers and N= 118 were patients, mostly hypertensives or those with renal impairment. A few cases were cirrhotic. Females made up 26% of the cases. Duration of exposure was between 7 and 30 days for N= 34 hypertensives and between 1 and 7 days for the rest.

2. NIS CC non- US Studies

N= 215 cases received NIS and N= 40 placebo. Of these, 17% were female. Most of the studies were of short duration, N= 7 days or less.

3. NIS IR US studies

N=83 healthy volunteers participated in these trials, which were all cross- over studies.

4. NIS IR non= US studies

N= 349 cases were exposed to NIS IR tablets and N= 297 to NIS IR solution. Females made up 24% of the total. N= 98 subjects were exposed at least 98 days; of these N+ 20 were exposed 61- 180 days.

B. Adverse event rates

1. NIS CC US Studies

Events occurring in at least 3% of cases included for PLAC and then for NIS, respectively, headache 48%; 64.5%; edema: 0%; 7.7%; dizziness/lightheadedness: 8%;4.9%; tachycardia: 0%; 4.4%.

N=7 NIS subjects discontinued in association with the following ADE: headache, tachycardia,peripheral edema(3), t-wave flattening, and tremor. All discontinuations occurred at NIS 60mgm or higher. The case with t- wave flattening occurred in the forced titration study discussed under ECG abnormalities, hypertension studies. ADE, characterized as "serious" also occurred in the same trial, were edema(2), ECG changes (2), tremor.

2. NIS CC non- US Studies

Headacne: 55%; Flushing 6%; tiredness 3.3%. Most of these studies had no placebo group so none is listed.

N= 7 of 215 NIS cases discontinued in association with angina, headache(5), transient atrial fibrillation, extrasystole.

3. NIS IR U.S. Studies

Headache occurred in 54% of subjects. Lightheadedness (13%) and drowsiness (3.6%) occurred. No discontinuations in association with ADE were reported.

4. NIS IR non- US Studies

Headache (5.7%) and "vasodilatation"(flushing)(4.9%) were two of the more frequently reported adverse effects. Headache was substantially less frequent than in the other groups of studies just discussed. N= 9 discontinuations in association with ADE were reported from among N= 646 cases treated with the IR formulation. These ADE were fluid retention and ankle swelling; headache; weakness and depression (2); and one case of arrhythmia. There were N= 4 additional discontinuations in cases receiving an IV formulation in doses of 0.12- 0.36 mgm. These ADEs were chest pain; "self-limiting" ventricular tachycardia; hypotension; and ECG ST changes.

C. Deaths

No deaths were reported in NIS CC studies in this category. One death occurred in a foreign study involving solution.

VI. Other safety information

A. Ongoing studies

1. Diabetic renal disease

About N= 1400 patients are involved in on- going, blinded studies. Most of these are taking part in a large U.S. trial of NIS CC in diabetic patients with or without hypertension and using enalapril as a positive control. Projected enrollment is N= 1200 per blood pressure category. The primary objective of the trial is to determine if intensive antihypertensive therapy is more effective than only moderate degrees of BP reduction in preventing deterioration of renal function. Additional antihypertensive drugs may be used if goal BP is not reached at doses of the assigned drugs causing intolerance. Note that in the material below data is still blinded.

a. Deaths

One case of suicide occurred in a subject with a history of two previous attempts. One death occurred at day 10 of active therapy in a case with a massive CVA.

b. "Serious" ADE

Most of these, N= 28, were cardiovascular (chest pain/angina: n=10; cardiac arrest/myocardial infarction: n= 4; CVA: n= 6; CHF: n= 2; deep vein thrombosis: 1; diagnostic procedure: n= 2).

c. Withdrawal "due to ADE"

There were N= 78 such ADE among N= 43 cases withdrawing. The most frequent events were edema (11), headache (9), chest pain/angina (5), flatulence (4), and hypertension (4). Cases withdrawing in association with angina/chest pain did not have antecedent headache noted in listings of multiple ADE.

2. Studies in various indications

These were foreign studies using CC IR in a total of N= 97 patients. Results were pooled over the indications for the safety review in the Sponsor's submission. Indications were peripheral vascular disease, post- MI, pulmonary hypertension, and renal hypertension.

a. Exposure/demography

About N= 90 cases were exposed for 1 month; N= 50 for 2- 6 months; and about N= 15 for more than 12 months. Only 25% of cases were females. About 58% of cases completed the study though the status is unknown for 37%. Only 1% are cited as dropping out for adverse effects

b. Adverse effects

Headache occurred in 7.2% of N= 97 NIS IR cases; vasodilatation in 5.2%; peripheral edema in 2.1%; skin rash in 2.1%.

There was one death reported- a patient in a post- MI study who had ventricular fibrillation on day 10 of treatment with NIS IR 10mg daily. Only N= 1 case dropped out due to ADE (for depression, headache, and rash on day 3 of treatment with NIS IR 20mg/day. N= 1 case dropped out (for malaise) of the trials called "supplemental" studies by the Sponsor consisting of trials in peripheral vascular disease in about N= 30 cases.

No biochemical data was available for these trials.

B. Post- marketing surveillance (PMS 1)

1. Exposure/demography

N= 8788 patients were treated in this German NIS IR study. About N= 6000 cases were treated for 2- 6 months. Women made up 40% of the patients.

2. Adverse events

a. Dropouts associated with ADE

Of N= 231 such dropouts 2% (7) had chest pain; 29% (67) had vasodilatation; 15.2%(35) had nausea; 13.4%(31) had peripheral edema; 0.4%(1) had lung edema; rash 1.3%(1). The Reviewer examined listings of associated signs/symptoms of the cases with chest pain/angina and found that 4/7 had either headache or vasodilation at the time of withdrawal. Biochemical data was not required in this study and none was presented.

b. Deaths

The following four pages provide a listing with comments on deaths in this study. N= 8 cases died from myocardial infarction; N= 4 from malignancy; N= 3 from heart failure; and N= 6 from other causes.

C. Post- marketing surveillance study (PMS 2)

1. exposure/demography

This second German study involve N= 640 NIS IR cases. Women comprised 38% of subjects. About 20% of cases were treated for more than one month and about 60 cases treated from 2- 6 months.

2. Dropouts associated with ADE

N= 18 cases dropped out under this condition. Of those 5.6% (1) had angina pectoris; 11.1%(2) had vasodilation; 16.7% (3) had hypotension. The patient with angina pectoris did not have cocomittant headache or vasodilatation in listings of multiple symptoms/signs. There were N= 6 deaths- N=3 due to myocardial infarction; N= 2 due to "cardiac insufficiency" with or without ventricular fibrillation. Clinical laboratory data was not assessed in this survey.

D. Post- marketing surveillance study (Japan)

1. exposure/demography

N= 1850 cases received NIS IR of whom about N= 1300 were treated from 2- 6 months. About half the cases were females.

2. Adverse effects

No deaths were reported from this study. Among the N= 50 dropouts for ADE 34% had headache, 26% had vasodilatation; 4% had chest pain; 4% hypotension;8% edema; 4% rash.

E. Experience after marketing

The Sponsor presents N= 10 Spontaneous Reports from marketing of the IR formulation in countries outside the U.S. These are one case each of anaphylaxis with edema of tongue and larynx; agranulocytosis; myocardial infarction (2); rash plus hyperglycemia; hyponatremia; jaundice with taste perversion and anorexia; photosensitivity; dypsnea plus headache; gastroenteritis with "non- cardiac pulmonary edema".

F. ADE literature search

The sponsor did a literature search using a database that periodically scans more than a dozen data bases. The cut- off date was June 15, 1992. Of N= 2941 articles, N=459 referred to adverse effects and of these N= 41 articles were selected as not containing data expected to overlap that in this submission. The Sponsor tabulated ADE by individual article (vol 322). Symptoms/signs were very nearly limited to those found in the NDA submission. One article dealt with increased insulin production.

004003

NIS CC SAFETY SUMMARY
10 February 1993

NARRATIVES OF POST-MARKETING SURVEILLANCE EVENTS
POOL 10A PMS1 (GERMANY)
PATIENTS WHO DIED

Twenty one of the 8788 patients enrolled in this surveillance died during or after discontinuing the study and details are available on the following patients.

1999 **Cause of death: Bladder carcinoma.** This 58-year old male patient had participated in the surveillance for 83 days when he died from bladder carcinoma. His final dose of NIS IR was 10mg daily.

2474 **Cause of death: Myocardial infarction.** This 77-year old male patient with a 10-year history of CHD, no previous MI, arterial occlusive disease, and bronchitis was concomitantly taking phenprocoumon, theophylline, and digitalis in addition to NIS IR. During therapy with NIS IR, He died after 30 days on NIS IR 10mg daily from a myocardial infarction.

2541 **Cause of death: Cardiogenic shock.** This 82-year old male patient with a 15-year history of CHD, one previous MI 15 years prior to study participation, concurrent heart failure was taking metoprolol and mononitrate. He was observed to have a reduction in on NIS IR 20mg daily. Ten days later, as a consequence of a myocardial infarction, this patient died because of cardiogenic shock.

2542 **Cause of death: Encephalomalacia during coronary angiography.** This 68-year old female patient died nine days after discontinuing 20mg daily of NIS IR (total of 17 days on NIS IR). No additional information is available.

2899 **Cause of death: Acute heart failure.** This 69-year old male patient had a 7-month history of CHD with a myocardial infarction nine months prior to participating in this surveillance. In addition to CHD, this patient also had hypertension, cardiac dysrhythmias (Lown IVa) and was concomitantly taking metoprolol. He had two follow-up visits, after 39 and 68 days on NIS IR 10mg daily, which noted He died 10 days later because of acute heart failure with suspected recurrence of myocardial infarction resulting in cardiac dysrhythmias.

2957 **Cause of death: Sudden cardiac death with calcifying coronary sclerosis.** This 60-year old male patient with a history of CHD, no previous MI, status post-renal transplant and with renewed renal impairment had received 10mg daily of NIS IR for 30 days when he was hospitalized and drug was discontinued. He died four days later.

004004

NIS CC SAFETY SUMMARY
10 February 1993

4008 **Cause of death: Breast cancer.** This 74-year old female patient with a history of died from breast cancer after having completed the surveillance with 10mg daily of NIS IR.

4373 **Cause of death: Myocardial infarction.** This 75-year old male patient with a 12-year history of and two prior MIs (2 and 8 years before study entry) was concomitantly taking digitalis and nitroglycerine spray along with his NIS IR 10mg daily. His first follow-up visit was done after 8 days on NIS IR
He died 3 days later as a result of a myocardial infarction.

4381 **Cause of death: Cardiac arrest.** This 48-year old male patient with a history of and no previous MI appears to have taken only one dose (10mg) of NIS IR during the study. He died two days later in the ambulance after cardiac arrest. No additional information is available.

4435 **Cause of death: Myocardial infarction.** This 66-year old male patient with a 6-year history of and a prior infarction 12 years prior to study participation also had hyperlipidemia and hypertension and was taking dinitrate, metoprolol and bezafibrate when he enrolled in the surveillance. His participation lasted for 61 days on a final daily dose of 10mg NIS IR. He died from a myocardial infarction 11 days after having completed the study.

6357 **Cause of death: Bronchial carcinoma.** This 54-year old male patient with a history of and no previous MI died from bronchial carcinoma after having participated in the study for 83 days on a daily dose of 10mg NIS IR.

6599 **Cause of death: Sudden cardiac death.** This 82-year old female patient with a 15-year history of and Type II diabetes mellitus was taking concomitant glibenclamide, digitalis, captopril, and furosemide. She participated in the study for 28 days on a daily dose of 10mg NIS IR. Thirty-nine (39) days after discontinuing NIS IR, she was admitted to the hospital (reason unknown) and died.

8016 **Cause of death: Car accident.** No additional information is available on this 74-year old female patient who had participated in the surveillance for 92 days and was taking 10mg NIS IR daily at the time of her death.

8601 **Cause of death: Post-operative after CABG, renal failure, and pneumonia.** This 76-year old male patient with a 3-year history of and no previous MI, was to undergo ACVB owing to the unstable condition of his at the start of surveillance. Follow-up visits were done 22 and 59 days after start of surveillance and showed no deterioration in . He was admitted to the hospital for purposes of the CABG and discontinued the NIS IR 20mg daily. Thirteen (13) days later, he died from sequelae of surgery.

Miles Inc.
Pharmaceutical Division

NIS CC SAFETY SUMMARY
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9891 **Cause of death: Status post-aneurysm of the abdominal aorta and circulatory failure.** This 86-year old female with a 10-year history of and no previous MI had follow-up visits done 64 and 78 days after starting NIS IR and completed the surveillance after 106 days with the final dose of NIS IR being 10mg daily. During the surveillance period, she had an operation for an aneurysm of the abdominal aorta and probably as a result of this surgery she died nine days after completing the study.

9916 **Cause of death: Myocardial infarction.** This 61-year old male patient with a 15-year history of wo previous MIs (15 and 6 years prior to study entry), and Type II diabetes mellitus was taking glibenclamide, nitroglycerine spray, mononitrate, and nifedipine/atenolol combination at the beginning of surveillance. During treatment with NIS IR 20mg daily,

He

died 23 days into study as a result of a myocardial infarction.

10704 **Cause of death: Renal carcinoma.** This 57-year old male patient died from renal carcinoma after having participated in the study for 47 days and having taken 20mg daily of NIS IR. No additional information is available.

1095 **Cause of death: Acute heart failure.** This 73-year old male patient with a 11-year history of with 2 previous MIs (11 and 10 years prior to study) also had hypertension, cerebrovascular processes, and Parkinson's disease At the start of surveillance, he was taking aspirin, potassium replacement, metoxen, diltiazem, and mononitrate in addition to NIS IR. His participation in the study lasted for 41 days hile on 10mg daily of NIS IR. Thirty-four (34) days after his last dose of NIS IR, he died from acute heart failure.

11499 **Cause of death: Posterior wall infarction.** This 76-year old male patient with a 10-year history of and previous MI (3 years prior to study participation), also had hyperuricemia, prostatic hyperplasia, and heart failure, and was taking dinitrate, flecainide, phenprocoumon, digitalis, and magnesium/potassium replacement along with NIS IR. Improvement in symptoms was noted at his first follow-up visit after 19 days on NIS IR 10mg daily. He was hospitalized the same day for acute cholecystitis and NIS IR was discontinued. Four (4) days later, despite showing clinical improvement in cholecystitis, the patient died from a myocardial infarction.

12650 **Cause of death: Malignant hypertension.** This 60-year old male patient with a 6-year history of also had hypertension, cerebrovascular insufficiency, and heart failure, and was taking mononitrate, clonidine, indapamide, enalapril, and digitalis. His participation in the study lasted 66 days with a final NIS IR dose of 20mg daily during

He died 3 days after completing the study from malignant hypertension.

NIS CC SAFETY SUMMARY
10 February 1993

13765 **Cause of death: Acute re-infarction.** This 51-year old male patient had a 4-year history of previous MI (4 years prior to study participation), and hypercholesterolemia and was taking mononitrate and metoprolol. His follow-up visits occurred 20 and 49 days after starting NIS IR and he completed the study on day 50 on a final dose of 20mg daily of NIS IR. He died 27 days later from acute re-infarction.

G. A pharmacodynamic- clinical model for adverse reactions

If, in fact, a drug increases cardiac work unfavorably at some phase of its administration in a subset of patients and that increase in cardiac work is the factor responsible for determining the onset of ischemia, it is possible that the distribution of times to drop out for angina or chest pain would approximate that for withdrawal due to "vasodilation" phenomena other than angina such as headache, tachycardia etc. Perhaps in some patients the increase in cardiac work associated with vasodilation might increase rather than improve ischemia. If so, then the timing of both phenomena should be similar.

For US controlled- trial angina patients Table 17 page 20 of this review shows that 3% of NIS CC patients discontinued due to angina or myocardial infarction versus 1% for placebo cases.
Table 18 on p21 of this review shows that for non- US placebo- controlled trial cases, the percentage of cases discontinuing for these indications is similar in NIS CC and placebo cases.

For US, placebo- controlled trials, the narrative comments (this review page 20) show that the times to discontinuation for worsening angina or MI were relatively soon after starting treatment. For the non- US trials the days to discontinuation were also relatively early.

The Kaplan- Meier "survival" curve for the endpoint headache (see this review page 5) shows that the percentage of patients without headache falls abruptly early (in about two weeks) to 85% but takes until week 30 to fall another 15%. This early discontinuation for headache would be occurring during dose escalation and/or early exposure to nisoldipine and is most probably a concomitant response to the pharmacologic action of the drug.

Note that in the long- term trials the times of discontinuation vary widely and may be a number of months. Such cases might represent the "background" incidence of events in a population with from which some cases with had been removed due to drug exposure during the short- term phases of long- term studies. In some patients may be due to sporadic increases in dosage. To reduce the complexity of multiple causes of withdrawal, this reviewer has used the short- term studies for the graphical analyses to be shown subsequently.

Note that either 1) the similarity of timing of withdrawal for ischemia and for "vasodilation" and 2) withdrawal for both in the same patient would be supportative of an association between the action of the calcium channel blocker and ischemia. Although instances were not infrequent of withdrawal for both in the same patient, they were not observed often enough to provide strong evidence. This may be due to a tendency to emphasize a single cause for withdrawal in clinical trials. Therefore, the reviewer reports on the similarity of the withdrawal- time distributions for ischemia and vasodilation.

Graphical analysis of withdrawal:

Pages
41
41A
3
C
D
E
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INFORMATION

Summary of Withdrawals from Hypertension Trials With Particular Reference to Chest Pain (N events due to this reviewer's tally using submitted safety pool data):

HYPERTENSION TRIALS

CONTROLLED TRIALS:

STUDY	NIS EVENT/N	PLAC EVENT/N	%NIS	%PLAC	DOSES
D89026	0/82	0/40	0.0	0.0	
D88054	0/91	0/31	0.0	0.0	10-30
D89029	2/189	0/62	1.1	0.0	20-60
D89039	3/167*	0/75	1.8	0.0	20-80
D90019	1/149	1/72	0.7	1.4	30-60

* N=1 with vasodilation (see definition below)

UNCONTROLLED TRIALS:

X89039	3/136	na	2.2	na	20-40
X90019	0/88	na	0.0	na	30-60

Comment: Even in the uncontrolled (long- term) trials withdrawal for angina/cad was relatively infrequent in the hypertension data. Probably this is a reflection of small rates of serious CAD in the population sampled such that any tendency for NIS Rx to accentuate manifestations was also minimal.

Overall Conclusions for Safety of Nisoldipine:

Two major findings in this submission are 1) a substantial incidence of withdrawal associated with signs/symptoms of vasodilation and 2) an increased rate of withdrawal for angina/cad in In the short- term trials the latter withdrawals tended to occur early and to be associated with signs/symptoms of vasodilation but, in the long- term trials they were primarily manifested by an increased rate of withdrawal.

In the US, placebo- controlled, hypertension trials there was a significant dose response for overall withdrawal of about 11% at 60mg NIS and 5.4% at 10 mg. The incidence of headache, not necessarily associated with withdrawal was about 20%. In the long- term, uncontrolled hypertension studies, about 20% of cases had withdrawn by 30 weeks and the cumulative incidence of peripheral edema was more than 40%. Thus whatever blood pressure reduction that is achieved is associated with substantial side effects, most of which are due to the pharmacologic action of the drug in causing vasodilation or to the compensatory mechanisms such as tachycardia.

One might expect some myocardial ischemic phenomena if the vasodilation and tachycardia increased cardiac work out of proportion due the benefits of reduction of afterload through lowering of blood pressure. There was little evidence that the balance was unfavorably affected since, in the hypertension studies the incidence of withdrawal due to angina/cad was about 1%. However, there were instances, especially in the phase 2 rapid, forced- titration trial, of the development of t wave abnormalities.

It is in patients that evidence for ischemic effects of nisoldipine are of greater concern. Even in the short- term, US placebo- controlled trials the rates of withdrawal for angina/chd exceed those on placebo. In addition, it is of particular interest to note that a high proportion of such withdrawals occurred very early at times close to those for withdrawal due to vasodilation. Thus the close similarity of the distribution of withdrawal for vasodilation and that for angina/cad supports a similar mechanism for both. Symptoms of vasodilation were not prominant in the but in those the number of events was higher, about 10%. Thus for the short- term trials one may use the rates, the timing of withdrawal and/or association of vasodilation; for the long- term trials only the rates are useful. Note that the use of timing of withdrawal and/or any associated vasodilation constitutes an "internal" control.

From a purely safety standpoint, this reviewer is not in favor of approval for the It is possible that a combination of a beta- blocker and nisoldipine would allow use of the latter drug in a single study (0702) carried out in Canada, US, and Israel had very few withdrawals due

to ADE. N= 1 subject withdrew for [redacted]g angina and N= 1 for myocardial infarction out of N= 200 NIS+ atenelol cases. The experience with this combination is as yet insufficient to recommend this combination but it may be a justifiable treatment to explore in future trials.

Note that if the NIS formulation dumps early, one would expect to see early withdrawal or the early occurrence of signs/symptoms of vasodilation.

The hypertension indication, at least for subjects without notable coronary heart disease, is supported by the safety data. However, if the spectrum of patients selected for treatment includes patients whose hypertension is associated with CHD or as hypertensive subjects develop CHD, some of these may be unable to tolerate NIS therapy. Alternative therapy should be considered in such cases.

The ECG findings in rapid- dose escalation (intervals of less than a week) in hypertensive subjects, while not clearly established as adverse, are in an unfavorable direction and suggest that titration be carried out over the longest intervals consistant with the patient's need for blood pressure control.

Summary of safety recommendations:

1. Nisoldipine, as studied, not suitable

2. Further studies with beta blocker + NIS of interest

3. Subjects with asymptomatic coronary disease constitute a somewhat difficult group with respect to suitability for NIS therapy since many hypertensives responding to this treatment undoubtedly have this condition. Perhaps some clinical judgement needs to be invoked here.

4. In view of the findings of ECG T abnormalities on rapid titration in hypertensive patients, it is suggested that dosage increments be made at the greatest intervals consistant with the need for BP control. The Sponsor's dosing recommendations do not specify the interval between dosage increments. Intervals of only a few days may be too frequent since they were associated with "adverse" ECG changes in hypertensives.

5. Although mono- therapy for hypertension with NIS appears justifiable due to the lack of serious drug- induced effects, there is still a substantial incidence of troublesome symptoms of vasodilation that might be diminished with combination of NIS an beta- blockade. This may also be a suitable and informative area for further trials.

6. Pharmacokinetic studies by the Sponsor show that the mean Cmax was 48% higher when NIS was administered with a meal. It is not clear whether this explains the very early occurrence of vasodilation after dosing and,
[redacted] It may not be correct to state that it is known that there are no clinical consequences from dose dumping.

7. Since elderly subjects have a 2- 3 fold higher plasma NIS concentration than younger ones, it may be best to follow these subjects more closely and increase dosage slower.

Philip L. Dern M.D.

cc:original
HFD-110
HFD-110/CSO
HFD-110/pld

JUL - 7 1994

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
MEDICAL OFFICER'S NDA REVIEW

NDA: 20- 356

DRUG: Nisoldipine

SPONSOR: Miles

DATE SUBMITTED: 17 August, 1993

DATE REVIEWED: 7 July, 1994

REVIEWER: Philip L. Dern M.D. Philip Dern

RESUME:

This is a 120- day safety review and includes both completed and uncompleted trials, foreign and domestic. For uncompleted trials, data is still blinded and treatment is listed as "either drug 1 or drug 2" if these are the possibilities for a particular patient.

I. Deaths
A. Completed US studies

One death is recorded for this update and was also included in the NDA for an on-going trial.

Study # D90- 029-06; Pt 6004.

This 44- yr- old female with a qualification, single- blind BP of 190/111 and a randomization BP of 141/97 died suddenly at home on day 24 of the study. She was on NIS 40 mg qd and HCTZ 25 mg qd. Serum potassium at baseline and last visit were, respectively, 3.9 and 3.6 mEq/l. Autopsy revealed moderate coronary atherosclerosis without thrombi.

B. Completed Non- US studies

Study 752. No deaths.

C. On-going Studies (all non- US)

Study 764; Pt 128.
This 83-yr- old female was being treated with either NIS or lisinopril (LIS). She died at home of acute pulmonary edema and has an associated abdominal infarction.

Study 769; pt 16002.

This 53 yr- old male was being treated with either NIS or atenelol (ATN). The patient had a TIA on 9/91. Baseline BP was 162/105 after three weeks of placebo. He was admitted to hospital on 5/18/92 after 53 days of treatment. He died the following day of a CVA. Bp at last clinic visit was 177/83.

Study 769; pt 17013.

This patient, a male aged 73, was being treated with either NIS or ATN. After 5 days of therapy the patient developed diarrhea, nausea, and vomiting, and, three days later, a fatal MI.

Study 769; pt 54002.

The patient, a male aged 66, was on either NIS or ATN. Baseline BP was 155/93. After 25 days of treatment he developed a CVA and died. BP at last clinic visit was 145/83.

II. Discontinuations due to adverse experiences

A. Completed US studies

REASON WITHDRAWN	DAY	DRUG & DOSE
Edema,erythema	24	NIS40
headache	2	NIS20
headache	1	NIS20
tachycardia,vasodil	1	NIS20
Dizzy, n & v,headach	2	NIS20
Gout	5	NIS20
Faint at phlebotomy	10	NIS40
edema	13	NIS40
edema	19	NIS40
Abn Liver function*	35	NIS40, HCTZ25
Cough	8	NIS40+ LIS20
card. arrest	24	NIS40, HCTZ25
sinus tachycardia	10	NIS40, HCTZ25

* Also abnormal during pre- NIS phase

n.b. There is a notable number of cases withdrawn relatively early in the active dose phase especially for those signs and/or symptoms likely to be due to the vasodilatory action of nisoldipine.

B. Ongoing trials (all non- US):

SELECTED SIGNS/SYMPTOMS	N*
edema	23
migraine	3
headache	28
angina	1
MI	2

* often sign/symptom occurred with others

The above table concentrates on typical findings on nisoldipine therapy (edema, headache) but notes occurrence of migraine. Number of cases of angina and MI is small. Total number of cases withdrawn due to syncope (1), postural hypotension (1), hypotension (1), suggests that excessive BP fall was uncommon. N=1 case was withdrawn for thrombocytopenia.

Among 1370 cases in these foreign studies, 100 were withdrawn due to adverse experiences.

Conclusions:

The withdrawals for adverse effects were often due to the pharmacologic action of nisoldipine and consequent to vasodilation (headache, vasodilation). In this hypertensive population few cases of angina or MI occurred. However, the data base for this report is not cumulative and the number of cases in the completed US trial is too small to provide a good estimate of the risk of angina/MI. The database for the foreign studies is larger and, even though treatment assignment is in doubt, the small number of angina/MI cases is notable (See this Reviewer's review of the hypertension safety segment of the NDA for comments on the association of symptoms/signs of vasodilation and angina/MI).

cc: HFD/110
HFD/110 orig
HFD/110 CSO ✓
HFD/110 pld

D d c d s

OCT 2 6 1993

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
MEDICAL OFFICER'S REVIEW

NDA: 20-356 (Nisoldipine Coat-Core tablets for exertional angina; Bay K 5552:

Sponsor: Miles Inc. Pharmaceuticals Div.

Submission: NDA 120 day Safety Update

Submission date: 17 August 1993.

Receipt date: 19 August 1993.

Review date: 26 October 1993.

Reviewer: N. Stockbridge, M.D., Ph.D.

1. US trials

There is only 1 ongoing US trial, #X90-015, an open-label, long-term follow-on to Study #90-015 (q.v.). There are no new safety data for this trial. However, two subjects who completed this trial and continued to receive nisoldipine coat-core under an individual investigator's IND experienced serious adverse experiences.

Subject was a 69 year old Caucasian female who had generally received 20 mg q.d. The dose was reduced because of dizziness and light-headedness. She experienced chest pain or discomfort for two weeks prior to admission at 734 days for unstable angina. Enzymes did not indicate myocardial infarction and she was discharged after 3 days. She was readmitted with similar history at 770 days, at which time hiatal hernia was diagnosed.

Subject was a 68 year old Caucasian who suffered myocardial infarction while receiving 30 mg q.d. Three months later he was readmitted for severe angina while on 40 mg.

2. Non-US trials

2.1. Study #697

This is a randomized, double-blind, parallel group study being conducted in Germany and Italy. The groups are nisoldipine 40 mg q.d. (n=138) and diltiazem 60 mg t.i.d. (n=136).

Subject #11-009: was a 72 year old female who discontinued after 8 months because of allergic exanthema.

Subject #41-005 was a 55 year old female who discontinued after 3 months because of resting tachycardia.

2.2. Study #701

This is a randomized, double-blind, parallel group study being conducted in Germany. The groups are nisoldipine coat-core 20 mg q.d. (n=70) and nisoldipine immediate release 10 mg b.i.d. (n=72).

Subject 0101 was a 58 year old female with a 2-year . She discontinued at 5 days with severe burning sensation of the skin, headache, and vasodilation, all of which began with the first dose.

Subject #0115 was a 68 year old female with a 6-month She discontinued at 4 months with nausea, inner "trembling", and pressure and heat sensation in hands and feet.

2.3. Study #718

This is an ongoing randomized, double-blind parallel group trial with nisoldipine 20 and 40 mg q.d., and diltiazem 60 and 120 mg b.i.d. and t.i.d.

Subject #303 was a 51 year old male with a 6-year He withdrew after

4 days because of chest pressure and palpitations.

Subject #306 was a 54 year old male with an 8-year . During month 7, he complained of flatulence. After 8 months, he withdrew because of hypotension, malaise, and fatigue.

Study #771

This is an ongoing randomized, double-blind, comparison of nisoldipine 20 to 40 mg q.d. with diltiazem 60 mg t.i.d. or q.i.d.

Subject #3307 was a 55 year old male with a 7 month and myocardial infarction 12 years previously. He discontinued at 5 months with atrial fibrillation which resulted in prolonged hospitalization; outcome unknown.

Subject #3602 was a 78 year old male with 2 month . He suffered cramps from the onset of treatment and discontinued after 4 weeks with the onset of fasciculations.

Subject #3703 was a 63 year old male with a 1-year h He discontinued after 3 weeks because of vertigo.

2.4. Study #781

This is an ongoing randomized, double-blind trial in Germany comparing 20 and 40 mg q.d. nisoldipine with ISDN 20 and 40 mg b.i.d.

Subject #213 was a 70 year old female with a She discontinued after 3 weeks because of severe headaches and nausea.

Subject #214 was a 62 year old female with a i. She discontinued after 6 weeks because of leg edema.

Subject #603 was a 64 year old female with a She discontinued after 2 weeks because of severe headaches, diarrhea, restlessness, and general malaise.

Subject #906 was a 69 year old female with a She discontinued after 2 months because of hair loss.

Subject #1004 was a 68 year old male with a and myocardial infarction 1 year previously. He was discontinued after 1 week because of non-compliance.

Subject #1016 was a 58 year old male with a d possibly 2 previous myocardial infarctions. He was discontinued after 2 months because of non-compliance.

2.5. Study #761

This is an ongoing open-label trial in Israel with nisoldipine 10 to 60 mg q.d.

Subject #122 was a 59 year old Caucasian male who discontinued after 6 months because of unstable angina.

Subject #404 was a 54 year old Caucasian male who suffered a myocardial infarction at 6 months.

Subject #414 was a 73 year old Caucasian male who discontinued after 4 months because of unstable angina.

Subject #617 was a 95 year old (?) Caucasian male who suffered a myocardial infarction after 7 months.

Subject #1004 was a 72 year old Caucasian male who discontinued for constipation after 6 months.

Subject #1017 was a 66 year old Caucasian male who discontinued after 4 months because of intermittent claudication and fissure following prostatectomy.

2.6. Study #762

This is an ongoing randomized, double-blind trial in Italy comparing 20 mg q.d. nisoldipine coat-core with 10 mg b.i.d. nisoldipine immediate release.

Subject #402 was a 21 year old Caucasian male with a who had a myocardial infarction at 1 month.

Subject #1504 was a 61 year old Caucasian male with an . He

discontinued after 4 weeks because of unstable angina.

Subject #605 , was a 69 year old Caucasian male with an He discontinued after 2 weeks because of unstable angina.

Subject #607 was a 64 year old Caucasian male with c He discontinued after 2 weeks because of rash and hypotension.

Subject #615 was a 58 year old Caucasian male with a He discontinued after 2 weeks with rhinitis, edema of the legs, erythema, and pruritus which began on the second or third day of treatment. Symptoms resolved 2 days after withdrawal.

Subject #810 was a 60 year old Caucasian male with a He dropped out after 3 weeks because of unstable angina.

2.7. Study #10011 (X90-010)

This is an ongoing open-label study in Israel with doses 10 to 60 mg q.d.

Subject #101 was a 56 year old Caucasian male who developed thyroiditis and tonsillitis after 4 months.

Subject #103 was a 63 year old Caucasian male who discontinued after 1 month because of unstable angina.

Subject #207 was a 64 year old Caucasian male who was hospitalized after 5 weeks because of unstable angina.

Subject #420 was a 69 year old Caucasian male who discontinued after 3 months because of pedal edema.

Subject #805 was a 63 year old Caucasian male who experienced severe prolonged angina and tachycardia 9 days after beginning treatment. He was off study drug for a short period and later completed 12 months.

Subject #910 was a 70 year old Caucasian female with a history of ophthalmological disease. She had intraocular hypertension for 9 months during study.

Subject #911 was a 65 year old Caucasian male was hospitalized for severe angina after 2 weeks. He subsequently completed 6 months, with complaints of decreased libido. The reason for discontinuation is not explicitly stated.

Subject #913 was a 61 year old Caucasian male with He complained of flank pain at week 2; the complaint resolved.

Subject #915 was a 52 year old Caucasian male who discontinued after 3 months because of facial flushing.

Subject #1004) was a 71 year old Caucasian male who was hospitalized for unstable angina at 7 months. He completed 12 months of treatment

Subject #1018 was a 60 year old Caucasian male. He was twice hospitalized for chest pain, the second was after about 12 months of treatment.

Subject #1107 , was a 57 year old Caucasian male who developed severe chest pain and underwent CABG 4 days after completing 12 months treatment.

3. Summary

Headache, vasodilation, and peripheral edema remained the common treatment-related adverse events.

Several of the cases described appear to represent acute worsening of angina during treatment. Subject #1107 in Study #10011 may represent a rebound phenomenon.

The information provided in this 120-day safety update do not materially affect conclusions made with the Medical Officer's review (2 August 1993) of safety and efficacy

CHEMISTRY REVIEW

SEP 16 1994

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: **20-356** **CHEM.REVIEW #:** 4 **REVIEW DATE:** 09-Sep-94

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
ORIGINAL	31-Mar-93	05-Apr-93	05-Apr-93
AMENDMENT	20-Jun-94	21-Jun-94	24-Jun-94
	29-Jul-94	03-Aug-94	05-Aug-94
	29-Jul-94	03-Aug-94	05-Aug-94

NAME & ADDRESS OF APPLICANT:
Miles Inc.
Pharmaceutical Division
400 Morgan Lane
West Haven, CT 06516-4175

DRUG PRODUCT NAME

Proprietary:	Not yet established
Nonproprietary/USAN:	Nisoldipine
Code Name/#:	BAY k 5552, CAS-63675-72-9
Chem.Type/Ther.Class:	1 S

Patent Status: Patents which claim the drug, Nisoldipine, (BAY 5552) and its use are as follows:

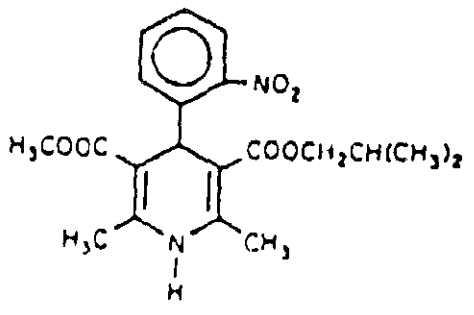
U.S. Patent No. 4,154,836	Expires May 15, 1996 and covers the compound, pharmaceutical compositions for increasing coronary perfusion; and claims methods for increasing coronary perfusion.
U.S. Patent No. 4,892,741	Expires January 9, 2007, covers the coat-core tablet.
U.S. Patent No. 4,600,778	Expires July 15, 2003, covers the preferred process for providing Nisoldipine.

PHARMACOL.CATEGORY/INDICATION: Hypertension

7/29/94 Angina indication was withdrawn

DOSAGE FORM: Coat core (extended release) Tablets
STRENGTHS: 10, 20, 30 and 40 mg
ROUTE OF ADMINISTRATION: Oral
DISPENSED: __X__ Rx _____ OTC

STRUCTURAL FORMULA, CHEMICAL NAME, MOLECULAR FORMULA, MOLECULAR WEIGHT:



Chemical name(s):

3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-methyl-2-methylpropyl ester, (±)

(±)-Isobutyl methyl 1,4-dihydro-2,6-dimethyl-4-(*o*-nitrophenyl)-3,5-pyridinedicarboxylate

Molecular Formula: $C_{20}H_{24}N_2O_6$ Molecular Weight: 388.42

SUPPORTING DOCUMENTS:

RELATED DOCUMENTS (if applicable):

CONSULTS: EA was requested on 6/11/93, amended 8/4/93.

REMARKS/COMMENTS:

The nisoldipine CC tablets consist of a tablet core with a rapid active ingredient release in a compressed press-coating with controlled, delayed active ingredient release. To achieve protection from light, the tablets are film-coated.

Nisoldipine, racemate, will be used in the preparation of the drug product. Studies using enantiomers of nisoldipine were performed. (+)-Nisoldipine was found to be 10-20 times more potent than (-)-nisoldipine in hypertensive rats. There was no relevant difference in oral efficacy between (+)-nisoldipine and the racemate in either rats or dogs. (+)-Nisoldipine binds to isolated membranes with an affinity 100 times higher than (-)-nisoldipine.

In general, (+)-nisoldipine (BAY R 1224) shows a spectrum of activities in standard safety pharmacology testing similar to the racemate, but at lower dose levels.

EER requested on 6/4/93. Acceptable on 12/16/93.

Methods validation - requested of DDA on 12/14/93. DO will be assigned when additional sample will be picked up. (Foreign manufacturing facility)

July 29, 1994 amendment - response to deficiencies.

July 29, 1994 amendment - change in dissolution specifications.

Proposed expiration date - 24 months.

Dissolution specifications (3 hours - 6 hours - 12 hours - nlt is acceptable if Biopharm reviewer agrees).

CONCLUSIONS & RECOMMENDATIONS:

Responses to the deficiencies were satisfactory.

cc:
Orig. NDA 20-356
HFD-110/Division File
HFD-110/CunninghamD/9/9/94
District
HFD-110/CSO
HFD-102/CKumkumian [#1 only]
R/D Init by: SUPERVISOR

Danute G. Cunningham, Review Chemist
filename: 20356R04.NDA

9-16-94

PHARMACOLOGY REVIEW

NDA 20-356

SEP - 2 1994

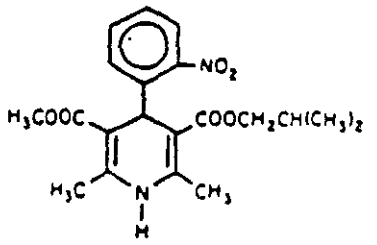
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Sidney Stolzenberg, Ph.D.
Xavier Joseph, D.V.M.

ORIGINAL NDA DATED: March 31, 1993
CENTER RECEIPT DATE: April 1, 1993
REVIEWERS RECEIPT DATE: April 9, 1993

SPONSOR: Miles Inc. Pharmaceutical Division
400 Morgan Lane, West Haven, CT 06516

DRUG: Proprietary name - not established
Generic name - nisoldipine
Code name - BAY k 5552



M.W. 388.4

FORMULATION: Coat core (extended release) tablets containing 10, 20, 30 or 40 mg of nisoldipine are formulated with following inactive ingredients: hydroxypropylcellulose, lactose, corn starch, crospovidone, microcrystalline cellulose, sodium lauryl sulfate, povidone and magnesium stearate (core and outer coat); hydroxypropylmethylcellulose, polyethylene glycol ferric oxide and titanium dioxide (film coat).

PHARMACOLOGICAL CLASS: Calcium channel blocker

PROPOSED INDICATION: Treatment of hypertension

PROPOSED DOSAGE REGIMEN: 10-40 mg once daily

IND UNDER WHICH CLINICAL TRIALS WERE CONDUCTED:

Table of Contents

SUMMARY OF PHARMACOLOGICAL STUDIES (X. Joseph)

A. Studies Related to Therapeutic Indications

1. Effects on Blood Pressure

a. Rats

The effects of nisoldipine on blood pressure and heart rate were studied and compared with appropriate reference drugs (nifedipine, nicardipine and hydralazine) in normotensive (NT) and spontaneously hypertensive (SH) rats. Single oral doses of nisoldipine (3-30 mg/kg) produced a dose-dependent decrease in blood pressure in normotensive rats. Although the hypotensive effect at 3 mg/kg was statistically not significant, doses of 9 and 30 mg/kg produced significant reductions in blood pressure lasting for 2 and 4 hr, respectively, after nisoldipine administration (Table 1). A significant dose-dependent increase in heart rate was observed in all nisoldipine treated groups for 2-6 hr postdose (Table 2). Reference drugs also produced similar dose dependent hypotensive effects and increased heart rates. However, in normotensive rats, hypotensive effects produced by reference drugs at 9 mg/kg were almost equivalent to the effect produced by the high dose level, 30 mg/kg, of nisoldipine (Table 1).

Nisoldipine produced a more pronounced antihypertensive effect in SH rats than in normotensive rats. Dose dependent significant reductions in blood pressure were seen at all levels of nisoldipine tested (3-30 mg/kg, po), beginning at 30 min and lasting until 4 hr (3 mg/kg) or 6 hr (9 mg/kg and above) postdose (Table 3). Heart rate was significantly increased up to 2 hr postdose in all nisoldipine treated groups and until 6 hr at the highest dose level (Table 4). At 24 hr, no significant differences in blood pressure or heart rate were seen between control and treated groups. Reference drugs also produced dose dependent decreases in blood pressure and increases in heart rates in SH rats.

Studies in other experimental animal models of induced chronic hypertension (renal hypertensive rats and deoxycorticosterone-NaCl hypertensive rats) also revealed a dose related hypotensive effect for nisoldipine.

The doses of nisoldipine and reference drugs required to decrease blood pressure by 20% or increase heart rate by 20% of the initial values (ED20 values) in normotensive and SH rats and also in other experimental animal models of hypertension are given in Table 5.

Table 1: Effects of nisoldipine and reference drugs on blood pressure in normotensive rats.

Drugs	Dose (mg/kg)	Mean blood pressure (mmHg) ± S.E.M.						
		Before	30 min	60 min	120 min	240 min	360 min	24 h
Control	-	108 ± 5	109 ± 5	110 ± 4	190 ± 5	180 ± 5	109 ± 5	109 ± 5
Nisoldipine	3	109 ± 6	99 ± 5	100 ± 5	101 ± 5	103 ± 6	107 ± 6	109 ± 6
	9	111 ± 3	94 ± 6	93 ± 5*	92 ± 4*	99 ± 5	103 ± 5	112 ± 3
	30	112 ± 5	87 ± 4**	83 ± 2**	85 ± 2**	89 ± 2**	97 ± 3	114 ± 3
Nifedipine	1	112 ± 1	103 ± 1	105 ± 1	107 ± 1	107 ± 1	108 ± 1	113 ± 2
	3	109 ± 3	92 ± 3*	91 ± 3**	94 ± 3*	92 ± 2*	94 ± 2*	109 ± 3
	9	107 ± 5	79 ± 4**	76 ± 2**	77 ± 3**	77 ± 3**	80 ± 2**	108 ± 4
Nicardipine	3	113 ± 2	104 ± 2	106 ± 2	106 ± 1	109 ± 2	113 ± 2	115 ± 2
	6	112 ± 3	98 ± 3*	98 ± 3*	98 ± 2	100 ± 2	103 ± 3	113 ± 3
	9	109 ± 3	75 ± 2**	77 ± 2**	84 ± 2**	91 ± 1**	98 ± 1	111 ± 1
Hydralazine	3	113 ± 1	95 ± 3*	99 ± 2	101 ± 1	105 ± 1	107 ± 1	113 ± 1
	6	113 ± 2	90 ± 2**	89 ± 2**	90 ± 1**	91 ± 2**	91 ± 2**	112 ± 1
	9	108 ± 3	82 ± 4**	84 ± 4**	84 ± 3**	87 ± 3**	89 ± 2**	107 ± 3

Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$.
In the control group, the vehicle alone (0.5% CMC suspension) was administered.

Table 2: Effects of nisoldipine and reference drugs on heart rate in normotensive rat.

Drugs	Dose (mg/kg)	Mean heart rate (beats/min) ± S.E.M.						
		Before	30 min	60 min	120 min	240 min	360 min	24 h
Control	-	361 ± 17	358 ± 17	358 ± 15	355 ± 12	350 ± 10	350 ± 7	356 ± 15
Nisoldipine	3	359 ± 9	417 ± 20*	405 ± 7*	400 ± 9*	391 ± 17	376 ± 15	356 ± 7
	9	359 ± 7	451 ± 24**	469 ± 24**	469 ± 25**	413 ± 22*	385 ± 13*	356 ± 9
	30	353 ± 10	498 ± 15**	500 ± 14**	484 ± 20**	468 ± 14**	433 ± 11**	342 ± 20
Nifedipine	1	349 ± 7	395 ± 17	389 ± 17	374 ± 17	367 ± 12	362 ± 9	349 ± 7
	3	351 ± 7	489 ± 13**	491 ± 14**	477 ± 14**	462 ± 20**	429 ± 15**	350 ± 7
	9	352 ± 17	487 ± 11**	491 ± 11**	483 ± 13**	476 ± 11**	463 ± 12**	349 ± 12
Nicardipine	3	363 ± 12	443 ± 20**	400 ± 16	389 ± 11	381 ± 10	368 ± 9	349 ± 12
	6	354 ± 20	461 ± 12**	451 ± 14**	429 ± 17**	413 ± 12**	387 ± 17	357 ± 17
	9	360 ± 9	532 ± 15**	513 ± 15**	476 ± 10**	446 ± 16**	402 ± 10**	364 ± 9
Hydralazine	3	361 ± 17	446 ± 12**	439 ± 12**	424 ± 10**	411 ± 22**	387 ± 17	361 ± 9
	6	357 ± 17	479 ± 22**	460 ± 12**	446 ± 13**	429 ± 12**	399 ± 22	359 ± 9
	9	366 ± 7	514 ± 18**	473 ± 17**	449 ± 15**	433 ± 13**	423 ± 12**	368 ± 9

Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$.

Table 3: Effects of nisoldipine and reference drugs on blood pressure in spontaneously hypertensive rats.

Drugs	Dose (mg/kg)	Mean blood pressure (mmHg) ± S.E.M.						
		Before	30 min	60 min	120 min	240 min	360 min	24 h
Control	-	181 ± 5	179 ± 7	183 ± 6	182 ± 5	175 ± 4	176 ± 5	184 ± 4
Nisoldipine	3	180 ± 5	157 ± 4*	159 ± 4*	159 ± 4**	158 ± 4**	166 ± 3	181 ± 5
	9	180 ± 5	140 ± 5**	144 ± 4**	144 ± 5**	146 ± 5**	150 ± 4**	181 ± 5
	30	180 ± 7	115 ± 6**	117 ± 6**	127 ± 5**	131 ± 5**	136 ± 5**	179 ± 7
Nifedipine	1	188 ± 3	171 ± 6	167 ± 5	172 ± 5	170 ± 6	171 ± 2	199 ± 5
	3	177 ± 2	137 ± 5**	152 ± 2**	152 ± 5**	153 ± 2**	162 ± 4	175 ± 4
	9	176 ± 4	137 ± 8**	134 ± 7**	131 ± 7**	144 ± 11*	140 ± 10**	168 ± 9
Nicardipine	3	180 ± 5	157 ± 8	160 ± 6*	162 ± 6*	159 ± 7	164 ± 6	180 ± 4
	6	180 ± 5	145 ± 6**	152 ± 7**	154 ± 7**	160 ± 5*	169 ± 4	180 ± 5
	9	180 ± 4	122 ± 9**	120 ± 7**	134 ± 7**	142 ± 5**	153 ± 5**	172 ± 4
Hydralazine	3	184 ± 8	160 ± 10	151 ± 5**	149 ± 5**	141 ± 7**	147 ± 7**	169 ± 6
	6	179 ± 2	133 ± 10**	132 ± 6**	133 ± 8**	132 ± 6**	139 ± 6**	163 ± 4
	9	178 ± 5	82 ± 8**	80 ± 7**	86 ± 7**	92 ± 7**	102 ± 5**	146 ± 4

Significantly different from the control group * $p < 0.05$, ** $p < 0.01$

Table 4: Effects of nisoldipine and reference drugs on heart rate in spontaneously hypertensive rats.

Drugs	Dose (mg/kg)	Mean heart rate (beats/min) ± S.E.M.						
		Before	30 min	60 min	120 min	240 min	360 min	24 h
Control	-	397 ± 12	357 ± 15	352 ± 9	356 ± 11	369 ± 15	363 ± 12	403 ± 15
Nisoldipine	3	390 ± 16	402 ± 13*	394 ± 12*	398 ± 10*	404 ± 10	387 ± 10	415 ± 6
	9	400 ± 11	445 ± 21**	446 ± 18**	426 ± 17**	400 ± 10	403 ± 16	403 ± 9
	30	400 ± 9	423 ± 25*	435 ± 17**	452 ± 19**	436 ± 9**	410 ± 14*	390 ± 19
Nifedipine	1	385 ± 18	341 ± 10	343 ± 14	344 ± 13	351 ± 15	363 ± 12	403 ± 15
	3	399 ± 10	403 ± 11*	400 ± 16*	360 ± 15	373 ± 16	369 ± 18	391 ± 12
	9	413 ± 22	424 ± 26*	403 ± 21*	425 ± 24*	400 ± 15	400 ± 18	393 ± 15
Nicardipine	3	400 ± 9	395 ± 22	397 ± 21	389 ± 22	369 ± 12	358 ± 15	381 ± 5
	6	400 ± 14	453 ± 16*	406 ± 21*	414 ± 10*	419 ± 5*	396 ± 5*	419 ± 11
	9	397 ± 13	541 ± 9**	515 ± 22**	504 ± 19**	491 ± 15**	433 ± 27**	385 ± 10
Hydralazine	3	404 ± 6	461 ± 11**	443 ± 13**	425 ± 8**	422 ± 6**	413 ± 7**	416 ± 7
	6	394 ± 13	472 ± 8**	446 ± 9**	453 ± 12**	445 ± 3**	430 ± 13**	423 ± 6
	9	429 ± 20	537 ± 9**	508 ± 12**	504 ± 18**	500 ± 15**	470 ± 12**	457 ± 13

Significantly different from the control group * $p < 0.05$, ** $p < 0.01$

Figure 1

Antihypertensive effect of nisoldipine (BAY k 5552) after oral administration to spontaneously hypertensive rats.

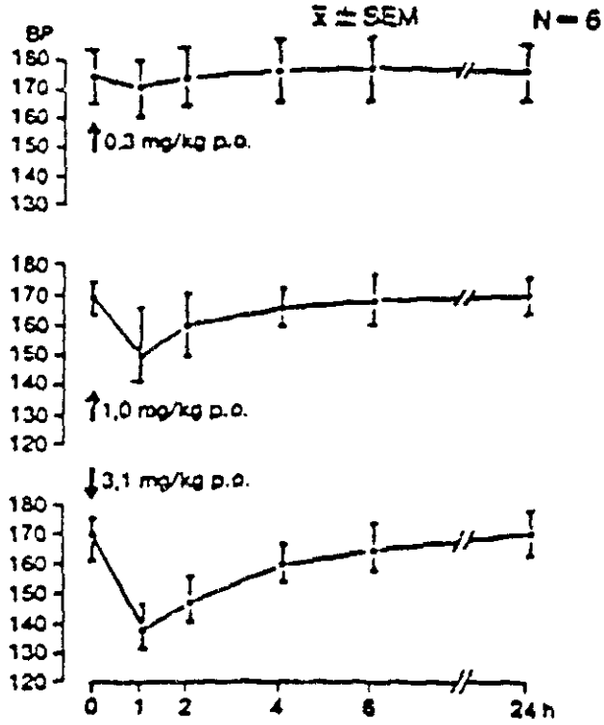


Figure 2

Antihypertensive effect of nisoldipine (BAY k 5552) by oral administration to one-kidney renal hypertensive rats.

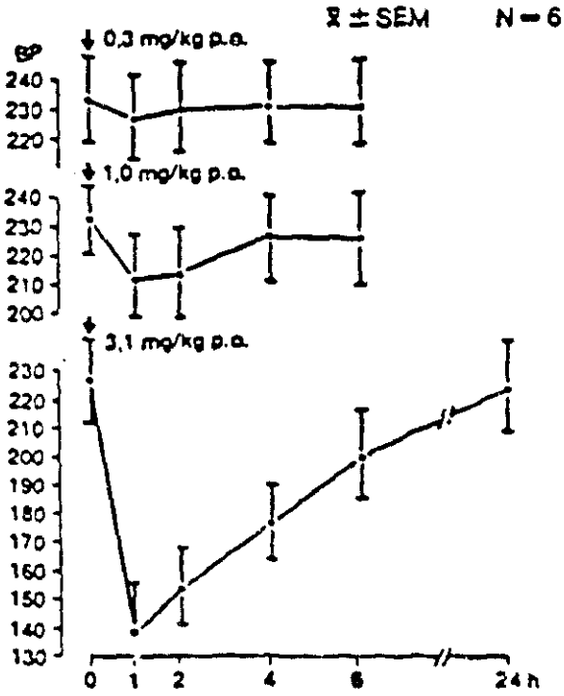


Table 5. Comparative effects (ED_{30}) of nisoldipine and reference drugs on blood pressure (BP) and heart rate (HR) in certain types of hypertensive rats and in normotensive rats.

Drugs	ED_{30} (BP) (mg/kg p.o.)				ED_{30} (HR) (mg/kg p.o.)			
	NR	SHR	DNR	RHR	NR	SHR	DNR	RHR
Nisoldipine	12.0 (1)	4.0 (1)	7.21 (1)	4.04 (1)	3.80 (1)	1.40 (1)	>30.00 (1)	1.60 (1)
Nifedipine	4.10 (0.34)	3.60 (0.90)	1.48 (0.21)	1.50 (0.37)	1.40 (0.37)	9.20 (1.46)	11.5 (0.38)	5.61 (0.37)
Nicardipine	6.80 (0.57)	4.00 (1)	2.52 (0.35)	1.93 (0.48)	3.00 (0.79)	5.00 (0.79)	9.68 (0.32)	7.80 (0.51)
Hydralazine	4.20 (0.37)	4.10 (1.03)	2.90 (0.40)	1.91 (0.47)	1.60 (0.42)	1.80 (0.29)	5.23 (0.17)	4.41 (0.29)

Relative values of ED_{30} are dipicted in parentheses (nisoldipine = 1).

NR=normotensive rat, SHR=spontaneously hypertensive rat, DNR= DOCA-NaCl hypertensive rat, RHR=renal hypertensive rat.

In terms of blood pressure lowering effect, nisoldipine was about equipotent to nifedipine, nicardipine and hydralazine in SH rats; however, it was less potent than the other drugs in other hypertensive models and in normotensive rats. The positive chronotropic effects of nisoldipine were less remarkable than those of reference drugs except in SH rats.

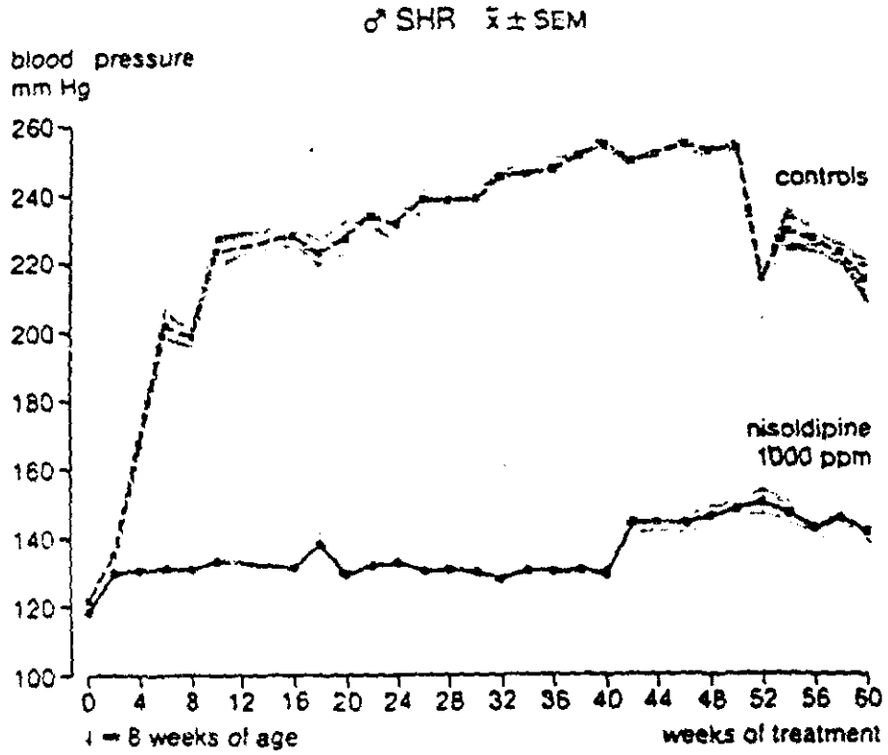
In another study, single oral doses of nisoldipine (0.315, 1.0 and 3.15 mg/kg) produced a dose dependent reduction of systolic blood pressure in conscious female SH rats (Fig.1). Although the lowest dose (0.315 mg/kg) produced only a slight reduction in blood pressure (3% reduction from the base value), doses of 1.0 and 3.15 mg/kg reduced blood pressure 1% and 18%, respectively. The maximum effect, at all dose levels, was seen at 1 hr after drug administration and the blood pressure returned completely or nearly to pretreatment level by 6 hr postdose. When the above doses of nisoldipine were given orally to one-kidney renal hypertensive rats, a significant decrease (39%) in blood pressure was seen at 3.15 mg/kg and moderate (9%) and slight (3%) reductions were observed at 1 and 0.315 mg/kg, respectively (Fig.2).

In conscious normotensive rats, nisoldipine (0.3 mg/kg po) significantly reduced systemic vascular resistance (0.58 to 0.38 mm Hg/kg/min/ml) and mean arterial pressure (122 to 108 mm Hg), and increased heart rate (395 to 447 beats/min), stroke volume (0.57 to 0.72 ml/beat/kg) and cardiac index (225 to 326 ml/min/kg). Left ventricular end-diastolic pressure (LVEDP) was slightly decreased (9.6 to 8.8 mm Hg, $p<0.05$) but no significant change in left ventricular systolic pressure was seen.

The effect of chronic dietary administration of nisoldipine on the development of hypertension was studied in SH rats.

Fig. 3

Effect of Long-term Treatment (60 weeks) with Nisoldipine on Systemic Blood Pressure in SH Rats



Shaded areas indicate SEM.

TABLE 6. *Preventive Experiment: The Effect of Long-term Treatment (60 weeks) with the Calcium Antagonist Nisoldipine on Systolic Blood Pressure, Plasma irANP, Relative Heart Weight, Body Weight, PRA, and Plasma Aldosterone Concentration in SHR and WKY*

Parameter measured after 60 weeks	SHR		WKY	
	Controls ($n = 7$)	Nisoldipine ($n = 10$)	Controls ($n = 8$)	Nisoldipine ($n = 8$)
SBP (mm Hg)	214 ± 7	141 ± 3‡	145 ± 3‡	137 ± 3
Plasma irANP (pg/ml)	470 ± 38	139 ± 35‡	88 ± 23‡	107 ± 29
Relative heart weight (mg/100 g body wt)	376 ± 29	313 ± 4*	277 ± 16†	284 ± 16
Body wt (g)	388 ± 8	377 ± 9	380 ± 16	381 ± 20
PRA (ng ANG I/ml/hr)	2.9 ± 0.3	1.9 ± 0.4	3.3 ± 0.4	2.4 ± 0.7
PAC (pg/ml)	332 ± 26	242 ± 16†	369 ± 30	454 ± 28

Values are means ± SEM. SBP = systolic blood pressure; irANP = immunoreactive ANP; ANG I = angiotensin I; PAC = plasma aldosterone concentration.
*$p < 0.025$; †$p < 0.01$, ‡$p < 0.001$, compared with values in untreated SHR.

Administration of nisoldipine to male SH rats (8 weeks old at the initiation of treatment) at 1000 ppm (50-100 mg/kg/day in diet) for 60 weeks prevented the development of hypertension during the treatment period [mean systolic blood pressure of 141 mm Hg in the treated group vs 214 mm Hg in the control group at the end of the study (Fig.3)]. The final blood pressure of treated SH rats was nearly the same as that of treated or untreated normotensive Wistar Kyoto (WKY) rats (Table 6). (However, it is noted that blood pressure in treated SH rats rapidly increased to the untreated control level when the treatment was stopped.) On the other hand, in untreated concurrent control SH rats, blood pressure increased progressively till week 48, to a maximum of 250 mm Hg, and declined thereafter to 214 mm Hg at the termination of the study. In WKY rats, no significant treatment related blood pressure changes were seen in the nisoldipine treated group compared to the control group. Furthermore, the results of the above study also showed that long term treatment with nisoldipine significantly decreased plasma atrial natriuretic peptide-like immunoreactivity (ANP-IR) and plasma aldosterone concentrations (PAC) and attenuated cardiac hypertrophy in SH rats (Table 6).

It was also shown that a 10 week dietary treatment with nisoldipine (50-100 mg/kg) in old SH rats (69 weeks old) with end-stage hypertensive disease caused significant reductions in systolic blood pressure [from 210 to 169 mm Hg (20%)], ANP-IR (20%) and relative heart weight (20%).

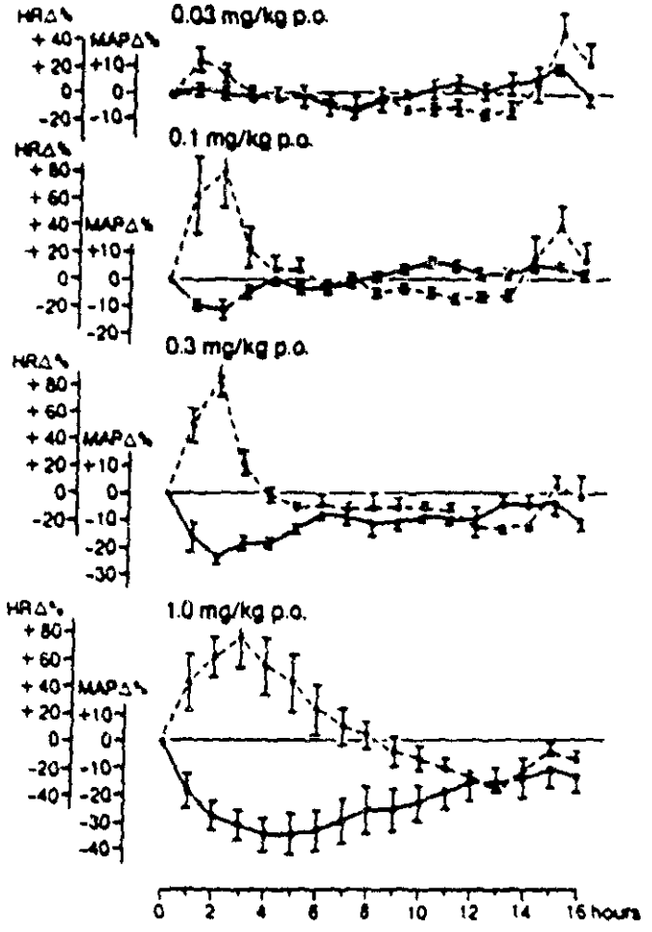
In inbred Dahl salt sensitive (DS) rats on a high salt diet (8% NaCl), dietary administration of nisoldipine at 1000 ppm (100 mg/kg/day) for 5 weeks produced significant reductions in systolic blood pressure (168 mm Hg in nisoldipine treated group vs 236 mm Hg in control DS rats) and plasma ANP IR and renin activities. No nisoldipine treatment related effects were seen in Dahl salt resistant rats. Although treatment with the arteriolar vasodilator minoxidil (10 mg/kg, in drinking water) caused reduction of blood pressure in DS rats, the plasma ANP-IR levels and heart weights were significantly increased in treated rats compared to control DS rats.

In diabetic SH rats (streptozotocin induced), nisoldipine (9 mg/kg po for 10 weeks) significantly reduced blood pressure and inhibited the progress of renal lesions. No nisoldipine treatment related effects were seen on blood glucose, body weight gain, heart rate and heart and kidney weights.

Nisoldipine (0.3-0.6 mg/kg in food for 20 weeks) prevented the development of hypertension in rats subjected to 5/6 th nephrectomy (147 mm Hg in treated vs 2[illegible] mm Hg in untreated group).

Nisoldipine infusion (0.7 µg/min) decreased mean arterial pressure in anesthetised normotensive rats from 107 to 76 mm Hg; and in conscious rats, a bolus administration of nisoldipine (1[illegible] µg) caused a reduction of blood pressure from [illegible] to 76 mm Hg.

Figure 4



Effect of nisoldipine on mean arterial blood pressure (MAP; ●——●) and heart rate (HR; ○- - -○) of conscious, unrestrained renal hypertensive dogs.

Fig.5

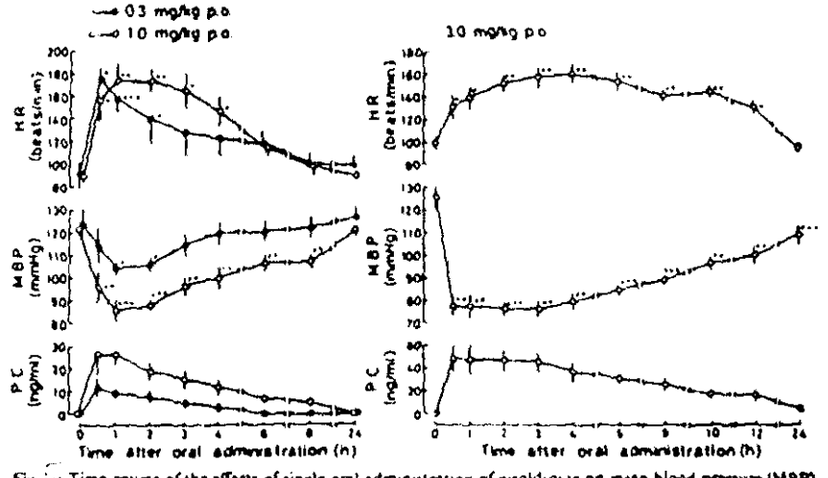


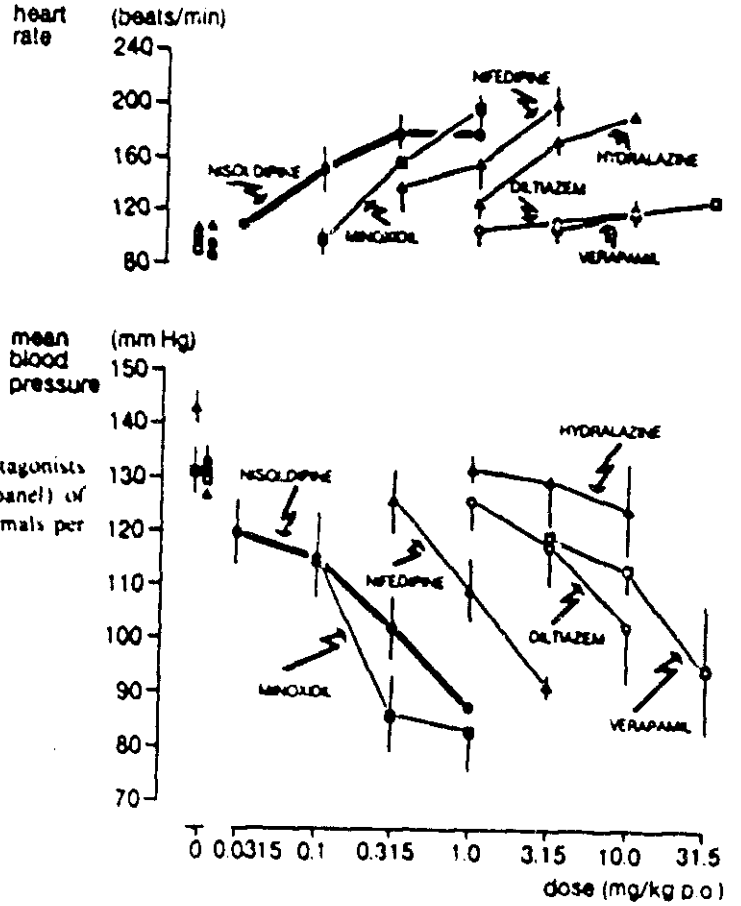
Fig. : Time course of the effects of single oral administration of nisoldipine on mean blood pressure (MBP), heart rate (HR) and plasma concentration (PC) in conscious renal hypertensive dogs. Values are expressed as the mean ± S.E.M. from 4 to 6 dogs. Asterisks indicate significant difference from the pre-drug values indicated at the zero time: *P < 0.05, **P < 0.01 and ***P < 0.001

b. Dogs

The effects of oral nisoldipine on blood pressure and heart rate were studied in conscious, unrestrained, renal hypertensive (unilateral renal artery stenosis) beagle dogs using a radio-telemetric method, and compared with effects of other calcium antagonists (nifedipine, diltiazem and verapamil) and vasodilators (hydralazine and minoxidil). Single oral doses of nisoldipine (0.03-1.0 mg/kg) produced a dose-dependent decrease in mean arterial blood pressure in renal hypertensive dogs (Fig.4). At 0.3 mg/kg, a marked reduction in blood pressure (24%) was produced within 2 hr after drug administration and the hypotensive action lasted for 12 hours. A reflex tachycardia, lasting for about 3 hr, occurred at the above dose level. A more pronounced hypotensive effect was seen at 1 mg/kg. Nifedipine produced about the same degree of hypotension as that produced by 0.3 mg/kg po of nisoldipine at a 10 fold higher dose level (3.15 mg/kg po). The hypotensive effect and the reflex tachycardia lasted for 6 hr. Diltiazem and verapamil produced comparable antihypertensive effects at higher dose levels with slight to moderate increase in heart rates. [At the highest tested dose level of verapamil (31.5 mg/kg po), 3/5 dogs showed marked bradycardia.] The anti- hypertensive effect of minoxidil was more pronounced (34% blood pressure reduction at 0.3 mg/kg po) than that of hydralazine (about 15% reduction at 10 mg/kg po), and persistent reflex tachycardia was seen for the entire period of blood pressure reduction in both cases.

The ED20 (mg/kg) values (the dose that causes a 20% reduction in mean blood pressure) and the dose response curves for nisoldipine and other calcium antagonists and vasodilators are given below.

	ED20 (mg/kg)
nisoldipine	0.14
nifedipine	1.68
diltiazem	6.21
verapamil	8.39
minoxidil	0.14
hydralazine	~10.00



Dose response curves for the influence of nisoldipine and some other calcium antagonists and vasodilators on heart rate (upper panel) and mean blood pressure (lower panel) of conscious, unrestrained renal hypertensive dogs. Given are means ± S.E. of 4-6 animals per dose. Pre drug levels of heart rate and blood pressure are indicated by 0.

The above ED20 data indicate that nisoldipine and minoxidil are more potent antihypertensive agents in dogs than the other drugs studied. However, the dose response curves show that the antihypertensive effect of minoxidil is markedly more steep than that of nisoldipine. While diltiazem, verapamil and hydralazine are shown to be much less potent than nisoldipine and minoxidil in reducing blood pressure in renal hypertensive dogs, the antihypertensive action of nifedipine is rated as intermediate between nisoldipine or minoxidil and the other reference drugs studied.

Nisoldipine (31-315 µg/kg po) decreased MAP and TPR, and increased HR in anesthetized normotensive dogs. One hour after 100 µg/kg nisoldipine, MAP and TPR were decreased 20 and 45%, respectively, HR increased 76%, and LVEDP was unchanged. In another study in anesthetized dogs, nisoldipine (0.3 µg/kg iv) decreased TPR by 20% and increased stroke volume 28% without decreasing MAP, however, a dose of 30 µg/kg iv decreased MAP by 14% and TPR by 66%, increased HR 110% and stroke volume by 38% without changing EDP.

The antihypertensive effects and the pharmacokinetics of nisoldipine were compared with those of nifedipine, nimodipine, nicardipine and hydralazine in conscious, renal hypertensive (one-clip, two-kidney type hypertension of Goldblatt et al) male mongrel dogs. Single oral doses of nisoldipine (0.3, 1.0 and 3.0 mg/kg with Cmax values of 13, 33 and 60 ng/ml, respectively, or AUC values of 36, 132 and 523 ng/ml/hr, respectively) produced dose-dependent reductions in mean arterial blood pressure, which were significantly different from pre-drug values at 30 min (1 and 3 mg/kg), with maximum effect seen at about an hour after dosing (Fig.5). At 1.0 and 3.0 mg/kg dose levels of nisoldipine, mean blood pressure reductions of 36 and 50 mm Hg, respectively, were observed. Significant antihypertensive activity lasted up to 24 hr after the 3.0 mg/kg dose. Although not dose dependent, increased heart rate was seen at all dose levels and remained significantly higher than pre-drug levels for 4 (1 mg/kg) to 12 hr (3.0 mg/kg) after dosing. Peak plasma concentrations of nisoldipine were seen 0.5 hr after oral administration and the antihypertensive activity significantly correlated with plasma concentrations of the drug ($r=0.727$, $p<0.001$). Like nisoldipine, other calcium antagonists (nifedipine, nimodipine or nicardipine) also dose-dependently lowered mean blood pressure, attaining peak effects at 1-2 hr after dosing. Hydralazine had a slow onset and its effect peaked 3 hr postdose. In the above study, it was found that nisoldipine was 5-6 times more potent than nifedipine, nicardipine and nimodipine and its antihypertensive effect lasted 3-6 times longer.

In another study in conscious renal hypertensive beagle dogs, single doses of nisoldipine (0.03-1.0 mg/kg po) produced the following dose-dependent decreases in mean arterial blood pressure (MAP) and increases in heart rate (HR).

Dose (mg/kg po)	MAP (% decrease)	HR (% increase)
0.03	n.s.	30
0.1	20	45
0.3	30	47
1.0	45	82

In conscious normotensive coronary artery occluded mongrel dogs, infusion of nisoldipine (1 and 3 µg/kg/min) for 15 min produced the following changes in blood pressure and heart rate.

	Control	(1 µg/kg/min)	(3 µg/kg/min)
SBP, mmHg	133±3	126±4	119±6*
DBP, mmHg	92±2	74±3*	59±5*
MAP, mmHg	105±2	91±3*	79±4*
HR, bpm	103±10	146±9*	163±12*

*Significantly ($p<0.05$) different from control.

In conscious, nonsedated, chronically instrumented mongrel dogs, iv administration of nisoldipine (10-100 µg/kg) decreased MAP by 11 and 29 mm Hg at 30 and 100 µg/kg dose levels, respectively.

In anesthetized mongrel dogs with cardiac tamponade, nisoldipine (3 µg/kg/min, iv for 15 min) caused mean blood pressure to fall from 120 to 71 mm Hg and reduced heart rate from 212 to 167 bpm.

c. Cats, Pigs and Sheep

In conscious cats, oral nisoldipine at 0.1 and 0.5 mg/kg dose levels produced 21 and 18% reductions in mean blood pressure and 23 and 63% increase in heart rates, respectively.

Nisoldipine (10, 30 and 60 µg, iv) dose-dependently reduced MAP and total peripheral resistance (TPR) and increased cardiac output in anesthetized cats.

In anesthetized Yorkshire pigs, infusions of nisoldipine (0.25, 0.5 and 1.0 µg/kg/min for 10 min) produced dose-dependent decreases in arterial blood pressure (30%), systemic vascular resistance (30%) and left ventricular filling pressure (15%), and increases in heart rate (25%) and LV dP/dt max (20%). Cardiac output was not significantly affected.

Nisoldipine (0.6 mg/kg/day iv for 4 days) prevented the development or reversed established hypertension induced by ACTH in sheep.

d. Antihypertensive Activity of Enantiomers

The antihypertensive activities of orally administered stereoisomers of nisoldipine were compared with the antihypertensive activity of the racemic compound in conscious SH rats and renal hypertensive dogs. In SH rats, (+)nisoldipine (BAY R 1224, ED20=2.1 mg/kg) was only slightly more potent (1.4 times) than the racemic compound but was about 20 times more potent than the (-)nisoldipine (BAY R 1223). No significant difference in antihypertensive activity was seen between (+)nisoldipine and the racemic compound in dogs.

In a study in anesthetized normotensive dogs, 10 and 30 µg/kg po (+)nisoldipine decreased MAP by 10 and 38% respectively, while (-)nisoldipine had no significant effects at doses up to 300 µg/kg.

e. Antihypertensive Activity of Metabolites

(Unless otherwise noted, the following studies were done in anesthetized normotensive dogs.)

BAY R 9590, a major metabolite of nisoldipine, showed weak peripheral vasodilator activity at iv doses of 1 mg/kg and above. No significant changes in blood pressure were seen at dose levels (0.3 to 3 mg/kg iv) tested in this study.

BAY O 3159, another metabolite, had relatively minor peripheral vasodilator activity at doses of 0.3 and 1.0 mg/kg iv in dogs. Slight blood pressure reduction was noted following the 1 mg/kg dose.

Metabolites BAY [illegible] and BAY S 4755 had no hemodynamic effects (total peripheral resistance, cardiac output and LV dP/dt) at 1 mg/kg iv and had only minor peripheral vasodilator effect at 3.0 mg/kg.

BAY R 9425, a dihydropyridine metabolite of nisoldipine, caused a dose-dependent drop in blood pressure at doses of 10 µg/kg iv and above, the effect lasting about 60 min at 30 µg/kg. Tachycardia and increased cardiac output were seen at the above dose level. BAY R 9425 (iv administration) was found to be 1/3rd to 1/10th as potent as nisoldipine in dogs.

In conscious renal hypertensive dogs, oral doses of BAY R 9425 (1 mg/kg) had a much weaker and shorter duration of action than the parent compound.

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3. **Mechanism of Action**

Nisoldipine is a dihydropyridine calcium channel blocker which binds with very high affinity to L-type calcium channels. By blocking calcium entry into vascular smooth muscle cells, it inhibits muscular contractions, thereby causing vasodilation of peripheral and coronary vasculatures.

The receptor binding characteristics of nisoldipine (BAY K 5552) and its optically pure enantiomers, BAY R 1224 (+ isomer) and BAY R 1223 (- isomer), were studied in rat cerebral cortical membranes using labelled nitrendipine. The concentration that causes 50% inhibition of ^{3}H nitrendipine binding (IC50), the inhibition constant (Ki) and the Hill Coefficient (nh) for the above compounds and the reference drugs are given below.

Substance	IC_{50} [nM]	K_i [nM]	nH	N
Bay K 5552 (Nisoldipine)	1.2 ± 0.1	0.769 ± 0.064	1.0 ± 0.05	34
Bay R 1224 ((+) enantiomer)	1.1 ± 0.08	0.705 ± 0.051	1.0 ± 0.03	34
Bay R 1223 ((-) enantiomer)	109 ± 0.15	70 ± 10	1.0 ± 0.01	34
Nifedipine (Reference)	2.7	1.7	1.15	
Bay K 8644 (Reference)	16	10	0.76	
Verapamil (Reference)	173	110	0.6	

Nisoldipine showed competitive displacement of ^{3}H nitrendipine with a Ki of 0.769 nM. The (+) enantiomer has a comparable Ki of 0.705 nM, while the affinity of the ( ) enantiomer was reduced 100 fold to 70 nM. The Hill Coefficient for all three compounds was same, representing a linear Scatchard Plot and a homogenous population of binding sites. The above data indicate that the biologically active component of nisoldipine seems to be the (+) enantiomer.

Further studies in guinea pig ileal smooth muscle have confirmed that the receptor binding was of high affinity, saturable, reversible and stereoselective with high structural specificity, and correlated well with the pharmacologic activities. Binding studies in partially purified rat brain membranes using ^{3}H

nimodipine showed Ki values of 0.24 and 7 nM for nisoldipine and nifedipine, respectively. Studies in rat and rabbit ventricular membranes showed that nisoldipine differs from nifedipine in its high affinity binding (about 20 times greater), slow dissociation rate and large partition coefficient. The binding characteristics of both drugs are given below.

Comparison of Binding Characteristics of *Nisoldipine* and *Nifedipine*

	(+)Nisoldipine	*Nifedipine*
K_d, nM	0.04	0.81
Dissociation		
$t_{1/2}$, min	12	1.2
B_{max}, pmol/mg	0.69	0.17
Association Rate ($\times 10^6 M^{-1}min^{-1}$)	6.7	3.1
Entropy of binding	large positive	negative
Partition coefficient into biological membrane	6,000-27,000	2,900

The above biochemical and biophysical differences can be expected to result in a longer duration of action for nisoldipine.

Studies in isolated rat aorta revealed that there is good agreement among binding affinity and the IC50 values both for inhibiting ^{45}Ca influx and aortic contraction. The IC50 values for the inhibition of rabbit aortic ring contraction (potassium stimulated) were 1.8, 0.15 and 81 nm for racemic nisoldipine, (+) isomer and (-) isomer, respectively, indicating that these findings were in reasonable agreement with the results of the ligand binding studies.

Voltage-clamp studies in isolated calf Purkinje fibers have shown that nisoldipine binding is one thousand times stronger to inactivated channels (Kd=1 nm) than to resting channels (Kd=1.3 µM), indicating that nisoldipine blocks calcium channel current in a voltage dependent manner. Nisoldipine (10 µM) completely blocked the slow inward current and contractile activation in the above tissue. Moreover, nisoldipine reduced the transient outward, but not the delayed rectifier K^+ current, in calf cardiac Purkinje fibers.

Nisoldipine was a more potent inhibitor of BHT 920 (α2-adrenoceptor agonist)-induced contraction of isolated aortic rings when these rings were taken from stroke prone SH rats than when they were taken from normotensive WKY rats (IC50=0.15 nM vs 7 nM).

Several studies have shown that the degree of nisoldipine inhibition of calcium channel currents (as well as contractions) increases with membrane depolarization. In patch-clamp studies in isolated smooth muscle cells from canine coronary artery, membrane depolarization from -80 to -30 mV increased the apparent affinity of nisoldipine binding about 9 fold (in the presence of 1 µM Bay K 8644, a calcium agonist). The calculated dissociation constant in this study was 0.07 nM, which was identical to the value obtained with radioligand binding

studies. In rabbit mesenteric artery, depolarization from -100 to -55 mV decreased the concentration of nisoldipine needed for 50% inhibition from 12 to 1.9 nM. Because of the very high affinity binding of nisoldipine to depolarized membranes, it is suggested that nisoldipine could preferentially bind to those depolarized arteries which increase total peripheral resistance of hypertension, and also to those producing coronary spasms.

B. **Additional Cardiovascular Studies**

1. Effects on Other Vascular Beds

Under conditions of controlled blood flow, nisoldipine infusion (1 µg/min) dilated the hindquarters vascular bed of anesthetized cats and inhibited vasoconstrictor responses to sympathetic nerve stimulation, norepinephrine, tyramine, methoxamine and BHT 933 (α2-adrenoceptor agonist).

The effects of nisoldipine on vascular resistance and vasoconstrictor responses were studied in the pulmonary vascular bed of anesthetized cats under conditions of controlled blood flow. Nisoldipine (1 µg/min) infused into the lobar artery caused only a small reduction in basal lobar resistance. It reduced pulmonary vasoconstrictor responses to methoxamine, BHT 933 and U46619 (thromboxane A2 mimetic).

2. Other Myocardial/Cardiovascular Effects

In isolated Langendorff-perfused rat hearts, nisoldipine (30 nm) prevented the ischemia-reperfusion-induced depletion of the cardiac stores of norepinephrine. In the above hearts, pretreatment with the drug (10 nm) 2 min before the onset of ischemia significantly improved cardiac output following global ischemia (20 min) and reperfusion (5 min). Pretreatment with nisoldipine (1 µM) for 5 min before ischemia prevented transcoronary macromolecular leakage after ischemia-reperfusion in isolated rat hearts.

In isovolumic coronary artery-perfused ferret hearts subjected to global ischemia for 3 min followed by 10 min reperfusion, nisoldipine (10 nm) significantly reduced the ischemia-induced rise in diastolic and systolic intracellular free ionized calcium (FIC, determined with the bioluminescent protein aequorin), and lessened the decrease in contractile function. Moreover, nisoldipine significantly accelerated the decline in FIC during reperfusion and improved recovery of contractility and relaxation.

Nisoldipine (5 µM) had no significant effect on dopamine-induced inhibition of nerve stimulated vasoconstriction of isolated perfused rabbit ear artery.

In anesthetized rats, nisoldipine (3 mg/kg po, 1-1.25 hr before acute coronary ligation) greatly reduced the duration of ventricular tachycardia and fibrillation occurring in the first 30 min postligation period. None of the treated animals died compared with a 40% mortality in controls.

Long-term dietary administration of nisoldipine (50-100 mg/kg for 22 weeks) prevented the rarefaction of myocardial capillarization in SH rats. Drug treated rats had lower arterial blood pressure and decreased left ventricular and septal weights compared to untreated rats.

In conscious chronically instrumented dogs, nisoldipine did not influence cardiac impulse formation or impulse conduction at 10 and 30 µg/kg iv, but at 100 µg/kg iv (strongly hypotensive dose), it produced reflex increase in the rate of arrioventricular conduction.

In dogs subjected to occlusion of the left anterior descending coronary artery for 1.5 hr followed by reperfusion, nisoldipine (3.5 µg/kg iv 10 min before the occlusion and again 10 min before reperfusion) suppressed the ischemia-induced increase in phospholipid breakdown as well as the increase in serum CPK activity. The drug also prevented ischemia-induced myocardial hemorrhage and premature ventricular contraction in a similar study.

Cumulative 10 min infusions of nisoldipine (0.05, 0.1, 0.25 and 0.5 µg/kg/min) in pigs with chronic left ventricular dysfunction (produced by the ligation of the left circumflex coronary artery 2-3 weeks before the study) improved ventricular function to the same extent as pimobendan, a phosphodiesterase inhibitor (2.5, 5, 12.5 and 25 µg/kg/min). Both drug normalized cardiac output and exhibited similar cardiovascular effects (systemic vasodilation, reduction in left ventricular filling pressure, and increased heart rate) except for the significantly greater increase in left ventricular dP/dt max with pimobendan (85%) than with nisoldipine (45%). Thus, nisoldipine, despite of its lack of inotropic properties, improved ventricular function to about the same extent as pimobendan.

Infusion of nisoldipine (10 µg/kg over a 5 min period, 30 min before coronary artery occlusion) in open-chest pigs subjected to the occlusion of the left anterior coronary artery, completely prevented the increase in lipid peroxidation products associated with ischemia.

C. General Pharmacological Studies

1. Central Nervous System Effects

The analgesic activity of nisoldipine (25-500 mg/kg po) was assessed in female Wistar rats by the failure of the animal to withdraw its tail within 20 seconds after exposure to a focused heat ray. Nisoldipine had no analgesic activity at 25 mg/kg; however, at higher dose levels (100 mg/kg and above) 40-60% of animals showed evidence of analgesic activity.

To study the effects of nisoldipine on orientation motility, mice were placed in cages in the dark and their orientation motility was assessed at 5 min intervals for a total of 25 min after the light was turned on. Nisoldipine (25, 100 and 250 mg/kg po) had no significant effect on orientation motility in this test. However, in a separate study in which mice were kept initially in a dark chamber and then exposed to daylight after drug treatment, nisoldipine at 10 or 31.5 mg/kg po, but not at 3.15 mg/kg, reduced orientation motility by 20% in mice. The above dose levels had no effect on spontaneous motility.

The balancing ability of male mice to maintain their position on a round horizontal wood bar (diameter 8 mm) was tested at 60 min after oral treatment with nisoldipine (3.15, 10 or 31.5 mg/kg). The drug inhibited the balancing ability by 10% at 31.5 mg/kg. The ability of the mice to grasp a horizontal metal bar (diameter 3 mm) was not inhibited at the above dose levels.

There was no evidence of any muscle relaxant or sedative effects for nisoldipine in mice, as assessed by the measurement of the holding and climbing ability on a horizontal bar with at least one hind paw within 5 sec after they were suspended on the bar by their front paws, at 25, 100 and 250 mg/kg po dose levels.

The anticonvulsant activity of nisoldipine was determined by the ability of the drug to antagonize either electroshock- or pentylenetetrazole (PTZ)-induced tonic convulsions in mice. Nisoldipine showed no anticonvulsant activity when administered 30 min before electroshock at 25, 100 or 250 mg/kg po. In the PTZ test, the drug (25-250 mg/kg po, 30 min before PTZ administration) had a dose-dependent anticonvulsant effect with an estimated ED50 of 98 mg/kg.

In a subsequent study, nisoldipine (3.15, 10 or 31.5 mg/kg po), given 60 min before electroshock or PTZ administration, antagonized the electroshock-induced tonic convulsions in 20% of mice at 3.15 and 10 mg/kg and in 30% at 31.5 mg/kg. PTZ-induced convulsions were antagonized by nisoldipine at 10 (30%) and 31.5 (80%) mg/kg, but not at 3.15 mg/kg.

The depth of hexobarbital-induced anesthesia was not affected by nisoldipine at 3.15, 10 and 31.5 mg/kg po, but the duration of

anesthesia was prolonged at 31.5 mg/kg.

To evaluate the possible tranquilizing effect of nisoldipine on defensive behavior, fighting episodes were induced in mice by weak electric foot shocks of 0.2 msec duration at a frequency of 5 per min. Nisoldipine at 25 mg/kg po was ineffective, but a dose-dependent antagonism of fighting was observed at higher doses (50 to 400 mg/kg po) with an ED50 of 82 mg/kg.

The (-)enantiomer (BAY R 1223) had no significant CNS effects in mice at oral doses of 3, 10 and 30 mg/kg. The (+)enantiomer (BAY R 1224) at 10 and 30 mg/kg po impaired motor coordination of mice in the balance rod test and increased the threshold dose of PTZ required to produce convulsions. The no-effect dose was 3 mg/kg. No significant effects were seen in mice on traction ability, analgesic and anticonvulsive responses or depth of hexobarbital-induced anesthesia at 3-30 mg/kg po dose levels. In rats, administration of BAY R 1224 (10 and 30 mg/kg po) produced ptosis, salivation, sedation, hypothermia, prone position, reduced muscle tone and ataxia during walking.

2. Gastrointestinal Effects

Nisoldipine (3, 10 and 30 mg/kg po) significantly reduced the intestinal transit time in mice, as measured by the length of the intestine covered by charcoal which was given orally 40 min after nisoldipine administration, at all tested dose levels with an estimated ED50 of 17.19 mg/kg.

(-)Nisoldipine, at the above dose levels, had no effect on intestinal transit time in the rat, whereas (+)nisoldipine significantly reduced transit time dose dependently.

Nisoldipine or its stereoisomers (10 or 30 mg/kg po) did not induce any gastric lesions in rats.

At 3, 10 and 30 mg/kg po, (-)nisoldipine had no effect on indomethacin induced ulcers in rats, while (+)nisoldipine significantly reduced those lesions at all dose levels.

Nisoldipine (3 or 30 mg/kg intraduodenal) or (+) nisoldipine (3, 10 or 30 mg/kg id) had no significant effects on basal gastric acid secretion, whereas (-)nisoldipine significantly reduced basal gastric acid secretion at 30 mg/kg id.

Nisoldipine antagonized acetylcholine and histamine induced spasms of isolated guinea pig ileum at 1 mg/L and barium chloride induced spasms at 3 mg/L.

3. Metabolic Effects

In fasted rats nisoldipine at 10 and 30 mg/kg po significantly and dose dependently elevated blood glucose at 1, 2 and 4 hr after drug administration, while the serum triglyceride

concentration was slightly reduced in a dose-dependent manner. At 3 mg/kg po, the drug had no significant effect on either blood glucose or triglycerides.

In fed rats nisoldipine elevated blood glucose and lowered serum triglyceride concentrations at 3, 10 and 30 mg/kg po.

4. Effects on Respiration

Nisoldipine (0.26 nm to 26 µM) had no effect on resting tone of the isolated guinea pig trachea. Histamine- and LTD4-induced contractions were significantly reduced by nisoldipine at 26 nM.

Nisoldipine (0.32, 1.0 and 3.2 µg/kg iv) had no significant effect on spontaneous respiration in anesthetized dogs.

The enantiomers of nisoldipine ((3, 10 and 30 mg/kg po) had no effect on airway resistance or lung compliance, and did not modify histamine-induced increases in lung resistance in anesthetized, spontaneously breathing guinea pigs.

5. Renal Effects

The diuretic activity of nisoldipine was tested in normotensive male Wistar rats loaded with 0.5% methylhydroxyethylcellulose (10 ml/kg). Nisoldipine at 1.0, 3.15, 10.0 and 31.5 mg/kg po produced no significant effects on urine volume or urinary excretion of Na^+ or K^+ over a 6 hr collection period. However, at 100 mg/kg, the drug reduced urinary volume and Na^+ and K^+ excretion. Urinary pH was not changed over the entire dose range.

In another study in liquid-loaded rats (with a solution containing 0.2% NaCl, 0.4% KCl and 0.1% tragacanth), nisoldipine (10, 30 and 100 mg/kg po) increased urinary volumes and excretion of Na^+ and K^+ at all dose levels, the effects being more pronounced at 10 mg/kg than at higher dose levels.

BAY R 1224, the (+)enantiomer of nisoldipine, had no significant effects on urine volume or electrolyte excretion in normotensive rats at 3 and 10 mg/kg po; however, at 30 mg/kg, it significantly reduced urine volume and the excretion of Na^+ and K^+. BAY R 1223, the (-)enantiomer, had no significant effects on the above parameters.

In stroke-prone SH rats, administration of nisoldipine (0.315 to 10 mg/kg po) significantly increased urine volumes and excretion of Na^+ at 3.15 and 10 mg/kg. Although excretion of K^+ was increased after 10 mg/kg of nisoldipine, the increase failed to achieve statistical significance.

To elucidate the mechanism of diuretic effects of nisoldipine in SH rats, renal clearance and micropuncture studies were carried out in moderately saline-loaded animals. At dose levels of 0.1, 0.15 and 0.3 µg/kg/min iv for 15 min, the drug produced a

significant fall in blood pressure accompanied by increased sodium excretion and glomerular filtration rate (GFR). This natriuretic effect was attributed to the suppression of distal tubular sodium reabsorption. In another study in SH rats, nisoldipine (10 µg/kg/hr iv) produced diuretic and natriuretic effects without any changes in GFR. It was also shown that the diuretic and saluretic effects were considerably more pronounced in hypertensive rats than in normotensive Wistar-Kyota rats.

Nisoldipine (10 nm) completely reversed the norepinephrine-induced reduction in GFR in the isolated perfused rat kidney.

In the rat acute renal failure model (glycerol induced), nisoldipine treatment (10 mg/kg b.i.d for 2 days) increased urine volumes and significantly reduced glycerol-induced increases in serum creatinine and urea and renal tissue calcium levels.

In conscious dogs, nisoldipine at 0.1 mg/kg po had no significant effect on renal function (GFR and inulin and paraaminohippurate clearances). However, at 0.3 mg/kg the drug reduced all measured parameters of renal function, the maximum effects observed 20 min after drug administration.

The interaction of nisoldipine with angiotensin II (AII) or norepinephrine (NE) was studied in anesthetized mongrel dogs. Intrarenal infusion of nisoldipine (2, 10 or 50 ng/kg/min for 20 min) produced a dose-dependent increase in urine flow and urinary excretion of sodium, chloride and potassium, although no significant change in GFR was seen. Renal blood flow was significantly increased only at the highest dose level. AII and NE reduced renal blood flow and urine volumes. The decreased urine flow induced by AII, but not by NE, was completely blocked by nisoldipine, while the effect of AII on renal blood flow was only partially antagonized.

6. Hematological Effects

Collagen-induced platelet aggregation, coagulation time, thrombus elasticity and partial thromboplastin time were not affected by nisoldipine administration (10, 30 or 100 mg/kg po; blood sampling 90 min postdose) in rats. Nisoldipine (2.6 nm) had no effect on factor XIII activity in bovine plasma.

Neither (+) nor (-) enantiomer (3, 10 and 30 mg/kg po) had effects (60 min postdose) on hematological parameters in rats (hemoglobin, hematocrit, platelet count, fibrinogen levels, sedimentation rate, thrombin or thromboplastin time and collagen-induced platelet aggregation).

7. Antiatherogenic Activity

In rabbits fed a cholesterol (2.5%) supplemented diet, administration of nisoldipine (1 mg/kg/day for 7 weeks) significantly reduced the serum and aortic concentrations of cholesterol and

preserved endothelium-dependent relaxation of the isolated aortic rings.

8. Antiinflammatory Effects

Oral administration of nisoldipine (5 to 315 mg/kg, 1 hr before kaoline injection) showed antiinflammatory activity against edema (caused by intraplantar injection of kaolin into the hind paw) in rats at dose levels of 10 mg/kg and above (ED50=46 mg/kg po).

9. Effects on Histamine Release

Nisoldipine (0.26 to 260 µM) had no significant effect on histamine release from rat peritoneal mast cells in vitro and also did not modify antigen-induced histamine release from these cells.

D. Antidote Studies

In anesthetized rats, 100 µg/kg/min iv infusions of nisoldipine produced pronounced decreases in arterial blood pressure, heart rate, cardiac output and peripheral resistance, followed by death within about 5 min after the initiation of the infusion. EKG changes included sinus bradycardia, partial or complete AV block and shifting of the pacemaker to the AV node. Infusion of calcium gluconate (15 mg/kg/min), isoproterenol (20 µg/kg/min) or dopamine (100 µg/kg/min) simultaneously with nisoldipine prolonged survival time by more than 100%. Norepinephrine (20 µg/kg/min) had no significant effect.

In anesthetized dogs, calcium gluconate (100 mg/kg iv) reversed the hypotension and tachycardia produced by 10 µg/kg iv nisoldipine, but exacerbated the hypotension produced by 30 and 100 µg/kg.

E. General Pharmacological Studies of Metabolites

Nisoldipine was shown to be about 21 times and the BAY R 9425 (a dihydropyridine metabolite of nisoldipine) was twice as potent as diphenhydramine (reference compound) in inhibiting histamine-induced spasms of isolated guinea pig ileum. The other metabolites (BAY 03199, BAY R 9590, BAY S 1869 and BAY S 4755) had no significant effect on the above parameter.

BAY R 9425 had no significant CNS (rat and mouse), pulmonary (guinea pig), hematologic (rat), gastrointestinal (rat) and urinary (rat) effects. This metabolite at 0.26 to 26 µM did not induce histamine release from rat peritoneal mast cells in vitro; however, at the same concentration range, it significantly inhibited the antigen induced histamine release from these cells.

BAY R 9425 inhibited LTD4-induced contraction of isolated guinea pig trachea at 260 nM, but not at 26 nM. This compound was about 10 times less potent than nisoldipine in inhibiting K^+-induced contractions of isolated pregnant (IC50=86 nM) or nonpregnant (IC50=138 nM) rat uterus. Other metabolites (BAY O 3199, BAY R 9590, BAY S 1869 and BAY S 4755) were effective only at very high concentrations (above 50 µM).

F. **Interaction of Nisoldipine with pranolol**

Administration of nisoldipine (0.315 mg/kg po) in conscious unrestrained renal hypertensive dogs after ß-adrenoceptor blockade by propranolol (3.15 mg/kg po) increased the maximal reduction in systolic blood pressure from 15 to 23% and reduced the maximal reflex increase in heart rate from 109 to 50%.

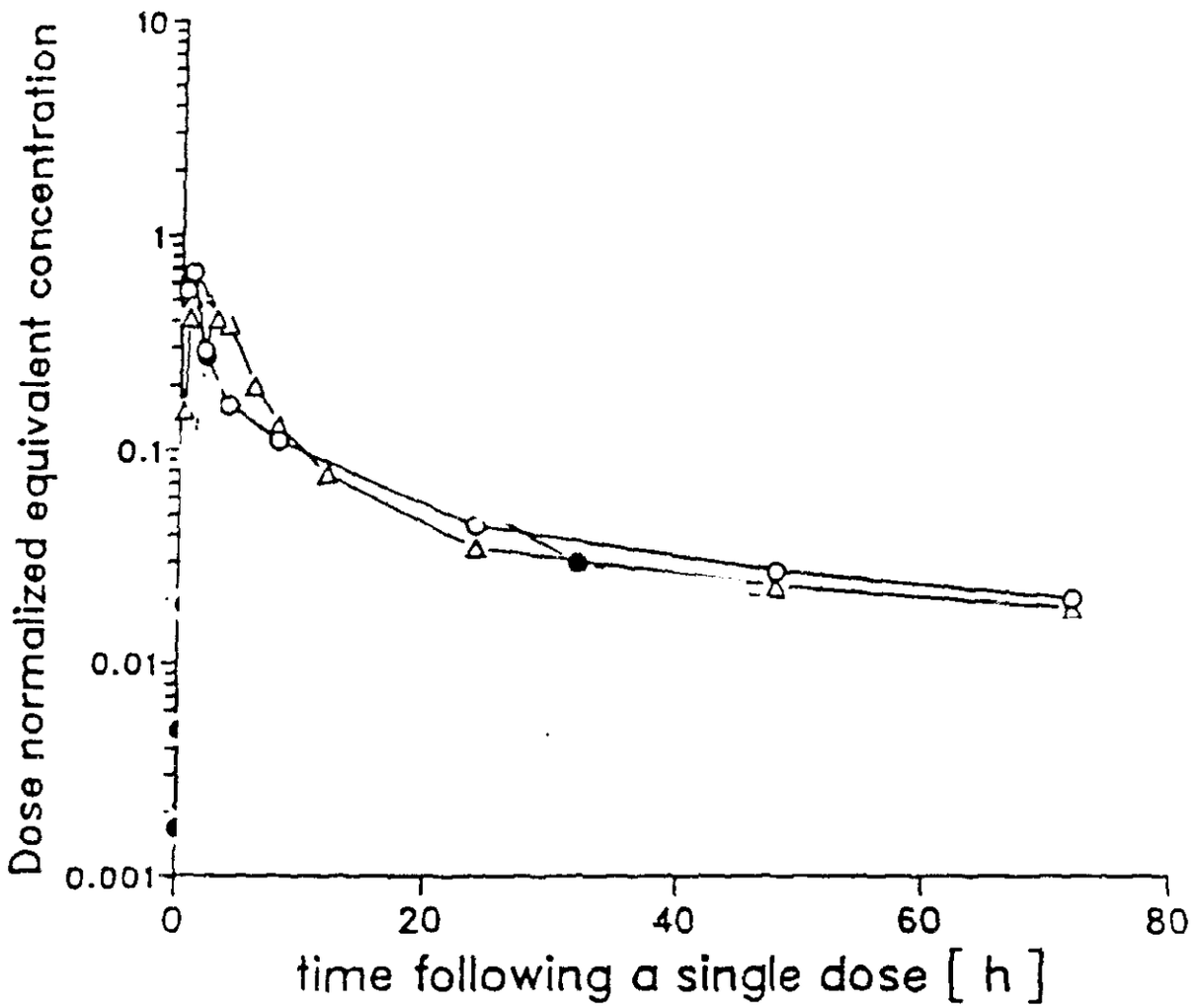
Propranolol (2 mg/kg iv) attenuated the reflex increase in heart rate produced by nisoldipine (2.5 to 25 µg/kg/min iv) in conscious instrumented dogs and prevented an increase in the heart rate-systolic pressure product (an index of myocardial oxygen consumption). Propranolol potentiated the hypotensive effect of nisoldipine (5-25 µg/kg/min), but did not further increase mean coronary blood flow.

Propranolol (4.4 mg/kg po) attenuated both the positive chronotropic and inotropic effects and the changes in systolic wall thickening caused by exercise in pigs with coronary artery stenosis. Nisoldipine (0.5 mg/kg po) alone did not modify cardiovascular effects of exercise, but it further improved wall function in the presence of propranolol.

SUMMARY OF PHARMACOKINETICS STUDIES (S. Stolzenberg)

1. Absorption and Excretion: Nisoldipine was measured by gas chromatography and electron capture. A summary of urinary, fecal and CO_2 recoveries following administration of a single dose of ^{14}C-labelled nisoldipine in four species, including man, is given on the page 30 of this review. In the rat, dog and pig, the primary route of excretion was the biliary-fecal route, whereas in the monkey and man, the urinary route predominated. Based on the ratio of the % of Dose Excreted in urine for i.v./p.o. routes multiplied by 100, nisoldipine was considered to be rapidly and almost completely absorbed in male rats (107%), dogs (107%) and man (89.7%). For the pig, monkey (rhesus) and rat, where i.v. doses were not given, the figure under this column represents the amount excreted in the urine, which is regarded as "the lower limit of absorption".

2. Plasma Pharmacokinetics: The figure which follows illustrates the plasma concentrations vs time, following a single oral dose in rats, dogs and monkeys. The table on selected plasma pharmacokinetics on page 31 reflects these observations and includes results from i.v. administration in the same 3 species and in man. After oral administration, no distinct species differences were noted in the rat, dog or monkey; $CEQ_{max,norm}$ (normalized to 1 mg/kg dose, based on radioactivity equivalence of parent compound) ranged between 0.49 - 0.79 $kg*l^{-1}$, but was 4.7 to 7.5 times higher in man (3.7 $kg*l^{-1}$). AUC_{norm} for total radioactivity was also similar in the 3 species, but was higher in man by a factor of 6 to 10. $C_{max,norm}$ and AUC_{norm} for unchanged nisoldipine following oral administration were very low in all four species, including man, which was attributed to an extensive first pass effect, known for this compound. In humans given immediate release tablets of 2.5, 5, 10 and 20 mg, or core coated tablets of 10, 20, 40 and 60 mg, dose proportionality was observed for plasma nisoldipine C_{max} and AUC_{0-24}. At steady state (8th day of dosing) in humans, both AUC_{0-24} and C_{max} showed a "dose dependent and broadly dose proportional increase" at 30, 60, 90 and 120 mg. Correspondingly, both systolic and diastolic blood pressures "showed a general dose related decrease from baseline at steady state". Despite the high rates of absorption, bioavailabilities (F) of parent compound after oral doses were correspondingly low; 2.7, 11.7 and 8.4% in the rat, dog and man, respectively. The core coated preparation had an F value of 5.5% in man. In the dog, it was shown that both the gut wall and the liver contributed to the first pass effect and resulting low F values for the parent compound. The plasma half-life of unchanged drug was essentially similar in dog, monkey and man (2.3 to 4 h). The shorter $t_{1/2}$ of unchanged compound listed in the table for rats following oral or i.v. dosing does not represent a true terminal half-life because plasma levels were measured for only 7 hours post-dosing. In rats, the pharmacologically more potent (+)- enantiomer had a five fold higher bioavailability than the (-)- enantiomer.



Dose-normalized equivalent concentrations of total radioactivity after single oral administration of [^{14}C]nisoldipine to male rats (n = 5), female dogs (n = 3) and female monkey (n = 1) (mean of each).

o = 5 mg·kg^{-1}, rat

Δ = 5 mg·kg^{-1}, dog

● = 10 mg·kg^{-1}, monkey

ABSORPTION/EXCRETION OF NISOLDIPINE IN ANIMAL SPECIES AND MAN FOLLOWING ADMINISTRATION OF [^{14}C]NISOLDIPINE.

Species	Route	Dose (mg/kg)	% of Dose[1] Excreted in: urine	feces	CO_2	% absorbed (minimum)
Rat (M)	p.o.	5.0	30.8 (2.4)[a]	75.0 (0.7)[a]	0.5[b]	107.0
	i.v.	1.0	28.7 (3.5)[a]	71.8 (6.5)[a]	0.6[b]	-----
Rat (F)	p.o.	5.0	41.9 (2.9)[a]	62.0 (2.8)[a]	-----	41.9
Dog (F)	p.o.	5.0	38.9 (5.1)[d]	55.2 (2.3)[d]	-----	107.0
	i.v.	0.5	36.3 (5.2)[d]	59.1 (8.2)[d]	-----	-----
Man (M)	p.o.	12.0 mg	73.7 (5.4)[f]	12.3 (2.4)[f]	-----	89.7
	i.v.	0.8 mg	82.2 (7.4)[f]	14.4 (9.5)[f]	-----	-----

[1]Values are arithmetic means (sd)

n = 5 (rat), 3 (dog) and 10 (man). For monkey and pig, n=1

Collection periods:

a) 48 h.
b) 24 h
c) 72 h
d) 144 h
e) 240 h
f) 96 h

SELECTED PLASMA PHARMACOKINETIC PARAMETERS IN SEVERAL SPECIES FOLLOWING ADMINISTRATION OF [^{14}C]NISOLDIPINE

Species	Rat (M)		Dog (F)		Monkey (F)	Man (M)	
Route Dose (mg/kg)	i.v. 1	p.o. 5	i.v. 0.5	p.o. 5		p.o. 12*	i.v. 0.8*
Radioactivity							
$CEQ_{max,norm}$ (kg*l^{-1})	---	0.49	---	0.59		3.7	---
t_{max}(h)	---	0.87	---	1.45		0.77	---
AUC_{norm}(kg*h*l^{-1})	13.6	4.0	4.2	5.9		40.1	50.2
$t_{½term}$(h)	23.9	14.9	37.9	54.4		80.3	85.8
Parent							
$C_{max,norm}$ (kg*l^{-1})	---	0.009	---	0.017		0.054	---
t_{max}(h)	---	0.5	---	1.0		0.42	---
AUC_{norm}(kg*h*l^{-1})	0.36	0.0097	0.46	0.054		0.077	0.954
$t_{½}$(h)	0.36	0.70	4.0	2.3		3.8	3.8
F(%)	---	2.7	---	11.7		8.4	---

n = 5 (rat), 3 (dog), 1 (monkey), 12 (man)
Rat data for parent (p.o.) from 1 mg/kg dose.

*Total mg dose per volunteer

Immediate Release

Dose (mg)	C_{max} (ng/mL)	AUC_{0-24} (ng.h/mL)
2.5	0.43 (58)	1.26 (84)
5	0.85 (46)	2.89 (48)
10	1.44 (59)	6.52 (46)
20	3.42 (57)	14.5 (42)

Coat Core

Dose (mg)	C_{max} (ng/mL)	AUC_{0-48} (ng.h/mL)
10	0.90 (43)	15.2 (33)
20	1.45 (39)	27.1 (37)
40	3.07 (49)	54.3 (47)
60	4.28 (52)	83.3 (43)

Study D90-022

Pharmacokinetic Parameters at Steady-State (Mean ± SD)

Dose (mg)	N	AUC(0-24h) (ng·h/ml)	C_{max} (ng/ml)	T_{max} (h)
30	18	74.28 ± 7.96	4.79 ± 0.68	7.22 ± 0.93
60	18	129.76 ± 12.74	8.48 ± 0.81	9.08 ± 1.97
90	9	199.31 ± 16.45	13.02 ± 1.20	6.78 ± 2.30
120	3	226.58 ± 12.41	14.92 ± 2.01	4.00 ± 1.00

Study D90-022

L.S. Mean Supine BP Change from Baseline at Steady-State (mmHg)

		Systolic		Diastolic	
Dose (mg)	N	8h post dose	24h post dose	8h post dose	24h post dose
30	18	-16.4	-14.0	-8.4	-10.2
60	18	-20.8	-16.8	-13.2	-15.0
90	9	-22.1	-23.0	-12.1	-13.4
120	3	-30.7	-44.3	-25.0	-19.0

3. Tissue Distribution: Quantitative tissue distribution in Sprague-Dawley rats was determined after 0.5, 1, 4, 8, 24, 48 and 72 hours (although the tables which follow provide 4, 24 and 72 hour values only). After oral administration, maximum concentrations in virtually all organs were reached within one hour, with liver, fat, kidney and adrenal gland generally containing the highest levels, brain and skeletal muscle the lowest. After a single dose, terminal elimination half-lives ranged from 42.2 h for plasma to 123 h for brain. With repeated daily dosing (5 mg/kg for 3 weeks), steady state was reached within 8 days. The CEQ at 24 h after the last (21st) dose and the $AUC_{1..8}$ (based on radioactivity) were increased by a factor of 5 to 9, compared to a single dose. After 21 days of dosing, a slower elimination phase, characterized by half-lives of 2.66 days for plasma, 6.13 days for lung and up to 38 days for brain (generally 6-11 days in most other organs), was found.

After a single dose, there was no indication for changes in organ distribution pattern at later time intervals (up to 72 hours). By 72 hours, 1.1% of the administered radioactivity remained in the body of the rat (excluding the intestinal tract). In beagle dogs, tissue levels were measured only after 72 hours and the pattern of distribution was found to be similar to that in rats. Corresponding residue values in the body of dogs after 3 days were around 1% (i.v.) or 2% (oral) of the administered dose.

In pregnant rats following single oral or intravenous dosing, placental transfer was observed, with total radioactivity in the fetus reaching 17% of maternal plasma and 29% of the average maternal tissue concentration within one hour.

In lactating rats, secretion of nisoldipine and its metabolites into milk was noted after an oral dose of 5 mg/kg, with concentrations in milk being lower than in plasma at all time periods up to 48 hours post-dosing.

Whole body autoradiography indicated rapid tissue distribution and penetration of the blood brain barrier within 5 minutes after an intravenous dose. In addition, autoradiography essentially confirmed the widespread tissue distribution that was observed with the quantitative tissue measurements, including placental transfer and secretion into milk.

Placental transfer and milk secretion studies in rats are summarized in the 11/7/88 review of [illegible] by X. Joseph, D.V.M., Ph.D.

QUANTITATIVE[1] TISSUE DISTRIBUTION OF TOTAL RADIOACTIVITY IN THE RAT AFTER ORAL (MALE AND FEMALE) AND INTRAVENOUS (MALE ONLY) ADMINISTRATION OF [^{14}C]NISOLDIPINE.

Time post-dose	4 h			24 h		
Route	i.v. (male)	p.o. (male)	p.o. (female)	i.v. (male)	p.o. (male)	p.o. (female)
Dose (mg*kg-1)	1	5	5	1	5	5
Organ/Tissue:						
body excl. g.i.t.	0.11	0.11	0.29	0.026	0.021	0.032
plasma	0.35	0.16	0.36	0.13	0.044	0.056
erythrocytes	0.09	0.05	0.14	0.038	0.015	0.018
liver	0.54	0.56	1.8	0.13	0.11	0.36
kidneys	0.26	0.19	0.44	0.057	0.034	0.031
lungs	0.17	0.11	0.26	0.068	0.027	0.034
heart	0.09	0.07	0.20	0.029	0.016	0.016
brain	0.04	0.03	0.046	0.012	0.011	0.0062
adrenal gland	0.13	0.15	0.32	0.11	0.034	0.043
testes	0.06	0.04	----	0.019	0.014	-----
ovaries	----	----	0.38	----	----	0.029
renal fat	0.33	0.32	0.70	0.031	0.025	0.025
skin	0.06	0.06	0.16	0.022	0.018	0.021
skel. muscle	0.05	0.03	0.11	0.011	0.011	0.0081
resid. carcass	0.10	0.09	0.22	0.018	0.015	0.015

[1]Mean dose-normalized equivalent concentrations ($kg*l^{-1}$). n = 5 per group.

QUANTITATIVE[1] TISSUE DISTRIBUTION IN THE RAT AND DOG AT 72 H FOLLOWING ADMINISTRATION OF [^{14}C]NISOLDIPINE.			
Species	Dog (F)		Rat (M)
Time after application	72 h		72 h
Route of administration	i.v.	p.o.	p.o.
Dose [mg*kg^{-1}]	0.5	5	5
body excl. g.i.t.	0.0095	0.017	0.012
plasma	0.011	0.017	0.020
erythrocytes	0.0083	0.011	0.0095
liver	0.078	0.090	0.057
kidney	0.020	0.034	0.017
lungs	0.016	0.024	0.009
heart	0.0057	0.014	0.009
brain	0.0016	0.0034	0.008
adrenal gland	0.032	0.072	0.025
renal fat	0.016	0.026	0.015
skin	0.0071	0.012	0.012
skel. muscle	0.0034	0.010	0.007
uterus	0.0080	0.018	-----
ovary	0.0090	0.016	-----

[1]Mean dose-normalized equivalent concentrations (kg*l^{-1}). n = 3 (dog) and 5 (rat) per group.

organs	CEO_{max} [$\mu g \cdot g^{-1}$]	t_{max} [h]	$AUC_{0.5-72\ h}$ [$mg \cdot h \cdot kg^{-1}$]	$t_{1/2}$ [h]
body excl. g.i.t.	1.75	1.0	12.3	59.5
plasma	3.35	1.0	21.4	42.2
erythrocytes	1.550	1.0	7.58	72.8
liver		0.5	66.7	50.6
kidney	1	0.5	30.4	48.0
lungs	2.6	0.5	13.0	45.5
heart	1.45	0.5	8.76	53.6
brain	0.265	0.5	4.8	123
adren. gland	1.55	1	16.5	74.9
testes	0.470		6.09	72.4
renal fat	4.40	2.	17.8	65.1
skin	1.15	1.0	8.54	82.1
skel. muscle	0.600	0.5	1.76	65.1
resid. carcass	1.60	2.0	1.44	58.6

Pharmacokinetic parameters in different organs and tissues obtained for total radioactivity after single oral administration of mg [^{14}C]nisoldipine per kg body weight to male Sprague Dawley rats (N = 5).

organ/tissue	parameters								
	CEQ (24 h) [$\mu g \cdot g^{-1}$]			AUC (1-3 d) [$mg \cdot h \cdot kg^{-1}$]			$t_{1/2}$ (1-3 d) [h]		
	21 doses	1 dose	F	21 doses	1 dose	F	21 doses	1 dose	F
body excl. g.i.t.	0.837±[illegible]	0.105±0.010	8.0	33.0	3.78	8.7	78.9	59.5	1.3
plasma	0.547±0.0	.220±0.035	2.5	17.1	7.08	2.4	35.8	42.2	0.85
erythrocytes	0.484±0.038	'5±0.010	6.5	19.8	2.91	6.8	96.6	72.8	1.3
liver	2.59 ±0.39	[illegible] 7.100	4.7	93.2	18.5	5.0	59.4	50.6	1.2
kidney	0.938±0.140	0.1 70	5.5	36.8	5.82	6.3	80.1	48.0	1.7
lung	0.650±0.052	0.135±	4.8	25.2	4.50	5.6	62.3	45.5	1.4
heart	0.435±0.049	0.080±0.[illegible]	[illegible].4	18.2	2.80	6.5	72.0	53.6	1.3
brain	0.285±0.032	0.055±0.005		12.8	2.30	5.6	109	123	0.89
adrenal gland	2.58 ±0.29	0.170±0.020	1	111	7.43	15	110	74.9	1.5
testes	0.350±0.028	0.070±0.005	5.[illegible]	15.1	2.57	5.9	90.2	73.4	1.2
renal fat	2.41 ±0.49	0.125±0.025	19.3	[illegible]	4.44	24	168	65.1	2.6
skin	0.826±0.076	0.090±0.010	9.2		3.48	9.4	79.0	82.1	0.96
muscle	0.492±0.056	0.055±0.005	8.9	[illegible]	1.91	11	61.7	65.1	0.95

Comparison of pharmacokinetic parameters for the total radio[illegible]ty in different organs and tissues between 1 and 3 days after single oral administration and termi[illegible] of repeated (21x) oral administration of 5 mg [^{14}C]nisoldipine per kg body weight to male [illegible]ue Dawley rats. Values represent means (± s.d.) of n = 5. Factors F give the ratios of the [illegible]eters after 21 doses and 1 dose.

organ/ti	parameters		
	CEQ_1 [/ug·g^{-1}]	$t_{1/2}$ [d]	AUC (1 - 10 d) [mg·h·kg^{-1}]
body excl. g.i.t.	0.753	9.74	109
plasma	0.548	2.66	35.8
erythrocytes	424	22.3	75.6
liver		4.49	262
kidney	0.	6.30	113
lung	0.61	6.13	73.2
heart	0.406	9.77	59.5
brain	0.266	28.0	49.7
adrenal gland	2.70	7.96	370
testes	0.335	7	50.4
renal fat	2.41		401
skin	0.709	15	118
muscle	0.414	17.8	72.7

Pharmacokinetic parameters for the total radioactiv · different organs and tissues between 1 and 10 days after termination o. ·ated (21x) oral administration of 5 mg [^{14}C]nisoldipine per kg body weigh ·ale Sprague Dawley rats.

4. Protein Binding: As determined by equilibrium dialysis, ^{14}C-nisoldipine was highly bound to plasma proteins of the rat (97.8-99.1%), dog (97.6-97.1%) and man (>99.4%) and was not influenced by sex in any of the three species. In humans, ^{14}C-labelled drug was bound predominently to the serum albumin, and the extent of binding was not influenced by plasma concentration over a broad range; i.e., between 0.1 and 10 ug/ml. Similar levels of protein binding were found in human plasma whether it was measured by equilibrium dialysis or ultrcentrifugation, and degree of binding of the (+) and (-) ^{14}C-enantiomers was similar (around 99.4%), with no indication of preferential stereospecific binding. In the "expert opinion" on pre-clinical pharmacokinetic studies, it is claimed that when protein binding was measured *ex vivo* (dialysis method) in rats and dogs after i.v. or oral administration, nisoldipine was highly bound initially, but the protein bound fraction dropped to 50 to 80% between 30 and 180 minutes after dosing, indicating lower binding affinities for the metabolites.

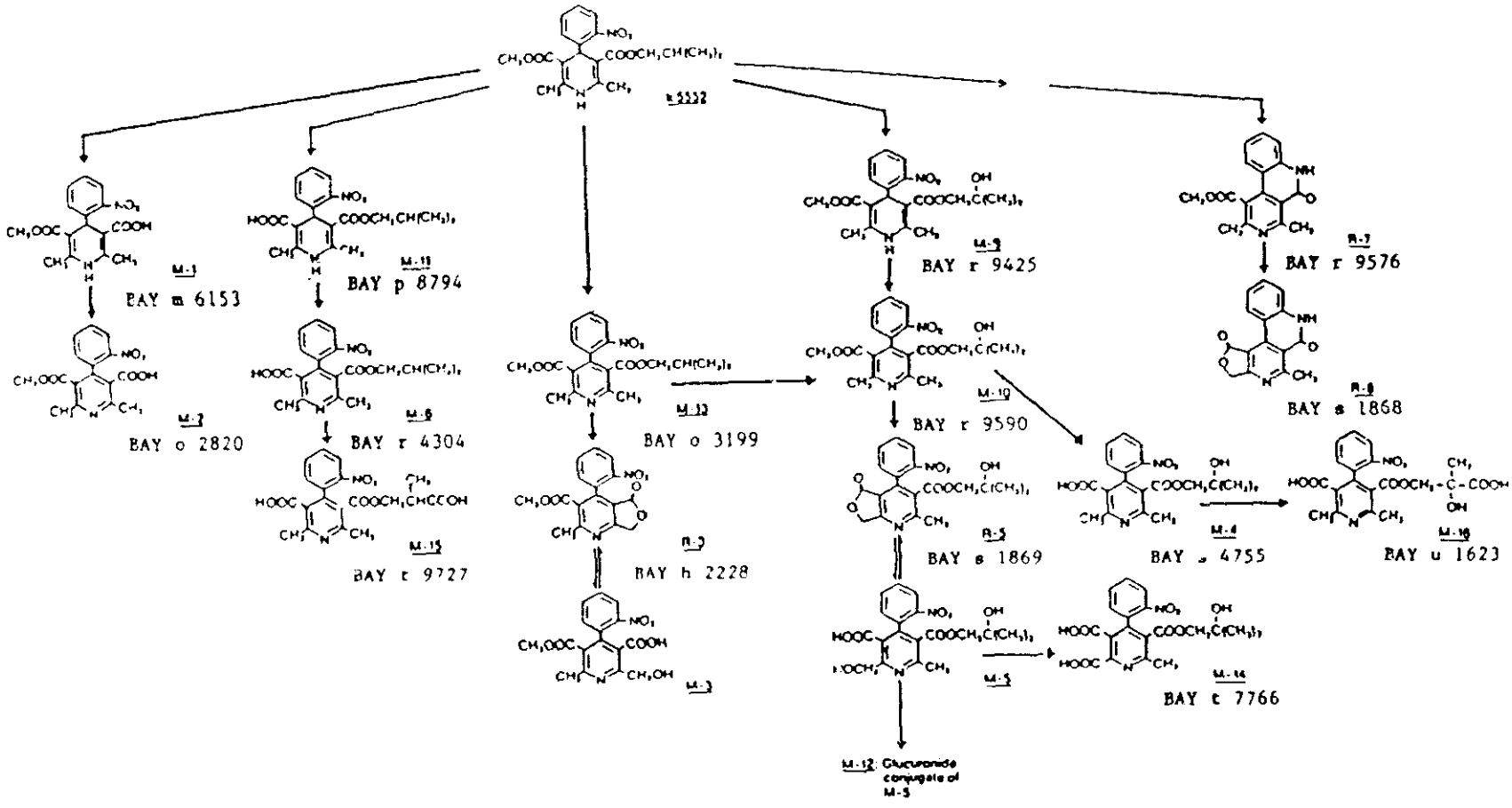
5. Metabolism

a. Biotransformation: Nisoldipine is rapidly and extensively metabolized in the rat, dog, monkey and man. Only a small percentage of unchanged ^{14}C-labelled substance could be found in the circulation of the rat or dog at 30 and 60 minutes after oral administration, when plasma radioactivity was at the maximum level. No unchanged drug was eliminated in the urine or feces of all 4 species, or in bile of bile duct cannulated rats that received the drug either by i.d. or i.v. route. Partial enterohepatic recirculation of metabolites was demonstrated in rats. A schematic for the biotransformation of the drug is shown on the page which follows. The investigators describe the biotransformation steps as follows:

hydroxylation of the isobutyl ester
-dehydrogenation to the pyridine derivative
-cleavage of the ester to form the carboxylic acid
reduction of the nitro group to the amino group
-glucuronidation (phase II enzymatic reaction)

In urine of all 4 species, at least 11 biotransformation products were detected, with 6 of them, M-1 to M-5 and M-12, accounting for 80% of radioactivity in urine; all other metabolites were minor products. M-5 was the major urinary metabolite, accounting for 24 to 46% of the renal eliminated radioactivity in all 4 species. Only 1 metabolite, M-12, was hydrolyzable with B-glucuronide, to give M-5 as the aglycone. Metabolic profile in urine was essentially similar in all 4 species.

In bile, metabolic profiles of rats "were quantitatively identical *in vitro* (isolated perfused rat liver model) and *in vivo* following intraduodenal administration". At least 24 metabolites have been detected, but only 8 of them, M-3 to M-5, M-10, M-12, M-14 to M-16, were quantitatively important. The



PROPOSED METABOLIC PATHWAYS OF NISOLDIPINE

major metabolic products in bile of rats were M-5 and its conjugate, M-12.

In serum, at least 12 metabolites were observed in rats within 30 minutes of dosing, and the main ones were M-2 and M-5, together with their gamma-lactones, R-3 and R-5. In dogs, at least 11 biotransformation products were isolated from the serum, and a similar pattern was seen as in rats, with M-2 and M-5 being the main products.

A total of 18 biotransformation products have been identified in urine and serum. The main biotransformation products in all 3 animal species and humans, based on findings in the urine and serum (also in the bile of rats), are M-5 plus M-12 which is the glucuronide of M-5, and R-5 which is the gamma-lactone of M-5.

main metabolites M-5, M-12 and R-5 in urine and serum

	rat serum	rat urine	dog serum	dog urine	monkey urine		
					0-7 h	7-11 h	11-24 h
	[%]	[%]	[%]	[%]	[%]		
M-5	13.3	34.9	46.5	44.9	27.2	23.7	26.7
M-12		3.1		11.7	30.8	31.1	15.5
R-5	11.7						
total:	25	38	46.5	56.6	58	54.8	42.2

In studies with dogs, the liver and gut wall were identified as the primary sites of biotransformation (Arzneim. Forsch./Drug Res. 38: 1093, 1988) after oral administration. It was estimated that around 60% is metabolized pre-hepatically in the gut and 30% in the liver.

b. Effects on Hepatic Enzymes: In two experiments with male rats, nisoldipine was administered orally at doses of 0, 10, 50 and 200 mg/kg for 2 weeks, followed by a 1 week recovery period in the second experiment. The positive control was phenobarbital at 25 mg/kg. The hepatic levels of cytochrome P-450, aminopyrine N-demethylase and aniline hydroxylase activities were decreased at mid and high dose, whereas phenobarbital caused significant increases in levels of all 3 enzymes. The decreases in all 3 enzyme levels were found to be reversible after a 1-week recovery period

SUMMARY OF TOXICOLOGICAL STUDIES

A. **Acute Toxicity Studies** (X. Joseph)

Acute oral and iv toxicity studies were done in mice, rats, rabbits and dogs at

For oral toxicity studies, the drug (suspended in a solution of glycerol, lutrol and demineralized water) was given via stomach tube (20 ml/kg) to mice, rats and rabbits; and in dogs the drug was given in gelatin capsules. For iv studies, the drug suspended in the above solution was given at a volume of 5 ml/kg for rodents and at 1 to 4 ml/kg for dogs. After drug treatment animals were observed for a period of 14 days. No clinical signs or mortality were seen after oral administration. However, tonic/clonic convulsions, gasping, cyanosis, exophthalmos and respiratory disturbances (all species) were seen after iv administration. All deaths occurred either during or within 10-20 min of drug administration. The surviving animals were free of symptoms within 1 (mice, rabbit and dog) to 48 hr (rat) postdose. The autopsies (dead or sacrificed at the end of the study) showed no pathological findings. The LD50 values for different species are below.

ACUTE TOXICITY OF NISOLDIPINE IN MICE, RATS, RABBITS, AND DOGS

Species	Sex	Route of Administration	LD_{50} - mg/kg (95% Conf. Limits)
Mouse (CFW1/W)	M	p.o.	> 10,000
Mouse (CFW1/W)	M	i.v.	2.20 (2.0-2.5)
Rat (Wistar)	M	p.o.	> 10,000
Rat (Wistar)	F	p.o.	> 10,000
Rat (Wistar)	M	i.v.	2.32 (2.06-2.65)
Rat (Wistar)	F	i.v.	1.86 (1.77-1.97)
Rabbit (Lge Chinchilla)	M,F	p.o.	> 5000
Rabbit (Lge Chinchilla)	M,F	i.v.	ca. 2.5
Dog (Beagle)	M,I	p.o.	> 5000
Dog (Beagle)	M,F	i.v.	ca. 2.0

A separate study showed that pretreatment with propranolol (1 mg/kg ip for 4 or 5 days) had no effect on the acute iv toxicity of nisoldipine in male Wistar rats (iv LD50 = 1.6 mg/kg with or without propranolol pretreatment).

B. **Subchronic, Chronic and Carcinogenicity Studies**

RAT STUDIES (X.Joseph)

a. Four Week Dietary Dose Rangefinding Study

Testing Facility:

Study Number: B/K 5552/024

Study Dates: August - September, 1980

GLP Compliance: Study was not conducted according to GLP regulations. The deviations were as follows: 1. no phase 1-3 GLP audits. 2. no checking of physico-chemical properties of test substance.

Animals: Wistar strain TNO-74 rats, individually housed in Macrolon cages, Type II, were 5-6 weeks old (average weights: males - 131 g; females - 112 g) at the initiation of dosing.

Dose Levels: BAY k 5552 (Batch No. 576,923) was mixed with powdered rat diet to obtain drug concentrations of 0, 300, 1000, and 3000 ppm.

Achieved Dose Levels:

Dose (ppm)	Average Drug Intake (mg/kg/day)	
	Male	Female
0	0	0
300	26	26
1000	86	89
3000	258	265

(Note: The drug intake was calculated from the average daily food intake for the whole duration of the study. No significant difference in drug intake was seen with time.)

Number of Animals: 10/sex/group

Parameters Evaluated: Appearance and behavior (at least once daily), body weight and food consumption (weekly), organ weights (heart, liver, kidneys and adrenal glands) and gross pathology. (No histopathological examinations were conducted.)

Results: No treatment-related clinical signs or mortalities were observed in this study. A significant reduction in body weight, compared to concurrent controls, was observed throughout the treatment period in high dose males (10-18%) and females (6-10%) except in females at week 4. No significant body weight differences were seen between control and mid or low dose groups (both sexes). Food consumption was reduced in the high dose group. Though not measured quantitatively, water consumption appeared to be increased in all treated groups. Organ weight findings (both absolute and relative) are given below.

Mean Organ Weights of Male and Female Rats

		Sex	Dose Group (ppm in diet)			
			0	300	1000	3000
Body Weight (g)		M	225	219	224	196*
		F	142	140	144	131
Adrenals	(Absolute, mg)	M	36	38	38	39
	(Relative, mg/100g)	M	16	18	17	21*
	(Absolute, mg)	F	50	49	53	49
	(Relative, mg/100g)	F	36	35	37	38
Heart	(Absolute, mg)	M	663	669	675	677
	(Relative, mg/100g)	M	295	304	301	349**
	(Absolute, mg)	F	490	517	555**	542
	(Relative, mg/100g)	F	346	371	387*	417**
Kidney	(Absolute, mg)	M	1489	1392	1409	1295**
	(Relative, mg/100g)	M	663	634	630	669
	(Absolute, mg)	F	986	978	1036	975
	(Relative, mg/100g)	F	697	702	719	749
Liver	(Absolute, mg)	M	8219	8074	8599	8699
	(Relative, mg/100g)	M	3650	3655	3825	4436**
	(Absolute, mg)	F	5300	5452	5857	6105*
	(Relative, mg/100g)	F	3761	3895*	4064*	4652**

** Significantly different from control at 0.05 level.*
*** Significantly different from control at 0.01 level.*

Relative mean heart and liver weights of the high dose group (both sexes) were significantly increased with no significant changes in absolute weights except for the mean liver weight of high dose females which was significantly higher (15%) than the control value. Both absolute and relative heart weights were increased in mid dose females.

It is stated that dietary dose levels, 0, 50, 300 and 1800 ppm, for the 2 year carcinogenicity study in rats were selected on the basis of the above study, and also based on the previous experience from long-term studies with other dihydropyridines [amendment (no serial number) dated May 31, 1994].

b. Three Month Oral (Gavage) Toxicity Study in Rats

Testing Facility:

Study Number: Bay k 5552/025

Study Dates: September - December, 1980

GLP Compliance: Not addressed.

Animals: Wistar (TNO/W 74, SPF) rats, individually housed in type II Makrolon cages, were 7-8 weeks old (males 115-155 g; females 120-145 g) at the initiation of dosing.

Mode of Administration: Bay k 5552 (Batch No. 576923) dissolved in a solvent mixture, containing Bay a 1040 placebo sclution (polyethylene glycol, glycerol and distilled water) and distilled water, was given by oral intubation. It is stated that the test formulation was stable at room temperature for over a week.

Dose Levels: 0 (vehicle control), 10, 30 and 100 mg/kg/day (5 ml/kg)

Number of Animals: 15/sex/group

Parameters Evaluated: Appearance and behavior (daily), body weight and food and water consumption (weekly), hematology, blood chemistry and urinalysis (5 rats/sex/group; weeks 4/5 and 13), major organ weights and gross and microscopic pathology (more than 20 different tissues/rat; control and high dose groups).

Major Findings: Two low dose females (on days 8 and 68) died during the study. Gross pathology findings in the above animals included enlarged kidneys, bladder and adrenals in one rat and discolored lung and pulmonary emphysema in the other. Labored breathing was noticed in high dose rats during the first 5 weeks of treatment. No significant differences in body weights were seen between treated and control groups except for the lower body weights observed in the high dose group, compared to concurrent controls, during the first one or two weeks of treatment. Food consumption was unaffected. Water intake of high dose females was about 20% higher than that of concurrent control. Although hemoglobin and hematocrit values in mid and high dose males were lower than concurrent control values at 13 weeks, it is stated that all values were within the historical control range for Wistar rats. High dose females had significantly higher plasma urea levels, compared to control, after either 4 or 13 weeks of treatment. In mid and high dose males, both absolute and relative thymus weights were significantly higher than control. Heart and liver weights (both absolute and relative) in high dose females were significantly higher than control; absolute and/or relative

weights of these organs in mid dose females and mid- and high-dose males were also higher than control. There were no significant histopathological findings in this study.

Mean Organ Weights of Male and Female Rats

		Sex	Dose Group (mg/kg)			
			0	10	30	100
Body Weight (g)		M	338	347	330	326
		F	206	207	200	204
Adrenals	(Absolute, mg)	M	36	39*	37	37
	(Relative, mg/100g)	M	11	11	11	11
	(Absolute, mg)	F	52	55	53	56
	(Relative, mg/100g)	F	25	27	26	27*
Brain	(Absolute, mg)	M	1788	1860*	1849	1843
	(Relative, mg/100g)	M	530	538	569	568**
	(Absolute, mg)	F	1652	1676	1649	1685
	(Relative, mg/100g)	F	802	815	829	829
Heart	(Absolute, mg)	M	955	1022*	1000*	1004
	(Relative, mg/100g)	M	283	295	304**	308**
	(Absolute, mg)	F	688	704	706	758**
	(Relative, mg/100g)	F	334	342	354**	372**
Liver	(Absolute, mg)	M	10966	11179	11777	11523
	(Relative, mg/100g)	M	3243	3220	3602*	3537*
	(Absolute, mg)	F	6394	6822	6907	7588**
	(Relative, mg/100g)	F	3100	3304	3460*	3716**
Lung	(Absolute, mg)	M	1172	1202	1000*	1004
	(Relative, mg/100g)	M	347	347	366	366*
	(Absolute, mg)	F	900	937	913	911
	(Relative, mg/100g)	F	436	455	457*	447
Thymus	(Absolute, mg)	M	196	222	235*	233*
	(Relative, mg/100g)	M	58	64	72*	72*
	(Absolute, mg)	F	206	213	180	185
	(Relative, mg/100g)	F	100	104	90	91

** Significantly different from control at 0.05 level.*
*** Significantly different from control at 0.01 level.*

c. Two Year Carcinogenicity Study in Rats

Testing Facility:

Study Number: T 1000876

Study Dates: November 1980 - November 1982

GLP Compliance: Study was conducted in accordance with GLP regulations

Animals: Wistar strain TNO/W 74 rats, individually housed in type II Makrolon cages, were 5-6 weeks old (mean body weights: males - 79 g; females - 75 g) at the initiation of the study.

Dose Levels and Mode of Administration: Bay k 5552 (Batch No.662836, purity - about 99.2%) was mixed, weekly, with powdered rat diet at concentrations of 0, 50, 300 and 1800 ppm. The stability and the concentration of the test substance in diet were determined pretest and then every three months. The concentrations of the drug in diet were found to be in good agreement with theoretical values. However, there is no indication that concentrations of drug in diet were adjusted periodically to maintain a constant mg/kg body weight exposure.

Number of Animals: 50/sex/group (An additional 10 rats/sex included in each group were sacrificed after 12 months of treatment - interim sacrifice.)

Observations/Measurements: Rats were observed at least once daily for general appearance, behavior and clinical signs. Body weights were recorded weekly until week 27 and biweekly thereafter. Food and water consumption were determined weekly and once every 3 months, respectively. Hematological [erythrocyte, leucocyte (total and differential), platelet and reticulocyte counts, hemoglobin, hematocrit, MCV, MCH and thromboplastin time (only at the termination of the study)] and blood chemistry (alkaline phosphatase, transaminases, creatine kinase, urea, creatinine, blood sugar, cholesterol, total bilirubin, total protein, corticosterone, aldosterone and serum electrolytes) evaluations and urinalyses were conducted on 10 rats/sex/group (selected at random) at 6, 12, 18 and 24 months. Complete autopsies were performed on animals that were sacrificed at 12 months and at study termination. Animals were examined grossly and heart, lung, liver, spleen, kidneys, adrenals and testes were weighed. All protocol specified tissues (more than 30 different tissues/rat) and gross lesions were fixed in buffered formalin. In addition, left liver lobe from all rats was fixed in formol-calcium, and lower jaw from 5 rats/sex/group was fixed in buffered formalin. Autopsies were also performed on rats that died or were sacrificed in extremis and all evaluable tissues were preserved. All protocol specified tissues from control and

high dose groups, all tissues from animals that died or were sacrificed moribund, as well as adrenals, genital organs, areas of skin change and kidneys (females) of low and mid dose animals and all grossly abnormal tissues were examined histologically.

Differences between treated and control groups were analyzed using the significance test (U-test) of Mann and Whitney and of Wilcoxon. Mortality and tumor data were analyzed by Fischer's exact test.

Achieved Dose Levels:

Dose (ppm)	Average Drug Intake (mg/kg/day)	
	Male	Female
0	0	0
50	2.15	2.78
300	13.13	18.04
1800	82.40	110.68

(Note: The drug intake was calculated, at the termination of the study, from the average daily food intake/animal/group for the whole duration of the study. Periodical drug intake determinations were not made in this study.

Results: No treatment-related clinical signs were seen in this study. The mortality data (cumulative) at different intervals are given below and it is presented graphically in Figures 6 and 7.

Mortality of Rats Receiving Nisoldipine in Diet for 24 Months

Daily Dose (ppm in diet)	Number of Rats (M/F)	Number of Dead (M/F)	% Mortality (M/F)
12 Months			
0	50/50	1/1	2/2
50	50/50	0/1	0/2
300	50/50	1/0	2/0
1800	50/50	0/2	0/4
18 Months			
0	50/50	2/3	4/6
50	50/50	0/2	0/4
300	50/50	2/5	4/10
1800	50/50	5/4	10/8
24 Months			
0	50/50	4/8	8/16
50	50/50	8/8	16/16
300	50/50	11/15	22/30
1800	50/50	11/13	22/26

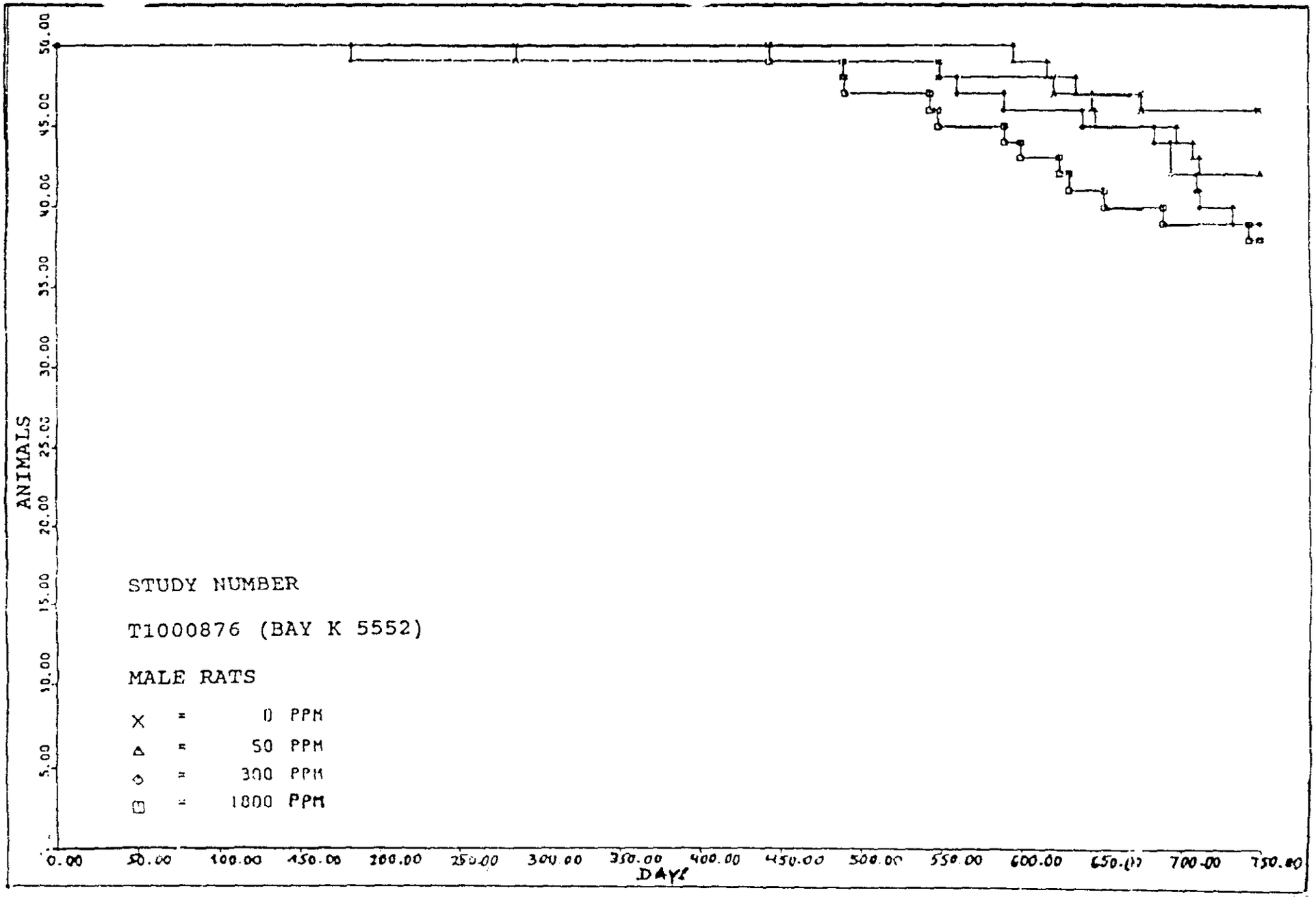


Fig. 6: Mortality curves of male rats which received Bay k 5552 for 24 months in their feed

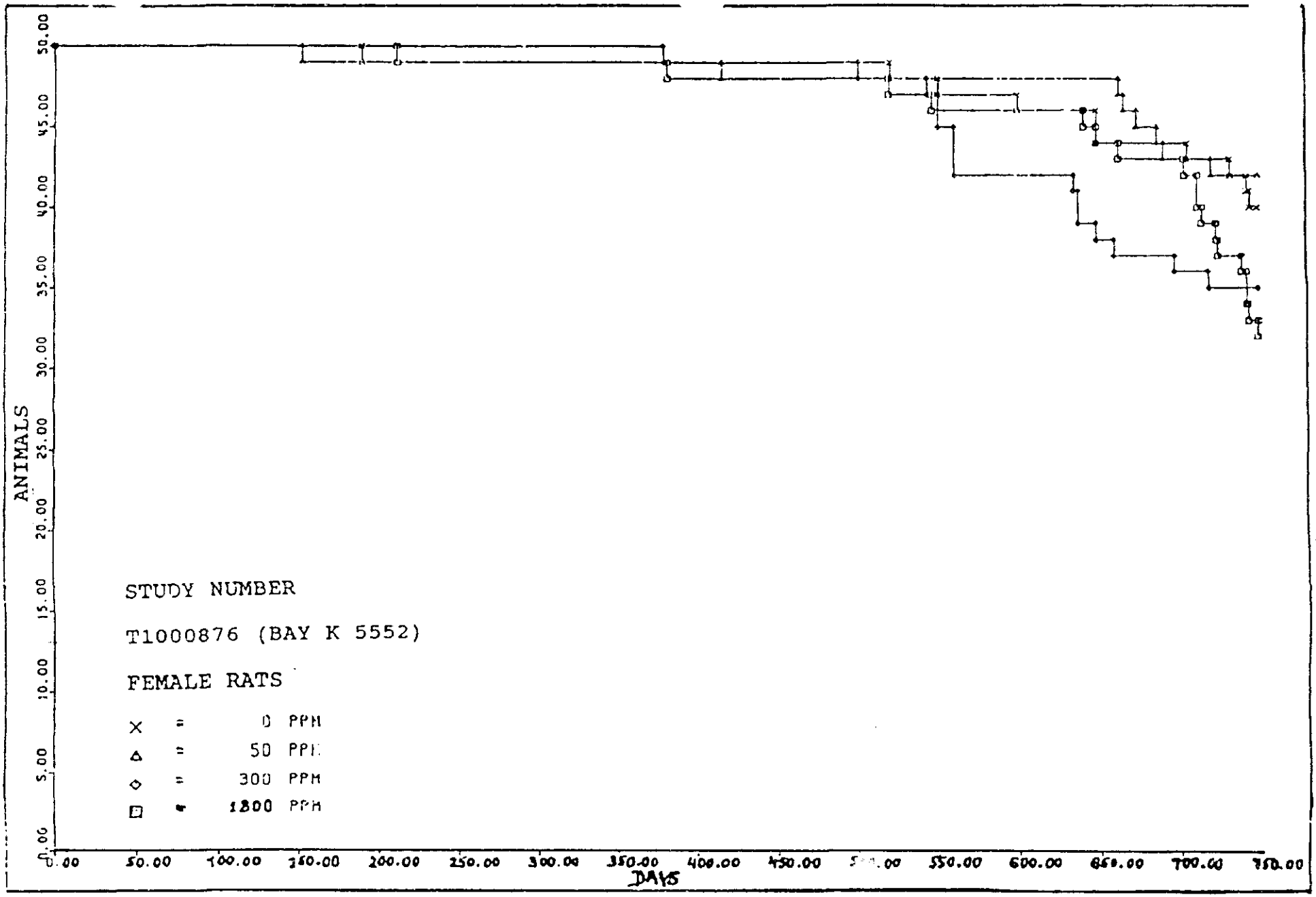


Fig. 7 : Mortality curves of female rats which received Bay k 5552 for 24 months in their feed

Although more animals (both sexes) died during the study in mid and high dose groups than in the concurrent control group, the differences were statistically not significant (sponsor's analysis - Fischer's exact test).

When tested for heterogeneity in survival distribution, FDA statisticians observed that there was no significant difference (at 0.05 level) in the survival distribution for either sex (both Cox test and generalized Wilcoxon test). Additionally, no significant linear trend (at 0.05 level) was seen in the intercurrent mortality rate for either males or females.

Mean body weight values (presented graphically in Fig.8) for the high dose group (both sexes) were significantly lower than control values for the whole duration of the treatment period, except on four occasions (weeks 25, 29, 51 and 53) in males. At the termination of the study, body weights of high dose males and females were 5.6% and 22%, respectively, lower than concurrent control. The body weight gain values of high dose males and females were 7% and 31%, respectively, lower than concurrent control. No significant treatment-related reductions in body weight were seen in low and mid dose groups except on a few occasions (weeks 13, 14 and 39 for males and weeks 77, 79, 85, 87 and 89 for females) at mid dose level. While food consumption was unaffected, water consumption in high dose animals, especially in females, was increased.

Although statistically significant hematological findings were occasionally observed in drug treated groups, no dose dependence or consistency at different intervals was observed. Statistically significant clinical chemistry findings and the time points of their occurrence are given on pages 48 & 49. In the high dose group, significant reductions in alkaline phosphatase levels (both sexes) and increases in GOT (males), GPT and CPK levels (females) were seen. Although a dose-dependent increase in bilirubin levels was seen in both sexes during the early part of the study, these levels in treated females were lower than that of control (no significant difference in males) at the termination of the study. Blood urea levels in mid and high dose females were significantly higher than control at the end of the study; however, these levels were lower than control during week 28. Decreased calcium levels were seen in treated animals, especially at the high dose level (both sexes). Plasma aldosterone levels in high dose males and plasma corticosterone levels in high dose females were significantly lower than respective control values at week 55.

A significant increase in urinary protein excretion was seen in high dose females. While urinary calcium excretion was decreased in treated female groups, especially in mid and high dose groups, urinary potassium levels were increased in high dose males. Urinary aldosterone excretion was significantly higher in high dose males than in control males.

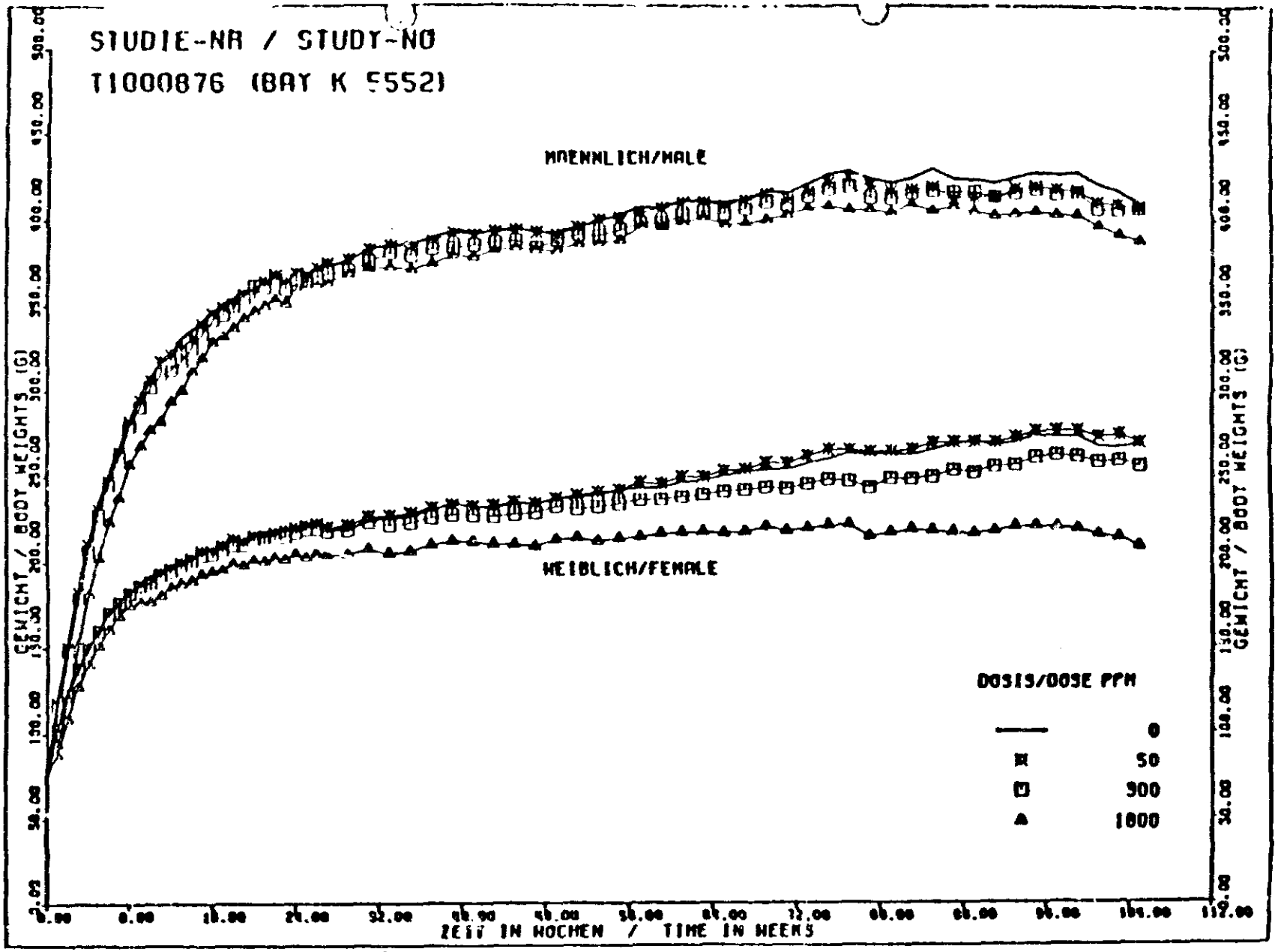


Fig.8 : Body weight curves for male and female rats which received BAY k 5552 with the feed for 24 months.

Mean Clinical Chemistry Parameters (Male Rats)					
		Dose Group (ppm in diet)			
Parameter	Week	0	50	300	1800
ALP	28	211	201	201	175*
U/L	54	182	174	186	145*
	79	180	156	176	136**
GOT		38.8	39.0	38.9	52.7*
U/L					
Bilirubin	28	3.6	3.1	4.0	4.8*
mcmol/L					
Creatinine	79	53	50	47*	51
mcmol/L	105	57	63	46**	50**
Urea	105	5.80	7.01*	5.69	5.27
mmol/L					
Cholesterol	28	1.98	2.16	2.21*	2.21
mmol/L					
Protein	54	66.5	64.2**	61.0**	61.4**
g/L	105	68.4	67.1*	66.3*	67.8
Sodium	28	142	143	140*	140*
mmol/L	54	141	142	142*	142
	79	140	139	138*	141
Potassium	79	4.8	5.0	5.1*	5.1
mmol/L					
Calcium	28	2.64	2.54*	2.49*	2.55*
mmol/L	79	2.76	2.66*	2.62**	2.63**
	105	2.69	2.66	2.63	2.56*
Aldosterone	55	349.7	360.2	334.9	245.1**
pg/mL					

** Significantly different from control at the 0.05 level*
*** Significantly different from control at the 0.01 level*

Mean Clinical Chemistry Parameters (Female Rats)					
		Dose Group (ppm in diet)			
Parameter	Week	0	50	300	1800
ALP	28	174	140	153	133*
U/L					
CPK	28	98	54*	72	85
U/L	79	43	75	64	77*
GPT	28	54.1	50.5	52.9	66.3*
U/L					
Bilirubin	54	3.0	3.1	3.2	4.3**
mcmol/L	105	4.7	2.9**	3.2*	2.9*
Creatinine	79	56	50	59	57*
mcmol/L	105	71	59	55**	61
Urea	28	7.54	7.27	6.56*	6.30**
mmol/L	79	6.22	5.96	6.51	7.56**
	105	6.09	6.43	6.67*	7.28*
Cholesterol	28	7.54	7.27	6.56*	6.30**
mmol/L	105	2.46	3.01*	2.76	2.96
Glucose	105	4.71	5.25	5.52*	5.22
mmol/L					
Sodium	54	140	138	135**	138
mmol/L					
Potassium	54	4.8	4.8	5.0	5.2*
mmol/L	105	4.5	4.6	4.8*	4.8*
Calcium	28	2.58	2.65	2.59	2.47*
mmol/L	54	2.71	2.65	2.58*	2.52**
Corticosterone	55	36.6	41.7	20.4	19.2*
mcg/DL					

** Significantly different from control at the 0.05 level*
*** Significantly different from control at the 0.01 level*

At the termination of the study, the relative mean weights of adrenals, heart, kidneys and liver of the high dose group (both sexes) were significantly higher than respective control values (page 50A). However, no significant differences were seen in the absolute weights of the above organs except for the increased mean kidney weight of the high dose males. At the interim sacrifice, relative heart and liver weights (high dose males and females) and relative adrenal and kidney weights (high dose females) were significantly increased without any significant changes in absolute weights.

No significant treatment related gross lesions were seen in this study.

At the interim sacrifice, no treatment-related histological findings were observed except for the moderate widening of the zona glomerulosa region of the adrenal cortex of high dose animals. The cells of this zone were large and contained a foamy cytoplasm. Four benign tumors [2 in control females (cystadenoma of thyroid in one and pituitary adenoma in the other) and 2 in high dose males (Leydig cell tumor of testis in one and meningioma of the cerebellum in the other)] were seen at the interim sacrifice.

[Note: The terms "blastoma" and "tumor" are used interchangeably in this NDA.]

The number of rats with benign and/or malignant tumors and the percent of these tumor carriers are given in Table 7. According to sponsor, no treatment-related increased incidence of tumor bearing animals was observed in this study. The incidence of various types of tumors observed at different locations are presented in Tables 8 and 9. Although the incidence of Leydig cell tumor of testes appears to be higher in treated male groups than in control, the differences were statistically not significant.

Analysis of the tumor data by FDA statisticians showed that there was a statistically significant (at 0.05 level) linear trend in brain granular cell tumor (listed also as meningioma in this NDA) in male rats ($p=0.0411$). The incidence of this tumor is as follows: control - 0/50, low dose - 0/50, mid dose - 0/50 and high dose - 3/50 (2 animals at the final necropsy and one at the interim sacrifice). However, pairwise comparison did not reveal any significant difference between control and high dose groups ($p=0.1594$). According to the sponsor, "the incidence rate for granular cell tumors among male rats at terminal kill in the study performed with BAY 5552 lay within the spontaneous range for male rats at terminal kill" (Table page 50e). Spontaneous tumors of meningeal origin (meningioma, meningeal sarcoma or granular cell tumor) were seen in 7 out of 30 studies in male Wistar rats (39-50 rats/study). In 2 studies, 2 rats each were diagnosed with such tumors at terminal kill, whereas in 4 other studies, only one rat each had above tumors.

Mean Organ Weights of Male and Female Rats (Final Sacrifice - 107 Weeks)

Organ		Sex	Dose Group (ppm in drinking water)			
			0	50	300	1800
Body Weight (g)		M	416	410	413	396*
		F	274	275	261	216**
Adrenals	Absolute, mg	M	49	49	43**	50
	Relative, mg/100g	M	12	12	10*	13*
	Absolute, mg	F	67	66	63	61
	Relative, mg/100g	F	24	24	25	29**
Heart	Absolute, mg	M	1215	1205	1199	1234
	Relative, mg/100g	M	294	295	290	312**
	Absolute, mg	F	912	927	898	934
	Relative, mg/100g	F	334	338	347	434**
Kidneys	Absolute, mg	M	2588	2600	2638	2791**
	Relative, mg/100g	M	625	634	640	706**
	Absolute, mg	F	1875	1823	1809	1745
	Relative, mg/100g	F	690	668	700*	810**
Liver	Absolute, mg	M	14277	14661	15213	14831
	Relative, mg/100g	M	3476	3715	3736	3937**
	Absolute, mg	F	8157	7912	8315	8087
	Relative, mg/100g	F	3327	3343	3509	3773**
Lung	Absolute, mg	M	1380	1368	1460	1373
	Relative, mg/100g	M	337	347	358	365
	Absolute, mg	F	1028	998	1004	1008
	Relative, mg/100g	F	419	423	425	472**
Spleen	Absolute, mg	M	656	656	673	684
	Relative, mg/100g	M	160	166	165	181*
	Absolute, mg	F	447	444	438	419
	Relative, mg/100g	F	183	188	185	196
Testicles	Absolute, mg	M	3371	3618	3661	3149
	Relative, mg/100g	M	823	917*	899	831

** Significantly different from control at 0.05 level*
*** Significantly different from control at 0.01 level*

Table 7 : Number of the blastoma carriers in the individual dose groups

Sex	♂				♀			
Dose (ppm)	0	50	300	1800	0	50	300	1800
Total number of rats investigated	49	50	50	48	48	48	48	48
Number of the blastoma carriers	31	16	21	26	33	21	21	28
Number of rats with exclusively benign blastomas	28	10	12	18	24	15	14	18
Number of rats with exclusively malignant blastomas	2	3	8	4	6	3	4	6
Number of rats with benign and malignant blastomas	1	3	1	4	3	3	3	4
Number of blastoma carriers as % all rats investigated	63	32	42	54	69	44	44	58
Number of blastoma carriers with exclusively benign blastomas as % all rats investigated	57	20	24	38	50	31	29	38
Number of blastoma carriers with exclusively malignant blastomas as % all rats investigated	4	6	16	8	13	6	8	13
Number of blastoma carriers with benign and malignant blastomas as % all rats investigated	2	6	2	8	6	6	6	8

Table 8 : List of all blastomas according to number, localisation, type and status (male rats)

(Dose ppm)	0	50	300	1800
Adenohypophysis*				
Adenoma	14	0	3	11
Carcinoma	0	2	0	0
Thyroids*				
C cell adenoma	6	0	0	0
C cell carcinoma	1	0	0	1
Follicular carcinoma	0	0	0	1
Adrenal cortex				
Adenoma unilateral	1	1	0	0
Adrenal medulla				
Phaeochromocytoma (b) unilateral	7	5	3	7
Phaeochromocytoma (b) bilateral	0	1	0	1
Phaeochromocytoma (m) unilateral	0	1	1	0
Parathyroids*				
Adenoma	3	0	0	0
Testes				
Leydig cell tumour (b) unilateral	4	5	6	7
Leydig cell tumour (b) bilateral	0	1	2	1
Pancreas* endocrine				
Adenoma	2	0	0	0
Pancreas* exocrine				
Adenoma	3	0	0	0
Heart*				
Endocardial fibromatosis (b)	2	0	0	1
Lung*				
Adenoma	0	0	1	0
Epididymis				
Sarcoma	0	0	0	1
Brain*				
Meningioma (b)	0	0	0	2
RHS*				
Malignant lymphoma	0	1	3	1
Histiocytary sarcoma	0	0	1	0
Skin*				
Cornified squamous cell carcinoma	0	1	1	1
Subcutis*				
Sarcoma	1	1	1	3
Haemangiosarcoma	0	0	1	1
Mesentery*				
Leiomyosarcoma	0	0	1	0
Abdomen*				
Fibrosarcoma	1	0	0	0
Sarcoma	0	1	0	0

b = benign
m = malignant
* This organ was not routinely histologically investigated in all the rats from the 50 and 300 ppm groups.

Table 9 : List of all blastomas according to number, localisation, type and status (female rats) (continuation)

(Dose ppm)	0	50	300	1800
Adenohypophysis				
Adenoma	16	3	5	7
Carcinoma	1	1	1	0
Thyroids*				
C cell adenoma	6	1	2	1
C cell carcinoma	1	0	0	0
Follicular adenoma	0	0	0	1
Follicular carcinoma	1	0	0	0
Adrenal cortex				
Adenoma unilateral	1	1	0	0
Adrenal medulla				
Phaeochromocytoma (b) unilateral	0	0	0	1
Phaeochromocytoma (m) unilateral	0	0	0	1
Ovary				
Granulosa-theca cell tumour (b)	0	0	1	1
Granulosa-theca cell tumour (m)	0	0	0	1
Uterus				
Endometrial stromal tumour (polyp) (b)	8	12	11	13
Endometrial stromal sarcoma	2	0	2	2
Adenocarcinoma	1	4	2	2
Mammary gland				
Adenoma	0	2	1	2
Adenocarcinoma	1	1	1	0
Kidneys				
Adenoma	1	0	0	0
Sarcoma	1	0	0	0
Urinary bladder*				
Adenoma	1	0	0	0
RHS*				
Malignant lymphoma	0	0	0	1
Histiocytary sarcoma	0	0	0	1
Intestine*				
Fibroma	0	0	0	1
Mesentery*				
Malignant mesothelioma	0	0	1	0
Skin				
Papilloma	0	0	0	1
Subcutis				
Haemangiosarcoma	1	0	0	0
Sarcoma	1	0	0	2

b = benign
m = malignant
* This organ was not routinely histologically investigated in all the rats from the 50 and 300 ppm groups.

Historical Control Data - Spontaneous Tumors in Wistar Rats (1981-1989)

NERVOUS S.

MALES

Experiment No.		1	2	3			6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	total
BRAIN																																
No. of rats examined		50	50	39	48	48			50	50	50	50	50	50	50	50	49	48	50	50	50	50	50	50	50	50	50	50	49	50	50	1464
meningioma	b	0	0	0	0	0	0			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
meningeal sarcoma	m	0	0	0	0	0	0			0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	6
glioma	m	1	0	0	1	0	1	0			0	0	0	0	0	1	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	8
granular cell tumour	b	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
granular cell tumour	m	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
sarcoma NOS	m	0	0	0	0	1	0	0	0			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
SPINAL CORD																																
No. of rats examined		50	50	39	40	40	49	50	50	50	50	50			50	50	49	48	50	50	47	50	47	50	50	50	50	50	49	50	50	1458
astrocytoma	m	0	0	0	0	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	2

FEMALES

Experiment No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15			18	19	20	21	22	23	24	25	26	27	28	29	30	total
BRAIN																																
No. of rats examined		50	49	40	40	39	50	50	50	50	50	50	50	50	49	50	49	50			50	50	50	50	50	50	50	50	49	50	50	1465
ependymoma	b	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0	0	0	1
meningioma	b	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0	0	0	1
meningeal sarcoma	m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				0	0	0	0	1	0	0	1	2
glioma	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				0	0	1	0	0	0	0	0	1
glioma	m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0			0	0	0	0	0	1	0	0	3
granular cell tumour	m	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0	1
neuroleonomal sarcoma	m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0			0	0	0	0	0	0	0	1

[Note: Granular cell tumors are believed to be of meningeal origin and are considered to be a subclassification of meningiomas.(Boorman et. al. 1990. eds. Pathology of the Fischer Rat. Academic Press, Inc.)]

Relevant nonneoplastic findings observed in this study included hypertrophy of the cells of the zona glomerulosa of the adrenal cortex of high dose males and females and increased incidence of progressive nephropathy in high dose females.

c Two-Week Intravenous Toxicity Study

Testing Facility:

Study Number: Not provided (Pharma Report No.7721)

Study Date: October, 1977

GLP Compliance: Not addressed

Animals: SPF Wistar albino rats, individually housed in Type II Macrolon cages, weighed 125 to 130 g at the initiation of dosing.

Dose Levels: 0, 0.1, 0.3 and 1.0 mg/kg. BAY K 5552, dissolved in a 10%:90% mixture of Cremophor EL and physiological saline, was administered as a single iv bolus injection (caudal vein; 1 ml/kg) daily for 14 consecutive days.

Number of Animals: 10/sex/group

Parameters Evaluated: Appearance and behavior (daily), body weight and food and water consumption (weekly), hematology and clinical chemistry (at the termination of the study; 5 rats/sex/group), urinalyses (after 10th treatment), major organ weights and gross and microscopic pathology (more than 30 different tissues/rat; 5 rats/sex from control and high dose groups).

Results: High dose animals showed inertia and dyspnea for about 5 to 15 min following drug administration. Two high dose females died during the study, one after the third dose and the other after the ninth dose. No treatment-related clinical signs or mortalities were seen in low and mid dose group animals.

Intravenous administration of nisoldipine had no significant effect on body weight, food or water consumption and hematologic, blood chemistry and urinalyses parameters. There were no treatment related gross or histopathological findings, or any evidence of local intolerance at dose levels tested in this study.

MOUSE STUDIES (X. Joseph)

a. 28-day Dietary Dose Rangefinding Study

Testing Facility:

Study Number: T 1003 576

Study Dates: June-July, 1981

GLP Compliance: Study was not conducted according to GLP regulations. The deviations were as follows: a. no phase 1-3 GLP audits, and b. no checking of physico-chemical properties of test substance.

Animals: SPF-bred NMRI mice, individually housed in Type I Macrolon cages, were 4-5 weeks old (average body weights: males-20.0 g, females-19.8 g) at the initiation of the study.

Dose Levels: Bay k 5552 (Batch No. 576,923) was mixed with powdered diet by the addition of peanut oil DAB 7 (1%) to obtain dietary drug concentrations of 0, 400, 800, 1200 and 1600 ppm.

Achieved Dose Levels:

Dose (ppm)	Average Drug Intake (mg/kg/day)	
	Male	Female
0	0	0
400	110	124
800	226	271
1200	348	383
1600	429	523

(Note: The drug intake was calculated from the average daily food intake/animal/group for the whole duration of the study.)

Number of Animals: 10/sex/group

Parameters Evaluated: Appearance and behavior (daily), body weight, food and water consumption (weekly), organ weights (heart, lungs, liver, spleen and kidneys) and gross pathology.

Results: No treatment-related clinical signs or mortalities were observed. Significant reductions in body weights (3-7%), compared to concurrent control, were seen in females throughout the study

at dose levels of 800 ppm and above except at 1200 ppm at the end of the study. In males, although body weights were lower than concurrent control (4-9%) at 1200 and 1600 ppm levels, the differences were statistically significant only at 1200 ppm. No significant differences were seen in food and water consumption between treated and control groups. Organ weight findings are given below.

Mean Organ Weights of Male and Female Mice

		Sex	Dose Group (ppm in diet)				
			0	400	800	1200	1600
Body Weight (g)		M	31.8	30.1	31.0	29.5*	30.3
		F	25.5	25.4	24.0*	24.7	24.5*
Heart	(Absolute, mg)	M	0.14	0.15	0.17**	0.15*	0.15
	(Relative, mg/100g)	M	0.44	0.52**	0.53**	0.53**	0.49*
	(Absolute, mg)	F	0.13	0.14	0.14	0.15*	0.14*
	(Relative, mg/100g)	F	0.51	0.54*	0.58**	0.61**	0.57**
Kidneys	(Absolute, mg)	M	0.46	0.46	0.49	0.49	0.48
	(Relative, mg/100g)	M	1.44	1.53	1.59	1.66*	1.58
	(Absolute, mg)	F	0.35	0.34	0.33	0.33	0.34
	(Relative, mg/100g)	F	1.35	1.32	1.38	1.33	1.37
Liver	(Absolute, mg)	M	1.93	1.84	1.82	1.74*	1.72*
	(Relative, mg/100g)	M	6.08	6.09	5.66	5.90	5.66*
	(Absolute, mg)	F	1.47	1.40	1.34	1.39	1.36
	(Relative, mg/100g)	F	5.72	5.53	5.55	5.64	5.56
Lung	(Absolute, mg)	M	0.23	0.26*	0.25	0.23	0.22
	(Relative, mg/100g)	M	0.72	0.88**	0.80**	0.78*	0.74
	(Absolute, mg)	F	0.22	0.21	0.20	0.23	0.22
	(Relative, mg/100g)	F	0.87	0.82	0.82	0.92*	0.89
Spleen	(Absolute, mg)	M	0.09	0.09	0.10	0.09	0.09
	(Relative, mg/100g)	M	0.28	0.31	0.32	0.30	0.29
	(Absolute, mg)	F	0.10	0.09	0.10	0.10	0.09
	(Relative, mg/100g)	F	0.38	0.37	0.41	0.38	(

** Significantly different from control at 0.05 level.*
*** Significantly different from control at 0.01 level.*

Relative heart weights in all treatment groups (both sexes) were significantly higher than control; however, absolute heart weights were significantly higher in males only at 800 and 1200

ppm and in females at 1200 and 1600 ppm levels. Both absolute and relative liver weights were lower than control in 1600 ppm males.

No significant gross findings were observed. Histopathological evaluations were not performed in this study.

Based on the results of this study, dietary dose levels of 100, 300 and 900 ppm were selected for the mouse carcinogenicity study.

b. 21 Month Carcinogenicity Study in Mice

Testing Facility:

Study Number: T7010709 (Sponsor's number)

Study Dates: Initiation of dosing - 10/7/81
Autopsy of last animal - 7/7/83

GLP Compliance: Studies were done in accordance with GLP regulations.

Animals:

Strain: Bor:NMRI (SPF HAN)
Sex: Both sexes
Age and Wt: 4 to 6 weeks old/20-22 g
Housing: Individually housed in Makrolon Type I cages.

Mode of Administration of Test Agent: Powdered diet.

Dose Levels: 0, 100, 300 and 900 ppm dosage levels of BAY k 5552 (Batch No. 662845, purity-98.1%) were used on the basis of results of the 28-day dietary dose rangefinding study. The stability and the concentration of drug in the diet were determined periodically. The concentrations of the drug in diet, at all intervals, were more than 89% of the theoretical values, and the compound was found to be stable in the diet for at least 10 days. However, there is no indication that concentrations of drug in diet were adjusted periodically to maintain a constant mg/kg body weight exposure.

No. of Animals: Equal numbers of males and females (50+20*/sex/dosage level) were used. *Additional 20 mice included in each group were sacrificed 12 months after the initiation of dosing for interim investigations.

Observations/Measurements:

Appearance/Behavior monitored twice daily and a detailed assessment of each individual animal was made on a weekly basis, with particular attention given to posture, general behavior, body surfaces, orifices and breathing and elimination products.

Body weight determinations were done at the beginning of the study, once a week until 27th week and every 2 weeks thereafter and also before the termination of the study.

Food intake was calculated on a weekly basis up to 23rd week and every two weeks thereafter.

Hematological (RBC, WBC, platelet and reticulocyte counts, differential white cell counts, hemoglobin, hematocrit, MCV, MCH and MCHC) and clinical chemistry [alkaline phosphatase, transaminases (ASAT and ALAT), plasma creatinine, urea, blood glucose, cholesterol, bilirubin and total plasma proteins] investigations were done at 12 months (interim sacrifice group) and also at the end of the study (10 animals/sex/treatment group).

Autopsies were done on all mice which died during the course of the study or that were killed in extremis, and also on those that were sacrificed at 12 months and at the termination of the study. Nine major organs were weighed and sections of various organs and tissues (about 38 different tissues/mouse) and gross lesions were preserved for histopathologic evaluation. At 12 months, these evaluations were done only on tissues from 0 and 900 ppm dosage groups and also on any tissue from 100 and 300 ppm groups which looked tumorous macroscopically. At the termination of the study, tissues from 0, 300 and 900 ppm dosage groups were examined histologically (only stomach, pituitary, uterus and liver were examined from 100 ppm group).

Statistical analysis on body weights, clinical laboratory values and organ weights were done using two-tailed U test according to Mann and Whitney, and Wilcoxon. The survival data were analyzed by the statistical software package using the generalized Wilcoxon test. Statistical analysis of tumor findings was done using the death rate method for malignant tumors and the prevalence method for benign tumors (Peto et al.). Because of the high mortality rate in high dose males, the death rate and the prevalence methods were used combined for the analysis of hepatocellular tumor data.

Interim Sacrifice:

Surviving animals from interim sacrifice groups (originally 20 mice/sex/dosage group) were killed at 12 months.

Achieved Dose Levels:

Average daily drug intake* (mg/kg body wt)

Sex	Dose (ppm) 0	100	300	900
Male		19.37	58.06	162.93
Female		24.99	74.36	217.28

(*The drug intake was calculated from the average daily food intake/animal/group for the whole duration of the study.)

Mortality:

Mortality data is summarized below and it is presented graphically in Figures 9 &10.

Mortality of Mice Receiving Nisoldipine in Diet for 21 Months

Daily Dose (ppm in diet)	Number of Mice (M/F)	Number of Dead (M/F)	% Mortality (M/F)
6 Months			
0	50/50	0/0	0/0
100	50/50	2/0	4/0
300	50/50	0/0	0/0
900	50/50	3/1	6/2
12 Months			
0	50/50	0/2	0/4
100	50/50	3/5	6/10
300	50/50	0/3	0/6
900	50/50	8/5	16/10
18 Months			
0	50/50	5/21	10/42
100	50/50	11/19	22/38
300	50/50	7/20	14/40
900	50/50	29/21	58/42
21 Months			
0	50/50	14/28	28/56
100	50/50	15/34	30/68
300	50/50	19/34	38/68
900	50/50	40/32	80/64

The mortality rates in treated females (all groups) were not significantly different from controls at any given interval. However, the incidence of deaths in females was high in all groups including controls from 12 months onward. The mortality rates in males from 900 ppm group, especially at 21 months, were significantly higher ($p<0.001$) compared to controls or other

Fig.9 : Mortality curves of male mice which received 21 months BAY k 5552 in the diet

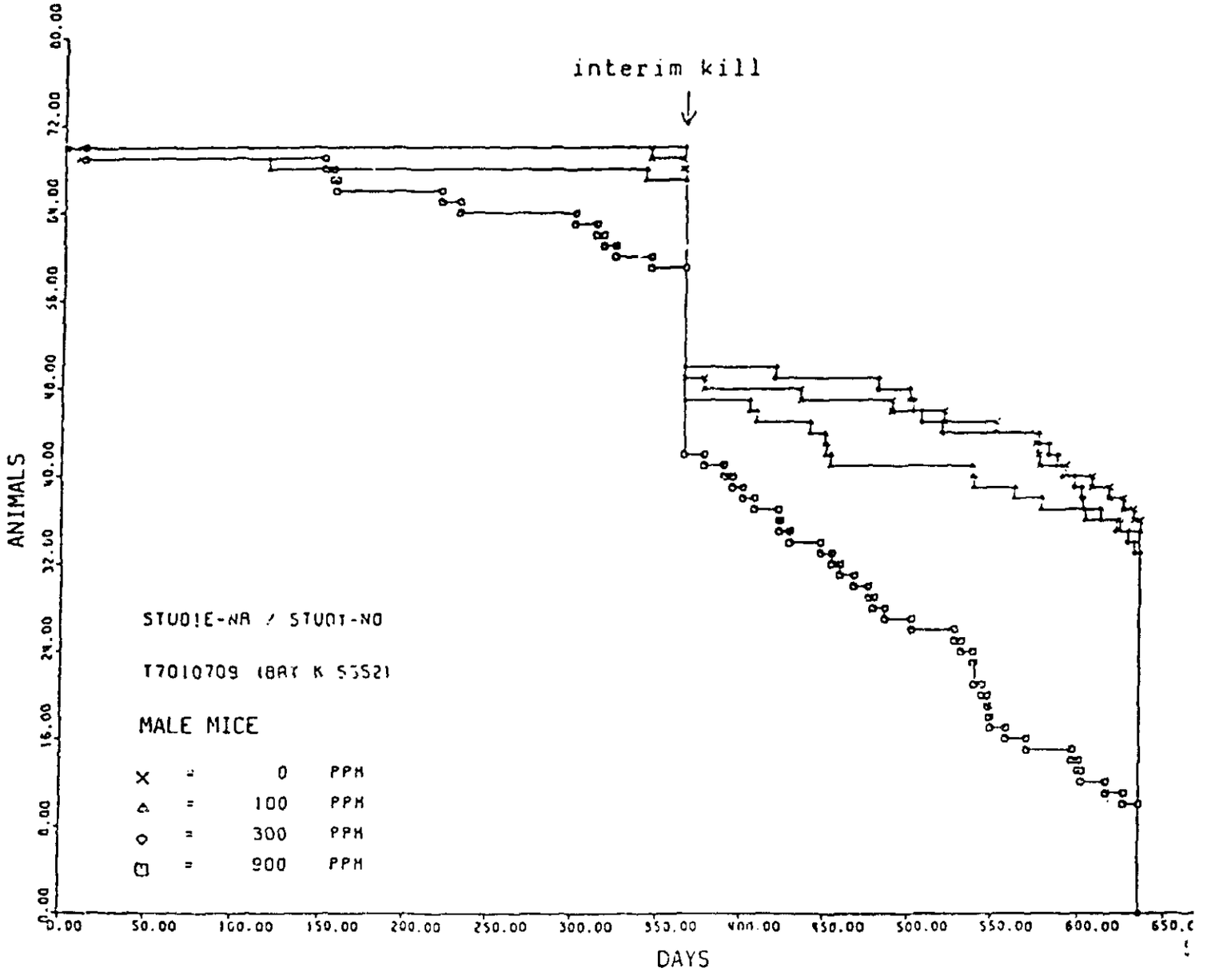
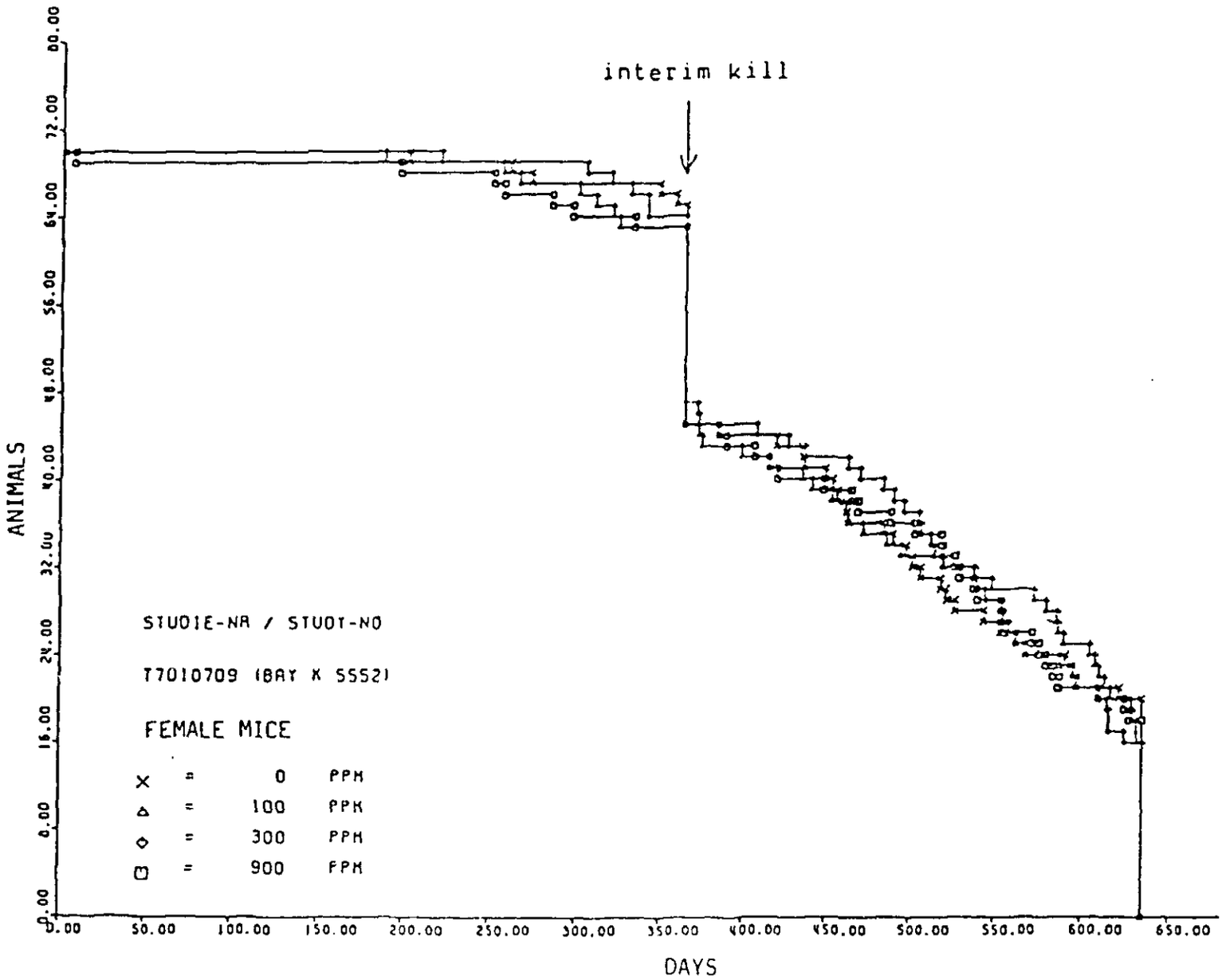


Fig. 10: Mortality curves of female mice which received 21 months BAY k 5552 in the diet



lower dosage groups. No significant difference noticed in this parameter between males of low or middle dosage groups and controls. The increased mortality of males in the high dose group is partly attributed to pharmacodynamically induced colonic atonies. Autopsy of animals that died or were killed in extremis frequently showed that the large intestine was tightly filled with solid faeces resulting from colonic atony.

Using the Cox and the generalized Wilcoxon methods for testing the heterogeneity in survival distribution, FDA statisticians observed a statistically significant difference (at 0.05 level) in the survival distribution in males, but not in females (for both of the above tests, the p values for males were <0.00001).

Drug Associated Findings:

No treatment related clinical signs were seen in this study. The food intake in males from the 900 ppm group was about 9% less than in control males. Average body weights are presented graphically in Figures 11 & 12. Statistically significant reductions in body weights at certain weeks were seen especially in males of 900 ppm group and to a lesser extent in 100 ppm group. However, at the termination of the study, no significant body weight differences were seen between treatment and control groups (both sexes). Leukocyte counts at 21 months were significantly lower ($p<0.01$) in males (900 ppm) and females (300 and 900 ppm groups) compared to respective controls. However, differential counts did not show any significant variations in the proportion of different cell types between treated and control mice. The hemoglobin and hematocrit values in mid and high dose males were significantly lower at 12 months, but not at 21 months. The blood glucose concentration was significantly higher ($p<0.01$) in males (300 and 900 ppm) at 12 months and also at 21 months (all treatment groups). Females showed a similar increase only at 12 months. All these values were reported to be within the range of historical control values. Significant elevations in blood urea levels were seen only at 12 months in all treated male groups and in high dose females.

Macroscopically, swollen gastric mucous membranes were observed more frequently in treated males than in controls (0 ppm - 5; 100 ppm - 12; 300 ppm - 14; 900 ppm - 14). The incidence of enlarged hearts was more in males of 900 ppm group (0 ppm - 7; 100 ppm - 2; 300 ppm - 11; and 900 ppm - 23). In mice that died or were killed in extremis, the incidence of large intestines impacted with solid feces was higher in both sexes at the highest dosage level (0 ppm - males 5 and female 0; 900 ppm - male 13 and female 9).

The heart and liver weights (absolute and relative) in males (21 months) were significantly increased at 300 and 900 ppm dose levels, but the weights of adrenals (absolute and relative) were significantly decreased in all treated male groups compared to controls. In females, the heart and liver weights were

Figure II : Body weight curves for male mice receiving BAY k 5552 in their food for 21 months

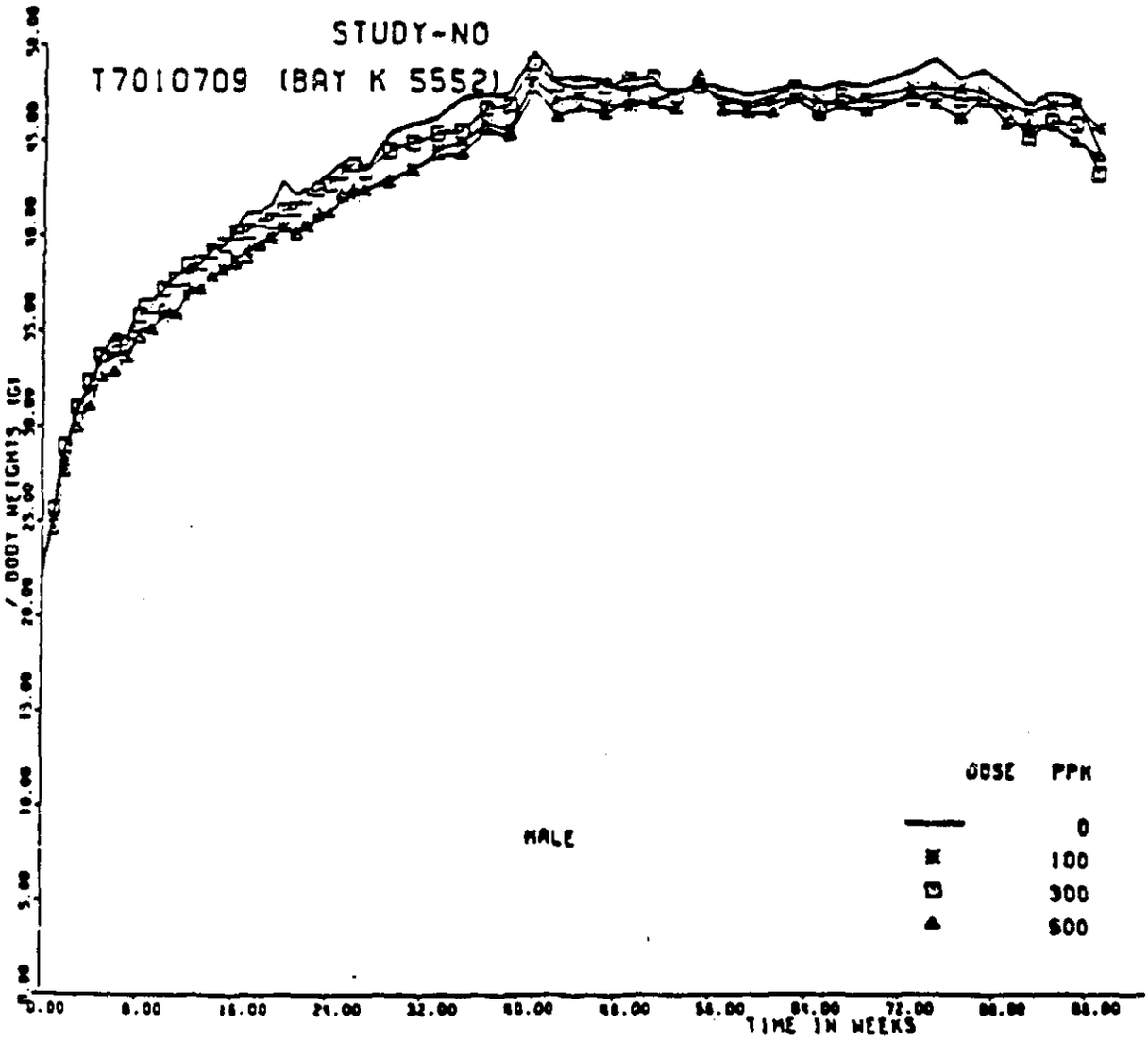
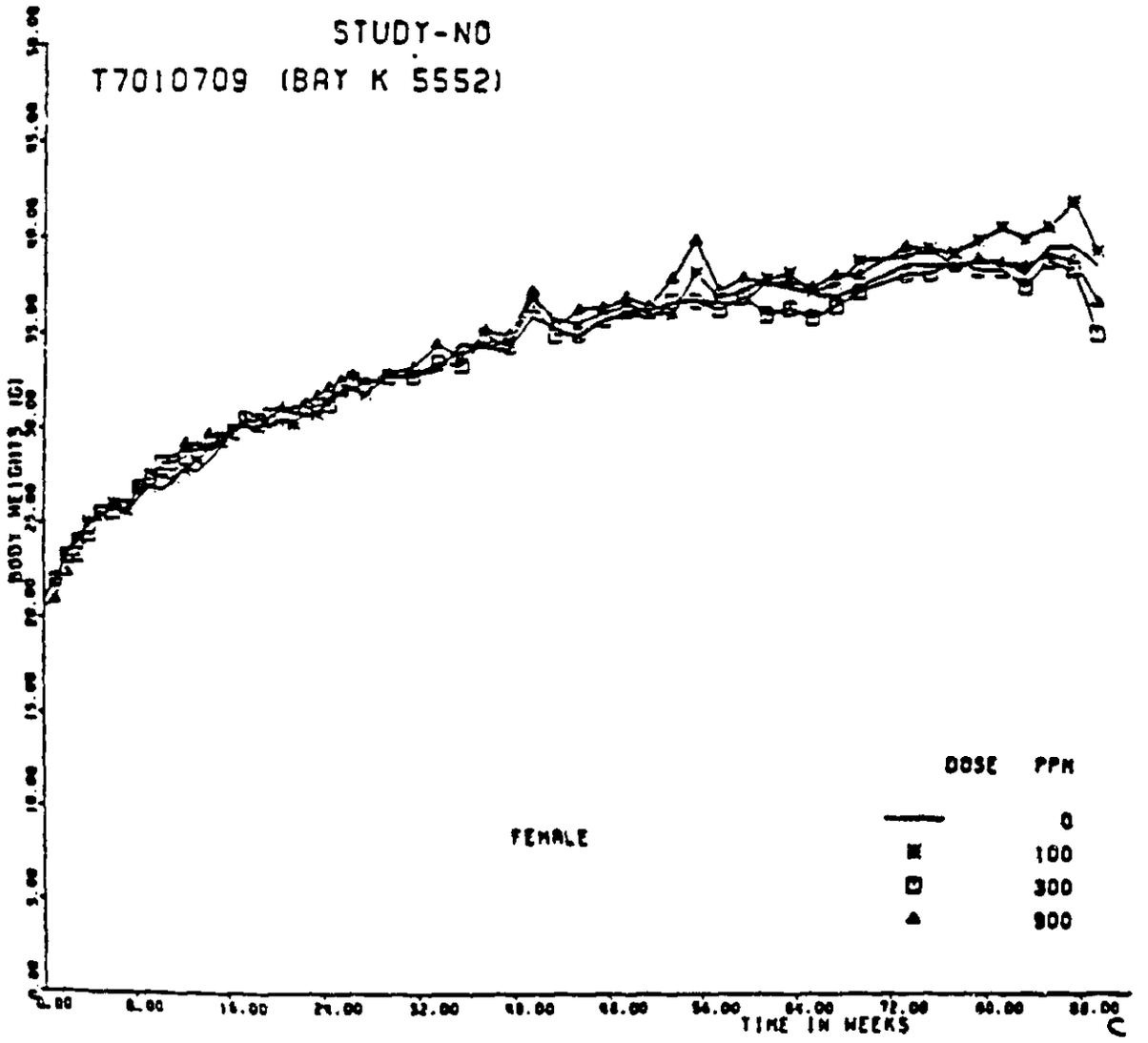


Figure12: Body weight curves for female mice receiving BAY k 5552 in their food for 21 months



significantly higher only at the highest dose level. Females that received either 300 or 900 ppm doses had significantly lower kidney weights at 21 months. Significantly increased liver weights (absolute and relative) were seen in mid and high dose females at the interim sacrifice.

Histologically, hyperplastic mucosa of the glandular stomach was found more frequent in treated males than in females. The incidence of this condition is given below.

Incidence of Hyperplastic Mucosa of the Glandular Stomach

Dose (ppm)	Percent affected	
	Male	Female
0	18	6
100	31	16
300	36	10
900	24	16

The above values are reported to be within the range of historical control values (Table 10).

A dose dependent increase in the occurrence of intracytoplasmic vacuoles near the nucleus was seen in the hepatic cells, more in females than in males. Round cell infiltrates in the kidney and senile nephropathy were predominantly found in females. The endometrium was often found to be hyperplastic (0 ppm - 13%; 100 ppm - 34%; 300 ppm - 33%; and 900 ppm - 28%). The above incidences are reported to be within the historical control ranges for this strain of mouse (Table). An increased incidence of pituitary hyperplasia was seen in females (0 ppm - 17%; 100 ppm - 13%; 300 ppm - 22%; 900 ppm - 42%).

The incidences of tumors observed at the interim sacrifice are given below.

Comparative Summary of Tumors at 12 Months According to Location, Type and Malignancy

Sex:	Males		Females	
Dose (ppm in diet):	0	900	0	900
Reticulocytary system: malignant lymphoma	1	1	2	4
Lung: alveologenic carcinoma (malignant)	0	2	1	2
Liver: hepatocellular carcinoma (malignant)	0	1	0	0
Stomach: kerato-acanthoma	0	0	1	0
Number of blastoma carriers	1	4	4	5
Number of malignant tumors	1	4	3	6
Number of benign tumors	0	0	1	0
Number of mice investigated	20	20	20	20

Table 10

Historic control values: NMRI mouse 1980 to 1984

	Number					
Test No.	1	2	3	4	5	6
Adenomatous gastric mucosal hyperplasias in males	9*	20	10	32	5	27
n	50	50	50	44	47	48
%	18	40	20	73	11	56
in females	1*	8	11	14	10	13
n	50	48	49	46	47	48
%	2	17	22	30	21	27
*classified as adenoma						
Liver tumour in males	7	3	9	5	1	6
n	50	50	50	45	46	48
%	14	6	18	11	2	12
Uterine hyperplasias	0	19	21	23	0	33
n	50	46	49	45	45	46
%	0	41	43	51	0	71

Glucose concentration in the plasma: 4.32 - 9.36 mmol/l (male)
4.51 - 7.75 mmol/l (female)
Urea concentration in the plasma: 5.96 - 15.12 mmol/l (male)
4.05 - 14.99 mmol/l (female)

n = Number of organs evaluated

The increased incidence of lymphoma of reticulohistiocytary system (RHS) observed in high dose females is considered to be incidental since the incidence of this tumor at 21 months was higher in the control group than in treated groups.

The overall incidences of benign and/or malignant tumors and the total number of tumor bearing animals (21 months) for both sexes at 0, 300 and 900 ppm dose levels are given in Table 11. There is no significant increase in the neoplasm incidence among treated animals of either sex compared to respective controls (sponsor's analysis). Moreover, there is also no difference in tumor occurrence between 300 and 900 ppm dosage groups. Incidence of tumors according to the location and the type is presented in Table 12. Because of the increased incidence of hepatocellular tumors in males especially in the 900 ppm group (only few females, 900 ppm group, had this type of tumor) at 21 months and also because of the occurrence of hepatocellular carcinoma in a male mouse from interim sacrifice, an additional investigation was carried out by examining more hepatic tissue sections (5 per animal) from male mice of each group for hepatocellular tumor occurrence. This second study showed additional cases of hepatocellular tumors as follows: 1 each from 100 and 900 ppm groups, 4 from 300 ppm and 1 from control groups. Combined incidences of these tumors (males) from the original and additional investigations (49-50 mice/group) and the p values from the trend test (death rate method) are given below. (The results of sponsor's statistical analyses of liver tumor data are summarized in Table 13.)

	Dose(ppm)				p value
	0	100	300	900	
hepatocellular adenoma	2	2	2	3	0.0735
hepatocellular carcinoma	3	4	5	8	0.0015
hepatocellular tumors (all)	5	6	7	11	0.0004

Thus, the sponsor's analysis showed significant positive linear trends (at 0.05 level) for the incidences of hepatocellular carcinoma and hepatocellular tumors (all) in male mice. Analysis of the tumor data by FDA statisticians showed that there were no significant positive linear trends for the incidences of hepatocellular carcinoma ($p=0.0762$) and hepatocellular tumors ($p=0.0514$) in male mice. According to FDA statisticians, the above discrepancies in p values observed in sponsor's and FDA analyses are attributed to "1. the sponsor did not apply the survival-adjusted method and 2. the ordinal dose levels 0, 1, 2 and 3 were used in sponsor's analysis."

Table II : Summary of number of male and female mice with benign and/or malignant tumours, as well as frequency of benign and malignant tumours-encountered

Sex	♂			♀		
Dose ppm	0	300	900	0	300	900
No. of animals investigated	50	50	49	48	50	50
No. of animals with tumours	27	28	24	34	31	30
No. of animals with only benign tumours	7	6	8	6	6	6
No. of animals with only malignant tumours	12	15	14	21	18	17
No. of animals with benign and malignant tumours	8	7	2	7	7	7
No. of animals with more than one primary tumour	15	11	5	13	9	8

Table 12 : Comparative summary of tumours occurring according to location, type, number and dignity $ (animals scheduled for terminal kill)

Sex	♂				♀			
Dose ppm	0	100	300	900	0	100	300	900
Lung:		-				-		
bronchiolo-alveolar adenoma	2		3	4	2		1	3
bronchiolo-alveolar carcinoma (malig.)	13		12	5	8		6	5
Stomach:								
papilloma	0	0	0	2	0	0	0	0
sarcoma (malig.)	0	0	2	1	0	0	0	0
Liver:								
hepatocellular adenoma	2	2	2	3	0	0	0	1
hepatocellular carcinoma (malig.)	3	4	5	8	0	0	0	1
haemangiosarcoma (malignant)	1	0	0	0	0	0	0	0
Kidneys:		-				-		
tubular carcinoma (malignant)	0		1	0	0		0	0
haemangiosarcoma (malignant)	0		1	0	0		0	0
Bladder:		-				-		
stromal tumour (benign)	1		1	2	0		0	0
stromal tumour (malignant)	0		1	1	0		0	0
Ovary:	-	-	-	-		-		
granulosa-theca cell tumour (ben.)					5		5	3
granulosa-theca cell tumour (malig.)					1		0	0
luteoma (benign)					2		2	0
tubular adeno-carcinoma (malig.)					1		0	0
Sertoli cell tumour (benign)					0		0	1
Uterus:	-	-	-	-				
adenoma					0	0	1	0
carcinoma (malig.)					1	0	0	0
fibroma					0	0	1	0
myoma					0	0	1	2
myosarcoma (malig.)					0	0	0	1
stromal tumour (benign)					3	0	2	2
stromal sarcoma (malignant)					1	3	2	2

Table 12 (continued):

Sex	♂				♀			
Dose ppm	0	100	300	900	0	100	300	900
Testes:		-			-	-	-	-
Leydig cell tumour (benign)	2		2	0				
adenoma of rete testis	1		0	0				
Pituitary:		-						
adenoma	2		0	0	0	3	3	1
Thyroid:		-				-		
follicle cell adenoma	0		1	0	0		0	0
papillary cyst-adenoma	1		0	0	0		0	0
Adrenals:		-				-		
cortical adenoma	3		3	1	2		0	0
phaeochromocytoma (benign)	1		0	0	1		0	2
phaeochromocytoma (malignant)	0		0	1	0		0	0
RH system:		-				-		
lymphoma (malig.)	7		3	1	18		12	14
lymph node sarcoma (malignant)	1		0	0	1		0	0
Skin/subcutis:		-				-		
epithelioma (malignant)	0		0	1	0		0	0
sarcoma (malig.)	1		0	0	0		3	0
Mammary gland:	-	-	-	-		-		
carcinoma (malig.)					3		0	0
adeno-ancanthoma (malignant)					0		1	1
Harder's gland:		-				-		
papillary adenoma	3		2	0	1		1	1
Spinal marrow:		-				-		
schwannoma (malig.)	0		0	0	0		1	0
Bones:		-				-		
osteosarcoma (malignant)	0		0	0	0		1	0
Abdomen:		-				-		
haemangiosarcoma (malignant)	0		0	0	0		1	0
Pelvic serosa:		-	-	-	-	-	-	-
sarcoma (malig.)	1							

- Organ not investigated $ Bilateral tumours counted twice

Table 13 : Statistical Analysis of Tumour Data

sex	target character	groups (ppm)	trend test	incidence	z	p
male	hepatocellular tumours	0/100/300/900	death-rate	5/ 6/ 7/11	3.321	0.0004
male	hepatocellular tumours	0/100/300/900	prevalence	5/ 6/ 7/11	1.833	0.0334
male	hepatocellular tumours	0/100/300	death-rate	5/ 6/ 7	0.821	0.2059
male	hepatocellular tumours	0/900	death-rate	5/11	3.430	0.0003
male	hepatocellular tumours	0/300	death-rate	5/ 7	0.855	0.1964
male	hepatocellular tumours	0/100	death-rate	5/ 6	0.374	0.3541
male	tumour, benign	0/300/900	prevalence	15/13/10	0.286	0.3876
male	tumour, malignant	0/300/900	death-rate	20/22/16	2.692	0.0035
male	hepatocellular adenoma	0/100/300/900	death-rate	2/ 2/ 2/ 3	1.450	0.0735
male	hepatocellular carcinoma	0/100/300/900	death-rate	3/ 4/ 5/ 8	2.970	0.0015
male	hepatocellular carcinoma	0/100/300/900	prevalence	3/ 4/ 5/ 8	1.931	0.0267
male	hepatocellular carcinoma	0/100/300	death-rate	3/ 4/ 5	0.876	0.1905
male	hepatocellular carcinoma	0/900	death-rate	3/ 8	3.067	0.0011
male	hepatocellular carcinoma	0/300	death-rate	3/ 5	0.919	0.1790
male	hepatocellular carcinoma	0/100	death-rate	3/ 4	0.434	0.3321
female	tumour, benign	0/300/900	prevalence	13/13/13	0.040	0.4842
female	tumour, malignant	0/300/900	death-rate	28/25/24	-0.257	0.6015

Historical Control Data - Spontaneous Tumors in NMRI Mice (1981-1988)

Experiment No.		1		2		3		4		5		6		7		8		9		10	
Sex		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f
Stomach																					
No. of mice examined		48	48	47	47	50	49	50	48	45	46	50	50	49	49	50	48	48	48	49	45
papilloma	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
adenomatous polyp	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
adenoma	b	0	0	0	0	0	0	0	0	0	0	9	1	0	0	0	0	0	0	0	0
adenocarcinoma	m	0	0	0	0	0	0	0	0	0	0	0	0	6	1	0	0	0	0	0	1
spindle cell carcinoma	m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Experiment No.		11		12		13		14		15		16		17		18		total		
Sex		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m + f
Stomach																				
No. of mice examined		48	47	49	50	50	50	49	48	50	49	49	47	47	45	47	46	875	860	1735
papilloma	b	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	2
adenomatous polyp	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
adenoma	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	1	10
adenocarcinoma	m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	2	8
spindle cell carcinoma	m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1

Historical Control Data - Spontaneous Tumors in NMRI Mice (1974-1979)

Number of tumors of the digestive system (salivary gland, liver, stomach, intestine).

Experiment No.	1		2		3		4		5		6		7		8		9		10		11		12	
	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f
Salivary gland																								
Squamous cell carcinoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Adenoma	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Adenocarcinoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Liver																								
Adenoma, hepatocellular	1	0	4	0	2	0	3	0	0	0	0	0	2	0	3	0	4	1	9	0	1	1	3	0
Carcinoma, hepatocellular	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
Sarcoma	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Forestomach																								
Papilloma	1	0	3	1	0	0	1	2	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	0
Squamous cell carcinoma	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0
Glandular stomach																								
Adenoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	0	0	0	0
Adenocarcinoma	0	0	0	0	0	0	0	0	2	0	0	0	1	1	2	0	1	0	0	0	1	1	0	1

Experiment No.	1	2	3	4	5	6	7	8	9	10	11	12
Number of male animals at start	58	75	75	40	40	40	50	50	50	51	50	50
Number of females animals at start	58	75	75	40	40	40	50	50	50	49	50	50
Number of male animals evaluated	56	75	75	40	40	36	48	47	46	51	50	50
Number of female animals evaluated	56	73	75	40	40	36	45	37	42	49	49	49

FDA analysis of tumor data showed a significant positive linear trend for inverted papilloma of pars cutanea of the stomach in male mice (p=0.0072). The incidence of the above tumor is as follows: 0 ppm - 0/50; 100 ppm - 0/49; 300 ppm - 0/50; and 900 ppm - 2/50. Pairwise comparison also showed significant difference between high dose and control groups (p=0.0435). Historical control data from 21 month studies in NMRI mice, conducted during a 7 year period from July 1981 to August 1988, showed that papillomas of the stomach occurred in 2 of the 18 studies evaluated (page 65e), in 1/49 males and 1/47 females examined (amendment to original application dated May 31, 1994). Moreover, incidence rates upto 4% were seen for the above tumor in NMRI control male mice in carcinogenicity studies conducted between 1974 and 1979 (page 65f). Although statistically significant, the incidence rate (4%) observed in the present study for the stomach papilloma is considered to be within the historical control range for NMRI mice.

Significant positive linear trends were also reported by FDA statisticians for the urinary bladder benign stromal tumor in male mice and RHS malignant lymphoma* in females. However, when the incidences of urinary bladder stromal tumors are combined (benign + malignant, benign + polypous, or benign + malignant + polypous tumors), no statistically significant trend was seen. In the case of RHS malignant lymphoma also, if all malignant lymphomas of different locations are combined, then, no significant linear trend was observed.

*Note: The sponsor has listed <u>all</u> malignant lymphomas, irrespective of locations, under <u>RHS system</u>; however, for some lymphomas, the anatomic site (organ) is specified (e.g. lymphoma of adrenal or heart etc.) but for others no site is given (listed only as lymphomas). By using the combined incidences of all lymphomas, no treatment-related increased incidence of this tumor was seen in sponsor's statistical analysis. [According to NTP guidelines (McConnel et al, 1986. Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. JNCI 76: 283-289), lymphomas of all types can be combined for statistical evaluation.]

DOG STUDIES (S.Stolzenberg)

a. 4-Week Oral Administration Study

Testing Facility:

Pharma-Report No: 7075
Study No: Not given

Study Dates: 11/8/76 to 12/9/76

GLP compliance: This study predates GLP compliance requirements.

Animals: Purebred beagles, 2 males and 2 females per group were used. At the start of dosing, the animals were 25 to 30 weeks old, with body weights of 7.4 to 11.8 kg.

Dose Levels/Mode of Administration: The test substance (Batch 2/76) was administered at doses of 0, 1, 3 and 10 mg/kg, once daily, 4 to 6 hours before feeding, in a vehicle of polyethylene glycol 400, glycerol and water, in gelatin capsules.

Observations/Measurements: Body weights were obtained before the start of treatment and weekly thereafter. Appearance, behavior, body posture, appetite and feces were checked daily. Neurological investigations (pupillary reflex, patellar reflex and extensor postural reflex) and body temperature measurements (rectal) were conducted pretreatment and after 2 and 4 weeks. Ophthalmoscopy (direct) was performed at pre-treatment and after 4 weeks. ECG measurements (Leads I, II and III) were recorded on the 1st, 11th and 23rd day, immediately before administration and 1 and 24 hours after administration. Femoral artery blood pressure was measured on the 1st, 11th and 23rd day, before administration and 1 and 24 hours after, via a Stratham element, Hellige measuring bridge and Hellige recorder. Blood and 6 hour urine samples were obtained before treatment, then after 1 and 4 weeks, for hematology, blood chemistry and urinalysis. Post-mortem examination included weights of 12 or 13 major organs (including gonads and prostate), gross pathology and complete histopathology (31 or 32 organs).

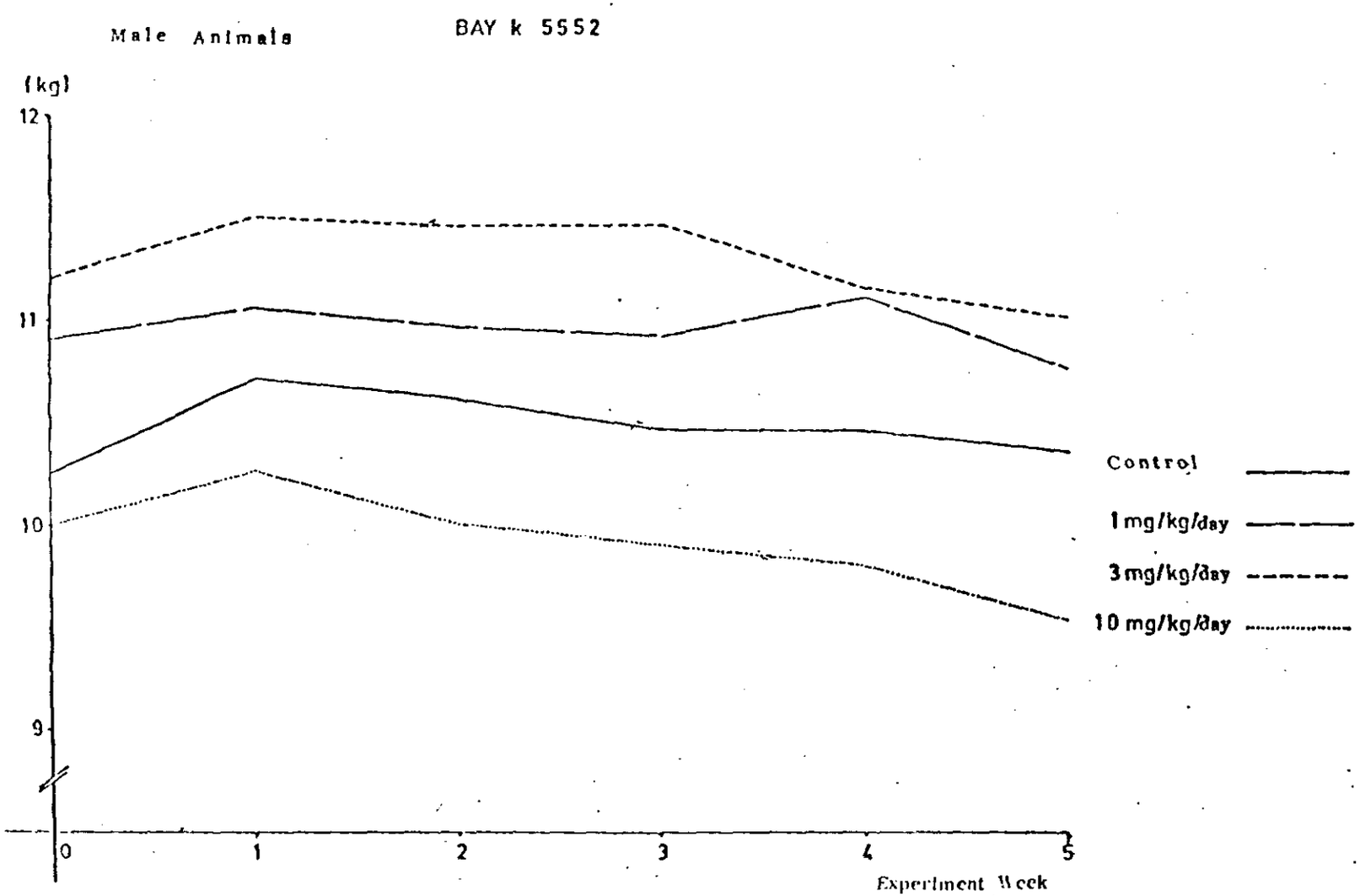
Mortality: There were no deaths.

Drug Associated Findings: Slightly reduced weight gain was observed in the high dose males, with a reduced food consumption in both high dose females and in one high dose male, from the middle of the third week to the end of the study. In the 10 mg/kg treated animals, a distinct ST drop (manifestation of a possible myocardial ischemia) was observed in one male 1 hour after the 1st and 23rd dose, and in one female 1 hour after the 1st dose. No treatment related effects on P or Q waves or QRS complex were observed at any time. Heart rates determined from

ECGs, showed dose dependent increases one hour after dosing on days 1, 11 and 23, and with the high dose, bradycardia was still evident 24 hours after dosing on days 11 and 23. Systolic and diastolic blood pressures at 1 hour post dosing were decreased by a mean of 30 to 50% in all treated groups (dose dependent) on days 1, 11 and 23. As a rule, blood pressures returned to pre-treatment levels by 24 hours after treatment, except after day 1, when they remained lower for the 1 and 10 mg/kg groups.

Although no gross pathology or organ weight changes due to treatment were noted, histopathology revealed that the hearts of both females and 1 of the 2 males on the high dose had myocardial scars in one or both left ventricular papillary muscles. The effect was attributed to hypoxic damage related to vasodilator-induced heart rate increase, "a known damage mechanism in the dog". The ST drops noted above were observed in two of the dogs with myocardial scars. The ST drops and the bradycardia (which was most pronounced in a male with the most severe lesions) were attributed to the heart muscle damage.

Weight Gains of the Male Dogs. The weights were measured in each case at the end of the experimental week.



BAY k 5552

Heart Rate (Beats per Minute)

(Average Values)

Dose mg/kg	Time of Investigation	Before Administration	1 Hour After Administration	% Deviation from 1-Hour Value	24 Hours After Administration
0	Preliminary Investigation	136			
	1st Administration	148	115	- 22	140
	11th Administration	118	108	- 8	125
	23rd Administration	123	123	0	118
1	Preliminary Investigation	158			
	1st Administration	143	253	+ 77	160
	11th Administration	120	235	+ 96	113
	23rd Administration	113	223	+ 97	110
3	Preliminary Investigation	128			
	1st Administration	133	193	+ 47	143
	11th Administration	113	213	+ 88	110
	23rd Administration	98	213	+ 117	90
10	Preliminary Investigation	133			
	1st Administration	133	210	+ 58	148
	11th Administration	68	223	+ 228	75
	23rd Administration	80	200	+ 150	81

Blood Pressure (mmHg)

(Average Values)

Dose mg/kg	Time of Investigation	Before Administration		1 Hour After Administration		% Deviation from 1-Hour Value		24 Hours After Administration	
		s	d	s	d	s	d	s	d
0	Preliminary Investigation								
	1st Administration	172	107	179	99	+ 4	- 7	181	101
	11th Administration	176	95	171	102	- 3	+ 7	174	110
	23rd Administration	181	78	191	96	+ 6	+ 24	187	97
1	Preliminary Investigation								
	1st Administration	171	95	115	63	- 33	- 34	116	83
	11th Administration	176	101	96	54	- 45	- 47	181	110
	23rd Administration	173	94	114	68	- 34	- 28	193	118
3	Preliminary Investigation								
	1st Administration	177	101	99	52	- 44	- 49	179	96
	11th Administration	178	106	78	49	- 56	- 54	176	109
	23rd Administration	178	99	107	53	- 40	- 46	188	96
10	Preliminary Investigation								
	1st Administration	177	94	114	51	- 36	- 47	149	74
	11th Administration	194	111	98	51	- 49	- 54	194	111
	23rd Administration	203	111	116	53	- 43	- 52	231	133

s = systolic pressure d = diastolic pressure

Histological Data

BAY k 5552, Oral, Dogs (2 Week Experiment)

Animal No.	Sex	Dose and Frequency of Administration	Heart	Lung	Liver	Spleen	Kidney	Adrenals
F 813	♂	Control (0 mg/kg)	0	Ici +	0	0	0	0
F 823	♂	"	0	Ici +	Ici +	0	0	0
F 800	♀	"	0	Ici +	Ici +	0	0	0
F 802	♀	"	0	Ici +	Ici +	0	0	0
F 807	♂	10 mg/kg	Fi2	Ici +	0	0	0	0
F 817	♂	"	0	Ici +	0	0	0	0
F 814	♀	"	Fi1-2	Ici1	Ici +	0	0	0
F 818	♀	"	Fi +	Ici2	Ici +	0	0	V+

List of Abbreviations

Histological Data

At	=	Atrophy
Cy	=	Cyst
Fi	=	Focal fibrosis with isolated mononuclear cells (Figures 4 and 5)
Ici	=	Cellular or inflammatory-cellular infiltration
P	=	Parasitic lesion (bore hole, granuloma, eosinophilic infiltration)
0	=	Finding within the normal variability, which, in particular, corresponds to the species and to the age of the experimental animals and to their conventional housing conditions
∅	=	Not investigated (section missing)
Pi	=	Yellow-green (hematogenous) pigment
Th	=	Thrombus
V	=	Cytoplasmic vacuoles

Intensity of the Changes

+	=	very slight, indicated
1	=	slight
2	=	moderate
3	=	severe

b. 13-Week Oral Administration Study in Dogs

Pharma-Report No: 10,380
Study No: B/K 5552/023

Performing Laboratory:

Dates Performed: 8/21/80 to 11/25/80

Quality Assurance: No statement of GLP compliance is included.

Test Animals: Purebred beagles, 3 males and 3 females per group, were used. At the start of dosing, the animals were 38 to 51 weeks old, with body weights of 6.8 to 10.8 kg.

Dose Levels/Mode of Administration: The test substance (Batch 576,923) was administered at doses of 0, 1, 2.5 and 6.25 mg/kg, once daily, 4 to 6 hours before feeding, in a vehicle of polyethylene glycol 400, glycerol and water, in gelatin capsules.

Observations/Measurements: Body weights were obtained before the start of treatment and weekly thereafter. Appearance, behavior, body posture, appetite and feces were checked daily. Neurological examinations (pupillary reflex, patellar reflex and extensor postural reflex), ophthalmoscopic examinations (direct) and body temperature measurements (rectal) were performed pretreatment and after 2, 5 and 12 weeks. Femoral artery blood pressure was measured at the time of the first dose, and in weeks 3, 6 and 13, before administration, and 1 and 24 hours after, via a Stratham element, Hellige measuring bridge and Hellige recorder; ECG measurements (Leads I, II and III) were recorded at the same time periods. Blood and 6 hour urine samples were obtained before treatment, then after 3, 6 and 13 weeks, for hematology, blood chemistry and urinalysis. Postmortem examination included weights of 12 (female) or 13 (male) organs, gross pathology and complete histopathology (31 or 32 organs for control and high dose, but all 3 doses for heart).

Mortality: There were no deaths.

Drug Associated Findings: Circumoral reddening of the skin and reddening of the conjunctiva in the mid and high dose groups, and ataxia in the high dose group, occurred regularly throughout the treatment period, around 1 hour after dosing. Blood pressure decreased (systolic decreased to a greater extent than the diastolic), and heart rate increased (data on heart rate not provided by sponsor) at 1 hour post dosing in all 3 treated groups. Neither of these two effects were considered to be dose related, and values returned to pretreatment levels by 24 hours post-treatment. No changes in ECG occurred at low and mid doses. One high dose male developed a ventricular tachycardia with a "bundle-branch-block-like deformation of the QRS complex",

diagnosed 1 hour after the first dose. For this animal, another ECG was taken on the following day 2 hours after dosing; the P wave was still elevated and the ST segment again showed sagging depression. "On the 19th day in this dog, no pathological finding in the ECG was observed" but this animal showed extra systoles and an elevated P wave. Serum chemistry effects included a small increase in GOT during week 6. The only compound related post-mortem finding noted was scaring of the left ventricular papillary muscles of 1 male and 1 female at the high dose, and 1 female at the mid dose. Histopathology revealed focal fibrosis with isolated mononucler cells and a cellular and inflammatory-cellular infiltration.

Study No.: B/K 5552/023

Blood Pressure (mm Hg) - Average Values

Dose mg/kg	Time of Examination	Before administration		1 hour after administration		% Deviation of 1 hour value		24 Hours after administration	
		s	d	s	d	s	d	s	d
0	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration	210	130	180	110	-14	-15	180	110
	in the 3rd week	200	120	190	120*	- 5	0	205	125**
	in the 6th week	225	135	170	90	-24	-33	165	105**
	in the 13th week	200	120	205	130	+ 2	+ 8	195	120*
1.0	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration	190	115	140	75*	-26	-35	175	115
	in the 3rd week	195	115	135	75	-31	-35	190	120
	in the 6th week	185	115	85	50	-54	-57	175	100**
	in the 13th week	190	120	130	80	-32	-33	195	115**
2.5	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration	190	105	145	70	-24	-33	180	105
	in the 3rd week	195	115	115	60	-41	-48	200	120
	in the 6th week	195	115	100	50	-49	-56	175	105
	in the 13th week	195	120	130	70	-33	-42	175	110
6.25	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration	185	115	110	60	-41	-48	185	125
	in the 3rd week	190	115	110	55	-42	-52	170	110**
	in the 6th week	185	115	80	45	-57	-61	170	105
	in the 13th week	195	120	100	60**	-49	-50	190	125

s = systolic blood pressure; d = diastolic blood pressure *n = 5; **r = 4

BAY k 5552/Study 023

Animal No.	Sex	Dose	Esophagus	Stomach	Intestine	Mesenteric Lymph Nodes	Thymus	Gall-bladder	Urinary Bladder
K 103	♂	Control	0	0	0	0	0	0	0
K 109	♂	Control	0	0	0	ø	At3	0	0
K 121	♂	Control	0	0	0	0	0	0	0
K 108	♀	Control	0	0	0	0	0	0	0
K 112	♀	Control	0	0	0	0	At2	0	0
K 120	♀	Control	0	0	0	0	ø	0	0
K 93	♂	6.25 mg/kg	0	0	0	0	ø	0	0
K 115	♂	6.25 mg/kg	0	0	0	0	0	0	0
K 117	♂	6.25 mg/kg	0	0	0	0	ø	0	0
K 102	♀	6.25 mg/kg	0	0	0	P/Ici1	At1	0	0
K 104	♀	6.25 mg/kg	0	0	0	P/Ici2	0	0	0
K 118	♀	6.25 mg/kg	0	0	0	0	0	0	0

BAY k 5552/Study 023

Animal No.	Sex	Dose	Heart	Animal No.	Sex	Dose	Heart
K 103	♂	Control	0	K 113	♂	2.5 mg/kg	0
K 109	♂	Control	0	K 119	♂	2.5 mg/kg	0
K 121	♂	Control	0	K 123	♂	2.5 mg/kg	0
K 108	♀	Control	0	K 122	♀	2.5 mg/kg	F11 P1+
K 112	♀	Control	0	K 124	♀	2.5 mg/kg	0
K 120	♀	Control	0	K 126	♀	2.5 mg/kg	0
K 93	♂	6.25 mg/kg	0	K 79	♂	1.0 mg/kg	0
K 115	♂	6.25 mg/kg	F12-3 Ic11	K 105	♂	1.0 mg/kg	0
K 117	♂	6.25 mg/kg	0	K 107	♂	1.0 mg/kg	0
K 102	♀	6.25 mg/kg	0	K 110	♀	1.0 mg/kg	0
K 104	♀	6.25 mg/kg	F11	K 114	♀	1.0 mg/kg	0
K 118	♀	6.25 mg/kg	0	K 116	♀	1.0 mg/kg	0

c. 52-Week Oral Administration Study in Dogs

Study No: T 20 10 506

Performing Laboratory:

Dates Performed: July 13, 1981 to July 11, 1982

Quality Assurance: No statement of GLP compliance is included.

Test Animals: Purebred beagles, 4 males and 4 females per group. At the start of dosing, the animals were 38 to 51 weeks old, with body weights of 6.9 to 11.2 kg.

Dose Levels/Mode of Administration: The test substance (batch 57 69 23) was administered at doses of 0, 0.3, 1.0 and 3.0 mg/kg, once daily, 7 days per week, 4 to 6 hours before feeding, in a vehicle consisting of 85.3% polyethylene glycol 400, 4.8% anhydrous glycerol and 9.9% water, contained in gelatin capsules.

Observations/Measurements: Body weights were obtained before the start of treatment and weekly thereafter. General appearance was checked "several times a day". Neurological exams were conducted and body temperatures were checked pretreatment and after 3, 6, 17, 29, 39 and 50 weeks. Ophthalmoscopy was performed pre-treatment and after 12, 31, 38 and 51 weeks. Blood pressure and ECG were measured pre-treatment and after 3, 6 and 17, 29, 39 and 50 weeks, before dosing, then 1 and 24 hours after dosing. The methods and instruments used were the same as in the preceding dog studies. Blood and 6 hour urine samples were obtained before treatment, then after 3, 6, 13, 26, 39 and 52 weeks, for hematology, blood chemistry and urinalysis. Postmortem examination included weights of 11 or 12 organs, gross pathology and complete histopathology (31 or 32 organs for all animals on test). Liver enzyme induction of O-demethylase, N-demethylase and cytochrome P_{450} content of liver homogenates were measured.

Mortality: No deaths occurred.

Drug Associated Findings: Slight reddening of the mucosa and skin, observed in all nisoldipine treated groups, was considered to be due to the vasodilator effect of the drug. Dose related decreases in blood pressure and resultant increases in heart rate were observed. Twenty-four hours after dosing, all values had returned to normal. Slight ST segment depression, T wave inversion and QT segment shortening were observed, which were all reversible (data on ECG could not be found) and considered to be due to increased heart rate.

Study No: T 20 10 506

BLOOD PRESSURE (mm Hg)

(Means n = 8)

DOSE	TIME	before admin.	1 h after admin.	% difference **	24 h after admin.
0.0 mg/kg	1st admin.	195/112	198/113	+1.5/+0.9	199/114
	17th admin.	194/112	193/119	-0.5/+6.3	
	38th admin.	184/102	187/106	+1.6/+3.9	
	114th admin.	210/116	205/116	-2.4/0.0	
	200th admin.	199/108	204/113	+2.5/+4.6	
	269th admin.	211/108	195/110	-7.6/+1.9	
	347th admin.	201/118	204/119	+1.5/+0.8	
0.3 mg/kg	1st admin.	208/119	*167/ 97	-19.7/-18.5	193/115
	17th admin.	203/111	164/ 98	-19.2/-11.4	
	38th admin.	180/ 98	163/ 88	- 9.4/-10.2	
	114th admin.	219/118	171/ 98	-21.9/-16.9	
	200th admin.	191/113	153/ 86	-19.9/-23.9	
	269th admin.	205/114	168/ 90	-18.0/-21.1	
	347th admin.	215/124	184/105	-14.4/-15.3	

* n = 7

** Calculation using unrounded figures

Study No: T 20 10 506

BLOOD PRESSURE (mm Hg)

(Means n = 8)

DOSE	TIME	before admin.	1 h after admin.	% difference**	24 h after admin.
1.0 mg/kg	1st admin.	179/104	141/ 76	-21.2/-26.9	173/104
	17th admin.	177/101	136/ 76	-23.2/-24.8	
	38th admin.	181/ 98	116/ 68	-35.9/-30.6	
	114th admin.	184/106	133/ 75	-27.7/-29.0	
	200th admin.	178/107	136/ 79	-23.6/-26.3	
	269th admin.	196/109	135/ 73	-31.1/-33.0	
	347th admin.	195/115	161/ 93	-17.6/-19.1	
3.0 mg/kg	1st admin.	195/114	116/ 64	-40.7/-43.9	194/115
	17th admin.	200/127	133/ 71	-33.5/-44.1	
	38th admin.	186/112	*106/ 59	-43.0/-47.3	
	114th admin.	212/126	124/ 66	-41.6/-47.6	
	200th admin.	197/119	133/ 76	-32.5/-36.1	
	269th admin.	218/123	115/ 62	-47.2/-49.6	
	347th admin.	206/124	134/ 69	-35.0/-44.4	

* n = 7

** Calculation using unrounded figures

Study No: T 20 10 506

HEART RATES (beats/min)

(means, n = 8)

(calculation using unrounded figures)

DOSE (mg/kg)	Initial Figure	TIME OF INVESTIGATION															
		1st admin.			17th admin.		38th admin.		114th admin.		200th admin.		269th admin.		347th admin.	
		before	1 h	24 h	before	1 h	before	1 h	before	1 h	before	1 h	before	1 h	before	1 h
0.0	134*	120	113*	134	128	132*	136	133	133	121	129	138	119	119	101**	112
Diff %			-5.9		+3.1		-2.2		-9.0		+7.0		0.0		+10.9	
0.3	138	136	172	141	134	190	137	194	123	177	128	205	118*	178	113*	161
Diff %			+26.5		+41.8		+41.6		+43.9		+60.2		+50.8		+42.5	
1.0	147	134	191	135	130	209	131	218	106	221	113	220	96	207	111	153
Diff %			+42.5		+60.8		+66.4		+108.5		+94.7		+115.6		+37.8	
3.0	146	134	211	128	120	234	113	201	101	224	99	215	90	209	93	186*
Diff %			+57.5		+95.0		+77.9		+121.8		117.2		+132.2		+100.0	

*n = 7
**n = 6

CHRONIC TOXICITY STUDY ON DOGS

B A Y K 5 5 3 2 STUD.T2 010 506

SYNOPSIS OF GROUP MEANS

		HEART / HERZ	LUNG / LUNGE	LIVER / LEBER	KIDNEYS / NIEREN	SPLEEN / MILZ	TESTES / HODEN	OVARIES / OVARIEN	THYROID / SCHILD-DRUESE	ADREN. / NEBEN-NIEREN	THYMUS / THYMUS	PROSTA. / PRO-STATA	BRAIN / GEHIRN	PANC. / PAN-KREAS
ABSOLUTE ORGANWEIGHTS (G) / ABSOLUTE ORGANGEWICHTE (G)														
MALES / MN.TIERE														
CNTR./KONTR.		98.6	96.8	438.3	57.0	30.3	17.30	-	0.825	1.277	5.15	8.087	78.8	33.0
GROUP/GRUPPE	I	108.0	91.8	423.0	56.5	29.8	18.38	-	0.727	1.162	5.32	6.910	75.5	35.0
GROUP/GRUPPE	II	103.3	100.5	484.3	66.3	37.3	20.92	-	0.860	1.335	5.20	5.707	82.3	36.0
GROUP/GRUPPE	III	111.3	93.8	472.0	61.3	68.0	20.42	-	0.805	1.310	5.65	7.340	78.5	33.5
FEMALES / WB.TIERE														
CNTR./KONTR.		95.8	86.0	409.8	53.0	26.3	-	0.937	0.867	1.385	6.96	-	76.5	25.5
GROUP/GRUPPE	I	102.8	96.5	385.5	57.5	57.5	-	0.832	0.797	1.550	7.27	-	73.3	29.5
GROUP/GRUPPE	II	96.8	82.5	393.5	53.3	38.0	-	1.123	0.807	1.672	7.67	-	77.8	32.3
GROUP/GRUPPE	III	103.0	90.3	393.8	59.3	41.0	-	1.787	0.860	1.587	7.17	-	77.0	31.0
BOTH SEXES / ALLE TIERE														
CNTR./KONTR.		97.3	91.4	424.3	55.0	28.3	17.30	0.937	0.846	1.331	6.05	8.087	77.6	29.3
GROUP/GRUPPE	I	105.4	94.1	404.3	57.0	43.6	18.38	0.832	0.762	1.356	6.30	6.910	74.4	32.3
GROUP/GRUPPE	II	100.0	91.5	438.9	59.8	37.6	20.92	1.123	0.834	1.504	6.44	5.707	80.0	33.9
GROUP/GRUPPE	III	107.1	92.0	432.9	60.3	54.5	20.42	1.787	0.832	1.449	6.41	7.340	77.8	32.3
RELATIVE ORGANWEIGHTS (G/KG) / RELATIVE ORGANGEWICHTE (G/KG)														
MALES / MN.TIERE														
CNTR./KONTR.		9.12	9.00	40.25	5.25	2.85	1.667	-	0.0780	0.1200	0.480	0.7577	7.42	3.05
GROUP/GRUPPE	I	10.17	8.62	39.77	5.32	2.85	1.740	-	0.0687	0.1100	0.514	0.6585	7.15	3.30
GROUP/GRUPPE	II	9.15	8.87	42.67	5.85	3.30	1.842	-	0.0757	0.1175	0.462	0.5085	7.30	3.20
GROUP/GRUPPE	III	10.02	8.45	42.75	5.52	6.25	1.842	-	0.0730	0.1180	0.510	0.6627	7.10	3.05
FEMALES / WB.TIERE														
CNTR./KONTR.		9.50	8.55	40.77	5.25	2.50	-	0.0912	0.0865	0.1372	0.667	-	7.65	2.55
GROUP/GRUPPE	I	9.37	8.85	39.88	5.25	5.20	-	0.0755	0.0722	0.1415	0.652	-	6.70	2.65
GROUP/GRUPPE	II	9.72	8.25	44.17	5.32	3.77	-	0.0869	0.0812	0.1690	0.767	-	7.82	3.22
GROUP/GRUPPE	III	9.87	8.62	37.32	5.65	3.85	-	0.1642	0.0805	0.1535	0.670	-	7.45	3.05
BOTH SEXES / ALLE TIERE														
CNTR./KONTR.		9.31	8.77	40.51	5.25	2.67	1.667	0.0912	0.0822	0.1286	0.556	0.7577	7.54	2.80
GROUP/GRUPPE	I	9.77	8.74	41.18	5.29	4.02	1.740	0.0755	0.0705	0.1257	0.575	0.6585	6.92	2.97
GROUP/GRUPPE	II	9.44	8.56	43.42	5.56	3.54	1.842	0.0869	0.0785	0.1432	0.615	0.5085	7.56	3.21
GROUP/GRUPPE	III	9.95	8.54	40.04	5.59	5.05	1.842	0.1642	0.0767	0.1358	0.590	0.6627	7.27	3.05

REPRODUCTIVE TOXICITY STUDIES (S. Stolzenberg)

1. Fertility and Reproduction Ability in Wistar Rats

Bayer Study No: T0002152

This report is accompanied by a "first amendment to report no. 12691", dated 8/11/93. Tables in the original English translation of the report were of very poor quality, not legible, contained errors in translation and typing, and a few tables were not logically organized. Some of these faults are listed in the amendment under the "rationale for the first amendment".

Performing Laboratory:

Dates Performed: 2/81 to 9/81

Quality Assurance: No statement on GLP compliance was found in the original report. In the amendment, it is claimed, "...there were no legally binding GLP regulations in force" during the time that this study was performed.

Test Animals: Mura:WIST (SPF 67 HAN), 24 males and 60 females per group. At the start of dosing, males were 5-7 weeks old, and weighed 74-110 g, females were 8-10 weeks old and weighed 158-190 g.

Procedure: The test substance (batch 576 923) was administered at doses of 0, 3, 10 and 30 mg/kg, once daily, by oral gavage in a vehicle consisting of polyethylene glycol 400:glycerol:water in a ratio of 969:60:100. Males were dosed starting 10 weeks before mating and during the 3 week mating period, females were dosed for 3 weeks prior to mating until the 7th day of pregnancy. Except during mating and lactation, both the males and females were kept in individual Makrolon cages. Each male was paired with 2 or 3 females, which were placed together in a Makrolon cage each night and the females were examined for vaginal sperm in the morning. Half the pregnant females in each group, selected by "statistical methods", were C-sectioned on day 20 of gestation, the remaining half were allowed to litter and raise their young to postpatum day (PPD) 21. All C-sectioned fetuses were examined for external anomalies, 1/3 from each dam were examined for soft tissue anomalies (modified Wilson method) and 2/3 for skeletal malformations (alizarin red S). In addition to examining the F_0 parents for reproductive performance, the F_0 females for lactational performance and the F_1 offspring for survival and weight gain during lactation, one male and one female from each litter of the control group and of the highest dose group were reared to sexual maturity to determine F_1 reproductive capacity. The mated F_1 dams were allowed to litter, and testicular weights for F_1 males were obtained after mating.

Test substance administered was Batch 576 923. It is claimed that the preparations for oral gavage were tested for stability and concentration but the data and details for these tests were not included in the report.

There is no statement on why these doses were selected for this study.

Effects on *F_0 Males*

All treated and control males survived to scheduled necropsy and no compound related clinical signs were evident in males of any treated group. There were no effects on weight gain, mating behavior or fertility in males of any treated group compared to controls. There were no effects on gross pathology observed at necropsy (presumably sacrificed after mating and while still on drug treatment). The drug had no effect on testicular weights (See page which follows).

STUDY ON FERTILITY BAY K 5552 T0002152

BODYWEIGHTS [G] OF THE MALES BEFORE MATING
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEEK 10	90.5	87.3	91.0	89.9
	9.6	7.7	7.9	7.4
WEEK 9	136.5	132.5	135.4	131.7
	12.7	11.0	10.0	9.2
WEEK 8	175.0	172.0	174.3	173.3
	16.8	14.8	14.3	13.6
WEEK 7	215.5	212.3	215.7	213.5
	22.0	17.0	16.7	17.8
WEEK 6	252.5	249.6	249.6	252.1
	26.4	19.5	18.6	20.2
WEEK 5	279.0	276.3	276.1	280.6
	29.9	21.6	21.9	23.4
WEEK 4	291.5	291.5	287.6	294.5
	31.9	23.4	26.1	23.6
WEEK 3	311.4	309.9	308.8	314.0
	33.5	25.2	25.1	26.0
WEEK 2	331.5	329.7	327.1	331.6
	34.8	27.1	28.5	28.5
WEEK 1	344.5	345.9	341.6	346.9
	35.0	28.8	29.8	30.0
WEEK 0	360.7	358.9	354.7	360.4
	35.6	29.5	31.4	30.8

TESTICLE WEIGHTS [G]

GROUP MEAN VALUES AND STANDARD DEVIATIONS

0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
3.23	3.26	3.17	3.26
0.30	0.20	0.31	0.25

Effects on *F_0 Females*

Mortality: Deaths are listed only in the narrative portion of this report. In both the original report and the amendment, there is no indication of the time of death; not even if the deaths occurred before mating, during pregnancy or lactation. Deaths occurred in two rats at 3 mg/kg, in one at 10 mg/kg and in two at 30 mg/kg, but none of the deaths were attributed to treatment. Based on scrutinization of tables in the original report, the 2 animals in the 30 mg/kg group which died had both been assigned to "rearing animals". Deaths were attributed to misintubation for a low and mid dose rat, "gastrointestinal disorders" for the second low dose rat, to a lung tumor and to pneumonia for the two high dose rats. In addition, one dam in the control group, which had littered 12 pups and died shortly after birth, was not included in the results because at necropsy only 4 nidation sites were found.

Even in the amended tables for individual animal data, there is no indication of which animals died and the time of the deaths. Numerous animals were dropped from the study for a variety of reasons, which included, "not inseminated", "not pregnant", and for a few, there is a statement "animal dropped from the study" but no reason is given. Most summary tables do not specify the number of animals per group upon which the data are based. Therefore, the following table lists the total number of females in each group that were included in the results, based on a count taken from the individual animal body weight data.

Dosage Group	# C-Sectioned*	# Littered*
Control	25	22
3 mg/kg/day	23	27
10 mg/kg/day	24	20
30 mg/kg/day	27	22

* There were 60 mated females per group at initiation of the study, 30 of which were designated for C-section or littering.

Body Weight and Body Weight Gain: Mean body weight gains and body weights of pregnant females, those that were C-sectioned and those that were allowed to litter, are given on the two pages which follow. Body weights 3 weeks before mating (prior to initiation of treatment) and during pregnancy, were significantly lower for the high dose group of the C-sectioned animals, but there was no effect on body weight gain. Although a small increase in body weight gain was noted for the low dose C-sectioned group between days 7 and 20 of gestation, there was obviously no effect that could be attributed to treatment. No effects on mean body weight or body weight gain were observed in the females selected for delivery of litters during the 3 weeks prior to gestation, during gestation or during lactation.

STUDY ON FERTILITY BAY K 5552 T0002152

WEIGHT DEVELOPMENT (G) OF THE FEMALES UNDERGOING CESAREAN SECTION
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEIGHT GAIN				
DAY 0 - 7 P.C.	22.2	23.9	21.7	23.0
	4.7	5.1	5.1	5.6
DAY 7 - 20 P.C.	73.3	82.1*	76.2	71.4
	10.1	12.6	10.8	17.1
DAY 0 - 20 P.C.	95.5	106.0*	97.9	94.4
	12.4	15.5	12.6	18.1
BODYWEIGHTS BEFORE MATING				
WEEK 3	173.6	172.3	170.3	168.6**
	7.0	6.8	6.8	6.3
WEEK 2	187.1	187.1	186.0	184.9
	8.7	9.5	8.7	9.1
WEEK 1	198.9	197.3	198.3	194.9
	10.0	9.3	9.5	9.7
WEEK 0	209.7	210.2	210.8	204.8
	10.9	11.3	11.6	9.7
BODYWEIGHTS DURING GESTATION				
DAY 0 P.C.	223.8	223.2	223.9	213.5**
	11.5	13.9	13.6	12.4
DAY 7 P.C.	245.9	247.0	245.6	236.5*
	13.2	14.3	13.4	13.5
DAY 20 P.C.	319.2	329.1	321.8	308.0
	17.2	23.2	17.4	23.3

* SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.01$
** SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.005$

T0002152

WEIGHT DEVELOPMENT [G] OF THE DAMS
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEIGHT GAIN				
DAY 0 - 7 P.C.	23.7	19.9	23.7	21.4
	6.2	5.1	5.2	4.4
DAY 7 - 20 P.C.	73.5	70.3	77.0	71.7
	13.3	13.8	13.0	14.2
DAY 0 - 20 P.C.	97.2	90.2	100.7	93.1
	14.6	14.5	11.7	15.0
BODYWEIGHTS BEFORE MATING				
WEEK 3	173.7	171.6	172.5	172.4
	8.2	7.9	8.2	8.9
WEEK 2	186.1	186.1	188.8	187.6
	9.2	10.2	10.1	10.1
WEEK 1	194.7	196.8	200.2	198.6
	11.1	11.1	10.3	12.4
WEEK 0	206.3	208.7	211.0	208.7
	13.1	12.8	10.8	14.2
BODYWEIGHTS DURING GESTATION				
DAY 0 P.C.	216.4	225.0	224.1	217.5
	16.4	16.8	13.6	18.7
DAY 7 P.C.	240.1	244.9	247.8	238.9
	19.4	16.5	11.0	20.1
DAY 20 P.C.	313.5	315.3	324.8	310.6
	25.3	26.1	19.6	31.2
BODYWEIGHTS DURING LACTATION				
DAY 1 P.P.	244.8	250.0	253.7	240.3
	18.9	18.1	15.1	21.3
WEEK 1 P.P.	272.3	277.8	281.6	267.1
	20.5	19.4	14.3	24.4
WEEK 2 P.P.	272.5	278.7	283.3	271.7
	18.8	16.8	14.0	20.1
WEEK 3 P.P.	258.6	265.5	266.7	259.4
	19.0	17.8	15.3	18.2

C-Section *F_0 Females*

As seen in the tables which follow, there were no effects of compound treatment on number or percentages of animals inseminated, with implantations and with live fetuses, mean corpora lutea count, nidations, average number of male or female live fetuses, sex ratio or fetal loss. From these data, it is evident that there were no effects on pre- or post-implantation losses.

The mean fetal weights were significantly increased (apparently dose related) in the 10 and 30 mg/kg groups, and the mean placental weight was slightly but significantly increased in the 3 mg/kg group. The investigators claimed that the mean placental weight increase was incidental, and that the mean fetal weights for the mid and high dose groups were within the norm for this strain (given as 3.5 ± 0.27, based on 268 litters).

There were no compound related effects on mean numbers of gross, visceral or skeletal malformations, nor were there any effects on "underdeveloped forms" (fetuses weighing <3 g). There were also no effects on minor skeletal variations.

STUDY ON FERTILITY BAY K 5552

NUMBER OF ANIMALS - RESULTS OF THE STUDY

ANIMALS UNDERGOING CESAREAN SECTION

DOSE (MG/KG)	USED	INSEMINATED N	INSEMINATED % OF THOSE USED	NUMBER OF FEMALES WITH IMPLANTATIONS N	NUMBER OF FEMALES WITH IMPLANTATIONS % OF THOSE INSEMINATED	FEMALES WITH FOETUSES N	FEMALES WITH FOETUSES % OF THOSE WITH IMPLANTATIONS
0	30	27	90.0	25	92.6	25	100.0
3	29	27	93.1	23	85.2	23	100.0
10	30	26	86.7	25	96.2	24	96.0
30	30	28	93.3	27	96.4	27	100.0

STUDY ON FERTILITY BAY K 5552 T0002152

RESULTS OF THE CESAREAN SECTION (MEAN VALUES)

DOSE MG/KG	WEIGHT GAIN [G] 0-20 P.C.	WEIGHT GAIN [G] 7-20 P.C.	NUMBER (PER DAM) OF CORP. LUTEA	IMPL.	MALE	FEM.	SUM	LOSS	MEAN-WEIGHT IN GRAMMS FETUSES	PLACENT.	NO. OF FOETUSES EXAMINED BY WILSON	DAWSON	FOETUSES WITH MINOR SKELETAL DEVIAT.	MALFORMATIONS	NO. OF RUNTS (<3G)
	95.5	73.3	12.4	11.9	6.3	4.9	11.2	0.7	3.49	0.50	3.3	7.9	3.52	0.04	0.52
1	106.0**	82.1**	12.4	12.0	6.3	5.0	11.4	0.6	3.58	0.53*	3.5	7.8	3.48	0.09	0.30
3	97.9	76.2	11.4	11.0	5.6	5.0	10.6	0.4	3.60*	0.50	3.4	7.6	2.67	0.00	0.25
10	94.4	71.4	11.6	11.0	5.4	4.9	10.3	0.7	3.63**	0.52	3.0	7.3	3.22	0.00	0.19

* SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.025
** SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.01

F_0 Females Allowed to Litter

Pregnancy duration was slightly increased in all 3 treated groups (statistically significant for the 3 and 30 mg/kg groups; see table below). This effect was considered to be "incidental" because the mean durations for these groups were within the norm for this strain. No effects during lactation were noted.

Postpartum Examination of Pups: There were no significant effects on total number of live pups at birth per group, nor on number of viable pups after 1, 2 or 3 weeks postpartum (See table below). It was claimed there were no treatment related effects on number of stillborn pups, and on sex ratio at birth or at the 3 weekly intervals. Mean birth weight was slightly higher for all 3 treated groups (statistically significant for low and high dose), but mean weight and weight increase during the 3 weekly intervals were not influenced by treatment (See table below).

Maturational Development: There were no effects on age of pinna unfolding of the ears, hair coat, eye opening or normal gait.

Function Tests: There were no effects on sight or pupillary reflexes to light, hearing ("pinna twitch reflex", tested by means of a Galton whistle with a set frequency and duration). In a proprioreceptive reflex test (running roller brought from stationary position to 10 revolutions per minute) there was a decrease in performance at 30 mg/kg during the first test but no effect in the second or third test. The age of the animals when these tests were done was not indicated.

Fertility Test of F_1 Generation: There was no effect of treatment with 30 mg/kg on mating, fertility, duration of pregnancy, litter size, live and dead pups, sex ratio, mean weights of the pups or external anomalies at birth.

T0002152

'AMS

DOSE [MG/KG]	USED	N	INSEMINATED % OF THOSE USED	NUMBER OF FEMALES WITH IMPLANTATIONS N	% OF THOSE INSEMINATED	N	THAT LITTERED % OF THOSE WITH IMPLANTATIONS	THAT REARED THEIR PUPS N	% OF THOSE THAT LITTERED
0	29	24	82.8	22	91.7	22	100.0	22	100.0
3	29	28	96.6	27	96.4	27	100.0	27	100.0
10	29	22	75.9	20	90.9	20	100.0	20	100.0
30	28	26	92.9	22	84.6	22	100.0	22	100.0

DURATION OF PREGNANCY IN DAYS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
21.9	22.2*	22.1	22.2**
0.5	0.6	0.6	0.4

* SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.05
** SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.025

NUMBER OF IMPLANTATIONS OF THE DAMS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
10.8	10.4	11.0	11.2
3.0	2.9	2.4	2.8

PRENATAL LOSS OF DAMS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
0.5	0.6	0.4	0.9
0.7	0.9	1.0	1.1

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NUMBER AND WEIGHT DEVELOPMENT OF THE VIABLE PUPS
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION		0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
NUMBER OF PUPS					
AT BIRTH	TOTAL	10.4 3.0	9.8 2.8	10.4 2.5	9.7 2.7
	MALES	5.2 1.8	5.2 2.3	5.2 1.6	4.9 1.8
	FEMALES	5.2 2.3	4.6 1.7	5.3 2.0	4.8 2.1
AFTER 1 WEEK	TOTAL	10.2 3.0	9.7 2.7	10.4 2.5	9.4 2.9
	MALES	5.0 1.9	5.2 2.3	5.2 1.6	4.8 1.8
	FEMALES	5.1 2.3	4.5 1.7	5.3 2.0	4.5 2.3
AFTER 2 WEEKS	TOTAL	10.0 3.0	9.6 2.7	10.3 2.5	9.3 3.0
	MALES	5.0 1.9	5.1 2.2	5.1 1.7	4.7 1.9
	FEMALES	5.0 2.3	4.5 1.7	5.1 2.0	4.5 2.3
AFTER 3 WEEKS	TOTAL	10.0 3.0	9.6 2.7	10.2 2.5	9.3 3.0
	MALES	5.0 1.9	5.1 2.2	5.0 1.7	4.7 1.9
	FEMALES	5.0 2.3	4.5 1.7	5.1 2.0	4.5 2.3
WEIGHT [G] OF THE VIABLE PUPS					
AT BIRTH		5.9 0.5	6.2** 0.5	6.1 0.6	6.2* 0.6
AFTER 1 WEEK		14.0 2.2	14.8 1.6	14.9 1.8	15.0 2.2
AFTER 2 WEEKS		24.7 4.4	26.1 3.5	26.1 3.3	26.4 4.4
AFTER 3 WEEKS		38.1 6.1	40.4 5.8	39.7 5.8	41.5 7.4

* SIGNIFICANT DIFFERENCE TO CONTROL, $P \leq 0.05$
** SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.025$

2. Embryotoxic and Teratogenic Action in Long-Evans Rats

Pharma Report No: 7596 Study No: T2012540

Performing Laboratory:

Thi study was originally presented as a translation from wit only a few brief summary tables; no individual animal data. Amendments received at CDER on 9/29/93 and 10/8/93 contain tables with individual animal findings and summaries. It is claimed that the study was carried out between January and May, 1977, "in accordance with FDA recommendations", but there is no statement of GLP compliance.

Procedure: Naturally inseminated Long-Evans female (strain FB 30) rats, 20 or 21 per group, 2.5 to 3.5 months of age and weighing 195 to 262 g prior to mating, received 0, 10, 30 or 100 mg nisoldipine/kg/day by oral gavage (batch 3/76, micronized), from days 6 to 15 of gestation. The drug was dissolved in polyethylene glycol 400/glycerol/water. A C-section was performed for each dam on day 20 of gestation and the fetuses were examined for external, visceral (Wilson technique) and skeletal (alizarin red stain) anomalies.

Effects on Survival and Body Weights of Dams: One control rat died on gestation day 13 or 14, due to improper intubation into lungs, and was excluded from results. There was no compound related effect on mortality, nor on "general appearance or behavior" of the dams, but there was a dose related decrease in mean weight gain (see table which follows).

Dose (mg/kg)	Weight Gain in Grams	
	Treatment Period	Total Pregnancy
0	62.3	152.4
10	56.1	140.3
30	53.0*	132.6*
100	49.6*	131.8*

*) Significant difference from the control, $P < 0.01$

C-Section of Dams: Of the 21 inseminated rats in each of the 3 compound treated groups, 20 were pregnant, and all 20 surviving rats in the control group were pregnant. All pregnant treated and control rats had live fetuses at necropsy on day 20 of gestation. Corpora lutea count for each rat was not determined in this experiment, but no statistically significant differences between the treated groups and control were found for mean number of implantations, mean number of fetuses, mean number of dead fetuses and resorbed embryos, mean fetal weight, underdeveloped forms (fetuses <3 g in weight), mean placental weight, frequency of fetuses with minor skeletal deviations, sex distribution (see page 94), nor on external, soft tissue or bone deformations (see table below on this page).

Group	Dam No.	Number of Malformed Fetuses	Malformation
Control	664	2	Rib dysplasia (hump formation)
10 mg/kg	681	1	Edematous head
30 mg/kg	—	—	None
100 mg/kg	605	1	Rib dysplasia (hump formation)
	639	1	Cryptorchidism
	647	1	Kinking of the tail
	683	1	Hydrops universalis, micrognathia, kinking of the tail

BAY k 5552 T2012540

Results of the Caesarean Section

Mean values of the groups and standard deviations

Note. The mean fetal and placental weights given in the report no. 7596 were calculated by adding all litter weights of the group and by dividing these sums by the number of fetuses or placentas per group. In the following table these mean values are marked with "a".
For the calculation of the standard deviation the mean fetal and placental weights per litter were calculated first and were used for further calculation. Mean fetal and placental weights obtained by this procedure are marked with "b".

Dose mg/kg	Weight gain [g] during pregnancy	Weight gain [g] during treatment period	Number [per dam] of fetuses impl.	male	female	total	res.**	Mean weight [g] of fetuses	Mean weight [g] of placentas	Number of fetuses with minor skeletal deviations	Number of fetuses with malformations	Number of fetuses runts < 3 g
0	152.4	62.3	11.6	5.8	5.4	11.1	0.4	4.26[a] 4.27[b]	0.57[a] 0.58[b]	2.95	0.10	0.00
	18.5	11.7	1.6	2.3	2.3	1.7	0.7	0.29	0.06	2.11	0.45	0.00
10	140.3	56.1	11.0	5.5	5.0	10.5	0.5	4.07[a] 4.07[b]	0.59[a] 0.59[b]	2.60	0.05	0.00
	23.9	9.7	2.9	1.8	1.6	2.8	0.8	0.29	0.11	2.19	0.22	0.00
30	132.6*	53.0*	11.3	5.3	5.1	10.3	0.9	4.08[a] 4.09[b]	0.57[a] 0.57[b]	3.50	0.00	0.05
	19.9	8.1	2.5	2.4	2.5	2.6	1.3	0.33	0.05	2.61	0.00	0.22
100	131.8*	49.6*	11.9	5.5	5.2	10.7	1.1	4.12[a] 4.14[b]	0.57[a] 0.57[b]	4.10	0.20	0.00
	21.2	11.2	2.7	2.4	2.1	2.7	1.4	0.23	0.05	2.45	0.41	0.00

* significant difference to control, $p < 0.01$ (WILCOXON-MANN-WHITNEY-U-TEST)

** Res. is the abbreviation for resorptions, which the sponsor defined as the total of resorbed embryos and dead fetuses

3. Teratology Study in Sprague Dawley (CD) Rats

Study No: Not provided. Report No. 87/0938

Performing Laborat

Sponsor:

Dates Performed: 8/5/87 to 12/2/87

Quality Assurance: A signed statement of GLP compliance is included.

Test Animals: Charles River CD (Sprague-Dawley derived) females, 9-10 weeks of age and weighing 200-248 g on the day of insemination, were mated on a 1:1 basis with stock males of the same strain.

Procedure: The test substance (batch number 500139) was administered to 32 inseminated females per group, once daily by oral gavage as a suspension in 0.5% aqueous Tylose, prepared fresh each day, from days 7 to 17 of gestation, at doses of 0, 10, 30 and 100 mg/kg. On day 20 of gestation, 21 dams per group were C-sectioned and the fetuses were examined for external anomalies; 1/2 from each dam were examined by free hand serial sectioning (Wilson technique) for soft tissue anomalies. The remaining half were first dissected (neck, thoracic and abdominal cavities) to evaluate for soft tissue anomalies, then were prepared by a modification of Dawson's alizarin staining technique for evaluation of skeletal malformations.

The remaining 11 dams/group were allowed to litter and raise their young to postpatum day 25. On PPD 4, litters with more than 8 were reduced to 8 by random culling, leaving, if possible, 4 of each sex per litter. After weaning, the offspring were housed on a litter basis, but the sexes were separated and there was a maximum of 5 of the same sex per cage. At approximately 5 weeks of age, following completion of behavioral and neuromuscular function tests, 20/sex/group were randomly selected for further assessment of physical, sexual maturation and reproductive performance; unselected ones were killed and grossly examined. At 9 or 10 weeks of age, F_1 males and females were paired 1:1 within treatment groups, avoiding sibling matings. All F_1 mated females were laparotomized on day 20 of gestation; the fetuses were examined only for external malformations and discarded. After gross examination of the F_1 females, F_1 males were killed, then examined externally and internally for macroscopic abnormalities.

Stability of Test Substance: Test formulations for all 3 concentrations, taken from the first and last weeks of treatment,

were generally found to be within approximately 82 to 90% of the target concentrations, and were stable for at least 4 hours after preparation.

Results

F$_1$ Females

Mortality and Clinical Signs: No data on mortality, and no statement in the text pertaining to mortality, were found; all rats apparently survived. It is claimed that one dam receiving 100 mg/kg showed flaccid muscle tone and piloerection during the early stages of treatment (possibly compound related), but other females in that group were not affected.

Body Weights/Food Consumption: Small reductions in body weight gain (not statistically significant) were evident in all 3 treated groups during the initial day or 2 of treatment (See table on the page which follows), and this was accompanied by significant reductions in food intake by the mid and high dose groups, limited to the first 3 days of treatrent. Subsequent body weight gains in all 3 treated groups were not affected by treatment, but the body weights remained below control to the day of necropsy. The lower mean body weights of the mid and high dose groups compared to controls were statistically significant only on day 18 of gestation.

Laparotomy Observations: There were no effects on mean corpora lutea counts, total implantatiions, viable males or females. There was a slight increase in total resorptions ($P< 0.05$) predominently due to number of late resorptions, and in percent post-implantation loss ($P< 0.05$), in the high dose group. Fetal weights were depressed in all 3 treated groups; statistically significant and dose related at mid and high dose (See table two pages ahead).

Fetal Evaluation: There was an increased incidence of small fetuses (<2.7 g) and litters with one or more small fetuses in the 100 mg/kg group. An increased number of fetuses with slightly increased dilatation of lateral ventricles and/or space between the body wall and organs, occurred mainly in two litters, and this was associated with fetuses of low body weight in these two litters. The investigators considered this to be indicative of fetal immaturity. Also associated with the fetuses weighing <2.7 g in 2 litters of the high dose group and considered to be due to fetal immaturity, was an increased incidence of incomplete ossification of basisphenoid, first thoracic vertebral centrum, sacral vetebral arches, ischia, metacarpals and metatarsals.

Group mean bodyweights (g) of females during gestation

Group	:	1	2	3	4
Compound	:	Control	---	BAY k 5552	--
Dosage (mg/kg/day)	:	0	10	30	100

Group		Day of gestation														
		0	3	7	8	9	10	11	12	13	14	15	16	17	18	20
1	Mean	219	238	255	261	266	272	278	284	291	298	307	317	330	346	378
	SD	8	9	12	11	12	12	12	13	13	13	14	15	16	17	18
	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
2	Mean	217	237	251	256	261	267	273	279	285	292	300	312	323	338	368
	SD	10	12	14	12	13	12	14	13	14	14	15	16	16	17	18
	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
3	Mean	217	237	253	255	260	266	272	278	284	291	299	311	323	338*	367
	SD	11	13	14	13	14	14	14	15	15	15	17	17	18	19	21
	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
4	Mean	215	235	253	250	252	261	267	272	278	285	293	303	316	327***	361
	SD	8	9	12	12	12	13	12	12	13	13	13	16	17	18	20
	n	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31

SD Standard deviation.
n Number of pregnant animals.
* Bodyweight gain from Day 7 significantly different from Controls, $P<0.05$ (one way analysis of variance and Student's t-test).
*** Bodyweight gain from Day 7 significantly different from Controls, $P<0.001$ (one way analysis of variance and Student's t-test).

Group mean litter data - females killed on Day 20 of gestation

Group	: 1	2	3	4
Compound	: Control	--- BAY k 5552 ---		
Dosage (mg/kg/day)	: 0	10	30	100

Group	Number of pregnant animals		Corpora lutea count	Implantations	Viable young			Resorptions			Implantation loss (%)		Foetal weight (g)	Placental weight (g)
					M	F	Total	Early	Late	Total	Pre-	Post-		
1	21	Mean	17.1	15.6	8.0	6.9	14.9	0.5	0.1	0.7	9.4	4.3	3.50	0.53
		SD	1.7	1.3	2.3	1.9	1.1	0.7	0.4	0.8			0.06	0.01
2	21	Mean	16.9	14.9	7.0	7.0	14.0	0.8^{NS}	0.1	0.9^{NS}	11.6	6.1^{NS}	3.45	0.53
		SD	1.5	1.4	1.8	2.0	1.5	0.9	0.4	1.0			0.06	0.02
3	21	Mean	16.4	14.9	6.4	7.1	13.5	1.2^{NS}	0.1	1.4^{NS}	9.8	9.3^{NS}	3.34*	0.54
		SD	1.7	1.4	2.3	2.3	2.4	1.1	0.4	1.2			0.06	0.02
4	20	Mean	16.9	15.1	6.7	6.8	13.5	0.9^{NS}	0.8^{NS}	1.6^{+}	11.2	10.6^{+}	3.19***	0.52
		SD	1.9	3.3	2.3	2.6	3.6	0.9	0.9	1.3			0.09	0.03
Background control (159 studies)														
Mean			15.9	14.5	6.7	6.9	13.7	0.69	0.18	0.87	8.7	6.0	3.32	0.50
Low			13.9	12.0	5.2	5.6	11.1	0.05	0.00	0.25	1.6	1.7	3.00	0.43
High			19.0	16.7	8.2	8.7	15.3	1.68	0.58	1.79	16.5	12.7	3.55	0.57

SD Standard deviation.
* Significantly different from Control, P<0.05 (Nested analysis of variance and weighted t-test).
*** Significantly different from Control, P<0.001 (Nested analysis of variance and weighted t-test).
NS Not significant (Mann Whitney 'U'-test).
+ Significantly different from Control P<0.05 (Mann Whitney 'U'-test)

Summary of foetal observations at necropsy

Group	: 1	2	3	4
Compound	: Control	---	DAY k 5552	--
Dosage (mg/kg/day)	: 0	10	30	100

Group :	1	2	3	4	Control data	
External examination						
Number of foetuses (litters) examined:	313(21)	294(21)	284(21)	269(20)	39809 foetuses	159 studies
Number of male : female foetuses:	168:145	147:147	135:149	134:135		
Observations: % incidence♦ (number of litters)					Mean	Study ranges
Small foetus (less than 2.70 g)	1.0(3)	0.7(2)	1.4(4)	11.9(6)	3.5	0 - 16.9
Large foetus (more than 4.00 g)	2.9(3)	1.4(3)	1.4(2)	-	1.3	0 - 8.7
Shiny pup	-	0.3(1)	-	1.1(1)	0.3	0 - 4.1
Pale pup	-	-	-	0.4(1)	0.02	0 - 1.1
Domed head	-	0.3(1)	-	-	0.01	0 - 0.4
Subcutaneous haemorrhage on chin	0.3(1)	-	-	-	0.1	0 - 0.7
Small placenta (less than 0.30 g)	-	-	-	0.4(1)	0.2	0 - 2.3
Large placenta (more than 0.70 g)	2.2(4)	2.0(6)	3.2(4)	3.0(3)	1.3	0 - 6.2
Conjoined placentae	-	0.3(1)	-	-	0.03	0 - 0.8
Dark green material surrounding placenta	-	0.3(1)	-	-	0.1	0 - 6.9
Short tail	-	-	-	0.4(1)	0.01	0 - 0.5
Threadlike tail	0.3(1)	-	-	-	0.02	0 - 0.8
Imperforate anus	0.3(1)	-	-	-	0.04	0 - 0.8

♦ One foetus may have more than one observation.

continued

Summary of foetal observations after free-hand serial sectioning

Group	:	1	2	3	4
Compound	:	Control	---	BAY k 5552	--
Dosage (mg/kg/day)	:	0	10	30	100

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined.*	156(21)	148(21)	143(21)	134(20)	12835	126
Number of males : females	82:74	78:70	70:73	68:66	foetuses	studies
Observations: % foetal incidence (number of litters affected)					Mean	Study ranges
Abdomen:						
Diaphragmatic hernia	0.6(1)	-	-	0.7(1)	0.1	0 - 1.9
Small additional liver lobe(s)	25.6(19)	32.4(18)	23.1(18)	28.4(17)	0.8	0 - 10.9
Hepatic haemorrhage(s)	9.0(8)	12.2(15)	7.7(7)	9.0(11)	10.3	0 - 27.7
Localised internal abdominal haemorrhage	1.9(3)	-	0.7(1)	2.2(3)	1.3	0 - 6.7
Haemorrhagic peritoneal fluid	0.6(1)	-	0.7(1)	-	2.4	0 - 18.0
Haemorrhagic abdomen	0.6(1)	0.7(1)	0.7(1)	-	1.7	0 - 8.0
Left kidney displaced slightly towards midline	-	-	-	0.7(1)	0.05	0 - 1.7
Small haemorrhage within capsule of right kidney	-	0.7(1)	-	-	0.02	0 - 1.0
Unilateral hydronephrosis	1.3(1)	3.4(3)	1.4(2)	1.5(2)	2.6	0 - 11.7
Bilateral hydronephrosis	-	1.4(1)	-	-	0.9	0 - 9.8
Unilateral hydroureter	13.5(12)	7.4(6)	7.7(6)	14.2(13)	6.7	0 - 24.2
Bilateral hydroureter	2.6(2)	7.4(4)	2.8(4)	4.5(4)	4.4	0 - 27.1
Testis(es) displaced slightly†	11.0(7)	12.8(7)	8.6(6)	10.3(7)	3.7	0 - 23.5
Fluid-filled vesicle at anal edge of genital tubercle	-	0.7(1)	-	-	*	*
Genital tubercle slightly elongated	-	-	-	3.0(2)	0.4	0 - 6.3
Blood in anus	-	1.4(2)	0.7(1)	1.5(2)	0.2	0 - 7.2
Threadlike tail; imperforate anus; displacement of adrenal glands and kidneys	0.6(1)	-	-	-	0.08	0 - 1.8
Tip of tail threadlike and hooked	0.6(1)			-	*	*

* One foetus may have more than one observation.

† Percentage calculated on number of male foetuses.

* No record in background control data.

continued

Summary of foetal observations after free-hand serial sectioning

Group	: 1	2	3	4
Compound	: Control	--- BAY k 5552		--
Dosage (mg/kg/day)	: 0	10	30	100

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined.*	156(21)	148(21)	143(21)	134(20)	12835	126
Number of males : females	82:74	78:70	70:73	68:66	foetuses	studies
Observations: % foetal incidence (number of litters affected)					Mean	Study ranges
Thorax:						
Oesophagus displaced to right of trachea	-	-	-	0.7(1)	0.01	0 - 1.2
Left lobe of thyroid gland very reduced in size/absent	-	-	-	0.7(1)	0.1	0 - 2.2
Space between bodywall and organs	-	2.0(2)	-	11.2(5)	10.9	0 - 47.4
Retro-oesophageal right subclavian artery and misshapen thymus gland	-	0.7(1)		-	0.02	0 - 1.2
Blood-filled thoracic lymph duct	0.6(1)	1.4(2)	0.7(1)	-	0.3	0 - 2.9
Innominate artery reduced in length/absent	-	1.4(2)	1.4(1)	0.7(1)	0.1	0 - 1.4
Gross cardiovascular abnormality with valve defects	-		-	0.7(1)	*	*
Gross cardiovascular abnormality with double outlet right ventricle	-	-	-	0.7(1)	*	*
Slightly increased amount of pericardial fluid	1.3(2)	0.7(1)	1.4(2)	0.7(1)	0.3	0 - 5.3
Slightly haemorrhagic pericardial fluid	-	0.7(1)	1.4(1)	-	0.2	0 - 9.1
Haemorrhages on edge of lung lobes	-	-	-	1.5(2)	0.1	0 - 2.2

* One foetus may have more than one observation.
* No record in background control data.

continued

Summary of foetal observations after free hand serial sectioning

Group	:	1	2	3	4
Compound	:	Control	---	BAY k 5552	
Dosage (mg/kg/day)	:	0	10	30	100

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined.*	156(21)	148(21)	143(21)	134(20)	12835	126
Number of males : females	82:74	78:70	70:73	68:66	foetuses	studies
Observations: % foetal incidence (number of litters affected)					Mean	Study ranges
Others:						
Subcutaneous haemorrhage(s):						
Lower/side of jaw	3.2(3)	7.4(4)	4.2(4)	5.2(3)	0.8	0 - 8.9
Submandibular	2.6(2)	3.4(5)	6.3(7)	6.0(6)	1.0	0 - 8.8
Nasal	1.3(2)	3.4(5)	0.7(1)	2.2(2)	1.1	0 - 5.9
Cranial	1.9(3)	4.1(6)	1.4(2)	2.2(3)	2.5	0 - 8.2
Ventral/dorsal cervical	0.6(1)	2.7(3)	1.4(2)	6.7(5)	1.1	0 - 5.8
Scapular	11.5(12)	17.6(11)	16.1(12)	15.7(11)	28.6	6.4 - 90.5
Lateral/ventral/dorsal thoracic	2.6(3)	5.4(7)	7.7(8)	5.2(5)	1.5	0 - 5.8
Fore-/hind limb(s)	17.3(18)	13.5(7)	10.5(7)	16.4(12)	*	*
Lateral/dorsal abdominal	0.6(1)	2.0(3)	0.7(1)	0.7(1)	0.6	0 - 3.8
Anal region	-	0.7(1)	-	0.7(1)	0.5	0 - 10.0
Tail	-	-	0.7(1)	0.7(1)	0.7	0 - 10.6
Subcutaneous oedema - trunk	2.6(2)	6.1(4)	4.2(5)	9.0(8)	3.6	0 - 17.5

* One foetus may have more than one observation.
* No record in background control data.

continued

Summary of foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	-----	BAY k 5552	-----
Dosage (mg/kg/day)	:	0	10	30	100

Group :	1	2	3	4	Control data	
Number of foetuses (litters) examined:	157 (21)	146 (21)	141 (21)	135 (20)	19316 foetuses	129 studies
Observations :Grand Mean % foetal incidence @ (number of litters)					Mean	Study ranges
Vertebrae,limbs and girdles						
Ossification of ventral arch of 1st cervical vertebra.	7.0 (7)	4.8 (6)	7.1 (5)	2.2 (3)	6.94	0.0 - 22.2
Incomplete ossification, one or more cervical vertebral arches.	0.6 (1)	1.4 (2)	0.7 (1)	0.0 (0)	0.50	0.0 - 5.2
1st thoracic vertebral centrum unossified.	0.6 (1)	1.4 (1)	0.7 (1)	1.4 (2)	1.10	0.0 - 5.5
Incomplete ossification, one or more thoracic vertebral centra.	27.4 (15)	19.9 (16)	25.5 (17)	27.4 (14)	26.66	8.6 - 58.3
Incomplete ossification of one or more lumbar vertebral centra.	0.0 (0)	0.7 (1)	0.0 (0)	0.7 (1)	0.41	0.0 - 2.5
Incomplete ossification of one or more lumbar vertebral arches.	0.0 (0)	1.4 (2)	0.0 (0)	0.0 (0)	0.12	0.0 - 1.8
Incomplete ossification of sacral vertebral centra.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	0.01	0.0 - 0.5
Incomplete ossification of one or more sacral vertebral arches.	1.9 (3)	2.1 (3)	3.5 (5)	9.6 (4)	1.17	0.0 - 6.2
Short tail, tip thickened.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	*	
25 pre-sacral vertebrae.	1.3 (2)	0.0 (0)	2.8 (4)	0.7 (1)	0.81	0.0 - 6.7
Incomplete ossification of caudal vertebrae (less tha).	1.3 (2)	1.4 (2)	1.4 (2)	14.1 (5)	2.96	0.0 - 14.5
Metacarpals/metatarsals 3/4.	69.4 (20)	56.2 (20)	80.1 (21)	74.1 (19)	67.30	28.6 - 86.9
Metacarpals/metatarsals 4/4.	29.3 (14)	43.2 (16)	19.9 (10)	16.3 (12)	30.65	6.2 - 71.4
Metacarpals/metatarsals incompletely ossified or unossified.	6.4 (5)	4.8 (5)	5.0 (6)	14.1 (6)	3.05	0.0 - 10.8
One or more phalangeal bones ossified.	1.3 (1)	6.2 (5)	2.1 (2)	0.7 (1)	1.89	0.0 - 8.1
Inner corners of one or both scapulae unossified.	5.7 (6)	2.7 (2)	9.2 (9)	5.2 (4)	3.59	0.0 - 14.4
Pubic bones incompletely ossified or unossified.	7.6 (6)	4.1 (6)	7.1 (5)	14.8 (8)	7.43	0.0 - 18.6
Incomplete ossification of one or both ischial bones.	1.9 (3)	2.1 (3)	5.0 (3)	5.2 (5)	0.90	0.0 - 4.7
Asymmetric pelvis, iliial bones associated with different sacral vertebrae.	0.0 (0)	0.0 (0)	1.4 (2)	0.7 (1)	0.49	0.0 - 3.7

@ One foetus may have more than one observation
* New parameter, no control data available

- continued

Summary of foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	-----	BAY k 5552	-----
Dosage (mg/kg/day)	:	0	10	30	100

Group :	1	2	3	4	Control data	
Number of foetuses (litters) examined:	157 (21)	146 (21)	141 (21)	135 (20)	19316 foetuses	129 studies
Observations :Grand Mean % foetal incidence @ (number of litters)					Mean	Study ranges
Sternebrae and ribs						
Incomplete ossification of 1 sternebra.	18.5 (13)	19.2 (12)	9.2 (8)	11.9 (9)	13.53	0.0 - 40.0
Incomplete ossification of 2 sternebrae.	66.9 (21)	65.1 (21)	75.9 (21)	48.9 (18)	66.88	43.3 - 84.8
Incomplete ossification of 3 sternebrae.	7.6 (8)	11.6 (7)	11.3 (11)	23.0 (14)	11.80	1.1 - 23.3
Incomplete ossification of 4 sternebrae.	4.5 (4)	3.4 (4)	1.4 (2)	7.4 (9)	3.68	0.0 - 17.5
Incomplete ossification of 5 sternebrae.	0.0 (0)	0.0 (0)	2.1 (1)	3.0 (2)	0.00	0.0 - 3.8
Incomplete ossification of 6 sternebrae.	1.3 (2)	0.0 (0)	0.0 (0)	5.2 (2)	0.53	0.0 - 6.7
1st sternebra cleft.	0.6 (1)	0.7 (1)	0.7 (1)	5.2 (4)	0.86	0.0 - 7.6
One or more sternebrae offset.	2.5 (4)	1.4 (1)	2.1 (3)	0.0 (0)	1.32	0.0 - 5.2
Ribs 13/13.	100.0 (21)	97.3 (21)	98.6 (21)	97.0 (20)	98.17	92.5 -100.0
Ribs 13/14.	0.0 (0)	1.4 (2)	1.4 (2)	1.5 (2)	1.20	0.0 - 4.2
Ribs 14/14.	0.0 (0)	1.4 (2)	0.0 (0)	1.5 (2)	0.51	0.0 - 3.5
14th rib enlarged.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	*	
13th rib or ribs reduced in length.	1.3 (2)	2.7 (3)	2.8 (3)	2.2 (2)	2.31	0.0 - 13.4
Slight medial thickening of one or more ribs.	0.0 (0)	0.7 (1)	0.0 (0)	0.0 (0)	0.02	0.0 - 1.4

@ One foetus may have more than one observation
* New parameter, no control data available

Summary of foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	------	BAY k 5552	-----
Dosage (mg/kg/day)	:	0	10	30	100

Group :	1	2	3	4	Control data	
Number of foetuses (litters) examined:	157 (21)	146 (21)	141 (21)	135 (20)	1931 foe	129 studies
Observations :Grand Mean % foetal incidence @ (number of litters)					Mean	Study ranges
Head						
Small anterior fontanelle.	1.9 (2)	0.0 (0)	0.7 (1)	0.0 (0)	1.33	0.0 - 11.2
Medium anterior fontanelle.	96.2 (21)	98.6 (21)	99.3 (21)	91.9 (19)	96.20	76.4 -100.0
Large anterior fontanelle.	1.9 (3)	1.4 (2)	0.0 (0)	8.1 (2)	2.46	0.0 - 23.6
Incomplete ossification of supra-occipital bone.	24.2 (12)	14.4 (10)	12.8 (11)	19.3 (11)	13.48	0.0 - 29.2
Incomplete ossification of interparietal bone.	49.0 (20)	40.4 (18)	40.4 (18)	41.5 (18)	28.40	4.9 - 91.0
Incomplete ossification of parietal bone.	0.6 (1)	1.4 (2)	0.0 (0)	2.2 (1)	1.30	0.0 - 7.1
Incomplete ossification of squamosal bone.	0.0 (0)	0.7 (1)	0.0 (0)	3.0 (2)	0.84	0.0 - 4.7
Incomplete ossification of frontal bone.	1.3 (2)	0.0 (0)	0.0 (0)	3.0 (2)	0.09	0.0 - 1.7
Discrete unossified area in frontal bone.	0.6 (1)	2.7 (3)	2.1 (3)	2.2 (2)	0.43	0.0 - 4.6
Incomplete ossification of nasal bone.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	0.02	0.0 - 0.6
Discrete unossified area in basioccipital bone.	0.0 (0)	0.0 (0)	0.7 (1)	0.0 (0)	0.12	0.0 - 2.4
Incomplete ossification of basioccipital bone.	0.0 (0)	0.7 (1)	0.0 (0)	0.0 (0)	0.01	0.0 - 0.6
Incomplete ossification of basisphenoid, craniopharyngeal canal enlarged.	0.0 (0)	0.0 (0)	1.4 (2)	2.2 (2)	0.19	0.0 - 14.3
Incomplete ossification of basisphenoid bone.	4.5 (7)	7.5 (8)	11.3 (9)	13.3 (8)	0.94	0.0 - 12.6
Presphenoid bone incompletely ossified or unossified.	0.6 (1)	0.0 (0)	0.0 (0)	4.4 (2)	0.12	0.0 - 5.4
Fronto-nasal suture enlarged.	1.9 (3)	0.7 (1)	0.7 (1)	4.4 (2)	1.13	0.0 - 5.3
Incomplete ossification of hyoid bone.	7.0 (6)	5.5 (6)	9.9 (3)	5.9 (4)	7.28	0.0 - 25.0
Hyoid bone unossified.	12.7 (8)	11.6 (9)	7.1 (8)	5.2 (6)	8.52	0.0 - 18.5

@ One foetus may have more than one observation

F_0 Dams; Postnatal Phase

There was a slight increase in gestation length in the 30 mg/kg (n.s.) and 100 mg/kg ($P< 0.05$) groups, from a mean of 22.5 days in control to a mean of 23.0 days in the 100 mg/kg group). Although body weights of the drug treated animals tended to be higher than control, no significant intergroup differences in body weight were observed during lactation.

F_1 Offspring to 5 Weeks of Age

There were no compound related effects at any dose level on number of stillbirths, litter size or sex ratio at birth, number of implantation sites, survival indices throughout lactation, body weight or body weight gain during lactation.

There were no compound related effects on physical development (time of pinna unfolding, hair growth, testis descent, tooth eruption, eye opening and vaginal opening).

There were no effects of compound treatment on auditory and visual function (tested on PPD 25), within-cage activity (measured by means of electronic detectors and infra-red light apparently on PPD 26 to 27), learning ability (water filled Y-maze on PPD 27) and neuromuscular function (traversing flat and round rods, rotorod treadmill, mid-air righting reflex, fore- and hind-limb wire hanging and grid-griping ability on PPD 28-30). There were no effects on body weight or body weight gain to 5 weeks of age.

F_1 Offspring Between 5 and 10 Weeks of Age

The 20 males and 20 females per group, selected at 5 weeks of age, were assessed primarily for physical and sexual maturation and reproductive performance.

There were no compound related effects in males or females on appearance, behavior, body weights, mating performance at 9 or 10 weeks of age or on fertility.

In F_1 females killed on day 20 of gestation, there was no evidence of effects on implantation, embryo/fetal survival, fetal weights or placental weights.

No gross pathology abnormalities were observed in F_1 males or females that were considered to be related to treatment of the F_1 females.

4. Embryotoxic and Teratogenic Effects in Rabbits

Report No: 7595
Study No.: Not given

Performing Laboratory:

Dates Performed: 10/77 to 1/78 (first test)
1/78 to 4/78 (second test)

Quality Assurance: These studies were performed prior to the time that GLP compliance was required. The investigators in Germany claim that to the best of their knowledge, the study was performed "according to the state of the art".

Test Animals: Sexually mature female Himalayan rabbits, around 2 to 3.5 kg body weight, inseminated twice by means of copulation with males of the same strain and similar age.

Procedure: There were two separate but related studies in which batch 1/77, micronized Bay k 5552 were used. In the first study, the test substance was administered once daily by stomach tube, as a suspension in a vehicle consisting of "60 g anhydrous glycerol, 100 g demineralized water and polyethylene glycol 400 to make up 1129 g". Twelve or 13 does/group were treated from days 6 to 18 of gestation with doses of 0, 3, 10 or 30 mg/kg. On day 29, a C-section was carried out and each doe was examined for number of implantations (no data on corpora lutea count), number of live and dead fetuses and embryos, weight of litter and placentae. Each fetus was sexed, then examined for external, visceral and skeletal anomalies. Because of diarrhea in 4 animals of the 30 mg/kg group and spontaneous abortion in 2 of these 4 does, a second study limited to 0 and 30 mg/kg (n = 12 or 13/group), with a vehicle consisting of 0.5% aqueous Tylose, was performed. It was suspected that the vehicle contributed to the diarrhea in the first study. In all other respects, the procedure was the same as in the first study.

Results of the First Study

Maternal Survival, Clinical Signs and Body Weight Gain

One death at high dose (day 27 p.c.) was attributed to diarrhea on multiple days; 1 death at mid dose (day 9 p.c.) was attributed to an intubation accident.

The 30 mg/kg dose "caused" diarrhea in 4 animals (1, with multiple days of diarrhea, died; the remaining 3 had diarrhea on only 1 day, either on day 16 or 17 p.c.). Spontaneous abortions occurred in 1 doe at 3 mg/kg (day 25 p.c.), 1 at 10 mg/kg (day 28 p.c.), and 2 at 30 mg/kg (days 18 and 27; both had diarrhea). None of the control does aborted. Although there was only one

more spontaneous abortion in the high dose group than in the low or mid dose groups, the investigators nevertheless suggested that the incidence in the high dose group was increased, and attributed this effect to diarrhea associated with the vehicle and treatment. The abortions at low and mid doses were considered to be neither treatment related nor significant because the observed rates were considered normal for the strain of rabbit used.

Only females found to be pregnant were included in calculation of mean body weight values (N = 13, 12, 12 and 10 for control, 3, 10 and 30 mg/kg groups). Decreases in body weight gain between days 6 and 18 and over the entire period of gestation in all 3 treated groups were not statistically significant (See table on page 105A).

Laparoscopic Observations

There were no significant effects on mean numbers of implantations or resorptions per doe. The mean number of live male fetuses per doe was reduced in the 30 mg/kg group vs control ($P< 0.05$) resulting in a reduction in ratio of males:females but it was considered to be a random occurrence. There was no effect on fetal or placental weights, and no increase in number of "underdeveloped forms" (i.e. fetuses lower than 2.5 g body weight). There was an increase in malformation rate in the high dose group; the high dose malformations occurred in the offspring of the three animals that had diarrhea and were suggested to be the result of maternal stress (see pages 104 to 105A).

Results of the Second Study:

Maternal Survival, Clinical Signs and Body Weight Gain

Diarrhea or other clinical signs did not occur in does of the 30 mg/kg group, but one doe on 30 mg/kg died of an intubation accident (sometime between days 18 and 29, p.c., based on individual animal weight gain tables). Final results were based on 11 surviving does in each group. There was a decrease in mean body weight in the treated group, compared to an increase in control, between days 6 and 18 (treatment period), resulting in a compound related decrease in body weight gain over the entire gestation period.

Laparoscopic Observations

There were no compound related effects on pregnancy rate, spontaneous abortion rate, mean number of implantations (corpora lutea were not counted) or live fetal count, but mean fetal weight and placental weight were lower ($P<0.05$) and the number of "underdeveloped forms" was higher in the treated group. One of the dams on 30 mg/kg had 5 fetuses with "reduced motility". In this second study, there was no effect on sex ratio at birth, as had been observed in the first study. There was no increase in

external, visceral or skeletal malformations in any treated group vs control (See tables on pages 105B, C & D).

Comment: There were indications of fetal toxicity at 30 mg/kg, a dose that was maternally toxic. For example, there was a reduction in live male fetuses per dam and suggestions of an increase in total number of fetuses with malformations and the total number of litters with fetuses that had malformations, compared to control, in the first study. The total number of runts was higher and mean fetal weights were lower than control in the second study. However, there was no increase in incidence of any specific form or class of terata and no clear indication that this substance was teratogenic in rabbits.

BAY k 5552 Report No. 7595

Incidence table of the findings of the fetuses#

Findings	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
MENINGOCELE	1(1)[a]			
TELENCEPHALON dysplasia	1(1)[a]			
CRAWN-HAND slight	1(1)[a]			
TONGUE small/sharp/thin		1(1)		
FORE-LIMB abnormal position				1(1)[a]
arthrogryposis	1(1)[b]			1(1)[a]/1(1)[b]
MULTIPLE MALFORMATION			1(1)	1(1)[a]/-
CLEFT PALATE				
MOTILITY reduced				5(1)[b]

# on individual basis; values in () or [illegible]er basis
a = first study / b = second study

BAY k 5552 Report No. 7595

Individual clinical findings of the dams

Note: animals without findings are not listed

Dose [mg/kg]	Dam-No.	Findings
0 (1st study)	914	on day 26 p.c. no stool, from day 27 p.c. very reduced stool
3	899	abortion on day 25 p.c.: 3 placentas
10	876	abortion on day 28 p.c.: 4 fetuses with placentas
	896	found dead on day 9 p.c. (lung application)
30 (1st study)	877	abortion on day 27 p.c.: 3 fetuses and 3 centas
	881	on day 13 p.c. red bordered eyes
		on day 16 p.c. scratch wounds
		on day 17 p.c. diarrhea
	885	on day 17 p.c. diarrhea
	905	on day 18 p.c. diarrhea
	921	on days 17 and 18 p.c. dia
		from day 24 p.c sick, re stool
		on day 25 p.c. bloody ion
		found dead on day 27
	925	abortion on day 18 3 fetuses
0 (2nd study)	-	-
30 (2nd study)	950	from d .c. sick, decreased feed consumption cool
		on ay between day 18 and 25 p.c.
		ea
		d dead on day 25 p.c

BAY k 5552 Report No. 7595

R e t s o f t h e C a e s a r e a n S e c t i o n

Mean v of the groups and standard deviations 1st study

Note: The mean fetal and pl weights given in the report no. 7595 were caculated by adding all litter weights of the group and by div hese sums by the number of fetuses or placentas per group. In the following two tables these mean values ked with "a". For the calculation of the deviation the mean fetal and placental weights per litter were calculated first and were used for furth ulation. Mean fetal and placental weights obtained by this procedure are marked with "b".

Dose mg/kg	Weight gain [g] during pregnancy	Weight gain [g] during treatment period	Number [per f e t u impl.	male	female	of	res.**	Mean - weight [g] of fetuses	Mean - weight [g] of placentas	Number of fetuses with minor skeletal deviations	Number of fetuses with malformations	Number of fetuses runts < 25g
0	166.2	25.8	7.6	3.2	3.5	6.7		36.64[a] 36.96[b]	4.37[a] 4.37[b]	0.00	0.08	0.62
	176.4	140.2	1.8	2.0	1.5	2.0	1.	.12	0.61	0.00	0.28	1.33
3	109.2	5.0	6.2	2.3	2.9	5.2	1.0	3b	4.50[a] 4.50[b]	0.00	0.08	0.17
	86.5	87.2	2.2	1.4	1.7	2.8	1.4	5.9	1.08	0.00	0.29	0.58
10	123.8	7.5	8.0	3.0	3.9	6.9	1.1	34.70[a] 35.11[b]	[a]	0.00	0.08	0.25
	133.1	78.9	1.6	1.5	1.9	2.5	2.2	3.92	0.	0.00	0.29	0.87
30	112.5	0.5	5.9	1.5*	2.4	3.9	2.0	37.27[a] 37.44[b]	4.76[a] 4.76[b]	0	0.40	0.00
	135.5	135.6	1.9	1.1	1.9	2.6	2.7	3.94	0.77		0.70	0.00

* significant difference to control, $p < 0.05$

** Res. is the abbreviation for resorptions, which onsor defined as the total of resorbed embryos and dead es

DAY k 5552 Report No. 7595

R e t s o f t h e C a e s a r e a n S e c t i o n

Mean v of the groups and standard deviations 2nd study

Dose mg/kg	Weight gain [g] during pregnancy	during treatment period	Num impl.	male	dam] of s e s total		res.**	Mean of fetuses	weight [g] of placentas	Number of fetuses with minor skeletal deviations	with mal-formations	runts < 25g
0	200.9	38.2	7.0	3.2	3.		0.6	36.94[a] 37.50[b]	4.25[a] 4.25[b]	0.00	0.09	0.00
	128.0	62.7	2.5	1.5	1.9		.7	3.49	0.45	0.00	0.30	0.00
30	131.8	-47.6	6.9	2.8	3.0	5.8		34.07[a]* .95[b]	3.77[a]* 3.77[b]	0.00	0.18	0.64*
	136.4	158.6	1.4	1.9	1.2	1.6	1.1	?	0.52	0.00	0.40	0.81

* significant difference to control, $p < 0.05$

** Res. is the abbreviation for reso ons, which the sponsor defined as the total of resorbed e s and dead fetuses

Individual necropsy findings of the dams

Note: animals without findings are not listed

Dose [mg/kg]	Dam-No.	Findings
0 (1st study)	-	-
3	879	gall bladder congested
	899	colon distended and filled with dark brown-red fluid, liver light and brittle, distinct lobulation, gall bladder congested
10	896	thoracic cavity filled with fluid, organs autolytic, pregnant
30 (1st study)	921	gall bladder congested, liver light brittle, intestines filled with g[illegible] liquid brown-red paste, pregnant
0 (2nd study)	-	-
30 (2nd study)	937	fatty tissue of the [illegible] chondroid hardened
	938	uterus anomaly - [illegible] excluded from the study
	949	gall bladder [illegible]ed, knobby surface
	950	died as a re[illegible]of lung application, pregnant
	955	subdermal [illegible] tissue chondroid hardened in the abdo[illegible] region
	956	subder[illegible]atty tissue chondroid hardened in the [illegible]nal region
	967	su[illegible]l fatty tissue chondroid hardened in [illegible]dominal region
	968	[illegible] bladder congested

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	Oral Dose (mg/kg)	Dam No.	No. of Malformed Fetuses	Malformation
First Test	0	902	1	Meningocele and telencephalic dysplasia.
	3	903	1	Dysplasia of the tongue.
	10	926	1	Abnormal skull form, twisting of the umbilical cord, dysplasia of a liver lobe, abnormal bone formation and dysplasia in the sternum.
	30	881	2	1st fetus: Cleft palate 2nd fetus: Abnormal position of the right front leg.
		885	1	Multiple malformation (including cleft palate, fissured chest and stomach, spina bifida, ectrodactyly, abnormality of the ears).
		905	1	Arthrogryposis of both front legs.
Second Test	0	951	1	Arthrogryposis of the left front leg.
	30	943	1	Abdomen, pelvic girdle, and rear limbs rudimentary; cleft palate, ectrodactyly or adactyly of the front.
		956	1	Arthrogryposis of the right front leg.

5. Teratogenicity Study in Cynomolgus Monkey

Bayer Study No: T 3 022 847

Performing Laborato[illegible]

Sponsor

Dates Performed: 2/23/87 to 6/16/87

Quality Assurance: A signed statement of GLP compliance was included. The statement notes two deviations from 21CFR 58: 1) "the stability of the test article/carrier mixture had not been determined at the time of the study", and 2) "the final report of the study does not contain all the information specified in subsection 58.185. In particular, no analytical data relating to the test article or test article/carrier mixture are included."

Justification for Species Selection: "...because of its similar hormonal profile during pregnancy to that of man and this particular non human primate submits itself as a favourable species for reproductive toxicology studies."

Doses Tested: 0, [illegible] and [illegible] mg/kg

Procedure: Female cynomolgus monkeys (Macaca fascicularis) were obtained from [illegible] The females were "sexually mature", quarantined for "at least [illegible] weeks" and acclimated to laboratory conditions for [illegible] weeks, before initiation of the study. There were [illegible] and 100 mg/kg per group that had tested positive for pregnancy by [illegible]; [illegible] day [illegible] post coitum. Pregnancy was subsequently confirmed by rectal palpation on specified days between [illegible]. Test substance was administered by [illegible] between gestation days (GD) [illegible], and [illegible] were done on GD 100 ±1 day. Examinations for fetal malformations included a comprehensive external examination, including head and body size measurements and appearance of externally visible organs. [illegible]

[illegible] prepared daily. The [illegible]

Results:

Spontaneous Abortions, Clinical Observations and Mortality: One control and 1 high dose monkey were found to be not pregnant, thus leaving 11, 10 and 11 monkeys in control, low and high dose groups, respectively. Incidence of abortions and deaths are summarized below.

Dose mg/kg	#/Gp*	# Abortions	# Deaths
Control	11	6 (GD 26-56)	3 (GD 21-99)
30	10	7 (GD 28-53)	1 (GD 48)
100	11	8 (GD 33-59)	5 (GD 27-88)

*Includes only pregnant animals.

Symptoms included heavy bleeding (followed by abortion), reduced food intake, diarrhea and vomiting. Symptoms were observed only during intervals of treatment, and included animals in the control group. Although all of the symptoms were generally more frequent or of longer duration in drug treated monkeys, they are considered to be due to treatment with the vehicle.

Two of the 3 control animals that aborted, subsequently died. The increased incidence of spontaneous abortions and deaths in the 100 mg/kg dose treated group were considered treatment related. (See comments on next page.)

The death of an additional animal in the high dose group which was found to be not pregnant and is not included in these results, was also considered to be treatment related. Causes of death in the 3 pregnant control monkeys included gastro-enteritis, catarrhal enteritis and "signs of asphyxia". Of the drug treated animals which died, the one in the 30 mg/kg group, and 4 of the 5 in the 100 mg/kg group, each had a volvulus (an intestinal obstruction due to twisting of the bowel); 3 of these animals (all high dose) exhibited abdominal distention shortly before death, and one of these 3 had acute catarrhal enteritis. A volvulus was considered to be a compound related cause of death; no volvulus was seen in any control animals.

Blood Analyses: Blood samples were taken from each monkey on GD 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90 and 97. However, data on blood analyses of any kind could not be found.

Fetal Examinations: External, visceral and skeletal findings for each surviving fetus are shown on the page which follows. In spite of the very few surviving fetuses (3 control, 2 mid dose and 1 high dose), the investigators concluded that the external and skeletal malformations seen in the sole surviving high dose fetus were drug related and were due to severe maternal toxicity. It was claimed that these malformations had never

before been observed in controls.

Fetal Organ Weights: Although weights of 10 or 11 different fetal organs with means (and standard errors only for controls) are presented in Table 4 and Appendix III of the report, statistical comparisons were obviously not possible (only one or two surviving fetuses in the treated groups).

Comments: It should be noted that the control incidence of symptoms, mortality and spontaneous abortions were "unusually high". Although the investigators attributed the higher incidence of abortions and deaths in the high dose group to treatment, there are no indications that the differences were statistically significant. The animals used in the present study were feral monkeys. It seems reasonable to suggest that there may have been an interaction between disease or parasitic infestations (often inherent in feral monkeys), stress of handling or control vehicle administration, and treatment with the high dose, which would confound the outcome of this study. The monkey is not a commonly used model for reproductive toxicity tests and there is limited background information. The limited number of offspring (usually one per monkey) is considered to be a disadvantage for using this species for this type of study. The high mortality and abortion rates, even in control animals, further limits the usefulness of this study. However, the only surviving fetus in the in the high dose group showed malformations "which were never before observed in control fetuses".

Group 1 - 0 mg/kg

Female number	Fetus alive	Type of defect		
		External findings	Visceral findings	Skeletal findings
33797	+	bent tail end	left adrenal severely enlarged	6th to 10th and 12th rib on the right side and 7th to 9th rib on the left side of uneven thickness; 4th to 6th sternebra not ossified, 7th sternebra incompletely ossified
33383	+	no abnormalities detected	no abnormalities detected	5th to 11th rib on the right side and 4th to 11th rib on the left side of uneven thickness; 1st to 7th sternebra not ossified
28980	+	no abnormalities detected	no abnormalities detected	5th to 11th rib on the right side and 6th to 10th rib on the left side of uneven thickness; 6th sternebra not ossified and 7th sternebra incompletely ossified
149	+	no abnormalities detected	no visceral investigation due to caesarian section on day 76 p.c.	no skeletal investigation due to caesarian section on day 76 p.c.

Group 2 - 30 mg/kg

Female number	Fetus alive	Type of defect		
		External findings	Visceral findings	Skeletal findings
34738	+	prepuce not patent	no abnormalities detected	displaced zygostyle; 5th to 11th rib on the right side and 8th to 11th rib on the left side of uneven thickness, 1st to 2nd and 6th to 7th sternebra not ossified
34733	+	bent tail end	no abnormalities detected	parietals incompletely ossified; 6th to 13th rib on the right side and 2nd to 3rd, 7th to 11th and 13th rib on the left side of uneven thickness; 2nd and 6th to 7th sternebra not ossified

Group 3 - 100 mg/kg

Female number	Fetus alive	Type of defect		
		External findings	Visceral findings	Skeletal findings
34040	+	left forelimb appears thinner than normal; tail shortened and inwards curved tail end; only three fingers on the left side and 3rd finger with two nails	no abnormalities detected	additional ossification site between the metacarpals of the 2nd and 3rd finger, additional fingernail on the 3rd finger, proximal phalanx of the 2nd and 3rd finger and medial phalanx of the 3rd finger on the left side abnormally developed; the last three coccygeal vertebrae asymmetrically and incompletely ossified; 5th to 11th rib on the right side and 7th to 12th rib on the left side of uneven thickness; 1st to 3rd and 6th to 7th sternebrae not ossified

6. Perinatal and Postnatal Effects Following Oral Administration to Rats

Study No: T1002153

This report is accompanied by a "first amendment to report no. 12801 A", dated 8/14/93. Tables in the original English translation of the report were of very poor quality, not well organized, not legible, and contained errors in translation and typing. Some of these faults are listed in the amendment under the "rationale for the first amendment".

Performing Laboratory: [redacted]

Dates Performed: May 1981 to October 1981

Quality Assurance: No statement on GLP compliance was found in the original report. In the amendment, it is claimed, "...there were no legally binding GLP regulations in force" during the time that this study was performed.

Doses Tested: 0, 3, 10 and 30 mg/kg.

Test Animals: Mura:WIST (SPF 67 Han) female rats, 11-14 weeks of age and weighing 177-240 g, were mated with stock males of the same strain.

Procedure: Presumed pregnant females were dosed orally by gastric intubation from gestation day (GD) 16 to postpartum day (PPD) 21. Half the females in each group were C-sectioned on GD 20, the remainder were allowed to litter and rear their young to PPD 21. Of the C-sectioned animals, approximately 1/3 of all the fetuses in each group were examined for visceral anomalies by the Wilson technique, the remainder were evaluated for bone anomalies by staining with Alizarin red. The abdominal and thoracic organs of animals selected for bone anomaly examination were removed and "evaluated".

Test Substance: Batch No. 576 923. The vehicle consisted of Lutrol 400, anhydrous glycerol and demineralized water in a ratio of 969:60:100. A preparation of 0.6% nisoldipine was tested for stability and was found to be stable after 7 days.

Effects on All Pregnant Females: There were two deaths, one at 3 mg/kg (cause of death not determined), and one at 10 mg/kg (died of pneumonia), but no treatment related effects on mortality or clinical signs were evident. A small but significant decrease in body weight gain was found for the dams at 30 mg/kg (both the C-sectioned and rearing groups) between the first day of administration (GD 16) and GD 20 (See tables on pages 113 & 113A). Body weights of the low dose group of the C-sectioned rats were significantly higher than control throughout gestation, even on GD 0, but obviously this was not a compound related effect.

There were no compound related effects on reproduction parameters (percent inseminated, percent with implantations, etc.; see table on a page 114), nor were there any effects on gross pathology.

Examination of C-Sectioned Females: There were no effects found on mean number of implantation sites, live or dead fetuses or resorptions per dam. Furthermore, there were no effects of treatment on sex distribution, mean placental weight, number of runts (fetuses <3 g), or frequencies of external, visceral or bone deformations. A decrease ($P< 0.01$) in mean fetal weight at the 30 mg/kg dose was evident (See table on page 115). The table which follows immediately below is a summary of malformations found in all 4 groups, copied from the original report.

Group	Dam No.	Number of changed foetuses	Changes
Control	-	-	-
3 mg/kg	2995	1	no tail
10 mg/kg	2849	1	Otocephaly
	2852	2	slight dilation of the lateral ventricle of the brain
30 mg/kg	2991	1	Cryptorchism

Effects on F_0 Dams Allowed to Litter: There were no effects on duration of pregnancy at any dose level. The report indicates a significantly higher number of implantations per dam in the high dose group (probably incidental and not treatment related), and no effect on prenatal loss (implantations - surviving and dead pups). Complete litter losses were reported for 2 dams in the 30 mg/kg group (both did not suckle their young), and for 1 in the 10 mg/kg group (devoured its young during the 3rd week), but normal lactational behavior was observed in all other dams (not shown in tables but indicated narratively in the original report).

Effects on F_1 Offspring: There was an increase in number of stillborn pups, and a dose related increase in mortality of the newborn pups during the first week postpartum in the 10 and 30 mg/kg groups (See table on page 115B; no statistical analysis). The birth weight and the weight increase during the 3 weeks postpartum were both significantly reduced in the 30 mg/kg group vs control (See table on page 115C). The report claims no compound related effects on appearance or clinical signs of the F_1 offspring. In the maturational development tests, there were no effects of treatment on time to pinna unfolding, appearance of fur or eye opening, but there was a slight delay in time for normal walking in the 30 mg/kg group. For the functional tests, there was no effect on pupillary reflex ("following a light in a darkened room"; age when given is not stated), and no effect on hearing (stimuli from a Galton whistle "at the end of the lactation period"). Running performance on a running roller

(sensomotor behavior or proprioreceptor reflexes) was considerably reduced in the 3 mg/kg group, but this was considered an incidental finding because there was no effect at the 10 and 30 mg/kg dose levels. For the F_1 generation fertility test, 1 male and 1 female in each litter of the control and high dose groups were reared to 10 weeks of age, then mated. There were no differences between the 2 groups in rate of insemination or fertilization, duration of pregnancy, total number of live male or female pups at birth, body weight of pups or pups with external deformities.

PERI- AND POSTNATAL-STUDY BAY K 5552 T1002153

WEIGHT DEVELOPMENT (G) OF THE FEMALES UNDERGOING CESAREAN SECTION
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEIGHT GAIN				
DAY 0 - 16 P.C.	59.8	64.4	60.1	57.8
	9.6	9.9	11.1	10.3
DAY 16 - 20 P.C.	38.4	40.2	39.2	3[illegible]
	10.2	8.0	6.6	
DAY 0 - 20 P.C.	98.3	104.5	99.3	[illegible].8
	16.4	16.3	14.6	12.5
BODYWEIGHTS DURING GESTATION				
DAY 0 P.C.	201.0	210.0*	[illegible].8	203.1
	11.8	12.2	[illegible]0.1	11.3
DAY 16 P.C.	260.8	274.4*	261.9	260.9
	12.9	16	16.9	16.5
DAY 20 P.C.	299.3		301.1	293.9
	20.8	.0	20.6	16.8

* SIGNIFICANT DIFFERENC[illegible] ONTROL, P < 0.025
** SIGNIFICANT DIFFERENC CONTROL, P < 0.01

PERI- AND POSTNATAL-STUDY BAY K 5552 T1002153

WEIGHT DEVELOPMENT (G) OF THE DAMS
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEIGHT GAIN				
DAY 0 - 16 P.C.	58.4	60.1	61.2	61.7
	11.6	7.8	12.8	10.7
DAY 16 - 20 P.C.	39.9	37.7	35.6	33
	8.2	7.6	8.0	
DAY 0 - 20 P.C.	98.4	97.8	96.8	.3
	15.2	12.5	16.4	13.6
BODYWEIGHTS DURING GESTATION				
DAY 0 P.C.	204.3	202.1	[illegible]	205.3
	10.6	10.0	1.6	11.1
DAY 16 P.C.	262.8	262.2	264.1	267.0
	17.6	15.[illegible]	20.1	15.6
DAY 20 P.C.	302.8	[illegible]	299.7	300.7
	21.0	[illegible]	23.1	16.8
BODYWEIGHTS DURING LACTATION				
DAY 1 P.P.	238.8	240.0	239.7	236.6
	15	11.7	17.7	17.7
WEEK 1 P.P.		267.6	264.1	262.6
	.0	16.8	21.3	18.8
WEEK 2 P.P.	270.8	274.5	272.8	276.3
	20.3	13.5	21.2	19.8
WEEK [illegible]	264.6	262.2	265.1	266.0
	15.4	13.5	17.5	14.6

.IGNIFICANT DIFFERENCE TO CONTROL, P < 0.005

PERI- AND POSTNA[illegible]UDY BAY K 5552 T1002153

NUMBER OF ANIMALS [illegible]SULTS OF THE STUDY

DAMS

DOSE (MG/KG)	USED	NUMBER OF FEMALES INSEMINATED N	% OF THOSE USED	WITH IMPLANTATIONS N	% OF THOSE INSEMINATED	THAT LITTERED N	% OF THOSE WITH IMPLANTATIONS	THAT REARED THEIR PUPS N	% OF THOSE THAT LITTERED
0	25	25	100.0	21		20	95.2	20	100.0
3	25	25	100.0	20	8[illegible]	20	100.0	19	95.0
10	25	25	100.0	23	92.[illegible]	23	100.0	22	95.7
30	25	25	100.0	20	80.0	20	100.0	17	85.0

ANIMALS UNDERGOING CESAREAN SECTION

DOSE (MG/KG)	USED	NUMBER OF FEM[illegible]S INSEMINATED N	% OF THOSE USED	WITH IMPLANTATIONS N	% OF THOSE INSEMINATED	WITH FO[illegible] N	% OF TH[illegible] IMPLANTA[illegible]
0	25	25	100.0	20	80.0	20	100.0
3	25	25	100.0	20	80.0	19*	95.0
10	25	25	100.0	21	84.0	19*	90.5
30	25	25	100.0	23	92.0	22	95.7

* ONE FEMALE DIED BEFORE CESAREAN SECTION

PERI- AND POSTNA. STUDY BAY K 5552 T1002153

RESULTS OF THE CESAR SECTION (MEAN VALUES)

DOSE (MG/KG)	WEIGHT GAIN [G] 0-20 P.C.	WEIGHT GAIN [G] 16-20 P.C.	NO. IMPL.	(PER DAM) FEM.	(PER DAM) SUM	O F LOSS	MEAN-WEIGHT IN GRAMMS FETUSES	MEAN-WEIGHT IN GRAMMS PLACENT.	NO. OF FOETUSES EXAMINED BY WILSON	NO. OF FOETUSES EXAMINED BY DAWSON	FOETUSES WITH MINOR SKELE-TAL DEVIAT	FOETUSES WITH MALFOR-MATIONS	NO. OF RUNTS (<3G)
0	98.3	38.5	11.0	5.1	10.3	0.7	3.50	0.50	3.1	7.1	4.80	0.00	0.60
3	104.5	40.2	11.4	5.1	5.	.6	0.8	3.52	0.52	3.3	7.2	6.11***	0.05	0.58
10	99.3	39.2	10.2	4.5	4.9		0.8	3.54	0.53	2.8	6.9	4.68	0.05	0.32
30	90.8*	33.0*	10.7	5.3	4.6	9.5		3.38**	0.49	3.1	7.2	4.73	0.05	0.91

* SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.
** SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.0
*** SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.0

PERI- AND POSTNATAL-STUDY BAY K 5552 T1002153

PRENATAL LOSS OF THE REARING ANIMALS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
0.7	0.6	0.7	1.2
1.3	0.9	0.8	1.2

NUMBER OF IMPLANTATIONS OF THE [illegible]S

MEAN [illegible]VALUES AND STANDARD [illegible]TIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	[illegible].0 MG/KG	DOSE 30 MG/KG
10.3	9.9	10.5	11.0*
2.8	0.9	0.8	1.2

* SIGNIFICANT DIFFEREN[illegible] CONTROL, P<0.005

DU[illegible]ATION OF PREGNANCY IN DAYS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
21.8	21.9	22.0	22.0
0.7	0.7	0.6	0.8

Dose (MG/KG)	Total number of young (surviving + dead)	Number of still-born young	Total number of dead young after 1 week	2 weeks	3 weeks
Control	203	3	4	5	5
3	186	4	6	6	6
10	226	4	11	13	23
30	196	28	34	34	34

NUMBER AND WEIGHT DEVELOPMENT OF THE VIABLE PUPS
GROUP MEAN VALUES PER LITTER AND STANDARD DEVIATIONS

INVESTIGATION		0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
NUMBER OF PUPS					
AT BIRTH	TOTAL	10.0**	9.6**	9.6**	9.6**
		10.0	9.6	9.7	8.8
		2.5	2.2	1.9	3.1
AFTER 1 WEEK	TOTAL	9.9**	9.5**	9.3**	o
		9.9	9.5	9.3	
		2.5	2.1	2.0	
AFTER 2 WEEKS	TOTAL	9.9**	9.5**	9.2	9.5**
		9.9	9.5	9	9.5
		2.5	2.1		2.2
AFTER 3 WEEKS	TOTAL	9.9**	9.5**	9.2**	9.5**
		9.9	9.5	9.2	9.5
		2.5	2	2.0	2.2
WEIGHT [G] OF THE VIABLE PUPS					
AT BIRTH		5.9	6.1	6.0	5.2
		0	0.5	0.6	0.6
AFTER 1 WEEK			14.2	14.0	11.7
		.2	1.1	1.7	1.6
AFTER 2 WEEKS		24.2	25.2	25.0	20.6
		3.6	2.3	3.3	2.6
AFTER 3 WEEK		37.5	39.8	40.0	32.6
		6.1	3.8	5.1	4.4

** OF OM DAMS WHICH SURVIVED UNTIL THE END OF THE EXPERIMENT
AGE VALUE)

PERI- AND POSTNATAL-STUDY BAY K 5552 T1002153

NUMBER AND WEIGHT DEVELOPMENT OF THE VIABLE PUPS
GROUP MEAN VALUES PER LITTER AND STANDARD DEVIATIONS

INVESTIGATION		0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
NUMBER OF PUPS					
AT BIRTH	TOTAL	10.0 2.5	9.6 2.2	9.7 1.9	8.8 3.1
	MALES	5.8 2.1	4.4 1.3	5.3 1.9	4.0 2.1
	FEMALES	4.2 1.6	5.2 1.8	4.4 1.6	4.8 2.1
AFTER 1 WEEK	TOTAL	9.9 2.5	9.5 2.1	9.3 2.0	9.5 2
	MALES	5.8 2.1	4.4 1.3	5.1 1.9	
	FEMALES	4.1 1.6	5.1 1.7	4.2 1.7	5.2 1.8
AFTER 2 WEEKS	TOTAL	9.9 2.5	9.5 2.1	9	9.5 2.2
	MALES	5.8 2.1	4.4 1.3	.8	4.4 2.2
	FEMALES	4.1 1.6	5.1 1.7	4.2 1.7	5.2 1.8
AFTER 3 WEEKS	TOTAL	9.9 2.5		9.2 2.0	9.5 2.2
	MALES	5.8 2.1	4 .3	5.0 1.9	4.4 2.2
	FEMALES	4.1 1.7	5.1 1.7	4.2 1.7	5.2 1.8
WEIGHT [G] OF THE VIABLE					
AT BIRTH		9 .6	6.1 0.5	6.0 0.6	5.2* 0.6
AFTER 1 WEEK		13.4 2.2	14.2 1.1	14.0 1.7	11.7* 1.6
AFTER 2 WEEKS		24.2 3.6	25.2 2.3	25.0 3.3	20.6*** 2.6
AFTER 3		37.5 6.1	39.8 3.8	40.0 5.1	32.6** 4.4

* SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.01
** SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.005
*** SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.001
* SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.0005

7. Supplemental Perinatal and Postnatal Study in Rats

Study No.: T30[illegible]

Deviation:

Dates Performed: February 1984 to March 1984

Quality Assurance: A statement of GLP compliance was included.

Dosage Regimen: [illegible] mg/kg/day. On the last 3 days of treatment, "reduced activity" received only [illegible] mg/kg due to an error, but the study results were not considered to have been affected by this error.

Procedure: Presumed pregnant Sprague-Dawley derived female rats [illegible] per group were used. Animals were received on GD 2[illegible] at the start of treatment. They were housed individually in [illegible] cages. Dosing was by oral gavage from GD 6 to parturition day (PPD) 14. All the pregnant rats were allowed to litter and rear their young to PPD 21. The dams underwent observations for tolerance of the compound, effects of nitrendipine on parturition and influence on post natal development (similar to the observations in the main study). However, this study differed from the main study because the treatment and observation period extended to PPD 21 in the main study and only to PPD 14 in the present study.

Test Substance: [illegible]. The vehicle was methocel, glycerol and water.

Effects on the Dams: Of [illegible] rats, 1 died during parturition (with a dead fetus). Treatment related effects were a highly significant decrease in weight gain between [illegible] (initial part of the treatment period), which resulted in a reduced mean body weight up to [illegible] days, also evident by PPD 14, mainly caused by reduced intake, no [illegible] evidence of parturition, and prolonged gestation [illegible] days in the treated groups [illegible].

Effects on the Offspring: There were no significant intergroup differences in number of pups per litter at birth or after 1 and 2 weeks. However, there was a higher incidence of stillbirths and a higher rate of neonatal mortality throughout the 2 week postpartum study period for offspring of nisoldipine treated dams. The body weights of pups in the nisoldipine treated group were lower than control at the time of birth, and after 1 and 2 weeks. There was no effect on sex ratio at birth or at PPD 14 (See table which follows).

DOSE (MG/KG)	TOTAL NO. OF YOUNG (ALIVE + DEAD)	TOT. NO. OF DEAD YOUNG AT BIRTH	UP TO TIME OF 1ST WEIGHING	AFTER 1 WEEK	2 WEEKS
CONTROL	208	2	2	7	11
30	227	22*	30**	42**	50**

* significant difference from the controls, $p < 0.01$
**significant difference from the controls, $p < 0.001$

Comment: The increase in mean duration of gestation, followed by a higher incidence of stillbirths and a higher rate of neonatal mortality throughout the 2 week postpartum study period, and the lower body weight at birth for offspring of nisoldipine treated dams, were observed in both the main study and in the present study. These observations are suggestive of dystocia. Dystocia is defined as abnormal labor, which is usually accompanied by increased duration of labor and an increaed incidence of stillbirths and neonatal mortality.

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DATA FOR ANIMALS ALLOWED TO REAR THEIR OWN YOU'

INVESTIGATION	CONTROLS	.G/KG
DURATION OF GESTATION IN DAYS	21.6 0.5	22.1+ 0.5
WEIGHT GAIN DAY 0 to DAY 20 (G)	93 '	78.9*** 13.3
WEIGHT GAIN DAY 0 to DAY 16 (G)	.5 8.9	57.7 6.7
WEIGHT GAIN DAY 16 to DAY 20 (G)	37.5 8.9	21.2++ 10.2
BODY WEIGHT IN G DAY 0 P.C.	195.6 7.2	196.6 8.6
DAY 16 P.C.	251.1 13.3	254.4 10.8
DAY 20 P.C.	288.6 20.2	275.5** 17.2
DAY 1 '	222.8 13.0	210.2*** 16.6
1 P.P.	240.3 10.5	231.1* 13.6
WEEKS P.P.	246.3 14.6	245.9 14.3
NUMBER OF IMPLANTATIONS	10.0 3.3	10.4 2.8

* Significant difference from the controls, $p < 0.01$
** Significant difference from the controls, $p < 0.025$
***Significant difference from the controls, $p < 0.005$
\+ Significant difference from the controls, $p < 0.001$
++ Significant difference from the controls, $p < 0.0005$

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DATA ON THE YOUNG

INVESTIGATION	CONTROLS	MG/KG
NO. OF YOUNG AT BIRTH	9.4 3.3	8.5 3.1
AFTER 1 WEEK	[illegible]	8.4 3.1
AFTER 2 WEEKS	[illegible].0 3.1	8.0 3.1
AFTER 3 WEEKS	0.0 0.0	0.0 0.0
WEIGHTS OF THE YOUNG AT BIRTH (G)	5.9 0.7	5.3** 0.6
AFTER 1 WEEK	11.7 1.9	10.2* 2.1
AFTER 2 WEE	20.3 4.2	18.3 3.9
T[illegible] DAYS P.P.) OF: [illegible]DING OF PINNAE	3.5 0.7	3.5 0.6
[illegible]EVELOPMENT OF COAT	10.4 0.6	10.7 1.0

* Significant difference from the controls, $p < 0.01$
**Significant difference from the controls, $p < 0.005$

MUTAGENICITY STUDIES (S. Stolzenberg)

1. Salmonella/Microsome Test

Study No.: Not provided. Pharma Report No. 9634

Performing Laboratory:

Date Performed: August, 1980 to September, 1980

Quality Assurance: A signed statement of compliance with GLP is not included.

Background: This widely used mutagenicity assay detects point mutations (base pair by TA 1535 and TA 100 and frame shift by TA1537 and TA 98), caused by chemical agents, *in vitro*. The reversion rates to prototrophy of histidine requiring (his-) mutants to the wild type histidine independent strain (his+), are evaluated in a medium with a low content of histidine. In the presence of test compound, an increase in reversion rate, measured by an increase in colony growth on the agar plate, is an indication of mutagenicity.

Procedure: The evaluation was performed with and without metabolic activation (provided by S-9 fraction of livers of Aroclor pretreated rats). *S. cyphimurium* strains TA1535, TA100, TA1537 and TA98 were used. Two studies with TA 1535 but only one with the remaining 3 strains were carried out with 4 plates per concentration of test substance, DMSO control or postive control substances. Concentrations tested were 0, 20, 100, 500, 2500 and 12500 ug/plate.

Positive Controls:

a). without S-9 activation
Endoxan (cyclphosphamide) for TA1535 and TA100
Trypaflavin for TA1537 and TA98

b). with S-9 activation
2- aminoanthracene for all 4 tester strains

Results: 2500 ug/plate was toxic to bacterial growth for all 4 strains, whereas 12500 was toxic and caused precipitation, making it not possible to evaluate colony growth. 500 ug/plate was toxic for TA1535. In the first test, the positive control for TA1535 without S-9 showed no response, and the negative controls, both and without S-9, were unusually low. Therefore, the test with TA1535 was repeated. In the second test with TA1535, no indication of mutagenicity was seen with or without S-9. Similarly, Bay k 5552 showed no mutagenic effects on TA100, TA1537 and TA98. Positive controls gave adequate responses; i.e. well over double those of the negative controls.

Salmonella/Microsome Test with BAY k 5552 on Salmonella typhimurium TA 1535.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment / M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
12500			Could not be evaluated		
2500	0	0.8	64.4**	-	0.80
500	8.5	6.5	126.9**	8.50[a]	6.50[a]
100	9.0	6.3	158.4	9.00[a]	6.30[a]
20	14.5	5.0	146.0	14.50[a]	5.00[a]
Negative Control 0	1.0	1.0	152.4	1.00	1.00
Positive Control Endoxan 435	0.5	1.0	163.7	0.50[a]	1.00[a]
Positive Control 2-Aminoanthracene 10	32.8	19.0	5.2**	32.80[a]	19.00[a]

** Bacteriotoxic effect

a) See the "Results" section

In this test system, Bay k 5552 was considered not mutagenic at non-toxic, soluble concentrations.

Salmonella/Microsome Test with BAY k 5552 on Salmonella typhimurium TA 100.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
12500			Could not be evaluated		
2500	4.3	19.5	2,0**	0,06	0,04
500	80.5	34,5	4.3	1.18	0,71
100	72,3	62,0	4,7	1,06	1.27
20	53.5	49,3	6.2	0,78	1,01
Negative Control 0	68,5	48.8	4.7	1.00	1.00
Positive Control Endoxan 435	273.8	82.0	3.9	4.00*	1.68
Positive Control 2-Aminoanthracene 10	1136.0	81,8	0.6**	16.58*	1.68

* Mutagenic effect
** Bacteriotoxic effect

Salmonella/Microsome Test with BAY k 5552 on Salmonella typhimurium TA 1537.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
12500			Could not be evaluated		
2500	0	0.5	45.9**	-	0.13
500	3.3	1.5	49.1	0.66	0.38
100	7.3	2.0	51.3	1.46	0.50
20	5.0	1.5	51.5	1.00	0.38
Negative Control 0	5.0	4.0	50.9	1.00	1.00
Positive Control Trypaflavine 200	162.8	114.8	44.7**	32.56*	28.70*
Positive Control 2-Aminoanthracene 10	37.3	15.3	0.3**	7.46*	3.83*

* Mutagenic effect

** Bacteriotoxic effect

Salmonella/Microsome Test with BAY k 5552 on Salmonella typhimurium TA 98.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
12500			Could not be evaluated		
2500	3.0	1.3	100.0**	0,18	0,27
500	22.0	3,0	127.3	1.31	0.63
100	19.3	4,8	151.70	1.15	1,00
20	14.0	5,5	-	0.83	1.14
Negative Control 0	16,8	4,8	121.5	1.00	1.00
Positive Control Trypaflavine 200	460,0	4,3	144,3	27.38*	0.90
Positive Control 2-Aminoanthracene 10	845.3	8,0	70,7**	50.32*	1.67

* Mutagenic effect
** Bacteriotoxic effect

Salmonella/Microsome Test with BAY k 5552 on Salmonella typhimurium TA 1535. Repeat.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment / M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
2500	0	0	toxic	-	-
500	0	0	toxic	-	-
100	6.5	6.0	non-toxic	1.08	1.33
20	6.8	7.0	non-toxic	1.13	1.56
Negative Control 0	6.0	6.5	non-toxic	1.00	1.00
Positive Control Endoxan 435	427.3	24.3	non-toxic	71.22*	5.40*
Positive Control 2-Aminoanthracene 10	266.0	8.5	non-toxic	44.33*	1.89

* Mutagenic effect

2. Salmonella/Microsome Test

Study No.: T 5027709

Performing Laboratory:

Date Performed: 1/29/88 to 3/11/88

Quality Assurance: A signed statement of compliance with GLP is included.

Procedure: Tester strains used were *S. typhimurium* TA1535, TA100, TA1537 and TA98. Two different forms of S-9 were used; from livers of Aroclor 1254 pretreated male NMRI mice, and from livers of NMRI male mice that had been pre-treated for 28 days with 2000 ppm Bay k 5552 in the diet. All the studies were carried out with 4 plates per concentration of test substance, control or postive control substances. Initially, concentrations tested both without and with metabolic activation were 0, 20, 100, 500, 2500 and 12500 ug/plate. Subsequently, concentrations tested were 0, 75, 150, 300, 600, 1200 and 2400 ug per plate. There is no explanation as to why two methods of enzyme activation were employed.

Positive Controls:

a). without S-9 activation
Sodium azide for TA1535
Nitrofurantoin for TA100
4-nitro-1,2-phenylene diamine for TA 1537 and TA98

With S-9 activation
2-aminoanthracene for all 4 tester strains

Results: No indication of mutagenicity was observed at any concentration tested. However, 20 ug/plate, the lowest concentration tested, was the only concentration at which bacteriotoxic problems were not encountered. Starting at 150 ug/plate, precipitation problems were also encountered. Positive controls gave adequate responses; i.e. well over double the colony count of the negative controls.

Bay k 5552 was considered not mutagenic in this test system, but this study is valid only at 20 ug/plate because it was the only concentration at which toxicity and/or precipitation problems were not encountered with all 4 bacterial strains.

BAY k 5552
Salmonella/microsome test
Study No. T 5027709

Tabulated summary of data

Summary of means from Tables 1-8 without S-9 mix

Tables +group mcg/pl.	Strain TA 1535	TA 100	TA 1537	TA 98
1-4				
0	17	126	11	17
20	20	128	10	15
100	17	94	10	14
500	16	71	8	14
2500	--	9	--	9
12500	--	---	--	--
Na-Azid	926			
NF		405		
4-NPDA			126	187
5-8				
0	15	80	7	18
75	13	72	5	20
150	10	72	7	18
300	11	56	8	15
600	9	59	6	16
1200	--	38	8	12
2400	--	--	-	13
Na-Azid	1089			
NF		370		
4-NPDA			118	96

BAY k 5552
Salmonella/microsome test
Study No. T 5027709

Summary of means from Tables 1-12 with S-9 mix of Aroclor induced male NMRI mice

Tables +group mcg/pl.	Strain TA 1535	TA 100	TA 1537	TA 98
1-4 30% S-9				
0	19	146	11	35
20	17	128	12	30
100	18	125	9	32
500	13	83	10	32
2500	--	46	7	18
12500	--	---	--	--
2-AA	81	812	138	1018
5-8 30% S-9				
0	13	118	10	24
75	16	105	7	24
150	14	86	10	16
300	10	85	8	17
600	14	82	4	20
1200	10	62	6	17
2400	--	23	--	8
2-AA	89	1505	102	1290
9-12 10% S-9				
0	15	99	10	33
75	12	76	10	24
150	9	74	8	27
300	11	70	9	22
600	7	64	10	28
1200	--	63	8	21
2400	--	4	--	9
2-AA	264	2510	505	2402

BAY k 5552
Salmonella/microsome test
Study No. T 5027709

Summary of means from Tables 13-20 with S-9 mix
of BAY k 5552 treated male NMRI mice

Tables +group mcg/pl.	Strain TA 1535	TA 100	TA 1537	TA 98
13-16 30% S-9				
0	12	105	11	24
75	11	100	8	15
150	14	102	7	13
300	12	76	5	18
600	11	45	8	9
1200	4	---	--	--
2400	--	---	--	--
2-AA	78	893	44	748
17-20 10% S-9				
0	15	95	7	26
75	13	93	6	19
150	13	88	7	25
300	8	56	3	20
600	8	77	7	25
1200	--	32	-	--
2400	--	--	-	--
2-AA	349	1628	274	1066

3. CHO HGPRT Forward Mutation Assay

Study No.: T 1023114 (sponsor's number)

Performing Laboratory

Sponsor:

Date Performed: 7/11/86 to 10/29/86

Quality Assurance: A signed statement of compliance with GLP is included.

Background: "The objective of this study was to evaluate the test article for its ability to induce forward mutation at the HGPRT locus in the CHO-K1-BH Chinese hamster cell line, as assessed by colony growth in the presence of 6-thioguanine (TG). Hypoxanthine guanine phosphoribosyl transferase (HGPRT) is a cellular enzyme that allows cells to salvage hypoxanthine and guanine from the surrounding medium for use in DNA synthesis. If a purine analog such as TG is included in the growth medium, the analog will be phosphorylated via the HGPRT pathway and incorporated into nucleic acids, eventually resulting in cellular death. The HGPRT locus is located on the X chromosome. Since only one of the two X chromosomes is functional in the female CHO cells, a single-step forward mutation from HGPRT+ to HGPRT- in the functional X chromosome will render the cell unable to utilize hypoxanthine, guanine, or TG supplied in the culture medium. Such mutants are viable as wild-type cells in normal medium because DNA synthesis may still proceed by de novo synthetic pathways that do not involve hypoxanthine or guanine as intermediates. The basis for the selection of HGPRT- mutants is the loss of their ability to utilize toxic purine analogs (e.g., TG), which enables only the HGPRT- mutants to grow in the presence of TG. Cells which grow to form colonies in the presence of TG are therefore assumed to have mutated, either spontaneously or by the action of the test article, to the HGPRT- genotype."

Procedure: In preliminary, range finding tests, concentrations of 50 and 100 ug/ml Bay w 5552 (batch #828305) without metabolic activation were found to be 100% cytotoxic to the cell culture, but in the presence of metabolic activation (provided by S-9 fraction of livers of Aroclor 1254 pretreated rats), 100 ug/ml caused only a 39% decrease in survival index.

Three tests without S-9 included duplicate cultures with concentrations between 10 to 40 ug/ml, and two tests with S-9 included duplicate cultures with concentrations of 5 to 85 ug/ml. Positive controls were 5-bromodeoxyuridine without S-9 and 3-methylcholanthrene in the presence of S-9.

Results: Decreases in relative cell survival were seen at 30 ug/ml or higher concentrations without S-9, and at 50 ug/ml and higher with S-9. There were sporadic, small but statistically significant increases in mutant frequencies in each of the 3 tests without S-9 and in one of the two tests with S-9. In every case the increase occurred in only one of the two duplicate cultures. In spite of a suggestion of a concentration relationship at 40 and 60 ug/ml without S-9 (see first 2 tables which follow), it was claimed that a concentration relationship did not exist. It should, however, be noted that even among the duplicate controls, there were wide variations in mutant frequencies in most of the tests.

Bay k 5552 was considered not mutagenic in this test system.

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: Bay k 5552 mikr ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: August 20, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants
Expression Time: 7 days

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
NONACTIVATION:																		
Vehicle Control	258.0 ± 13.5	100.0	100.0	0	1	0	0	0	0	0	0	0	0	0	0	1	118.5 ± 8.7	0.4
	275.0 ± 1.0			2	2	0	1	0	1	2	1	2	3	0	4	18	108.8 ± 13.9	6.9*
Positive Control	145.7 ± 1.5	54.7	67.4	26	20	22	17	26	17	26	24	18	20	22	23	261	88.5 ± 1.3	122.9**
(50 µg/ml BrdU)[b]	149.3 ± 9.1	56.0	68.4	23	22	25	18	35	27	18	23	31	31	18	25	296	86.8 ± 7.3	142.1**
TEST ARTICLE																		
10.0 µg/ml	242.7 ± 6.5	91.1	169.6	NOT CLONED														
10.0 µg/ml	251.7 ± 12.7	94.4	159.6	NOT CLONED														
12.5 µg/ml	237.3 ± 7.6	89.1	131.4	NOT CLONED														
12.5 µg/ml	263.7 ± 32.0	98.9	139.6	NOT CLONED														
15.0 µg/ml	243.3 ± 7.5	91.3	127.3	0	2	0	0	0	0	0	0	1	C	0	0	3	83.5 ± 3.6	1.6
15.0 µg/ml	245.0 ± 22.6	91.9	164.6	0	0	1	0	0	0	0	0	0	0	0	0	1	80.5 ± 6.1	0.5
20.0 µg/ml	243.0 ± 21.7	91.2	143.7	NOT CLONED														
20.0 µg/ml	232.7 ± 11.6	87.3	136.2	NOT CLONED														
25.0 µg/ml	215.7 ± 10.2	80.9	124.1	1	1	1	1	3	1	2	0	2	0	1	0	13	84.0 ± 4.0	6.4
25.0 µg/ml	206.7 ± 28.6	77.5	115.2	2	0	1	1	0	0	1	1	1	1	2	0	10	100.3 ± 2.3	4.2
30.0 µg/ml	249.0 ± 6.6	93.4	99.7	C	C	C	C	C	C	C	C	C	C	C	C	-	100.0 ± 0.0†	-
30.0 µg/ml	227.3 ± 10.0	85.3	66.1	0	0	0	0	4	0	1	1	2	0	1	1	10	113.3 ± 4.6	3.7
35.0 µg/ml	237.7 ± 21.5	89.2	59.8	C	C	C	C	C	C	C	C	C	C	C	C	-	122.5 ± 6.4[x]	-
35.0 µg/ml	227.7 ± 11.7	85.4	55.1	0	0	0	0	2	0	0	0	0	1	0	1	4	133.7 ± 4.4	1.2
40.0 µg/ml	193.3 ± 9.3	72.5	39.6	4	2	1	1	4	3	3	0	0	3	0	0	21	105.2 ± 5.5	8.3**
40.0 µg/ml	193.0 ± 13.0	72.4	42.3	0	0	1	0	0	0	0	0	0	0	0	1	2	109.8 ± 3.3	0.8

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: Bay k 5552 mikron ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: September 19, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants

Expression Time: 7 days

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
NONACTIVATION:																		
Vehicle Control	193.7 ± 6.0	100.0	100.0	0	3	1	1	0	2	2	0	1	0	0	0	10	102.3 ± 4.9	4.1
	187.0 ± 15.4			0	2	1	1	2	2	0	1	0	1	0	0	10	111.3 ± 13.7	3.7
Positive Control (50 µg/ml BrdU)[b]	148.3 ± 15.8	77.9	37.6	12	14	16	10	13	13	15	14	13	16	17	15	168	103.3 ± 4.0	67.8**
	139.7 ± 7.8	73.4	32.9	20	12	18	16	20	15	22	14	16	13	14	10	190	105.0 ± 6.6	75.4**
TEST ARTICLE																		
10.0 µg/ml	147.3 ± 12.1	77.4	43.6	0	4	0	1	0	1	0	0	0	1	0	0	7	129.0 ± 5.3	2.3
10.0 µg/ml	156.3 ± 11.8	82.1	59.5	3	0	2	2	1	3	3	0	3	3	1	0	21	125.8 ± 6.4	7.0
15.0 µg/ml	152.0 ± 17.3	79.9	44.0	1	1	1	1	1	1	1	0	2	0	1	2	12	127.0 ± 14.1	3.9
15.0 µg/ml	151.3 ± 11.7	79.5	39.8	1	0	1	0	0	4	1	0	3	1	3	0	14	142.7 ± 14.7	4.1
20.0 µg/ml	137.0 ± 23.1	72.0	44.8	0	0	2	0	1	0	0	0	1	0	2	0	6	112.3 ± 7.4	2.2
20.0 µg/ml	151.3 ± 11.2	79.5	31.8	1	2	4	5	2	2	1	2	1	0	1	1	22	141.3 ± 2.8	6.5
30.0 µg/ml	125.3 ± 3.2	65.8	42.9	0	2	1	1	0	0	0	1	2	0	2	0	9	110.2 ± 1.2	3.4
30.0 µg/ml	135.7 ± 8.6	71.3	49.9	3	1	0	1	1	2	2	2	3	5	2	0	22	115.5 ± 4.4	7.9*
40.0 µg/ml	42.7 ± 9.0	22.4	7.0	0	0	1	0	0	0	1	0	0	1	2	1	6	129.3 ± 15.7	1.9
40.0 µg/ml	42.3 ± 8.1	22.2	7.4	0	3	2	4	3	0	4	3	4	3	7	3	44	96.3 ± 1.5	19.0**
60.0 µg/ml	0.0 ± 0.0	0.0	< 0.1	NOT CLONED														
60.0 µg/ml	0.0 ± 0.0	0.0	< 0.1	NOT CLONED														

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: Bay k 5552 mikron. ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: October 10, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants
Expression Time: 7 days

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES/DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
NONACTIVATION:																		
Vehicle Control	152.3 ± 11.2	100.0	100.0	1	2	0	0	1	2	0	0	0	0	0	3	9	112.8 ± 6.8	3.3
	172.0 ± 8.5			1	2	0	0	2	3	3	3	2	1	3	1	21	126.5 ± 11.0	6.9
Positive Control (50 µg/ml BrdU)[b]	84.7 ± 1.2	52.2	42.9	24	25	27	15	40	25	20	21	27	24	21	28	297	101.3 ± 11.5	122.2**
	84.7 ± 14.5	52.2	41.1	23	22	21	21	24	26	17	13	28	20	30	20	265	90.7 ± 5.5	121.7**
TEST ARTICLE																		
10.0 µg/ml	140.0 ± 6.1	86.3	76.0	2	2	1	3	5	2	2	3	1	2	2	0	25	108.7 ± 1.3	9.6*
10.0 µg/ml	145.7 ± 4.0	89.8	95.6	0	3	0	1	3	1	2	0	2	1	2	5	20	101.8 ± 11.3	8.2
15.0 µg/ml	146.7 ± 13.1	90.4	85.1	0	0	1	0	0	1	0	2	0	2	3	1	10	120.5 ± 1.7	3.5
15.0 µg/ml	136.7 ± 12.0	84.3	72.4	2	2	3	3	2	1	3	0	3	0	1	1	21	115.8 ± 13.8	7.6
20.0 µg/ml	138.7 ± 11.5	85.5	67.7	0	2	1	0	1	0	0	2	0	1	0	1	8	124.2 ± 6.6	2.7
20.0 µg/ml	128.0 ± 20.9	78.9	73.9	0	2	3	3	3	4	2	3	1	2	1	3	27	100.3 ± 9.4	11.2**
30.0 µg/ml	102.7 ± 7.4	63.3	65.8	1	2	1	1	1	3	2	1	1	1	3	3	20	104.2 ± 3.2	8.0
30.0 µg/ml	108.3 ± 9.0	66.8	57.7	T	T	T	2	2	1	1	5	2	1	4	0	18	122.3 ± 11.0	8.2
40.0 µg/ml	45.7 ± 12.1	28.2	8.2	6	0	0	1	2	4	4	0	1	0	3	1	22	116.8 ± 3.3	7.8
40.0 µg/ml	47.0 ± 5.3	29.0	9.1	1	0	1	0	1	1	3	0	1	1	2	0	11	113.5 ± 3.9	4.0
60.0 µg/ml	0.3 ± 0.6	0.2	< 0.1	NOT CLONED														
60.0 µg/ml	0.3 ± 0.6	0.2	< 0.1	NOT CLONED														
80.0 µg/ml	0.0 ± 0.0			NOT CLONED														
80.0 µg/ml	0.0 ± 0.0			NOT CLONED														
100.0 µg/ml	0.0 ± 0.0			NOT CLONED														
100.0 µg/ml	0.0 ± 0.0			NOT CLONED														

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: Bay k 5552 mikron. ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: September 10, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants

Expression Time: 7 days S9 batch: 1-186

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES/DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
S9 ACTIVATION:																		
Vehicle Control	294.3 ± 12.9	100.0	100.0	0	2	2	2	1	0	4	1	0	2	0	0	14	110.3 ± 5.1	5.3
	396.3 ± 122.1			0	0	0	2	1	1	0	0	0	0	0	0	4	110.2 ± 5.8	1.5
Positive Control (5 µg/ml 3-MCA)[d]	276.0 ± 20.2	79.9	55.4	69	55	53	88	53	70	69	60	61	63	61	52	754	97.2 ± 9.0	323.2**
	256.7 ± 12.0	74.3	37.8	86	81	65	64	80	64	54	62	63	77	68	83	841	117.3 ± 5.1	298.7**
TEST ARTICLE																		
10.0 µg/ml	272.7 ± 16.3	79.0	194.8	NOT CLONED														
10.0 µg/ml	267.3 ± 4.5	77.4	167.8	NOT CLONED														
20.0 µg/ml	295.3 ± 11.7	85.5	111.4	NOT CLONED														
20.0 µg/ml	324.7 ± 8.4	94.0	145.5	NOT CLONED														
35.0 µg/ml	305.0 ± 26.6	88.3	140.3	1	0	1	0	1	0	0	0	1	0	0		4	96.2 ± 10.2	1.7
35.0 µg/ml	293.0 ± 9.5	84.9	153.9	0	1	0	0	0	0	0	0	1	0	0	0	2	94.5 ± 5.9	0.9
50.0 µg/ml	680.3 ± 43.7	197.0	108.1	0	0	3	0	1	2	2	3	0	3	1	1	16	94.7 ± 3.8	7.0*
50.0 µg/ml	250.0 ± 11.5	72.4	94.4	1	0	2	0	0	1	0	0	0	0	0	0	4	87.8 ± 5.0	1.9
60.0 µg/ml	215.3 ± 13.6	62.4	77.2	0	0	0	0	0	0	0	0	0	0	0	0	0	91.7 ± 6.0	0.0
60.0 µg/ml	250.0 ± 13.0	72.4	37.5	0	0	0	0	1	0	1	0	3	2	0	0	7	95.7 ± 4.5	3.0
70.0 µg/ml	124.7 ± 2.1	36.1	17.3	0	2	0	0	0	0	0	0	0	0	1	0	3	81.3 ± 5.9	1.5
70.0 µg/ml	119.7 ± 12.7	34.7	17.8	0	0	0	0	1	0	1	2	0	1	0	0	5	79.8 ± 1.3	2.6
85.0 µg/ml	22.0 ± 1.7	6.4	2.9	0	0	0	0	0	0	0	0	0	0	0	0	0	102.2 ± 5.5	0.0
85.0 µg/ml	24.0 ± 3.6	7.0	3.0	0	0	0	0	0	0	0	0	0	0	0	0	0	98.3 ± 4.8	0.0
100.0 µg/ml	6.3 ± 1.5	1.8	0.3	NOT CLONED														
100.0 µg/ml	5.0 ± 2.6	1.4	0.3	NOT CLONED														

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: Bay k 5552 mikron. ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: October 1, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; 2×10^5/dish for mutants

Expression Time: 7 days S9 batch: 1-106

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES: DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
S9 ACTIVATION:																		
Vehicle Control	287.3 ± 12.7	100.0	100.0	0	0	0	0	0	0	0	1	2	1	0	1	5	101.8 ± 3.5	2.7
	347.3 ± 31.5			1	0	0	1	1	1	2	1	3	2	2	1	15	125.8 ± 9.5	5.0
Positive Control	259.7 ± 1.5	81.8	49.4	0	71	0	94	0	80	69	93	71	72	73	73	696	80.2 ± 7.2	482.1**
(5 µg/ml 3-MCA)[d]	266.3 ± 24.0	83.9	46.4	80	84	87	89	79	82	70	70	73	62	65	74	915	85.8 ± 7.7	444.3**
TEST ARTICLE																		
20.0 µg/ml	332.0 ± 26.5	104.6	90.3	0	0	1	0	1	0	2	2	1	0	1	0	8	119.7 ± 4.0	2.8
20.0 µg/ml	283.0 ± 13.8	89.2	80.6	2	2	0	1	1	1	1	0	1	0	1	3	13	127.3 ± 2.1	4.3
35.0 µg/ml	341.7 ± 13.1	107.7	99.2	0	1	0	0	2	0	1	0	0	1	0	0	5	122.3 ± 6.8	1.7
35.0 µg/ml	266.0 ± 16.6	83.8	132.5	0	0	0	0	0	0	0	1	0	0	1	0	2	129.2 ± 10.2	0.6
50.0 µg/ml	268.7 ± 7.6	84.7	C	NOT CLONED														
50.0 µg/ml	272.0 ± 7.8	85.7	C	NOT CLONED														
60.0 µg/ml	278.7 ± 39.4	87.8	75.4	2	0	0	1	3	2	3	0	1	1	1	C	14	117.3 ± 3.8	5.4
60.0 µg/ml	280.7 ± 4.6	88.5	82.3	1	1	>0	0	1	0	0	0	1	0	1	1	6	141.5 ± 4.8	1.8
70.0 µg/ml	169.3 ± 14.5	53.4	22.9	1	1	0	1	0	1	0	0	0	1	0	0	5	116.8 ± 9.4	1.8
70.0 µg/ml	142.7 ± 10.1	45.0	31.6	0	0	1	0	2	0	0	1	0	0	0	0	4	99.5 ± 4.8	1.7
85.0 µg/ml	46.0 ± 5.6	14.5	3.3	3	1	0	2	1	2	0	2	1	0	1	0	13	109.5 ± 2.8	4.9
85.0 µg/ml	34.0 ± 3.6	10.7	2.3	2	0	1	0	0	0	0	0	0	0	0	0	3	125.7 ± 0.6	1.0
100.0 µg/ml	5.0 ± 0.0	1.6	0.2	NOT CLONED														
100.0 µg/ml	3.3 ± 0.6	1.0	0.2	NOT CLONED														

EXPLANATION OF TABLES, ASSAY NO. E-9510

a = Mutant Frequency = Total mutant clones/ (No. of dishes x 2 x 10^5 x abs. C.E.)

b = BrdU = 5-Bromo-2'-deoxyuridine

d = 3-MCA = 3-Methylcholanthrene

** = Significant increase, p≤0.01

* = Significant increase, p≤0.05

C = Contaminated

T = Lost dishes due to a technical error

÷ = Two dishes lost due to contamination

⅄ = One dish lost due to contamination

4. Mouse Hepatocyte Primary Culture DNA Repair Assay

Study No.: T2 023 386 (sponsor's number)

Performing Laborator

Sponsor

Date Performed: 8/14/86 to 2/25/87

Quality Assurance: A signed statement of compliance with GLP is included.

Background: Freshly obtained rodent, metabolically active hepatocytes are capable of limited biotransformation activity. Chemically induced damage to nucleic acid of the mammalian cells results in an effort by the enzyme systems to repair the defect(s), resulting in unscheduled DNA synthesis.

Procedure: Freshly prepared hepatocyte primary cell cultures from adult $B6C3F_1$ males were used, according to the method of Williams et al, Cancer Letters 6: 119-306, 1982. Eight concentrations between 1 mg/ml and 5×10^{-4} mg/ml (listed in the table which follows) were tested in triplicate against $5X10^5$ hepatic cells. DNA repair was determined by ^{3}H-thymidine uptake, to determine the net increase in nuclear grains induced by the test compound. Benzo(a)pyrene served as positive control and DMSO and pyrene served as negative controls. A test compound was considered positive when the mean net nuclear grain count exceeded that of the DMSO control by more than 2 standard deviations.

Results: Cytotoxicity was observed at concentrations of 1 and $5X10^{-1}$ mg/ml. No net increase in nuclear grain count was observed at concentrations between $5X10^{-4}$ and 10^{-1} mg/ml or with pyrene and DMSO (negative controls); the positive control, benzo(a)pyrene, was highly genotoxic, indicating that the hepatocytes were capable of metabolic transformation and DNA repair.

Under the conditions of this test system, Nisoldipine was considered to be not genotoxic at concentrations up to 10^{-1}M

NDI/IN VITRO Systems Facility
Report on: UDS/DNA Repair Assay

Date: November 21, 1986
Expt # H112186
Chemical Nisoldipine
Molecular wt
Source/Purity
Lot #
Solvent/vehicle DMSO
Volatility
Precautions
Positive control Benzo(a)pyrene (BaP)
Negative control Pyrene

Assay method Autoradiography
Species/strain/sex/age/wt House/B6C3F$_1$/Male/Adult/28-32g
Organ or tissue/condition Liver
Cells/primary or line Primary
Medium used: Williams Medium E
Duration of chemical exposure 18 hrs.
Chemical dose range 1 to 5 x 10^{-4} mg/ml
Exposure method In Situ
Label/uCi/ml or Ci/mM, final ^{3}H-thymidine, 10uCi/ml
Interval between exp. and label Simultaneous
Duration of label 18 hrs.

CONTROLS			TEST RESULTS				COMMENTS:
Positive	Conc.	Autoradiog. grains/nucleus (NET)*	Conc. mg/ml	Autoradiog. grains/nucleus (NET)*	Cytoxicity	Evaluation	* Mean ± standard deviation of triplicate coverslips.
BaP	10^{-5}M	43.7 ± 5.0	1		Toxic		**Mean of duplicate coverslips.
			5×10^{-1}		Toxic		
			10^{-1}	-11.6 ± 1.7	Subtoxic	Negative	
			5×10^{-2}	-15.2**	Subtoxic	Negative	
			10^{-2}	-16.5 ± 1.8	Nontoxic	Negative	
			5×10^{-3}	-15.3 ± 2.4	Nontoxic	Negative	
Negative	conc.	grains/nucleus (NET)	10^{-3}	-16.5 ± 2.1	Nontoxic	Negative	
Pyrene	10^{-5}M	-11.1 ± 4.2	5×10^{-4}	-12.8 ± 0.9	Nontoxic	Negative	
DMSO	1%	-13.6 ± 1.3					
Cell Control		-13.9 ± 3.7					

5. Micronucleus Test in Mice

Study No.: T 1010 875

Performing Laboratory:

Date Performed: 11/6/81 to 12/1/81

Quality Assurance: A signed statement of compliance with GLP is not included.

Background: The micronucleus test permits the recognition of a mutagenic action on a somatic tissue, the femoral bone marrow, of an intact animal. Erythrocytes are normally anuclear. The increased occurrence of micronuclei (chromosome fragments) in polychromatic erythrocytes, compared to negative controls, indicates that the test substance brings about chromosome breaks, or has effects on the spindles in the erythroblasts.

Procedure: Bor: NMRI (SPF Han) mice, 8 to 12 weeks of age, weighing 23 to 32 g, 5 of each sex per group, received 0, 100 and 200 mg Bay k 5552/kg/day, p.o., on two consecutive days; positive controls received cyclophosphamide (Endoxan^{R}) orally. All animals were sacrificed 6 hours after the second dose and femoral bone marrow samples were removed for the purpose of determining the frequency of micronuclei observed, by examination of 1000 polychromatic erythrocytes per animal.

Results: Since there was no difference between males and females, the results obtained for both sexes were pooled. No deaths or clinical signs were observed. The significant decrease in normochromatic erythrocytes in the 100 mg/kg group was considered "not biologically significant" (see table on page which follows). It is therefore claimed that Bay k 5552 produced no inhibition of erythropoiesis.

There was no increase in polychromatic erythrocytes with micronuclei. The positive control caused a high level of response.

It was concluded that there was no indication of a mutagenic effect by this test.

Survey of the experimental evaluation he micronucleus test

with BAY k 5552

Experimental group	Number of investigated polychromatic erythrocytes	Number of normochromatic erythrocytes per 1,000 polychromatic erythrocytes	Number of cells with micronuclei	
			per 1,000 normochromatic erythrocytes	per 1,000 polychromatic erythrocytes
I Negative control	10000	682.6	1.0	1.6
II BAY k 5552 2 x 100 mg/kg	10000	465.6*	1.9	1.8
III BAY k 5552 2 x 200 mg/kg	10000	543.1	1.2	1.8
IV Positive control Endoxan 2 x 87 mg/kg	10000	724.0	1.2	31.4

* $P \leq 0.05$ in the distribution-free test of ranks according to NEMENIY related to the negative control (I)

6. Dominant-Lethal Test in Mice

Study No.: T 50 10 239

Performing Laboratory:

Date Performed: 5/11/81 to 11/10/81

Quality Assurance: A signed statement of compliance with GLP is not included.

Background: This test permits detection of mutations in germ cells, particularly stage-specific effects, during meiotic maturation of sperm cells, and is usually performed in mice or rats. A mutagenic test substance causes severe chromosomal damage or lethal germ cell mutations, resulting in embryo or fetal mortality. This is determined by increased occurrence of pre- or post-implantation loss in females (untreated) that were mated to test substance treated males.

Procedure: Bor: NMRI (SPF Han) mice, 8 to 12 weeks of age, body weights at initiation of study of 30-44 g for males and 25-30 g for females, were used. Males in the treated group (50/group) received 200 mg/kg Bay w 5552/kg in aqueous hydroxyethylmethyl-cellulose, single oral dose (Batch No. 576923); control males received vehicle. Starting on the day of drug administation, a new, untreated female was placed in the cage of each treated or control male at the start of each of 12 mating periods, lasting 4 days each (48 days total). About 14 days after the middle of the relevant mating period, the uterus of each female was examined for live and dead embryos, resorption sites and corpora lutea.

Criteria for Dose Selection: Claimed to be based on a preliminary study with female mice, 5/group, which received 200, 500 or 1000 mg/kg, p.o., and in which "200 mg/kg was tolerated with only slight symptoms".

Results: There were no effects of treatment on impregnation rate, fertility, pre- or post-implantation loss. Because there was no indication of early death of embryos at any stage of mating due to compound treatment, there was no indication of a dominant lethal effect (see tables on next 2 pages).

In conclusion, Bay K 5552 was considered to be not mutagenic in this test system.

DOMINANT-LETHAL-TEST

SINGLE TREATMENT OF MALE MICE WITH DAY X 5552

STUDY .: 15610739

DOSE GROUP 200 MG/KG (P.O.)

PREIMPLANTATION LOSS

MATING PERIOD	CORPORA LUTEA				IMPLANTATIONS*				PREIMPLANTATIONS LOSS			
	TOTAL		PER IMPREGNATED FEMALE		TOTAL		PER IMPREGNATED FEMALE		TOTAL		PER IMPREGNATED FEMALE	
	CONTROL	DOSE GROUP	CONTROL	DOSE GROUP	CONTROL	DOSE GROUP	CONTROL	DOSE GROUP	CONTROL	DOSE GROUP	CONTROL	DOSE GROUP
1	621	584	12.9	13.3	577	453	12.0	11.9	44	51	0.92	1.34
2	472	535	12.8	12.4	442	502	11.9	11.7	30	33	0.81	0.77
3	543	596	12.3	13.0	518	566	11.8	12.3	25	30	0.57	0.65
4	572	425	13.6	12.1	532	380	12.7	10.9	40	45	0.95	1.29
5	419	433	13.1	12.4	408	400	12.8	11.4	11	33	0.34	0.94
6	518	558	12.6	13.0	474	503	11.6	11.7	44	55	1.07	1.28
7	531	544	14.0	13.6	504	503	13.3	12.6	27	41	0.71	1.02
8	591	503	13.7	13.2	536	469	12.5	12.3	55	36	1.28	0.89
9	541	512	12.9	13.1	507	463	12.1	11.9	34	49	0.81	1.26
10	624	514	13.0	13.5	581	462	12.1	12.2	43	52	0.90	1.37
11	568	561	13.5	13.7	543	527	12.9	12.9	25	34	0.60	0.83
12	604	525	14.4	12.5	568	491	13.5	11.7	36	34	0.86	0.81
1 -12	6604	6218	13.2	13.0	6190	5719	12.4	12.0	414	491	0.83	1.03

* Since it can occur that a placenta is found with two embryos on one implantation site, the number of implantations can be smaller than the total of living and dead implants.

DOMINANT - LETHAL - TEST
SINGLE TREATMENT OF MALE MICE WITH BAY K 5552
STUDY : T5910219

DOSE GROUP 200 MG/KG (P.O.)

POSTIMPLANTATION LOSS

MATING PERIOD	LIVING IMPLANTS		PER IMPREGNATED FEMALE		DEAD IMPLANTS		PER IMPREGNATED FEMALE	
	CONTROL GROUP	DOSE GROUP	CONTROL GROUP	DOSE GROUP	CONTROL GROUP	DOSE GROUP	CONTROL GROUP	DOSE GROUP
1	547	427	11.4	11.2	31	26	0.65	0.68
2	414	481	11.2	11.2	31	24	0.84	0.56
3	492	531	11.2	11.5	27	36	0.61	0.78
4	480	346	11.4	9.9	52	38	1.24	1.09
5	338	361	12.1	10.3	22	40	0.69	1.14
6	446	461	10.9	10.7	29	46	.71	1.07
7	472	479	12.4	12.0	33	25	0.87	0.63
8	497	440	11.6	11.6	42	29	0.98	0.76
9	485	429	11.5	11.0	24	34	0.57	0.87
10	546	434	11.4	11.4	38	31	0.79	0.82
11	501	481	11.9	11.7	43	46	1.02	1.12
12	544	463	13.0	11.0	27	30	0.64	0.71
1 -12	5812	5333	11.6	11.2	399	405	0.80	0.85

7. In Vitro Chinese Hamster Ovary Cell (CHO) Test for Clastogenic Potency

Study No.: 2528 MIC

Performing Labora

Sponsor:

Date Performed: Not indicated. The report is dated 1/19/88

Quality Assurance: A signed statement of compliance with GLP is included.

Procedure: "CHO described by Puck" (Genetics 55:513-518, 1967), were used. After preliminary cytotoxicity tests, concentrations of Bay K 5552 tested without metabolic activation were 10, 20 and 30 uM, and with metabolic activation (provided by S-9 fraction of livers from Aroclor induced rats) they were 500, 600 and 800 uM. The highest dose levels were selected to give about a 50% inhibition of mitotic index, but allowed sufficient numbers of cells at the metaphase stage for analysis of chromosome and chromotid aberrations (breaks, gaps and exchanges); generally, a requirement for dose selection with this test. Positive control compounds were methyl-methane-sulfonate (MMS) without S-9 and cyclophosphamide (CP) with S-9. An 18 hour incubation period, which corresponds to one cell cycle, was selected for the main study. Aberrations were analyzed in 100 cells arrested at the metaphase stage of cell division for each concentration level. The assays with and without S-9 were performed twice.

Results: There were no increases in chromosome or chromatid aberrations at any concentration (see tables which follow). The positive controls (MMS or CP exposed cells) showed statistically significant, large increases in rates of aberration.

In conclusion, Bay K 5552 was considered to be not clastogenic in this test system.

ASSAYS WITHOUT S-9 MIX
Mean of both assays

A. Number of aberrations per 100 analysed metaphases

Chemical	Dose (µM)	Gap	Break	Chromatid Exchanges	Chromo-some Exchanges	Multiple aberra-tions	Total number of aberrations	
							Including gaps	Excluding gaps
-	-	8.5	2.0		1.0		11.5	3.0
DMSO	-	5.5	0.5		1.0		7.0	1.5
BAY K 5552	10	4.5	2.0			0.5	6.5	2.0
	20	2.5	5.5	1.0	2.5		11.5	9.0
	30	5.5	2.0				7.5	2.0
MMS	605	15.5	29.5	36.0	7.0	28.5	88.0	72.5

B. Percentage of cells containing aberrations

Chemical	Dose (µM)	Gap	Break	Chromatid Exchanges	Chromo-some Exchanges	Multiple aberra-tions	Percentage of cells containing aberrations	
							Including gaps	Excluding gaps
-	-	7.0	2.0		1.0		9.5	3.0
DMSO	-	5.5	0.5		1.0		6.5	1.5
BAY K 5552	10	4.5	2.0			0.5	7.0	2.5
	20	2.5	4.0	1.0	1.5		7.0	5.0
	30	4.5	1.5				6.0	1.5
MMS	605	13.0	18.0	26.5	6.5	28.5	73.0	65.0***

Break : chromatic and chromosome
Chromatid exchanges : inter and intrachange
Chromosome exchanges : inter and intrachange
Multiple aberrations : complex rearrangement
Statistical test used : X^2 *** $p \leq 0.001$

ASSAYS WITH S-9 MIX
Mean of both assays

A. Number of aberrations per 100 analysed metaphases

Chemical	Dose (µM)	Gap	Break	Chromatid Exchanges	Chromosome Exchanges	Multiple aberrations	Total number of aberrations	
							Including gaps	Excluding gaps
-	-	8.0	1.0	1.0	1.5	2.0	11.5	3.5
DMSO	-	6.0	1.0	1.0	1.5		9.5	3.5
BAY K 5552	500	3.5	2.0		0.5		6.0	2.5
	600	2.0	0.5		1.0		3.5	1.5
	800	3.5	2.5	1.5	1.0	0.5	8.5	5.0
CPA	130	17.0	29.5	24.5	6.5	3.0	77.5	60.5

B. Percentage of cells containing aberrations

Chemical	Dose (µM)	Gap	Break	Chromatid Exchanges	Chromosome Exchanges	Multiple aberrations	Percentage of cells containing aberrations	
							Including gaps	Excluding gaps
-	-	8.0	1.0	1.0	1.5	2.0	13.0	5.0
DMSO	-	6.0	0.5	1.0	1.5		8.0	2.5
BAY K 5552	500	3.5	2.0		0.5		6.0	2.5
	600	1.5	0.5		1.0		3.0	1.5
	800	3.5	2.0	1.0	1.0	0.5	7.5	4.0
CPA	130	15.0	25.5	20.0	5.5	3.0	53.5	44.0***

Break : chromatid and chromosome
Chromatid exchanges : inter and intrachange
Chromosome exchanges : inter and intrachange
Multiple aberrations : complex rearrangement

Statistical test used : X^2 *** $p \leq 0.001$

8. Test for Inhibition of Liver Cell Culture Intercellular Communication

Study No.: T2 023 386 (sponsor's number)

Performing Laboratory:

Sponsor:

Date Performed: 5/18/87 to 5/27/88

Quality Assurance: A signed statement of compliance with GLP is included.

Background Information: In this assay, the transfer of the toxic metabolite, 6-thioguanine (TG), from metabolically competant freshly isolated rat hepatocytes ($HGPRT^+$) to a mutant rat liver cell culture, ARL14-TG^R, which is purine analog resistant ($HGPRT^-$), is measured. When exposed to TG, the presence of the primary hepatocytes results in a reduction of TG^R colonies. The TG kills primary hepatocytes and ARL-TG^R cells to which the metabolite is transferred. If a test chemical inhibits membrane contact or intercellular communication (also referred to as metabolic cooperation), the reduction of TG^R colonies in the flasks containing primary hepatocytes will be diminished, leading to an increased survival and formation of colonies. It is claimed that "many but not all tumor promotors inhibit metabolic cooperation".

Procedure: Nisoldipine was tested at concentrations which ranged between $5X10^{-5}$ mg/ml and $5X10^{-4}$ mg/ml, to determine if it inhibited metabolic cooperation between rat primary hepatocytes (wild type cells) and ARL14-TG^R cells. It is claimed that toxicity had been previously observed at $5X10^{-2}$ mg/ml, but the data were not shown. Positive control used was DDT.

Results: A concentration dependent inhibition of metabolic cooperation was not observed with nisoldipine. DDT caused an inhibition of metabolic cooperation. (See table which follows).

It was concluded that Bay k 5552 did not inhibit metabolic cooperation in this test system.

Chemical	Concentration	Number of Colonies[a] No Hepatocytes	Number of Colonies[a] 1.25×10^6 Hepatocytes
None	-	233 ± 38 (226,276,185,245)	97 ± 19 (98,122,93,75)
DMSO	0.1%	221 ± 22 (252,222,206,205)	118 ± 11 (103,117,123,129)
DDT	5×10^{-5}M	157 ± 10 (145,163,153,167)	151 ± 14 (132,156,151,164)
Nisoldipine	5×10^{-4} mg/ml	187 ± 54 (235,230,160,124)	118 ± 7 (111,118,125)
	10^{-4} mg/ml	181 ± 9 (177,171,192,183)	118 ± 9 (114,112,129)
	5×10^{-5} mg/ml	202 ± 17 (227,197,198,187)	89 ± 28 (114,69,112,60)

[a] Mean ± standard deviation of three to four flasks.

LABELING

Under PRECAUTIONS, the first sentence of the subsection "Carcinogenesis, Mutagenesis and Impairment of Fertility" presently reads:

Nisoldipine was administered orally to mice and rats for 21 and 24 months respectively, and was not shown to be carcinogenic.

We recommend the following revision :

Dietary administration of nisoldipine at doses up to 111 mg/kg/day for 24 months to rats or at doses up to 217 mg/kg/day for 21 months to mice (125 to 250 times the maximum recommended human dose of 40 mg/kg, based on a mg/kg comparison assuming a patient weight of 60 kg) revealed no evidence of a tumorigenic effect.

Under the same subsection "Carcinogenesis, Mutagenesis and Impairment of Fertility", the statement, "_____nisoldipine did not interfere with fertility at a dose more than 30 times the maximum recommended human dose" is meaningless. This should be changed to indicate the dosage in terms of mg/kg, then converted to dosage based on surface area, i.e. in terms of mg/m^2, which may then be compared to human dosage.

Based on the outcome of all the *in vitro* and *in vivo* tests, the statement in labeling, "The results of *in vitro* and *in vivo* mutagenic studies were negative" is reasonable.

Under *Pregnancy Category C*, we agree with the Category C classification, even though animal studies are only suggestive of potential fetal risk. The labeling should be modified to indicate that the fetal toxicity observed in the studies with animals are suggestive, not conclusive. It should specify that the monkey study was confounded by 1) the use of feral monkeys which are particularly susceptible to the stress of handling, and 2) the high rate of abortion and mortality, in both treated and control groups, resulting in only one surviving fetus in the 100 mg/kg group (which presented with anomalies) and only 3 surviving control fetuses. It should be stated that although it cannot be concluded that nisoldipine was teratogenic in the monkey study, such a possibility cannot be ruled out because the teratogenic effects observed (multiple left forelimb, finger and tail abnormalities observed externally, and related forelimb and vertebral abnormalities observed with skeletal examination) had not been previously observed in untreated animals of this species.

The proposed labeling does not specify the form of maternal toxicity that was observed in either the rat or rabbit. The phrase which states that nisoldipine caused a slightly increased malformation rate in rabbits should be omitted from the labeling. Of the two rabbit studies, slightly increased malformation rate was attributed to the stress of diarrhea, which occurred in one

of these two studies. There was no increase in any specific form of malformation.

The statement on fetotoxicity in rats and rabbits should be revised to read as follows:

Fetotoxicity in rats and rabbits was usually observed only at doses which caused a decrease in body weight gain of dams compared to control. In rats, an increase in post-implantation loss was observed at 100 mg/kg, and a decrease in fetal weight was observed at 30 and 100 mg/kg. In rabbits, decreases in fetal and placental weights were observed at 30 mg/kg.

For the rat and rabbit Segment II studies, the dosages cited as "*30 (or 100) times the maximum human dose*", should be expressed in terms of both mg/kg and mg/m^2.

Under a new section, *Labor and Delivery*, the labeling should state that the drug caused a slight prolongation of pregnancy in rats. The prolongation of pregnancy is presently noted under the *Pregnancy Category* section but should be moved here.

Under *Nursing Mothers*, it should be pointed out that "nisoldipine was found in the milk of lactating rats at concentrations which were lower than levels found in the plasma".

OVERALL SUMMARY AND EVALUATION

Nisoldipine coat core (Nis CC) tablet, proposed for the treatment of hypertension (alone or in combination with other antihypertensive agents) on a once-a-day dosage regimen, is an extended release formulation of the dihydropyridine calcium channel blocker nisoldipine. Nis CC tablet has an external coat of slow release formulation and an internal core of fast release formulation of nisoldipine.

Calcium channel blockers have recently emerged as a promising new class of antihypertensive agents. By blocking the channels which mediate calcium entry into smooth muscle cells, these agents decrease intracellular calcium levels, thereby inhibiting vascular smooth muscle contractions, resulting in a decrease in peripheral vascular resistance and reduction in blood pressure. Because calcium channel blockers inhibit coronary vasoconstriction and reduce vascular resistance and increase coronary blood flow, thereby protecting the heart against ischemia and reperfusion damage, they are also effective in the treatment

Nisoldipine was developed with the aim of improving the pharmacologic properties of nifedipine. Despite its chemical similarity to nifedipine, nisoldipine is a more potent dilator of coronary as well as peripheral blood vessels. Nisoldipine is 3 to 10 times more potent than nifedipine in increasing coronary blood flow and coronary venous oxygen saturation in dogs. In isolated vascular preparations, nisoldipine inhibits calcium- and potassium-induced contractions at concentrations 4-10 times lower than that of nifedipine. A negative inotropic effect, an adverse effect usually observed with other calcium channel blockers, has not been shown with nisoldipine in its therapeutic dose range.

An oral dose of 10-40 mg once daily is proposed for the treatment of hypertension and a dose of once daily is proposed for the treatment

This new drug application is supported by fairly extensive preclinical studies.

Nisoldipine was shown to produce dose-dependent reductions in blood pressure and total peripheral resistance in rats, dogs, cats and pigs. In SH rats, single oral doses of 0.315, 1.0 and 3.15 mg nisoldipine/kg produced 3, 12 and 18% reductions in blood pressure, respectively. The maximum effect was seen at 1 hr after drug administration and the blood pressure returned to the pretreatment level at 6 hr postdose. The antihypertensive effect of nisoldipine was much more pronounced in renal hypertensive rats than in SH rats; a dose of 3.15 mg/kg po produced a 39% reduction in blood pressure in renal hypertensive rats compared to an 18% reduction in SH rats. Long term treatment with nisoldipine (50-100 mg/kg/day in the diet for 60 weeks)

prevented the development of hypertension in SH rats (mean systolic blood pressure of 141 mm Hg in the drug treated group vs 214 mm Hg in the control group at the termination of the study) and other rat models. Chronic treatment with the test compound also significantly reduced the atrial natriuretic peptide and aldosterone concentrations in plasma and attenuated cardiac hypertrophy in SH rats.

The (+) enantiomer was found to be only slightly more potent than the racemic compound in its antihypertensive activity in SH rats, but it was about 20 times more potent than the (-) enantiomer.

In terms of antihypertensive effect, nisoldipine was about equipotent to nifedipine, nicardipine and hydralazine (ED20 = 4 mg/kg po) in SH rats; however, it was less potent than the other drugs in other hypertensive rat models.

Single oral doses of nisoldipine (0.03-1.0 mg/kg) produced dose-dependent decreases in mean arterial blood pressure in conscious renal hypertensive dogs. At 0.3 mg/kg, a 24% reduction in blood pressure was produced within 2 hr after drug administration and the hypotensive action lasted for about 12 hours. A tachycardia, lasting for about 3 hr, also occurred at the above dose level. Nifedipine produced about the same degree of hypotension (lasting about 6 hr) as that produced by an oral dose of 0.3 mg nisoldipine/kg at about a 10 fold higher dose level (ED20 - 0.14 mg/kg for nisoldipine vs 1.68 mg/kg for nifedipine). The antihypertensive activity of orally administered nisoldipine in renal hypertensive dogs significantly correlated with plasma concentrations of the drug.

Low doses of nisoldipine increased coronary blood flow and protected the heart against ischemia and reperfusion damage in various experimental models. In isolated rat hearts subjected to ischemia and reperfusion, nisoldipine (3 nM) doubled coronary blood flow. At 1 nM, nisoldipine increased coronary blood flow 31% and improved the recovery of contractile function and tissue ATP levels. Radioactive microsphere studies in conscious rats showed that oral administration of nisoldipine (0.3 mg/kg) produced a marked increase in coronary blood flow as well as a decrease in coronary vascular resistance. In anesthetized dogs, nisoldipine (5 µg/kg iv) increased coronary sinus blood flow by 111% and coronary sinus oxygen content by 50%. In dogs with acute myocardial infarction, nisoldipine (5 µg/kg iv 15 min, 2 and 4 hr after coronary artery occlusion) reduced myocardial infarct size by 31%. In pigs with gradual coronary occlusion, the test drug (30 µg/kg po, every 6 hr for 1 month) significantly reduced infarct size and increased endocardial and transmural blood flow by enhancing endocardial collateral circulation.

Nisoldipine did not depress cardiac function at doses needed to increase coronary blood flow or lower blood pressure in hypertensive animals. The drug reduced or abolished ventricular fibrillation and reperfusion arrhythmias, improved cardiac

output, reduced mortality, and improved ventricular function in several animal models. The beneficial effects are attributed to increases in perfusion of the ischemic zone, and a reduction in afterload through a decrease in peripheral resistance.

In vitro studies have shown nisoldipine to be twice as potent as nifedipine in inhibiting contractions of isolated pig coronary artery; however, it was only 1/3 as potent as nifedipine in inhibiting femoral artery contractions, indicating its selectivity for coronary vasculature.

Nisoldipine produced diuretic and natriuretic effects in rats, the effects being more pronounced in hypertensive than in normotensive rats. The natriuretic effect was attributed to the suppression of distal tubular sodium reabsorption.

Nisoldipine binds with very high affinity (Kd=<0.1 nM) to L-type calcium channels of various cell types. Compared to nifedipine, nisoldipine is found to have 2 to 30 times higher binding affinity depending on the cell type and experimental conditions. Several studies have shown that there is good agreement among binding affinity and the IC50 values both for inhibiting ^{45}Ca influx and aortic contraction. Moreover, the degree of nisoldipine inhibition of calcium channel current was found to increase with membrane depolarization.

Isolated membrane studies showed that the (+) isomer had a binding affinity 100 times higher than that of the (-) isomer.

General pharmacological studies in rodents showed analgesic and anticonvulsant effects, prolongation of anesthesia, attenuation of aggressive behavior, elevation of blood glucose and reduction of intestinal motility at dose levels 15 to 150 times the maximum recommended human dose on a body weight basis. (It is noted that the above effects were seen at dose levels 33 to 333 times the dose that produced the desired pharmacological effects in rats.)

Bay R 9425, an active metabolite with a dihydropyridine structure, exhibited pharmacological effects similar to the parent compound but was 1/3 to 1/10 less potent. The other metabolites had no significant effects.

Combined administration with propranolol prolonged the anti-hypertensive action of nisoldipine and reduced the reflex tachycardia in dogs.

Based on the ratio of percent of administered radioactivity excreted in urine for the i.v./oral doses, nisoldipine has been shown to be virtually completely absorbed in rats, dogs and humans, following oral doses of 5 mg/kg in the animals or 10 to 40 mg Nisoldipine CC tablets, in man. Despite the high rates of oral absorption, bio-availabilities (F) of parent compound were low, averaging 11.7% or less in the 3 species, which was attributed to an extensive first pass effect (shown in the dog to

be due to metabolism in both the gut and liver). In man, the F value for immediate release compound was 8.4%, but for the core-coated tablet, it was 5.5%. When administered by the oral route, $CEQ_{max,norm}$'s (C_{max} normalized to 1 mg/kg dose, based on radioactivity equivalence) for parent compound were similar in rat, dog, monkey and man, whereas $CEQ_{max,norm}$'s for total radioactivity (which includes parent compound plus all metabolites) were 4.7 to 7.5-times higher in man than in the 3 animal species. AUC_{norm}'s for parent compound were also similar in all 4 species whereas AUC_{norm}'s for total radioactivity (parent compound plus metabolites) were 6 to 10 times higher in man than in the three animal species. The plasma half-lives for parent compound following oral administration were only 0.7, 2.3, 3.8 and 4.0 hours, respectively, for the 4 species, whereas the plasma half lives for total radioactivity (parent compound plus metabolites) were 14.9, 54.4, 41.8 and 80.3 hours for the rat, dog, monkey, and man, respectively.

At steady state in humans, dose proportionality was seen for both immediate release and coat core tablets, based either on AUC or C_{max}. Correspondingly, decreases in systolic and diastolic blood pressures showed a general dose related decline from baseline.

Tissue distribution of total radioactivity following a single oral dose of 5 mg/kg in rats, determined between 0.5 and 72 hours post-dosing, reached maximum values at 1 hour, with liver, fat, kidney and adrenal glands generally containing the highest levels, brain and skeletal muscle the lowest. There was no indication of a change in organ pattern distribution with time. In dogs, tissue distribution (measured only 72 hours) after oral dosing was similar to that observed in rats. Placental transfer in pregnant rats, and secretion into milk of lactating rats, were observed. Whole-body autoradiography in rats indicated rapid tissue distribution and penetration of the blood-brain barrier within 5 minutes after i.v. dosing.

Protein binding, determined by equilibrium dialysis, was greater than 97.5% in rats and dogs and around 99.4% in humans. Nisoldipine was rapidly and extensively metabolized in the rat, dog, monkey and man; only a small percentage of unchanged labelled test substance could be found in the circulation within 30 or 60 minutes after an oral dose. Partial enterohepatic recirculation of metabolites was detected in rats. Hepatic enzyme levels of cytochrome P_{450}, aminopyrine N-demethylase and aniline hydroxylase were decreased following oral administration for 2 weeks, but these decreases were found to be reversible within one week following treatment termination.

At least 18 biotransformation products have been identified in urine and serum of rat, dog, monkey or man, 6 of which account for 80% of radioactivity in urine of all 4 species. After oral administration, there were no important differences in plasma or urinary metabolic profile between the 4 species. The investigators describe the biotransformation steps in all 4

species as follows:

-hydroxylation of the isobutyl ester
-dehydrogenation to the pyridine derivative
-cleavage of the ester to form the carboxylic acid
-reduction of the nitro group to the amino group
-glucuronidation (phase II enzymatic reaction)

In acute toxicity studies, oral LD50 values of nisoldipine were greater than 10,000 mg/kg for mice and rats and greater than 5000 mg/kg for rabbits and dogs. Acute iv LD50 values for the above four species ranged from 1.9 to 2.5 mg/kg. Propranolol pretreatment had no effect on the iv LD50 in the rat.

In the rat three month oral (gavage) toxicity study (0, 10, 30 and 100 mg nisoldipine/kg/day), elevation of plasma urea levels was seen in the high dose female group. Although absolute and/or relative weights of heart and liver (mid and high dose rats of both sexes) and thymus (mid and high dose males) were significantly higher than control, no significant histopathological findings were observed. The "no effect level" for liver and heart weight effects in the above study was found to be 10 mg/kg/day.

In reply to a request for justification of dose selection for the three dog studies, we were informed by the sponsor that doses were selected for the initial 4 week study on the basis of previous experience with the pharmacologically similar dihydropyridines, nifedipine and nitrendipine. The rationale for selection of the highest dose in the 52-week study was the avoidance of papillary muscle scars that were observed with the highest doses employed in the 4 and 13 week experiments. Such lesions were considered life threatening by the sponsor. Myocardial scars in one or both left ventricular papillary muscles, observed at 10 mg/kg in the 4 week study, and 6.25 mg/kg in the 13-week study, are generally attributed to hypoxic damage associated with vasodilator induced heart rate increase, a known damage mechanism in dogs. In the 4-week study, the associated ST drops and tachycardia were most pronounced in the dog with the most severe lesions. The pharmacologic effects (decreases in blood pressure and increases in heart rate) were usually reversible within 24 hours after treatment. No other treatment related effects were noted in the 4 and 13 week studies. The doses selected for the 52-week study were 0.3, 1.0 and 3 mg/kg,which caused dose-related decreases in blood pressure and increases in heart rate. Also noted were slight ST segment depressions, T-wave inversions and QT segment depressions, but no gross or histologically observable heart muscle damage or other indications of toxicity. In humans, nisoldipine is known to cause ST segment depression along with a decrease in peripheral vascular resistance, and side effects include palpitation and tachycardia.

The two year oral (dietary) carcinogen bioassay in the rat at doses of 0, 50, 300 and 1800 ppm nisoldipine (2.15, 13.13 and 82.40 mg/kg/day, respectively, in males and 2.78, 18.04 and 110.68 mg/kg/day, respectively, in females) did not show any evidence of a treatment-related increased incidence of tumors according to sponsor's analysis. However, a statistically significant (at 0.05 level) linear trend was reported by FDA statisticians for brain granular cell tumor (p=0.0411) in male rats; occurrence of the above tumor was limited to 3 (of 50) high dose males. Pairwise comparison showed no significant difference between high dose and control groups (p=0.1594). According to the sponsor, the incidence rate for the brain granular cell tumor is within the spontaneous range for male rats. It is noted that the above tumor incidence was observed at a dose level which is about 125 times the maximum recommended human dose of 40 mg/day, based on a mg/kg comparison assuming a patient weight of 60 kg. Hypertrophy of the zona glomerulosa cells of the adrenal cortex (high dose males and females) and increased incidence of progressive nephropathy (high dose females) were the major nonneoplastic findings of the above study. Although mean body weights for the high dose group (both sexes), for the duration of the study, were significantly lower than control values except on few occasions, the body weight decrement in high dose males was less than 10% throughout the study. In high dose females, beginning week 45 of treatment, the weight decrement was 10% or more, compared to control, till the end of the study. (The terminal weight decrements for high dose males and females were 5.6% and 22%, respectively.) There were no statistically significant differences in the survival of drug treated and control rats (male or female) in the above study.

In the mouse, dietary administration of nisoldipine at 0, 100, 300 and 900 ppm (19.37, 58.06 and 162.93 mg/kg/day, respectively, in males and 24.99, 74.36 and 217.28 mg/kg/day, respectively, in females) for 21 months showed no evidence of a drug related carcinogenic effect except for significant positive linear trends (at 0.05 level) for hepatocellular carcinoma and hepatocellular tumors (all) in male mice (sponsor's analysis). Analysis of the tumor data by FDA statisticians failed to confirm these trends. However, FDA analysis showed a significant positive linear trend (p=0.0072) for stomach papilloma in male mice [occurrence limited to 2 (of 50) high dose males]. Pairwise comparison showed the difference between high dose and control groups to also be significant (p=0.0435). The incidence rate for the stomach papilloma is reported to be within the historical control range for this tumor in NMRI mice. It is noted that the above tumor incidence occurred at dose level that is about 250 times the maximum recommended human dose on a body weight basis. Relevant non-neoplastic findings observed in this study included increased incidences of gastric mucosal hyperplasia (treated males and females - all groups) and pituitary hyperplasia (treated females - all groups). Chronic drug treatment had no significant effect on body weight in this study. The mortality rate of high dose males was significantly higher ($p<0.001$) than control (80%

in the high dose male group vs 28% in control). Though the mortality rate in high dose females (64% vs 56% in control) was also higher than control, the difference was not statistically significant.

Since the incidences of brain granular cell tumor in male rats and stomach papilloma in male mice are within the historical control range, and because nisoldipine has been shown not to be genotoxic, the drug-tumor association is considered to be not biologically relevant.

Only two of the 7 reproductive toxicology studies submitted were performed in accordance with GLPs; the Sprague-Dawley rat and the cynomolgus monkey teratology studies. All of the studies appear to be scientifically valid, but in the non-GLP studies, even with the amended tables, it was frequently not possible to determine the times of death.

In spite of the fact that the test substance had very low acute toxicity (LD_{50}> 10 g/kg) in Wistar rats, and could be administered chronically to Wistar rats in the carcinogenicity study at a dose as high as 220 mg/kg, doses given in the modified Segment I and III studies were only 3, 10 and 30 mg/kg. The only justification given for selection of these low doses was the undocumented statement, "The doses were chosen on the basis of toxicological results of other studies". Both studies included C-section of a proportion of the dams on day 20 of gestation.

In the Segment I study, nisoldipine produced no observable effects in the males (treatment started 70 days prior to mating) or females (treatment started 21 days prior to mating) in terms of clinical signs, body weight gain, mating, fertility and pregnancy rate. There were small but significant and dose related increases in mean fetal weight at 10 and 30 mg/kg but weights were said to have remained within normal limits for this strain. There were no differences from control in fetuses with external, soft tissue or skeletal malformations. In dams allowed to give birth, pregnancy duration was slightly, but significantly increased at all 3 dose levels.

In the Segment III study, nisoldipine administration was associated with a slight but statistically significant decrease in body weights (compared to control) of the high dose (30 mg/kg) dams of both the C-sectioned and rearing groups, after only the 4th day of treatment (day 20 of gestation). The only indication of toxicity to the offspring of the C-sectioned dams was a statistically significant decrease in mean fetal weight at the high dose. In the dams allowed to give birth, there was an increase in number of stillborn pups, and an apparent dose related increase in mortality of the newborn pups during the first week postpartum in the mid and high dose groups, but no statistical analysis was performed. The birth weight and the body weight gain of pups during lactation was reduced in the 30 mg/kg group compared to control.

In the supplemental Segment III study, where only the 30 mg/kg dose was tested, reduced maternal body weight gain was evident by day 20 of gestation (after only 4 days of treatment), with weights remaining below control weights through the first week of lactation. Gestation length was significantly prolonged, a finding that was interpreted by the sponsor as a "pharmacologically-induced tocolytic effect" (inhibition of uterine contractions), and there was a large increase in number of pup deaths at birth and during the first 2 weeks of lactation. Also, a decrease in pup weight was noted at birth and during the first week of lactation. These findings of maternal and fetal toxicity at 30 mg/kg confirm the observations noted for the 30 mg/kg group of the main Segment III study. Prolongation of gestation, which was seen in the supplemental Segment III study, had not been observed in the primary Segment III study but was observed in the Segment I study as well as in a Segment II study in another strain of rat (see table which follows and table on page 156).

DOSAGE THRESHOLDS FOR FERTILITY-REPRODUCTION STUDY AND PERINATAL-POSTNATAL STUDIES IN RATS

Report No.	T0002152	T1002153	T3008898
Study Type	Fertil-Reproduction	Peri- post-natal	Peri- post-natal
Strain	Wistar	Wistar	Wistar
Dose (mg/kg)	0, 3, 10, 30	0, 3, 10, 30	0, 30
Vehicle	glycerol-water-PEG	Glycerol-water-Lutrol	Glycerol-water-Lutrol
Days Administered	From 10 wks (males) or 3 wks (females) prior to mating to GD 7	GD 16 to PPD 21	GD 16 to PPD 14
C-Section Day (GD)	20	20	Not done
No. Fem/gp. C-Sectioned	23-27	25	N/A
No. Fem/gp. Littered	22-27	20-23	25
Maternal toxicity			
1. Decr. weight gain	>30 mg/kg	>10 $\leq$30 mg/kg#	$\leq$30 mg/kg#
2. Decr. food intake	>30	>30	>30
3. Prolonged Gestation	>30	>30	$\leq$30
Fetal (C-sect) toxicity			
1. Decr. survival	>30 mg/kg	>30 mg/kg	N/A
2. Decr. fetal weight	>30	>30	N/A
3. Decr. placental wt	>30	>30	N/A
4. Incr. malformation	>30	>30	N/A
Neonatal Toxicity			
1. Incr. stillborn	>30 mg/kg	>3 $\leq$10 mg/kg	$\leq$30 mg/kg
2. Decr. survival	>30	>3 $\leq$10 (1st week)	$\leq$30 (1st wk)
3. Decr. birth weight	>30	>10$\leq$30	$\leq$30
4. Decr. wt gain	>30	>10$\leq$30	$\leq$30

Limited to GD 16-20; effect was slight and barely significant in the first study, highly significant in the second.

Two Segment II studies were performed with rats, the first one with Long-Evans rats which received the drug in a polyethylene glycol-glycerol-water vehicle, and the second one with Sprague-Dawley rats which received the drug in an aqueous-Tylose vehicle. In both tests, the doses administered were 10, 30 and 100 mg/kg. In the second test with Sprague-Dawley rats (but not in the first one with Long-Evans rats), half the pregnant dams on test were allowed to litter and raise their young until 25 days postpartum; then selected males and females in each litter were monitored to sexual maturity. In both studies, a dose related decrease in body weight gain was noted for dams at the 2 highest doses. In the Long-Evans rat, there was no effect of nisoldipine on fetal weight, but in the Sprague-Dawley study, a dose related decrease was noted (significant at the 2 highest doses). There was an increase in postimplantation loss in the high dose group of the Sprague-Dawley study. There were indications of fetal immaturity in the 100 mg/kg group (more clearly noted with the Sprague-Dawley rat), as indicated by increased incidence of incomplete ossification of various bones and of stunted fetuses. Also in the Sprague-Dawley study (not shown in the following table), an increased number of fetuses with slightly increased (relative to normal control) dilatation of lateral ventricles and/or space between the body walls and organs occurred mainly in 2 litters of the 100 mg/kg group and was associated with low body weights of the fetuses in these two litters. Prolongation of gestation length was noted for the high dose group. Curiously, the birth weights of pups from the mid and high dose dams were slightly higher than control (the same observation was made for the 10 and 30 mg/kg groups of the Segment I study, but the opposite observation, i.e. a decrease in pup weight at birth, was made in the Segment III main and supplemental studies), and there was no increase in stillbirths and neonatal deaths, as had been observed in the Segment III studies. It is also pertinent to point out that in contrast to the Segment III studies where treatment was continued through the time of expected delivery, in the Segment II study with Sprague-Dawley rats, treatment was limited to days 7 to 17 of gestation. Thus, prolongation of pregnancy occurred a few days after treatment with nisoldipine had been discontinued.

DOSAGE THRESHOLDS FOR ADVERSE EFFECTS IN RAT DEVELOPMENTAL STUDIES

Report No.	7596	87 BAG0520/938
Strain	Long-Evans	Sprague-Dawley
Dose (mg/kg)	0, 10, 30, 100	0, 10, 30, 100
Vehicle	Glycerol-water-PEG	Aqueous tylose
Days Administered (GD)	6-15	7-17
Day of C-Section (GD)	20	20
No./Gp. C-Sectioned	20-21	21
No./Gp. Littered	N/A	11
Maternal Toxicity 1. Mortality 2. Decr. weight gain 3. Decr. food intake 4. Prolonged gestat	 >100 mg/kg >10 ≤30 Not measured N/A	 >100 mg/kg >10 ≤30 >10 ≤30 >30 ≤100
Fetal Toxicity 1. Incr. post-implant loss 2. Decr. fetal wt 3. Decr. placental wt	 >100 mg/kg >100 >30 ≤100	 >30 ≤100 mg/kg >10 ≤30 >100
Neonatal & postnatal toxicity (to time of breeding)	N/A	>100 mg/kg

In both Segment II rabbit studies, there was a compound related decrease in body weight gain of the pregnant does. In the first study, where the doses administered were 3, 10 and 30 mg/kg and the vehicle used was glycerol-water-PEG, a decrease in mean number of live male fetuses per doe ($P< 0.05$) resulted in a decrease in ratio of live male:female fetuses in the 30 mg/kg group. There was also a small increase in incidence of fetuses with anomalies (e.g. forelimb abnormalities and cleft palate) in the 30 mg/kg (high dose) group, but this was not associated with an increased incidence of any specific malformation. In the second study, where only the 30 mg/kg dose was tested and the vehicle used was aqueous tylose, mean fetal and placental weights were decreased and "underdeveloped forms" (defined as fetuses weighing $\leq$ 2.5 g), were increased vs control. The investigators attributed the increase in malformations in the first rabbit study to the increase in maternal stress. They suggested that the combination of the glycerol-water-PEG vehicle and the drug resulted in an increased incidence of diarrhea in the high dose dams, and that the diarrhea caused an increased incidence of does which aborted their entire litters (4 at high dose had diarrhea; 2 of these aborted and 1 died). However, diarrhea and abortion, also observed in 1 low dose dam and 1 mid dose dam, were considered "normal" for this strain of rabbit.

In both the rat and rabbit studies, increased incidence of malformations, increased fetal lethalities and/or depressed fetal weights were observed only with maternally toxic doses.

DOSAGE THRESHOLDS FOR ADVERSE EFFECTS IN RABBIT DEVELOPMENTAL STUDIES

Report No.	7595	7595 (Suppl)
Strain	Himalayan	Himalayan
Doses (mg/kg)	0, 3, 10, 30	0, 30
Vehicle	Glycerol-water-PEG	Aqueous tylose
Days Administered (GD)	6-18	6-18
Day of C-section (GD)	29	29
No./Group C-Sectioned	10-13	11
Maternal Toxicity		
1. Decr. body wt gain	>10 ≤30 mg/kg	≤30 mg/kg
2. Lethality	>10 ≤30*	>30
3. Diarrhea	>10 ≤30*	>30
4. Spontan. abortion	>10 ≤30*	>30
Fetal Toxicity		
1. Decr. survival	>10 ≤30 mg/kg#	>30 mg/kg
2. Decr. fetal wt.	>30	>10 ≤30
3. Decr. placental wt	>30	>10 ≤30
4. Incr. malformation	>10 ≤30**	>30

* In the first study with glycerol-water-polyethylene glycol vehicle, there were 4 high dose does with diarrhea, 2 of which aborted and 1 of which died. There was also 1 at low dose with diarrhea which aborted and one at mid dose which aborted but did not have diarrhea; none of the controls aborted. In the second test with aqueous tylose vehicle, there were no deaths and none of the treated animals aborted or had diarrhea. The high dose deaths and abortions in the first study were attributed by the sponsor to diarrhea, caused by an interaction of vehicle and compound, although the 2 vehicles were not directly compared in the same experiment.

The mean number of male live fetuses was significantly reduced in the 30 mg/kg group, resulting in a reduction in ratio of males:females in the first study. The sponsor considered the reduction in ratio of males:females a spontaneous occurrence and the overall decrease in number of live fetuses was attributed to the stress of diarrhea.

** Malformations in fetuses of the high dose group occurred in 3 dams. There was an increase in total number of fetuses with malformations and total number of dams that had fetuses with malformations (no statistical analysis), but no increase in any specific malformation. The investigators attribute the increase in malformation rate to increased stress due to diarrhea.

In the study with cynomolgus monkeys, clinical signs, which included heavy bleeding (followed by abortion), reduced food intake, diarrhea and vomiting, occurred during the treatment period in all the groups, including vehicle control. Although the symptoms were generally more frequent and of longer duration in the 100 mg/kg treated monkeys, they were still considered by the investigators to be due to treatment with the vehicle (polyethylene glycol-glycerin-water). In the opinion of this reviewer, the stress of handling these feral monkeys (rather than, or in addition to, the vehicle), contributed to the observed symptoms. Of the 10 or 11 pregnant monkeys on test in each group, 6 to 8 of them aborted in every group, and deaths occurred in 3 control, 1 mid-dose (30 mg/kg) and 5 high dose (100 mg/kg) monkeys. The single death at the mid dose, and 4 of the 5 deaths at the high dose, were associated with a volvulus (twisting or knotting of the intestine), which was not seen in control animals which died. Malformations were observed in the only surviving fetus of the 100 mg/kg group (left forelimb and tail anomalies, observed externally and by skeletal examination). In spite of the very few surviving fetuses that could be examined for malformations (3 controls, 2 low dose and 1 high dose) it is claimed, "The skeletal abnormalities in this one fetus are considered to be related to treatment with BAY k 5552 because similar defects were never observed in control fetuses of previous studies of the same type in Macaca fascicularis". No historical control data or details on how many previous studies or the number of control monkeys that were examined for teratogenicity are provided to support this statement. It should be pointed out that malformations occurred only at a dose level that was highly maternally toxic.

Bay k 5552 (nisoldipine) was tested for mutagenicity by five *in vitro* test systems (Salmonella/microsome, CHO HGPRT forward mutation assay, mouse hepatocyte primary culture DNA repair, a CHO test for clastogenicity, and by a test for inhibition of intercellular communication between two types of liver cells, 1) a primary rat culture ("wild type cells") and 2) the ARL14-TG^R cell line. It was further tested for mutagenicity in two *in vivo* systems (mouse micronucleus and mouse dominant-lethal). All the tests for mutagenicity appeared to be adequately performed, and positive controls in all of them confirmed the acceptability of the studies. Based on the outcome of these *in vitro* and *in vivo* studies, nisoldipine was not found to be mutagenic.

In conclusion, the preclinical studies summarized above have demonstrated the efficacy of the test drug as an antihypertensive and Adverse effects are seen only at high multiples of the maximum recommended human dose.

RECOMMENDATION

The NDA is approvable with suggested changes in labeling.

Sidney Stolzenberg, Ph.D

Xavier Joseph, DVM
August 29, 1994

ATTACHMENTS (3)

cc:
Orig.NDA
HFD-502
HFD-345/GJames
HFD-110
HFD-110/CSO
HFD-110/SStolzenberg
HFD-110/XJoseph
CAR 8/31/94

ATTACHMENT

CDER Statistical Review of

Carcinogenicity Studies

Statistical Reviews

Statistical Review and Evaluation

IND #: Date: DEC 3 1992

Applicant: Miles Inc.

Name of Drug: Nisoldipine (Bay K 5552)

Documents Reviewed:

1. IND Submission Volume 19.1 & 19.2, Information Amendment Serial No. 031, "Carcinogenicity study on NMRI mice (feeding study over 21 months)", Pharma. Report no. 16329 (E), Date: Dec. 1987, Date of Document, August 11, 1988.

2. IND Submission Volume 9.1, Additional Bay K 5552 (Nisoldipine) Reports, "Chronic Toxicological investigations on rats (Feeding Study over 24 months)", Pharma Report No.: 13016(E), Date: May 6, 1985, Date of Document, Dec. 1985.

3. IND Special Submission, Data diskettes and print-outs for two animal tumorigenicity studies, Date of Document, April 1, 1992.

I. Background

Two animal carcinogenicity studies (one in mice and one in rats) were included in this IND submission. The purpose of this study was to evaluate the tumorigenic potential of Nisoldipine (Bay K 5552), when administered in the diet to mice and rats for two years, respectively. Dr. Xavier Joseph, HFD-110, who is the reviewing pharmacologist of this IND has requested the Division of Biometrics to perform the statistical review and evaluation of these two studies. The data submitted on computer floppy diskettes were used in the reviewer's independent analyses.

II. The Mouse Study

II. a. Design

In this study, four groups of 50 male and 50 female SPF-bred NMRI mice (strain Bor:NMRI(SPF HAN), breeder Winkelmann, Borchen) were admixed with 0, 100, 300, or 900 ppm Nisoldipine (Bay K 5552) (converted to 0, 19.37, 58.06, 162.93 mg/kg/day for males or 0, 24.99, 74.36, 217.28 mg/kg/day for females, respectively) in their diet for up to 21 months. In each dose and sex group, 20 additional mice were treated for up to 12 months and then sacrificed for interim investigations. The dosages were selected on the basis of the results of a previous subacute study with the administration of the substance in the food. Food consumption was monitored on a weekly basis up to 23rd week and every two weeks thereafter. Autopsies were done on all animals which died during the course of the study or that

were killed in extremis, and also on those that were sacrificed at 12 months and at the termination of the study. At the end of the study, some tissues from 0, 300, and 900 ppm groups were examined histopathologically. In addition, non-blastomatous lesions in the alimentary canal, the pituitary and the liver of animals in the 900 ppm group were recorded. Of the animals in the 100 ppm group (low dose group), the stomach, pituitary, and liver were examined histopathologically.

II. b. Sponsor's Analyses

The sponsor indicated that "no neoplasms were entered for the female animals in the 100 ppm group; therefore, the data for this group were not recorded." The following four animals (animal no. 333 and 343 in female control group, animal no. 550 in female high dose group, and animal no. 131 in male low dose group) were not included in the analysis due to autolysis. The pituitary was not examined in all animals. The stomach and liver were examined only in male animals from the 100 ppm group, hence, the low dose group is taken into consideration only in the evaluation of the mortality data and of tumors with the relevant location.

Mortality rates of female and male mice at 6, 12, 18, and 21 months reported by the sponsor are presented in Table 1. The survival curves for female and male mice are graphed in Figures 1-2, respectively. For male animals, the survival curves are significantly different at 0.05 level, regardless of whether all the groups are taken into consideration or the 100 ppm group is excluded. The significant difference in survival curve is due to the high mortality in the high dose group. For female animals, the survival curves of control, medium, and high dose groups are not significantly different ($p = 0.8192$).

The above survival data were analyzed by applying the BMDP statistical software package which uses the generalized Wilcoxon test in the life table and survival functions programs.

The sponsor indicated that the methods described in Peto et al. ("Guidelines for Simple, Sensitive Significance Tests for Carcinogenic Effects in Long-Term Animal Experiments", In Long-Term and Short-Term Screening Assays for Carcinogens: A Critical Appraisal, International Agency for Research on Cancer Monographs, Annex to Supplement 2, World Health Organization, 311-426, 1980) were used to test the linear trend in the tumor data. Since the data do not have information relating the tumors with the death of an animal, hence, malignant tumors are in general evaluated with the help of the death-rate method and benign tumors with the help of the prevalence method. The ordinal dose levels 0, 1, 2, 3 are used as the weighing factors for the control, low, medium, and high dose groups, respectively.

Table 2 summarizes the tumor bearing animals according to location

and tumor type. Table 3 lists the number of male and female mice with benign and/or malignant tumors for control, medium, and high dose groups. Table 4 lists the statistical analyses of selected tumor data. The results of the above analyses showed that there were significant (at 0.05 level) positive linear trends in hepatocellular carcinoma (death rate: $p = 0.0015$, prevalence: $p = 0.0267$), hepatocellular tumors (death rate: $p = 0.0004$, prevalence: $p = 0.0334$), and malignant tumors (death rate: $p = 0.0035$) in male mice.

Based on the above results, the sponsor concluded that "hepatocellular tumors were found in male mice from all four groups. If the incidences determined in these groups are compared with the historical control values which stem from six long-term studies with animals of this strain between 1980 and 1984 (see Table 5), the occurrence of 7 hepatocellular tumors in male mice from the 300 ppm group (medium dose) lies within the upper range of the norm. The number of hepatocellular tumors in male animals from the highest dose group is markedly higher than the values of the 0 ppm group and historical control values. This increased incidence is a result of a higher rate of hepatic tumors in mice which died, while animals from the terminal kill were not affected more frequently than the other groups. .... The increased incidence of hepatocellular tumors in males of the 900 ppm group is interpreted as a secondary effect of chronic liver overloading. A primary carcinogenic effect was not determined."

II.c. Reviewer's analyses and Comments

The sponsor did not include data of female low dose group in the computer diskettes. Hence, female low dose group was not included in the following survival and tumor data analyses. The Cox test and the generalized Wilcoxon test described in the paper of Thomas, Breslow, and Gart ("Trend and Homogeneity Analyses of Proportions and Life Table Data", Computers and Biomedical Research, 10, 373-381, 1977) were used to test for heterogeneity in survival distributions. The p-values of the Cox test were <0.00001 and 0.6081 for males and females, respectively. Hence, there was a statistically significant difference (at 0.05 level) in the survival distribution in male mice. No significant difference in the survival distribution was detected in female mice. A similar conclusion was obtained in the generalized Wilcoxon test. The p-values of the test were <0.00001 and 0.8213 for males and females, respectively.

The intercurrent mortality rates for both male and female mice (see Table 6) were tested for linear trend according to the death rate method described in the paper of Peto et al. (1980) using the time intervals 0-50, 51-80, 81-103 weeks. The actual dose levels 0, 100, 300, and 900 ppm were the scores assigned to the control, low, medium, and high dose groups, respectively. The results of the analyses showed that there was a significant (at 0.05 level) linear trend in the intercurrent mortality rate in male mice ($p < 0.00001$), but not in female mice ($p = 0.2716$).

The methods described in Peto et al. (1980) and the methods of age-adjusted exact permutation trend test were used to test the linear trend in the tumor data. The results of the above analyses showed that there were significant (at 0.05 level) positive linear trends in reticulo-histiocytary system malignant lymphoma ($p \leq 0.00001$) in female mice, and urinary bladder stromal tumor ($p = 0.0337$), lung bronchiolo-alveolar adenoma ($p = 0.0358$), and stomach inverted papilloma of pars cutanea ($p = 0.0072$) in male mice.

However, the prevalence rates of lung bronchiolo-alveolar adenoma in male mice in the concurrent control group is greater than one percent. They are considered as a common tumor in this strain of mice. For a common tumor, we consider a positive linear trend not to occur by chance of variation only if the p-value is smaller than 0.01. Therefore, we do not regard the positive linear trend in lung bronchiolo-alveolar adenoma in male mice as statistically significant. The incidence rates of reticulo-histiocytary system malignant lymphoma in female mice, and urinary bladder stromal tumor and stomach inverted papilloma of pars cutanea in male mice are given in Tables 7 to 9.

If all of the malignant lymphoma in different organ of female mice were combined, then there is no significant linear trend in malignant lymphoma of combined organ in female mice.

The sponsor's analyses showed that there were significant (at 0.05 level) positive linear trends in hepatocellular carcinoma and hepatocellular tumors in male mice. However, the reviewer found that there were not significant positive linear trends in hepatocellular adenoma ($p = 0.2641$), hepatocellular carcinoma ($p = 0.0762$) and hepatocellular tumors ($p = 0.0514$) in male mice. Tables 10 and 11 listed the incidence rates of hepatocellular adenoma and carcinoma in male mice. The different results of p-values are due to (1) the sponsor did not apply the survival-adjusted method and (2) the ordinal dose levels 0, 1, 2, and 3 were used in sponsor's analyses.

III. The Rat Study

III. a. Design

In this study, three groups of 50 male and 50 female SPF-bred rats (Wistar strain TNO/W 74, Winkelmann (Breeder), Borchen) received Nisoldipine (Bay K 5552) in doses of 50, 300, and 1800 ppm administered in their feed for up to 2 years. The dose levels can be converted to 2.78, 18.04, and 110.68 mg/kg/day for female, and 2.15, 13.13, and 82.4 mg/kg/day for male rats, respectively. An additional 50 male and 50 female rats received untreated diet and were designated as controls. The study lasted for 105 weeks (Nov. 1980 to Nov. 1982). The subsequent autopsy on the surviving animals extended over approximately further 2 weeks. The body weights of the experimental animals were recorded at the start of the study, then

each week up to the 27th week of the study, and thereafter at intervals of 2 weeks. A histopathological evaluation was carried out on selected organs or tissues from all the animals which died spontaneously, and those sacrificed in a moribund condition or at the end of the study from the control and the highest dose group (1800 ppm). For low and medium dose groups, the adrenals, the genital organs (testes, epididymis or ovaries, uterus) and skin changes suspected of being tumorous on gross inspection, as well as the kidneys from the females, were processed for microscopic assessment.

III. b. Sponsor's Analyses

The following are summaries of results of sponsor's analyses included in Volume 9.1 submitted on Dec. 9, 1985. It seems that second volume of rats study was missing. The reviewer has discussed this with the reviewing CSO, Mr. David Roeder, HFD-110.

Table 12 lists the mortality rates for each dose/sex group after 12, 18, and 24 months (at the end of the 52nd, 79th, and 105th weeks of the study). Figures 3 and 4 plot the cumulative mortality rates for each sex/dose group over the entire study period.

Based on the above mortality tables and graphs, the sponsor stated that "there was no significant statistical or biological effect on the mortality at any time".

The number of the blastoma carriers in the individual dose group was summarized in Table 13. The sponsor indicated that the numbers of blastoma carriers in the female rats from the control and the highest dose groups which had malignant or both malignant and benign tumors were approximately the same. In contrast, more male rats from the highest dose group had malignant blastomas compared with control rats. This is a finding which, considering the marked variability of the occurrence of spontaneous blastomas, is considered to be unrelated to the treatment. Table 14 lists all blastomas according to location, type, and status. The sponsor also indicated that no oncogenic activity of the test substance can be deduced from the table, which shows rather the variability of spontaneously arising blastomas.

Based on the above analyses, the sponsor concluded that "the type, localization, time of appearance and frequency of the benign and malignant tumors found provided no evidence of an oncogenic action of Bay K 5552. Therefore, under the conditions described, the 50 and 300 ppm doses are regarded as being tolerated without ill effects."

III.c. Reviewer's analyses and Comments

The Cox test and the generalized Wilcoxon test described in the paper of Thomas et al. (1977) were used to test for heterogeneity in

survival distribution. The p-values of the Cox test were 0.66 and 0.2525 for males and females, respectively. Hence, there was no statistically significant difference (at 0.05 level) in the survival distribution in either male or female rats. A similar conclusion was obtained in the generalized Wilcoxon test. The p-values were 0.7856 and 0.4237 for males and females, respectively.

The intercurrent mortality rates for both male and female rats (see Table 15) were tested for linear trend according to the death rate method described in the paper of Peto et al. (1980) using the time intervals 0-50, 51-80, and 81-105 (female)/81-109(male) weeks. The actual dose levels 0, 50, 300, and 1800 ppm were the scores assigned to the control, low, medium and high dose groups, respectively. The results of the analyses showed that there was no significant (at 0.05 level) linear trend in the intercurrent mortality rate in male or female rats.

The methods described in Peto et al. (1980) and the methods of age-adjusted exact permutation trend test were used to test the linear trend in the tumor data. The tumor intervals 0-50, 51-80, 81-105(female)/81-109(male) and terminal sacrifice were used in those methods. The results of the above analyses showed that there was a statistically significant (at 0.05 level) linear trend in brain granular cell tumor ($p = 0.0411$) in male rats. The incidence rates of this tumor are given in Table 16.

IV. Summary

IV. a. The Mouse Study

The oncogenic potential of nisoldipine was evaluated in this mouse study when administered in the diet continuously to the animals at dosage levels of 0, 100, 300, or 900 ppm for up to 21 months.

Noted that the sponsor did not include data of female low dose group in the computer diskettes. Hence, female low dose group was not included in the following survival and tumor data analyses.

The Cox and the generalized Wilcoxon methods were used to test the heterogeneity in survival distribution. The test results revealed that there was no statistically significant difference (at 0.05 level) in the survival distribution in female mice. However, there was a statistically significant difference (at 0.05 level) in the survival distribution in male mice.

The statistical methods given in the paper of Peto et al. (1980) and an exact permutation trend test were used to test the positive linear trend in intercurrent mortality and incidental tumor rates. Applying the above methods to the data on sponsor's computer diskettes, the results of the analyses showed that there was a significant (at 0.05 level) linear trend in the intercurrent mortality rate in male mice

($p < 0.00001$), but not in female mice ($p = 0.2716$). Results of tumor data analyses showed that there was a significant (at 0.05 level) positive linear trend in urinary bladder stromal tumor ($p = 0.0337$), and stomach inverted papilloma of pars cutanea ($p = 0.0072$) in male mice.

The sponsor's analyses showed that there were significant (at 0.05 level) positive linear trends in hepatocellular carcinoma and hepatocellular tumors in male mice. However, the reviewer found that there were not significant positive linear trends in hepatocellular adenoma ($p = 0.2641$), hepatocellular carcinoma ($p = 0.0762$) and hepatocellular tumors ($p = 0.0514$) in male mice. The different results of p-values are due to (1) the sponsor did not apply the survival-adjusted method and (2) the ordinal dose levels 0, 1, 2, and 3 were used in sponsor's analyses.

IV. b. The Rat Study

The oncogenic potential of nisoldipine was evaluated in this rat study when administered orally and continuously to the animals at dosage levels of 50, 300, & 1800 ppm for 105 weeks.

The Cox and the generalized Wilcoxon methods were used to test the heterogeneity in survival distribution. The test results revealed that there was no statistically significant difference (at 0.05 level) in the survival distribution in female or male rats.

The statistical methods given in the paper of Peto et al. (1980) and an exact permutation trend test were used to test the positive linear trend in intercurrent mortality and incidental tumor rates. Applying the above methods to the data on sponsor's computer diskettes, the results of the analyses showed that there was not significant (at 0.05 level) linear trend in the intercurrent mortality rate in male or female rats.

Results of tumor data analyses showed that there was a significant positive linear trend in brain granular cell tumor ($p = 0.0411$) in male rats.

Daphne Lin

Daphne Lin, Ph.D.
Mathematical Statistician

Concur: Karl K. Lin 11/27/92

Karl K. Lin, Ph.D., Group Leader, SARB

Figure 1

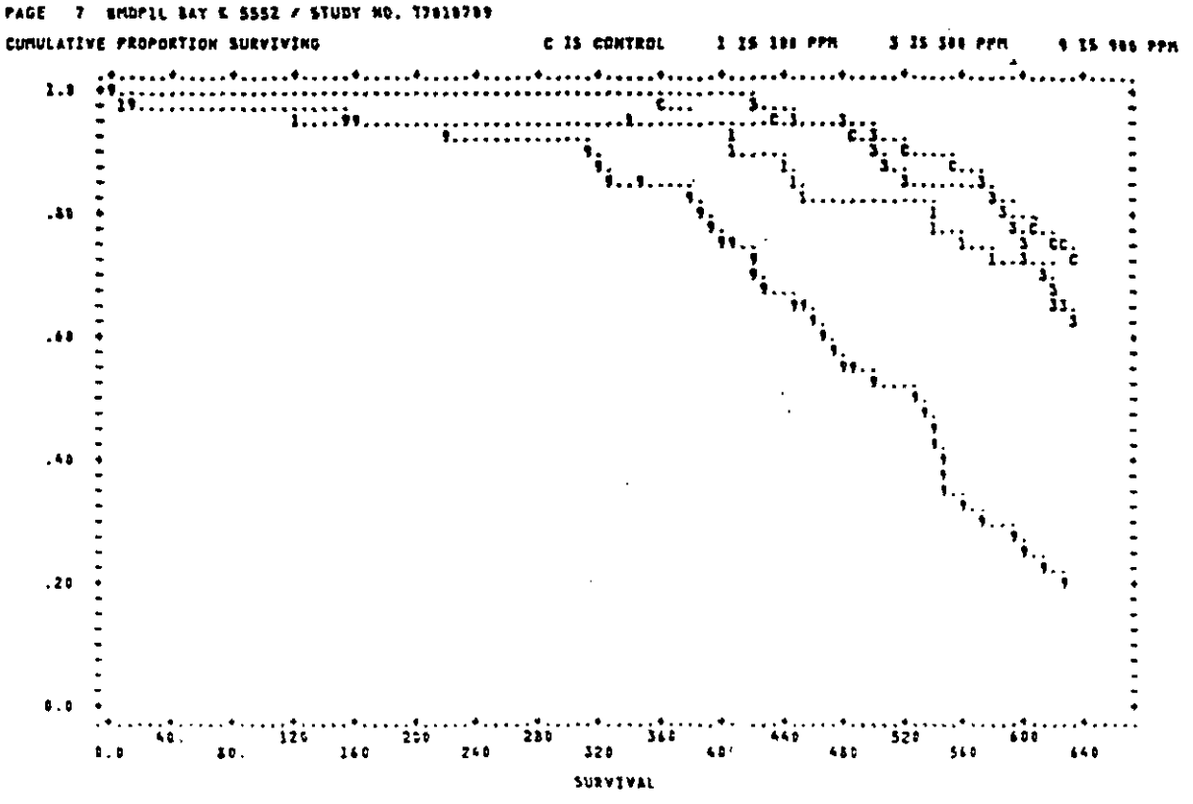
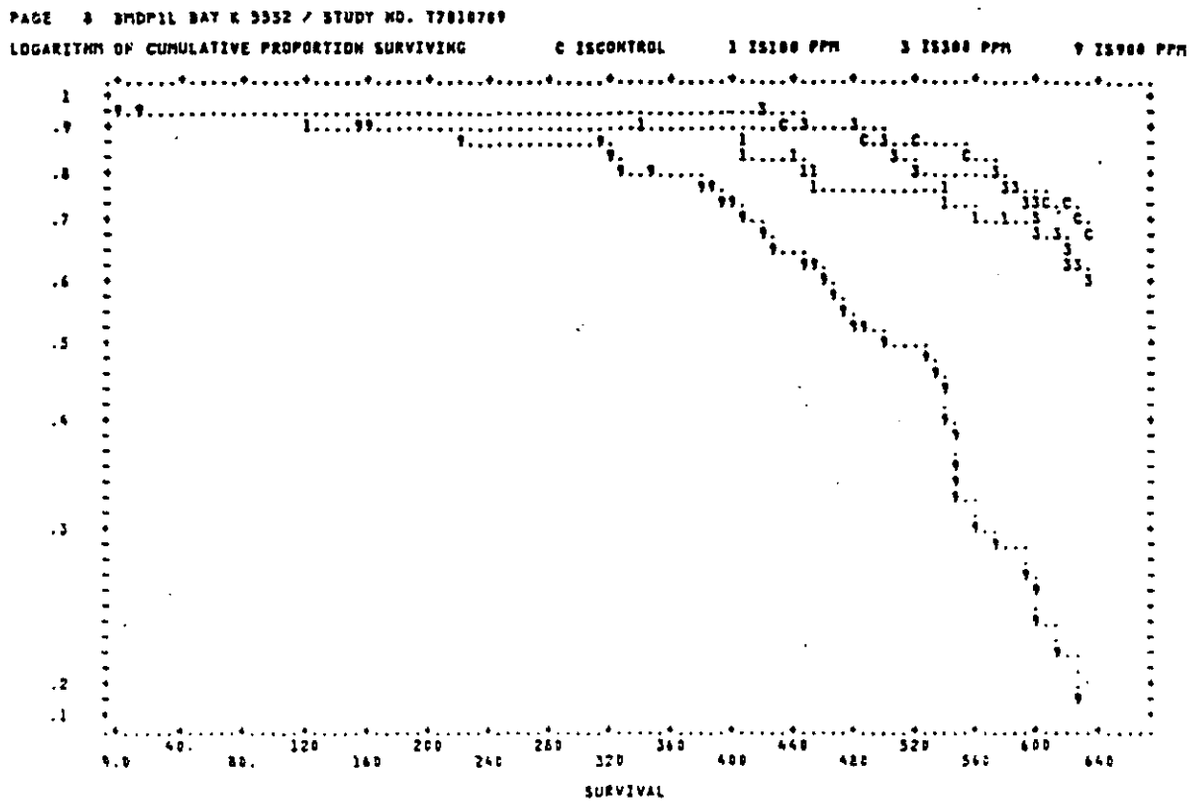

PAGE 7 EMDPIL BAY K 5552 / STUDY NO. T7818789
CUMULATIVE PROPORTION SURVIVING
C IS CONTROL
1 IS 188 PPM
3 IS 388 PPM
9 IS 986 PPM
1.0
.80
.60
.40
.20
0.0
0.0
40.
80.
120
160
200
240
280
320
360
400
440
480
520
560
600
640
SURVIVAL

Figure 2



NUMBER OF INTEGER WORDS OF STORAGE USED IN PRECEDING PROBLEM 3838
CPU TIME USED 0.314 SECONDS

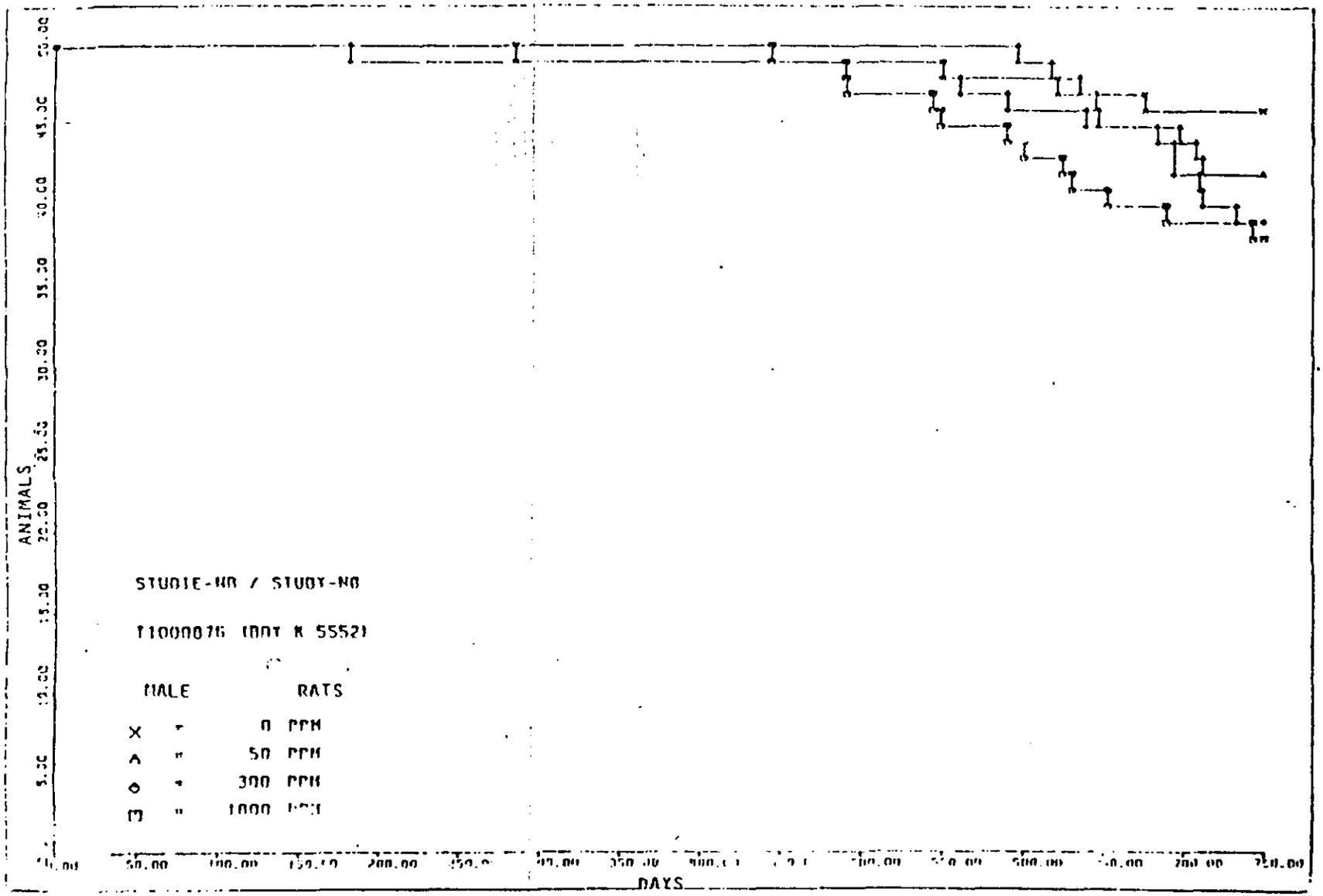


Fig. 2: Mortality curves of male rats which received BAY k 5552 with the food for 24 months.

001

Figure 3

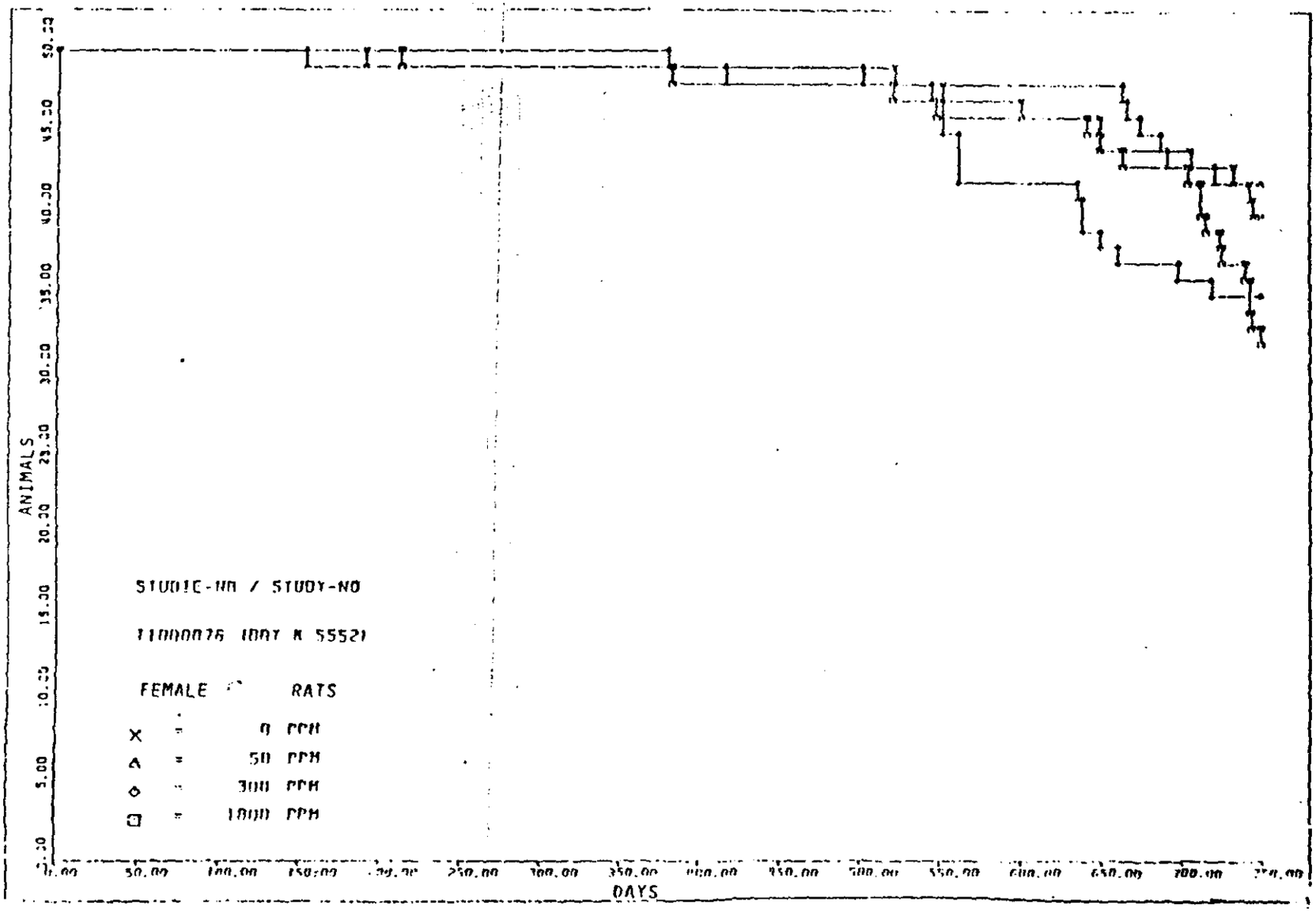


Fig. 3: Mortality curves of female rats which received BAY k 5552 with food for 24 months

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Figure 4

Table 1 Appendix 1

Table 2

MORTALITY

DOSE PPM	NUMBER USED *	NUMBER DIED	MORTALITY %
	6 MONTHS		
	MALE		
0	50	0	0.0
100	50	2	4.0
300	50	0	0.0
900	50	3	6.0
	FEMALE		
0	50	0	0.0
100	50	0	0.0
300	50	0	0.0
900	50	1	2.0
	12 MONTHS		
	MALE		
0	50	0	0.0
100	50	3	6.0
300	50	0	0.0
900	50	8	16.0
	FEMALE		
0	50	2	4.0
100	50	5	10.0
300	50	3	6.0
900	50	5	10.0
	18 MONTHS		
	MALE		
0	50	5	10.0
100	50	11	22.0
300	50	7	14.0
900	50	29	58.0
	FEMALE		
0	50	21	42.0
100	50	19	38.0
300	50	20	40.0
900	50	21	42.0
	21 MONTHS		
	MALE		
0	50	14	28.0
100	50	15	30.0
300	50	19	38.0
900	50	40	80.0
	FEMALE		
0	50	28	56.0
100	50	34	68.0
300	50	34	68.0
900	50	32	64.0

* Animals scheduled for terminal kill

Table 18: Comparative summary of tumours occurring according to location, type, number and dignity $ (animals scheduled for terminal kill)

Sex	♂ (M)				♀ (F)			
Dose ppm	0	100	300	900	0	100	300	900
Lung:		-				-		
bronchiolo-alveolar adenoma	2		3	4	2		1	3
bronchiolo-alveolar carcinoma (malig.)	13		12	5	8		6	5
Stomach:								
papilloma	0	0	0	2	0	0	0	0
sarcoma (malig.)	0	0	2	1	0	0	0	0
Liver:								
hepatocellular adenoma	2	2	2	3	0	0	0	1
hepatocellular carcinoma (malig.)	3	4	5	8	0	0	0	1
haemangiosarcoma (malignant)	1	0	0	0	0	0	0	0
Kidneys:		-				-		
tubular carcinoma (malignant)	0		1	0	0		0	0
haemangiosarcoma (malignant)	0		1	0	0		0	0
Bladder:		-				-		
stromal tumour (benign)	1		1	2	0		0	0
stromal tumour (malignant)	0		1	1	0		0	0
Ovary:	-	-	-	-		-		
granulosa-theca cell tumour (ben.)					5		5	3
granulosa-theca cell tumour (malig.)					1		0	0
luteoma (benign)					2		2	0
tubular adeno-carcinoma (malig.)					1		0	0
Sertoli cell tumour (benign)					0		0	1
Uterus:	-	-	-	-				
adenoma					0	0	1	0
carcinoma (malig.)					1	0	0	0
fibroma					0	0	1	0
myoma					0	0	1	2
myosarcoma (malig.)					0	0	0	1
stromal tumour (benign)					3	0	2	2
stromal sarcoma (malignant)					1	3	2	2

Table 2 (Continued)

Table 18 (continued):

Sex	♂				♀			
Dose ppm	0	100	300	900	0	100	300	900
Testes:		-				-	-	-
Leydig cell tumour (benign)	2		2	0				
adenoma of rete testis	1		0	0				
Pituitary:		-						
adenoma	2		0	0	0	3	3	1
Thyroid:		-				-		
follicle cell adenoma	0		1	0	0		0	0
papillary cyst-adenoma	1		0	0	0		0	0
Adrenals:		-				-		
cortical adenoma	3		3	1	2		0	0
phaeochromocytoma (benign)	1		0	0	1		0	2
phaeochromocytoma (malignant)	0		0	1	0		0	0
RH system:		-				-		
lymphoma (malig.)	7		3	1	18		12	14
lymph node sarcoma (malignant)	1		0	0	1		0	0
Skin/subcutis:		-				-		
epithelioma (malignant)	0		0	1	0		0	0
sarcoma (malig.)	1		0	0	0		3	0
Mammary gland:	-	-	-	-		-		
carcinoma (malig.)					3		0	0
adeno-ancanthoma (malignant)					0		1	1
Harder's gland:		-				-		
papillary adenoma	3		2	0	1		1	1
Spinal marrow:		-				-		
schwannoma (malig.)	0		0	0	0		1	0
Bones:		-				-		
osteosarcoma (malignant)	0		0	0	0		1	0
Abdomen:		-				-		
haemangiosarcoma (malignant)	0		0	0	0		1	0
Pelvic serosa:		-	-	-	-	-	-	-
sarcoma (malig.)	1							

- Organ not investigated $ Bilateral tumours counted twice

Table 17: Summary of number of male and female mice with benign and/or malignant tumours, as well as frequency of benign and malignant tumours encountered

Sex	♂			♀		
Dose ppm	0	300	900	0	300	900
No. of animals investigated	50	50	49	48	50	50
No. of animals with tumours	27	28	24	34	31	30
No. of animals with only benign tumours	7	6	8	6	6	6
No of animals with only malignant tumours	12	15	14	21	18	17
No. of animals with benign and malignant tumours	8	7	2	7	7	7
No. of animals with more than one primary tumour	15	11	5	13	9	8

Table 15: Statistical Analysis of Tumour Data

sex	target character	groups (ppm)	trend test	incidence	$\hat{z}$	p
male	hepatocellular tumours	0/100/300/900	death-rate	5/ 6/ 7/11	3.321	0.0004
male	hepatocellular tumours	0/100/300/900	prevalence	5/ 6/ 7/11	1.833	0.0334
male	hepatocellular tumours	0/100/300	death-rate	5/ 6/ 7	0.821	0.2059
male	hepatocellular tumours	0/900	death-rate	5/11	3.430	0.0003
male	hepatocellular tumours	0/300	death-rate	5/ 7	0.855	0.1964
male	hepatocellular tumours	0/100	death-rate	5/ 6	0.374	0.3541
male	tumour, benign	0/300/900	prevalence	15/13/10	0.286	0.3876
male	tumour, malignant	0/300/900	death-rate	20/22/16	2.692	0.0035
male	hepatocellular adenoma	0/100/300/900	death-rate	2/ 2/ 2/ 3	1.450	0.0735
male	hepatocellular carcinoma	0/100/300/900	death-rate	3/ 4/ 5/ 8	2.970	0.0015
male	hepatocellular carcinoma	0/100/300/900	prevalence	3/ 4/ 5/ 8	1.931	0.0267
male	hepatocellular carcinoma	0/100/300	death-rate	3/ 4/ 5	0.876	0.1905
male	hepatocellular carcinoma	0/900	death-rate	3/ 8	3.067	0.0011
male	hepatocellular carcinoma	0/300	death-rate	3/ 5	0.919	0.1790
male	hepatocellular carcinoma	0/100	death-rate	3/ 4	0.434	0.3321
female	tumour, benign	0/300/900	prevalence	13/13/13	0.040	0.4842
female	tumour, malignant	0/300/900	death-rate	28/25/24	-0.257	0.6015

Table 5

Historic control values: NMRI mouse 1980 to 1984

	Number					
Test No.	1	2	3	4	5	6
Adenomatous gastric mucosal hyperplasias in males	9*	20	10	32	5	27
n	50	50	50	44	47	48
%	18	40	20	73	11	56
in females	1*	8	11	14	10	13
n	50	48	49	46	47	48
%	2	17	22	30	21	27
*classified as adenoma						
Liver tumour in males	7	3	9	5	1	6
n	50	50	50	45	46	48
%	14	6	18	11	2	12
Uterine hyperplasias	0	19	21	23	0	33
n	50	46	49	45	45	46
%	0	41	43	51	0	71

Glucose concentration in the plasma: 4.32 - 9.36 mmol/l (male)
4.51 - 7.75 mmol/l (female)
Urea concentration in the plasma: 5.96 - 15.12 mmol/l (male)
4.05 - 14.99 mmol/l (female)

n = Number of organs evaluated

Table 6
Intercurrent Mortality Rates
Male Mice

Weeks	Control			Low			Medium			High		
	S	D	%	S	D	%	S	D	%	S	D	%
0-50	50	0	0	49	2	4.0	50	0	0	50	4	8
51-80	50	3	6.0	47	7	14.8	50	2	4	46	16	34
81-103	47	11	23.4	40	5	12.5	48	17	35.4	30	20	66
Term.	36			35			31			10		

Female Mice

Weeks	Control			Medium			High		
	S	D	%	S	D	%	S	D	%
0-50	48	2	4.1	50	1	2	49	3	6.1
51-80	46	10	21.7	49	9	18.3	46	9	19.5
81-103	36	14	38.8	40	24	60	37	19	51.3
Term.	22			16			18		

Notes: S: Number of animals starting during the period
D: Deaths
%: Percent of death during the period

Table 7
Tumor Incidence Rates
Female Mice, Reticulo-histiocytary System Malignant Lymphoma

Weeks	Control		Medium		High	
	T	N	T	N	T	N
0-50	0	2	0	1	0	3
51-80	0	10	0	9	3	9
81-103	0	14	0	24	8	19
Terminal	0	22	0	16	2	18
Total	0	48	0	50	13	49

Table 8
Tumor Incidence Rates
Male Mice, Urinary Bladder Stromal Tumor

Weeks	Control		LOW		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	0	7	0	2	0	16
81-103	0	11	0	5	0	17	1	20
Terminal	0	36	0	35	0	31	1	10
Total	0	50	0	49	0	50	2	50

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 9
Tumor Incidence Rates
Male Mice, Stomach inverted Papilloma of Pars Cutanea

Weeks	Control		LOW		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	0	7	0	2	0	16
81-103	0	11	0	5	0	17	0	20
Terminal	0	36	0	35	0	31	2	10
Total	0	50	0	49	0	50	2	50

Table 10
Tumor Incidence Rates
Male Mice, Hepatocellular Adenoma

Weeks	Control		LOW		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	0	7	0	2	1	16
81-103	1	11	0	5	0	17	2	20
Terminal	1	36	2	35	2	31	0	10
Total	2	50	2	49	2	50	3	50

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 11
Tumor Incidence Rates
Male Mice, Hepatocellular Carcinoma

Weeks	Control		LOW		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	2	7	0	2	2	16
81-103	0	11	0	5	2	17	5	20
Terminal	3	36	2	35	3	31	1	10
Total	3	50	4	49	5	50	8	50

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 3

STERBLICHKEIT / MORTALITY *			
DOSE DOSIS PPM	NUMBER USED EINGESETZTE TIERE	NUMBER DIED VERENDETE TIERE	MORTALITY STERBLICHKEIT %
12 MONATE / 12 MONTHS			
MAENNLICH/MALE			
0	50	1	2.0
50	50	0	0.0
300	50	1	2.0
1800	50	0	0.0
WEIBLICH/FEMALE			
0	50	1	2.0
50	50	1	2.0
300	50	0	0.0
1800	50	2	4.0
18 MONATE / 18 MONTHS			
MAENNLICH/MALE			
0	50	2	4.0
50	50	0	0.0
300	50	2	4.0
1800	50	5	10.0
WEIBLICH/FEMALE			
0	50	3	6.0
50	50	2	4.0
300	50	5	10.0
1800	50	4	8.0
24 MONATE / 24 MONTHS			
MAENNLICH/MALE			
0	50	4	8.0
50	50	8	16.0
300	50	11	22.0
1800	50	11	22.0
WEIBLICH/FEMALE			
0	50	8	16.0
50	50	8	16.0
300	50	15	30.0
1800	50	13	26.0

* The animals selected for the interim autopsy are not considered in this Table.

Table 31: Number of the blastoma carriers in the individual dose groups

Sex	♂				♀			
Dose (ppm)	0	50	300	1800	0	50	300	1800
Total number of rats investigated	49	50	50	48	48	48	48	48
Number of the blastoma carriers	31	16	21	26	33	21	21	28
Number of rats with exclusively benign blastomas	28	10	12	18	24	15	14	18
Number of rats with exclusively malignant blastomas	2	3	8	4	6	3	4	6
Number of rats with benign and malignant blastomas	1	3	1	4	3	3	3	4
Number of blastoma carriers as % all rats investigated	63	32	42	54	69	44	44	58
Number of blastoma carriers with exclusively benign blastomas as % all rats investigated	57	20	24	38	50	31	29	38
Number of blastoma carriers with exclusively malignant blastomas as % all rats investigated	4	6	16	8	13	6	8	13
Number of blastoma carriers with benign and malignant blastomas as % all rats investigated	2	6	2	8	6	6	6	8

Table 32: List of all blastomas according to number, localisation, type and status (male rats)

(Dose ppm)	0	50	300	1800
Adenohypophysis*	14	0	3	11
Adenoma	0	2	0	0
Carcinoma				
Thyroids*	6	0	0	0
C cell adenoma	1	0	0	1
C cell carcinoma	0	0	0	1
Follicular carcinoma				
Adrenal cortex	1	1	0	0
Adenoma unilateral				
Adrenal medulla	7	5	3	7
Phaeochromocytoma (b) unilateral	0	1	0	1
Phaeochromocytoma (b) bilateral	0	-	1	0
Phaeochromocytoma (m) unilateral				
Parathyroids*	3	0	0	0
Adenoma				
Testes	4	5	6	7
Leydig cell tumour (b) unilateral	0	-	2	1
Leydig cell tumour (b) bilateral				
Pancreas* endocrine	2	0	0	0
Adenoma				
Pancreas* exocrine	3	0	0	0
Adenoma				
Heart*	2	0	0	1
Endocardial fibromatosis (b)				
Lung*	0	0	1	0
Adenoma				
Epididymis	0	0	0	1
Sarcoma				
Brain*	0	0	0	2
Meningioma (b)				
RHS*	0	1	3	1
Malignant lymphoma	0	0	1	0
Histiocytary sarcoma				
Skin*	0	1	1	1
Cornified squamous cell carcinoma				
Subcutis*	1	1	1	3
Sarcoma	0	0	1	1
Haemangiosarcoma				
Mesentery*	0	0	1	0
Leiomyosarcoma				
Abdomen*	1	0	0	0
Fibrosarcoma	0	1	0	0
Sarcoma				

b = benign
m = malignant
* This organ was not routinely histologically investigated in all the rats from the 50 and 300 ppm groups.

Table 32: List of all blastomas according to number, localisation, type (continuation) and status (female rats)

(Dose ppm)	0	50	300	1800
Adenohypophysis				
Adenoma	16	3	5	7
Carcinoma	1	1	1	0
Thyroids*				
C cell adenoma	6	1	2	1
C cell carcinoma	1	0	0	0
Follicular adenoma	0	0	0	1
Follicular carcinoma	1	0	0	0
Acrenal cortex				
Adenoma unilateral	1	1	0	0
Acrenal medulla				
Phaeochromocytoma (b) unilateral	0	0	0	1
Phaeochromocytoma (m) unilateral	0	0	0	1
Ovary				
Granulosa-theca cell tumour (b)	0	0	1	1
Granulosa-theca cell tumour (m)	0	0	0	1
Uterus				
Endometrial stromal tumour (polyp) (b)	8	12	11	13
Endometrial stromal sarcoma	2	0	2	2
Adenocarcinoma	1	4	2	2
Mammary gland				
Adenoma	0	2	1	2
Adenocarcinoma	1	1	1	0
Kidneys				
Adenoma	1	0	0	0
Sarcoma	1	0	0	0
Urinary bladder*				
Adenoma	1	0	0	0
RHS*				
Malignant lymphoma	0	0	0	1
Histiocytary sarcoma	0	0	0	1
Intestine*				
Fibroma	0	0	0	1
Mesentery*				
Malignant mesothelioma	0	0	1	0
Skin				
Papilloma	0	0	0	1
Subcutis				
Haemangiosarcoma	1	0	0	0
Sarcoma	1	0	0	2

b = benign
m = malignant
* This organ was not routinely histologically investigated in all the rats from the 50 and 300 ppm groups.

Table 15
Intercurrent Mortality Rates
Male Rats

Weeks	Control			Low			Medium			High		
	S	D	%	S	D	%	S	D	%	S	D	%
0-50	50	1	2	50	0	0	50	1	2	50	0	0
51-80	49	1	2.0	50	0	0	49	0	0	50	3	6
81-109	48	3	6.2	50	8	16	49	10	20.4	47	8	17.
Term.	45			42			39			39		

Female Rats

Weeks	Control			Low			Medium			High		
	S	D	%	S	D	%	S	D	%	S	D	%
0-50	50	1	2	50	1	2	50	0	0	50	2	4
51-80	49	2	4.0	49	1	2.0	50	8	16	48	2	4.1
81-105	47	5	10.6	48	6	12.5	42	7	16.6	46	9	19.5
Term.	42			42			35			37		

Notes: S: Number of animals starting during the period
D: Deaths
%: Percent of death during the period

Table 16
Tumor Incidence Rates
Male Rats, Brain Granular Cell Tumor

Weeks	Control		LOW		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	1	0	0	0	1	0	0
51-80	0	1	0	0	0	0	1	3
81-109	0	3	0	8	0	10	0	8
Terminal	0	45	0	42	0	39	2	39
Total	0	50	0	50	0	50	3	50

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

pairwise : C v.s H
$P = 0.1594$

Statistical Review and Evaluation
(An Addendum)

IND #: Date: MAR -8 1994

Applicant: Miles Inc.

Name of Drug: Nisoldipine

I. Background

The two animal carcinogenicity studies (one in rats and one in mice) included in this IND submission were reviewed and a statistical review report was issued on Dec. 3, 1992 by the Division of Biometrics. Our analyses showed that there were statistically significant positive linear trends in urinary bladder benign stromal tumor ($p = 0.0337$) and stomach inverted papilloma of para cutanea ($p = 0.0072$) in male mice and in reticulo-histiocytary system malignant lymphoma ($p < 0.00001$) in female mice. However, in the last case, if all of the malignant lymphomas in different organs of female mice are combined, then the linear trend became not significant. In rats study, results of tumor data analyses showed that there was a significant positive linear trend in brain granular cell tumor ($p = 0.0411$) in male rats. Dr. Xavier Joseph, HFD-110, who is the reviewing pharmacologist of this IND, requested the Division of Biometrics to test if the positive dose-response relationship is significant in any possible combinations of urinary bladder malignant stromal tumor, benign polypous stromal tumor, and benign stromal tumor in male mice. He also requested the reviewer to check the difference in the incidence rates of reticulo-histiocytary system malignant lymphoma in female mice between the sponsor's and the reviewer's results. Pairwise comparisons were also requested for tumors which have significant positive linear trends.

II. The Mice Study

The methods described in Peto et al. (1980) and the methods of age-adjusted exact permutation trend test were used to test the positive linear trend in different combinations of urinary bladder tumors in male mice and in malignant lymphoma in female mice. The time intervals 0-50, 51-80, 81-103 weeks, and terminal sacrifice were used in those methods. The actual dose levels 0, 100, 300, and 900 ppm were the scores assigned to the control, low, medium, and high dose groups, respectively.

The results showed that there was a statistically significant positive linear trend in urinary bladder benign stromal tumor (trend: $p = 0.0337$; pairwise: high vs control: $p = 0.1403$) in male mice. There was no statistically significant (at 0.05 level) trend in any other urinary bladder tumors in male mice. Noted that there are two male mice (animal numbers 68 and 189) developed urinary bladder polypous stromal tumors, one male mice (animal number 207) developed

urinary bladder malignant stromal tumor, and two male mice (animal numbers 234 and 242) developed urinary bladder benign tumors. The following table lists the p-values of the test results in urinary bladder tumors in male mice:

Tumors	P-values
Benign Stromal Tumor	0.0337 *
Polypous Stromal Tumor	0.5793
Malignant Stromal Tumor	0.6226
Benign Stromal Tumor & Polypous Stromal Tumor	0.0606
Benign Stromal Tumor & Malignant Stromal Tumor	0.0725
Polypous Stromal Tumor & Malignant Stromal Tumor	0.4289
Benign Stromal Tumor & Polypous Stromal Tumor & Malignant Stromal Tumor	0.1036

The incidence rates of urinary bladder benign stromal tumor, urinary bladder malignant stromal tumor, and urinary bladder benign polypous stromal tumor in male mice are given in Tables 1 to 3.

For the malignant lymphoma, as mentioned in the previous review, there was a statistically significant positive linear trend in reticulo-histiocytary system malignant lymphoma (trend : $p < 0.00001$; pairwise: high vs control: $p = 0.0001$; pairwise: high vs medium: $p < 0.0001$) in female mice. However, if all of the malignant lymphomas in different organs of female mice were combined, then the linear trend became not significant ($p = 0.2307$). Table 4 lists the incidence rates of reticulo-histiocytary system malignant lymphoma in female mice. Table 5 lists the incidence rates of reticulo-histiocytary system malignant lymphoma and all the malignant lymphomas in different organs of female mice. Noted that the sponsor did not include data of female low dose group in the computer diskettes. Hence, female low dose group was not included in the analysis of malignant lymphoma.

In the sponsor's submission dated on August 11, 1988, the tumor bearing animals were summarized according to location and tumor type and shown in Table 18 of their submission (see attached Table 6). Under "RH system malignant lymphoma", there are 18, 12, and 14 female mice developed this tumor in control, medium, and high dose groups, respectively. However, the reviewer found that the numbers are 0, 0, and 13 female mice in control, medium, and high dose groups,

respectively, recorded in the data diskette submitted on April 1, 1992. If the female animals developed malignant lymphoma in reticulo-histiocytary system and all different organs are combined, then the numbers are 18, 12, and 14 female mice in control, medium, and high dose groups, respectively.

There was a statistically significant positive linear trend in stomach inverted papilloma of para cutanea ($p = 0.0072$) in male mice. The pairwise comparison also showed a statistically significant difference between control and high dose male mice in this tumor ($p = 0.0435$). The incidence rates of this tumor are given in Table 7.

III. The Rats Study

Results of tumor data analyses showed that there was a significant positive linear trend in brain granular cell tumor ($p = 0.0411$) in male rats. However, the pairwise comparison between control and high dose male rats did not show any significant difference ($p = 0.1594$) in tumor rate between the two groups. The incidence rates of this tumor are given in Table 8.

IV. Summary

The statistical methods given in the paper of Peto et al. (1980) and an exact permutation trend test were used to test the positive linear trend in malignant lymphoma in female mice and in any possible combinations of urinary bladder malignant stromal tumor, benign polypous stromal tumor, and benign stromal tumor in male mice. The time intervals 0-50, 51-80, 81-103 weeks, and terminal sacrifice were used in those methods. The actual dose levels 0, 100, 300, and 900 ppm were the scores assigned to the control, low, medium, and high dose groups, respectively.

The results showed that there was a statistically significant positive linear trend in urinary bladder benign stromal tumor ($p = 0.0337$) in male mice. However, the pairwise comparison between control and high dose male mice did not show any difference ($p = 0.1403$) in urinary bladder benign stromal tumor rates between the two groups. There was no statistically significant (at 0.05 level) trend in any other urinary bladder tumors in male mice.

There was a statistically significant positive linear trend in reticulo-histiocytary system malignant lymphoma (trend : $p < 0.00001$; pairwise: high vs control: $p = 0.0001$; pairwise: high vs medium: $p < 0.0001$) in female mice. However, if all of the malignant lymphomas in different organs of female mice were combined, then the linear trend ($p = 0.2307$) became not significant. In Table 18 of the sponsor's submission dated on August 11, 1988, the "RH system malignant lymphoma" in female mice actually is the "combination of malignant lymphomas in reticulo-histiocytary system and all different organs".

There was a statistically significant positive linear trend in stomach inverted papilloma of para cutanea (p = 0.0072) in male mice. The pairwise comparison also showed a statistically significant difference between control and high dose male mice in this tumor (p = 0.0435). For the rat study, there was a significant positive linear trend in brain granular cell tumor (p = 0.0411) in male rats. However, the pairwise comparison between control and high dose male rats did not show any difference (p = 0.1594) in tumor rate between the two groups.

Daphne Lin

Daphne Lin, Ph.D.
Mathematical Statistician

Concur: Karl K. Lin 3/3/94

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HFD-502/Assistant Director (Pharmacology)
HFD-715/DRU 2.1.1, Nisoldipine, Miles Inc.

Table 1
Tumor Incidence Rates
Male Mice, Urinary Bladder Benign Stromal Tumor

Weeks	Control		LOW		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	0	7	0	2	0	16
81-103	0	11	0	5	0	17	1	20
Terminal	0	36	0	35	0	31	1	10
Total	0	50	0	49	0	50	2	50

Trend test: p = 0.0337
Pairwise comparison: High vs Control p = 0.1403

Table 2
Tumor Incidence Rates
Male Mice, Urinary Bladder Malignant Stromal Tumor

Weeks	Control		LOW		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	0	7	0	2	0	16
81-103	0	11	0	5	1	17	0	20
Terminal	0	36	0	35	0	31	0	10
Total	0	50	0	49	1	50	0	50

Trend test: p = 0.6226

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 3
Tumor Incidence Rates
Male Mice, Urinary Bladder Polypous Stromal Tumor

Weeks	Control		Low		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	0	7	0	2	0	16
81-103	0	11	0	5	0	17	0	20
Terminal	1	36	0	35	1	31	0	10
Total	1	50	0	49	1	50	0	50

Trend test: p = 0.5793

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 4
Tumor Incidence Rates
Female Mice, Reticulo-histiocytary System Malignant Lymphoma

Weeks	Control		Medium		High	
	T	N	T	N	T	N
0-50	0	2	0	1	0	3
51-80	0	10	0	9	3	9
81-103	0	14	0	24	8	19
Terminal	0	22	0	16	2	18
Total	0	48	0	50	13	49

Trend test: $p < 0.00001$
Pairwise comparison: high vs control: $p = 0.0001$
: high vs medium: $p < 0.0001$

Table 5
Tumor Incidence Rates
Female Mice, Reticulo-histiocytary System Malignant Lymphoma & All Possible sites of Malignant Lymphoma

Weeks	Control		Medium		High	
	T	N	T	N	T	N
0-50	0	2	0	1	0	3
51-80	5	10	1	9	3	9
81-103	9	14	7	24	8	19
Terminal	4	22	4	16	3	18
Total	18	48	12	50	14	49

Trend test: $p = 0.2307$

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Appendix 2

Table 18: Comparative summary of tumours occurring according to location, type, number and dignity $ (animals scheduled for terminal kill)

Sex	♂ (M)				♀ (F)			
Dose ppm	0	100	300	900	0	100	300	900
Lung:		-				-		
bronchiolo-alveolar adenoma	2		3	4	2		1	3
bronchiolo-alveolar carcinoma (malig.)	13		12	5	8		6	5
Stomach:								
papilloma	0	0	0	2	0	0	0	0
sarcoma (malig.)	0	0	2	1	0	0	0	0
Liver:								
hepatocellular adenoma	2	2	2	3	0	0	0	1
hepatocellular carcinoma (malig.)	3	4	5	8	0	0	0	1
haemangiosarcoma (malignant)	1	0	0	0	0	0	0	0
Kidneys:		-				-		
tubular carcinoma (malignant)	0		1	0	0		0	0
haemangiosarcoma (malignant)	0		1	0	0		0	0
Bladder:		-				-		
stromal tumour (benign)	1		1	2	0		0	0
stromal tumour (malignant)	0		1	1	0		0	0
Ovary:	-	-	-	-		-		
granulosa-theca cell tumour (ben.)					5		5	3
granulosa-theca cell tumour (malig.)					1		0	0
luteoma (benign)					2		2	0
tubular adeno-carcinoma (malig.)					1		0	0
Sertoli cell tumour (benign)					0		0	1
Uterus:	-	-	-	-				
adenoma					0	0	1	0
carcinoma (malig.)					1	0	0	0
fibroma					0	0	1	0
myoma					0	0	1	2
myosarcoma (malig.)					0	0	0	1
stromal tumour (benign)					3	0	2	2
stromal sarcoma (malignant)					1	3	2	2

Table 18 (continued):

Sex	♂				♀			
Dose ppm	0	100	300	900	0	100	300	900
Testes:		-				-	-	-
Leydig cell tumour (benign)	2		2	0				
adenoma of rete testis	1		0	0				
Pituitary:		-						
adenoma	2		0	0	0	3	3	1
Thyroid:		-				-		
follicle cell adenoma	0		1	0	0		0	0
papillary cyst-adenoma	1		0	0	0		0	0
Adrenals:		-				-		
cortical adenoma	3		3	1	2		0	0
phaeochromocytoma (benign)	1		0	0	1		0	2
phaeochromocytoma (malignant)	0		0	1	0		0	0
RH system:		-				-		
lymphoma (malig.)	7		3	1	18		12	14
lymph node sarcoma (malignant)	1		0	0	1		0	0
Skin/subcutis:		-				-		
epithelioma (malignant)	0		0	1	0		0	0
sarcoma (malig.)	1		0	0	0		3	0
Mammary gland:	-	-	-	-		-		
carcinoma (malig.)					3		0	0
adeno-acanthoma (malignant)					0		1	1
Harder's gland:		-				-		
papillary adenoma	3		2	0	1		1	1
Spinal marrow:		-				-		
schwannoma (malig.)	0		0	0	0		1	0
Bones:		-				-		
osteosarcoma (malignant)	0		0	0	0		1	0
Abdomen:		-				-		
haemangiosarcoma (malignant)	0		0	0	0		1	0
Pelvic serosa:		-	-	-	-	-	-	-
sarcoma (malig.)	1							

- Organ not investigated § Bilateral tumours counted twice

Table 7
Tumor Incidence Rates
Male Mice, Stomach Inverted Papilloma of Pars Cutanea

Weeks	Control		Low		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	0	7	0	2	0	16
81-103	0	11	0	5	0	17	0	20
Terminal	0	36	0	35	0	31	2	10
Total	0	50	0	49	0	50	2	50

Trend test: p = 0.0072
Pairwise comparison: high vs control: p = 0.0435

Table 8
Tumor Incidence Rates
Male Rats, Brain Granular Cell Tumor

Weeks	Control		Low		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	1	0	0	0	1	0	0
51-80	0	1	0	0	0	0	1	3
81-109	0	3	0	8	0	10	0	8
Terminal	0	45	0	42	0	39	2	39
Total	0	50	0	50	0	50	3	50

Trend test: p = 0.0411
Pairwise comparison: high vs control: p = 0.1594

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

ATTACHMENT

Histopathology Incidence

Two-Year Dietary Study in the Rat

A Brief Coversheet for Carcinogenesis Study Review

NDA 20-356
Drug: Nisoldipine
Sponsor: Miles Inc. Pharmaceutical Division

1. Species & Strain	Rat, Wistar (TNO/W 74)
2. Name of Laboratory	
3. No./sex/group	50 (additional 10 rats/sex included in each group were sacrificed after 12 months of treatment)
4. Doses (C, L, M & H)	0, 50, 300 & 1800 ppm in diet
5. Basis for dose selection stated: yes (x) no ( )	4-week dietary dose rangefinding study in mice
6. Interim sacrifice	yes
7. Total duration	Two year
8. No. alive at termination (C, L, M & H)	Males: 46, 42, 39 & 39 Females: 42, 42, 35 & 37
9. Statistical methods used	U-test of Mann and Whitney and of Wilcoxon, and Fischer's exact test

10. Tumor and non-tumor data for each tissue attached.

List of neoplasms in the rats at the interim sacrifice

Control group, ♀

Animal No. 71 Thyroid: cystadenoma
No. 80 Pituitary: adenoma

Dose group 1800 ppm BAY k 5552, ♂

No. 399 Testis : Leydig cell tumour (benign)
No. 414 Brain (cerebellum): Meningioma (benign)

Table : Number of the blastoma carriers in the individual dose groups

Sex	♂				♀			
Dose (ppm)	0	50	300	1800	0	50	300	1800
Total number of rats investigated	49	50	50	48	48	48	48	48
Number of the blastoma carriers	31	16	21	26	33	21	21	28
Number of rats with exclusively benign blastomas	28	10	12	18	24	15	14	18
Number of rats with exclusively malignant blastomas	2	3	8	4	6	3	4	6
Number of rats with benign and malignant blastomas	1	3	1	4	3	3	3	4
Number of blastoma carriers as % all rats investigated	63	32	42	54	69	44	44	58
Number of blastoma carriers with exclusively benign blastomas as % all rats investigated	57	20	24	38	50	31	29	38
Number of blastoma carriers with exclusively malignant blastomas as % all rats investigated	4	6	16	8	13	6	8	13
Number of blastoma carriers with benign and malignant blastomas as % all rats investigated	2	6	2	8	6	6	6	8

Table ___ : List of all blastomas according to number, localisation, type and status (male rats)

(Dose ppm)	0	50	300	1800
Adenohypophysis*				
Adenoma	14	0	3	11
Carcinoma	0	2	0	0
Thyroids*				
C cell adenoma	6	0	0	0
C cell carcinoma	1	0	0	1
Follicular carcinoma	0	0	0	1
Adrenal cortex				
Adenoma unilateral	1	1	0	0
Adrenal medulla				
Phaeochromocytoma (b) unilateral	7	5	3	7
Phaeochromocytoma (b) bilateral	0	1	0	1
Phaeochromocytoma (m) unilateral	0	1	1	0
Parathyroids*				
Adenoma	3	0	0	0
Testes				
Leydig cell tumour (b) unilateral	4	5	6	7
Leydig cell tumour (b) bilateral	0	1	2	1
Pancreas* endocrine				
Adenoma	2	0	0	0
Pancreas* exocrine				
Adenoma	3	0	0	0
Heart*				
Endocardial fibromatosis (b)	2	0	0	1
Lung*				
Adenoma	0	0	1	0
Epididymis				
Sarcoma	0	0	0	1
Brain*				
Meningioma (b)	0	0	0	2
RHS*				
Malignant lymphoma	0	1	3	1
Histiocytary sarcoma	0	0	1	0
Skin*				
Cornified squamous cell carcinoma	0	1	1	1
Subcutis*				
Sarcoma	1	1	1	3
Haemangiosarcoma	0	0	1	1
Mesentery*				
Leiomyosarcoma	0	0	1	0
Abdomen*				
Fibrosarcoma	1	0	0	0
Sarcoma	0	1	0	0

b = benign
m = malignant
* This organ was not routinely histologically investigated in all the rats from the 50 and 300 ppm groups.

Table : List of all blastomas according to number, localisation, type (continuation) and status (female rats)

(Dose ppm)	0	50	300	1800
Adenohypophysis				
Adenoma	16	3	5	7
Carcinoma	1	1	1	0
Thyroids*				
C cell adenoma	6	1	2	1
C cell carcinoma	1	0	0	0
Follicular adenoma	0	0	0	1
Follicular carcinoma	1	0	0	0
Adrenal cortex				
Adenoma unilateral	1	1	0	0
Adrenal medulla				
Phaeochromocytoma (b) unilateral	0	0	0	1
Phaeochromocytoma (m) unilateral	0	0	0	1
Ovary				
Granulosa-theca cell tumour (b)	0	0	1	1
Granulosa-theca cell tumour (m)	0	0	0	1
Uterus				
Endometrial stromal tumour (polyp) (b)	8	12	11	13
Endometrial stromal sarcoma	2	0	2	2
Adenocarcinoma	1	4	2	2
Mammary gland				
Adenoma	0	2	1	2
Adenocarcinoma	1	1	1	0
Kidneys				
Adenoma	1	0	0	0
Sarcoma	1	0	0	0
Urinary bladder*				
Adenoma	1	0	0	0
RHS*				
Malignant lymphoma	0	0	0	1
Histiocytary sarcoma	0	0	0	1
Intestine*				
Fibroma	0	0	0	1
Mesentery*				
Malignant mesothelioma	0	0	1	0
Skin				
Papilloma	0	0	0	1
Subcutis				
Haemangiosarcoma	1	0	0	0
Sarcoma	1	0	0	2

b = benign
m = malignant
* This organ was not routinely histologically investigated in all the rats from the 50 and 300 ppm groups.

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group: 0 ppm, sex ♂

Animal No.	8	Pituitary	Adenoma
		Endocrine pancreas	Islet cell adenoma
No.	11	Testes	Leydig cell tumour, benign (b), unilateral
		Thyroid	C cell adenoma
No.	13	Pituitary	Adenoma
No.	14	Testes	Leydig cell tumour (b), unilateral
No.	18	Adrenals	Cortical adenoma, unilateral
No.	19	Pituitary	Adenoma
No.	20	Heart	Endocardial fibromatosis (b)
		Adrenals	Phaeochromocytoma (b), unilateral
		Parathyroids	Adenoma, unilateral
		Exocrine pancreas	Adenoma
No.	23	Testes	Leydig cell tumour (b), unilateral
		Thyroid	C cell carcinoma
No.	24	Pituitary	Adenoma
No.	26	Pituitary	Adenoma
No.	27	Pituitary	Adenoma
No.	30	Adrenals	Phaeochromocytoma (b), unilateral
		Parathyroids	Adenoma, unilateral
No.	33	Adrenals	Phaeochromocytoma (b), unilateral
No.	35	Pituitary	Adenoma
No.	36	Thyroid	C cell adenoma
No.	37	Pituitary	Adenoma
No.	39	Pituitary	Adenoma
No.	41	Adrenals	Phaeochromocytoma (b), unilateral
No.	42	Thyroid	C cell adenoma
No.	43	Subcutis (ear)	Sarcoma
No.	44	Thyroid	C cell adenoma
No.	45	Pituitary	Adenoma
No.	46	Endocrine pancreas	Islet cell adenoma
		Adrenals	Phaeochromocytoma (b), unilateral
No.	47	Pituitary	Adenoma
		Parathyroids	Adenoma, unilateral

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group: 0 ppm, sex ♂

Animal No.	49	Testes	Leydig cell tumour (b), unilateral
No.	50	Exocrine pancreas	Adenoma
		Pituitary	Adenoma
		Thyroid	C cell adenoma
No.	54	Pituitary	Adenoma
		Adrenals	Phaeochromocytoma (b), unilateral
No.	55	Heart	Endocardial fibromatosis (b)
No.	56	Pituitary	Adenoma
		Adrenals	Phaeochromocytoma (b), unilateral
No.	57	Abdomen	Fibrosarcoma
No.	60	Exocrine pancreas	Adenoma
		Thyroid	C cell adenoma

Dose group 50 ppm, sex ♂

Animal No.	124	Adrenals	Phaeochromocytoma (b), unilateral
No.	128	Adrenals	Phaeochromocytoma (b), unilateral
No.	132	Testes	Leydig cell tumour (b), unilateral
		Skin (head)	Cornifying squamous cell carcinoma
No.	136	Adrenals	Phaeochromocytoma (b), unilateral
No.	142	Testes	Leydig cell tumour (b), unilateral
No.	145	Abdomen	Sarcoma
No.	149	Adrenals	Cortical adenoma, unilateral
No.	151	Adrenals	Tumour. The neoplastic tissue has penetrated the organ capsule. The origin of the tumour cells cannot be determined with certainty due to autolysis. Presumably it is a malignant unilateral phaeochromocytoma.
		Reticulohistiocytary system (RHS)	Malignant lymphomas in the spleen, liver, lung and bone marrow
No.	153	Adrenals	Phaeochromocytoma (b), unilateral
No.	163	Testes	Leydig cell tumour (b), unilateral

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group: 50 ppm, sex ♂		
Animal No. 165	Pituitary	Adenocarcinoma, infiltrating the brain
No. 168	Testes	Leydig cell tumour (b), unilateral
No. 169	Testes Subcutis (hea[illegible]	Leydig cell tumour (b), bilateral Sarcoma
No. 170	Adrenals Pituitary	Phaeochromocytoma (b), unilateral Adenocarcinoma with multiple focal necroses
No. 175	Adrenals	Phaeochromocytoma (b), bilateral
No. 180	Testes	Leydig cell tumour (b), unilateral
Dose group: 300 ppm, sex ♂		
Animal No. 252	Adrenals	Phaeochromocytoma, the tumour cells have penetrated the organ capsule, malignant, unilateral
No. 253	RHS	Malignant lymphoma in the thymus (?) and spleen
No. 257	Testes	Leydig cell tumour (b), unilateral
No. 259	Adrenals RHS	Phaeochromocytoma, (b), unilateral malignant lymphoma in the spleen, lymph nodes, kidneys, lung, mesentery, muscles, urinary bladder, pancreas and bone marrow
No. 260	Pituitary	Adenoma
No. 262	Testes	Leydig cell tumour (b), bilateral
No. 263	Subcutis (rear limb)	Sarcoma infiltrating the skeletal muscle
No. 265	Pituitary	Adenoma
No. 267	Testes	Leydig cell tumour (b), unilateral
No. 272	Adrenals Testes	Phaeochromocytoma (b), unilateral Leydig cell tumour (b), bilateral

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group 300 ppm, sex ♂

Animal No. 273	Testes	Leydig cell tumour (b), unilateral
No. 274	Mesentery	Leiomyosarcoma which has infiltrated the pancreas and the skeletal muscle (diaphragm)
No. 276	HS	Histiocytary sarcoma with foci in the heart, spleen, liver, lung, pancreas, bones, bone marrow, stomach, orbit, oesophagus, trachea, thyroid, epididymis and adrenals
No. 285	Testes	Leydig cell tumour (b), unilateral
No. 288	Adrenals	Phaeochromocytoma (b), unilateral
No. 289	RHS	Malignant lymphomas in the spleen, lymph nodes, liver, lung, pancreas, kidneys, mesentery, mediastinum and heart
No. 290	Skin (lower abdomen)	Cornifying squamous cell carcinoma
No. 291	Testes	Leydig cell tumour (b), unilateral
No. 296	Pituitary Lung	Adenoma Adenoma
No. 297	Testes	Leydig cell tumour (b), unilateral
No. 299	Subcutis (penis)	Haemangiosarcoma

Dose group 1800 ppm, sex ♂

Animal No. 361	Testes Adrenals	Leydig cell tumour (b), unilateral Phaeochromocytoma (b), unilateral
No. 363	Heart Pituitary	Endocardial fibromatosis (b) Adenoma
No. 368	Testes Pituitary	Leydig cell tumour (b), unilateral Adenoma
No. 370	Adrenals	Phaeochromocytoma (b), bilateral
No. 372	Subcutis (lower leg)	Haemangiosarcoma

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group 1800 ppm, sex ♂

Animal No. 374	Adrenals	Phaeochromocytoma (b), unilateral
No. 375	Subcutis (head)	Sarcoma. The soft-tissue tumour has infiltrated the periorbital and retro-orbital tissue and the pituitary.
No. 376	Adrenals	Phaeochromocytoma (b), unilateral
No. 377	Thyroid	C cell carcinoma
	Brain	Meningioma (b)
No. 378	Testes	Leydig cell tumour (b), unilateral
No. 379	Pituitary	Adenoma
No. 380	Pituitary	Adenoma
No. 382	Pituitary	Adenoma
	Adrenals	Phaeochromocytoma (b), unilateral
No. 383	Testes	Leydig cell tumour (b), unilateral
No. 384	Pituitary	Adenoma
	Adrenals	Phaeochromocytoma (b), unilateral
No. 385	Subcutis (Femur, ear)	Sarcoma with infiltrates or metastases in the skeletal muscle, bone, bone marrow, and lung
No. 390	Testes	Leydig cell tumour (b), bilateral
No. 391	Pituitary	Adenoma
No. 392	RHS	Malignant lymphomas in the thymus, spleen and lymph nodes
	Pituitary	Adenoma
	Adrenals	Phaeochromocytoma (b), unilateral
No. 393	Epididymides	Sarcoma
No. 398	Pituitary	Adenoma
	Adrenals	Phaeochromocytoma (b), unilateral
No. 400	Testes	Leydig cell tumour (b), unilateral
	Thyroid	Follicular carcinoma
	Brain	Meningioma (b)
	Subcutis (base of tail)	Sarcoma
No. 404	Testes	Leydig cell tumour (b), unilateral
No. 410	Testes	Leydig cell tumour (b), unilateral
No. 415	Pituitary	Adenoma
No. 416	Pituitary	Adenoma
	Skin (head)	Cornifying squamous cell carcinoma

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group 0 ppm, sex ♀

Animal No. 61	Pituitary	Adenoma
No. 66	Thyroid	C cell adenoma
No. 68	Uterus	Endometrial stromal tumour (polyp, b)
	Thyroid	C cell adenoma
No. 69	Uterus	Endometrial stromal tumour (polyp, b)
	Pituitary	Adenoma
No. 72	Pituitary	Adenoma
No. 74	Pituitary	Adenoma
No. 75	Uterus	Adenocarcinoma with infiltrates in the mesentery
No. 76	Uterus	Endometrial stromal tumour (polyp, b)
No. 77	Pituitary	Adenoma
	Mammary gland	Adenocarcinoma
No. 82	Uterus	Endometrial stromal tumour (polyp, b)
No. 84	Urinary bladder	Adenoma
No. 85	Pituitary	Adenoma
No. 89	Kidney	Sarcoma
	Thyroid	C cell adenoma
No. 90	Uterus	Endometrial stromal tumour (polyp, b)
	Thyroid	C cell adenoma
No. 92	Pituitary	Adenoma
No. 93	Uterus	Endometrial stromal tumour (polyp, b)
	Thyroid	C cell adenoma
No. 94	Pituitary	Adenoma
No. 97	Uterus	Endometrial stromal tumour (polyp, b)
No. 98	Thyroid	Follicular carcinoma
	Subcutis (head)	Haemangiosarcoma
No. 99	Thyroid	C cell carcinoma
No.100	Pituitary	Adenoma
	Adrenals	Cortical adenoma, unilateral
No.102	Uterus	Endometrial stromal sarcoma infiltrating the urinary bladder
No.104	Pituitary	Adenoma

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group 0 ppm, sex ♀

Animal No.106	Pituitary	Adenoma
No.107	Pituitary	Adenoma
	Subcutis (inguinal region)	Sarcoma
No.110	Kidney	Adenoma
	Pituitary	Adenoma
No 111	Pituitary	Carcinoma with infiltrates in the brain
No.113	Pituitary	Adenoma
No.114	Thyroid	C cell adenoma
No.117	Uterus	Endometrial stromal sarcoma with infiltration into the mesentery
No.118	Uterus	Endometrial stromal tumour (polyp, b)
No.119	Pituitary	Adenoma
No.120	Pituitary	Adenoma

Dose group 50 ppm, sex ♀

Animal No. 181	Uterus	Endometrial stromal tumour (b)
No. 187	Uterus	Endometrial stromal tumour (polyp, b) Infiltrating adenocarcinoma with metastases in the liver, pancreas and mesentery
No. 188	Uterus	Endometrial stromal tumour (polyp, b)
No. 192	Adrenals	Cortical adenoma, unilateral
No. 196	Uterus	Adenocarcinoma
No. 199	Thyroid	C cell adenoma. Tentative diagnosis because of the severe autolysis.
No. 212	Mammary gland	Adenoma
No. 213	Mammary gland	(Fibro)adenoma with pericanalicular and intracanalicular growth
No. 219	Uterus	Endometrial stromal tumour (polyp, b)
No. 220	Pituitary	Adenoma

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group 300 ppm, sex ♀

Animal No.333	Pituitary	Carcinoma infiltrating the brain
	Thyroid	C cell adenoma
No.334	Uterus	Endometrial stromal tumour (polyp, b)
	Pituitary	Adenoma
No.335	Uterus	Endometrial stromal tumour (polyp, b)
	Mammary gland	Adenoma
No.336	Uterus	Infiltrating adenocarcinoma with squamous metaplasia and sarcomatous tissue areas
	Pituitary	Adenoma
No.340	Thyroid	C cell adenoma
No.341	Uterus	Endometrial stromal tumour (polyp, b)
No.345	Uterus	Endometrial stromal sarcoma infiltrating the mesentery and pancreas
No.347	Uterus	Endometrial stromal tumour (polyp, b)
No.348	Uterus	Endometrial stromal tumour (polyp, b)
No.350	Ovaries	Granulosa-theca cell tumour (b), unilateral
	Pituitary	Adenoma

Dose group 1800 ppm, sex ♀

Animal No.425	Thyroid	C cell adenoma
No.426	Ovaries	Granulosa-theca cell tumour (m)
No.427	Uterus	Endometrial stromal tumour (polyp, b
	Pituitary	Adenoma
No.429	Skin (head)	Papilloma (b)
No.431	Ovaries	Granulosa-theca cell tumour (b)
	Pituitary	Adenoma
	Adrenals	Phaeochromocytoma, unilateral. The tumour has penetrated the organ capsule,m
No.435	Uterus	Adenocarcinoma with infiltrates in the mesentery
No.445	Uterus	Endometrial stromal tumour (polyp, b)
	Pituitary	Adenoma
No.446	Uterus	Endometrial stromal tumour (polyp, b)
	Pituitary	Adenoma

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group 50 ppm, sex ♀

Animal No.221	Uterus	Endometrial stromal tumour (polyp, b) Adenocarcinoma
No.222	Uterus	Endometrial stromal tumour (polyp, b)
No.223	Uterus	Endometrial stromal tumour with squamous metaplasia (polyp, b)
	Pituitary	Carcinoma infiltrating the brain
No.224	Uterus	Adenocarcinoma with metastases in the liver, pancreas, stomach, intestir and mesentery
No.230	Uterus	Endometrial stromal tumour (polyp, b)
No.231	Pituitary	Adenoma
No.233	Uterus	Endometrial stromal tumour (polyp, b)
	Pituitary	Adenoma
No.234	Mammary gland	Adenocarcinoma
No.236	Uterus	Endometrial stromal tumour (polyp, b)
No.237	Uterus	Endometrial stromal tumour (polyp, b)
No.240	Uterus	Endometrial stromal tumour (polyp, b)

Dose group 300 ppm, sex ♀

Animal No.301	Uterus	Endometrial stromal sarcoma
No.305	Pituitary	Adenoma
No.308	Uterus	Adenocarcinoma
No.310	Uterus	Endometrial stromal tumour (polyp, b)
No.312	Pituitary	Adenoma
No.313	Uterus	Endometrial stromal tumour (polyp, b)
No.314	Uterus	Endometrial stromal tumour (polyp, b)
No.318	Mesentery	Malignant mesothelioma of the serous lining of the adrenals, uterus, ovarie spleen, stomach, intestines and urinar bladder
No.325	Uterus	Endometrial stromal tumour (polyp, b)
No.326	Uterus	Endometrial stromal tumour (polyp, b)
	Mammary gland	Adenocarcinoma
No.330	Uterus	Endometrial stromal tumour (polyp, b)

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group 1800 ppm, sex ♀

Animal No.447	Uterus	Endometrial stromal tumour (polyp, b)
No.450	Uterus	Endometrial stromal tumour (polyp, b)
No.453	Pituitary	Adenoma
No.457	Uterus Adrenals	Endometrial stromal tumour (polyp, b) Phaeochromocytoma (b), unilateral
No.459	Intestines Uterus	Fibroma Endometrial stromal sarcoma
No.460	Subcutis (flank)	Sarcoma infiltrating through the muscles of the flank and extending into the mesentery.
No.463	Thyroid	Follicular adenoma
No.464	Uterus Subcutis (ear)	Endometrial stromal tumour (polyp, b) Sarcoma
No.465	Uterus Mammary gland	Endometrial stromal tumour (polyp, b) Adenoma
No.466	RHS	Histiocytary sarcoma in the mesentery liver, pancreas, skeletal muscle and subcutis
No.467	RHS	Malignant lymphoma in the spleen, lymph nodes, liver, lung, kidneys, urinary bladder, uterus, thyroid, skeletal muscle, bone marrow, mediastinum and mesentery
No.469	Uterus	Endometrial stromal tumour (polyp) with squamous metaplasia (b) Adenocarcinoma with metastases in the lung, liver, pancreas, kidneys, ovaries and mesentery
No.471	Uterus	Endometrial stromal sarcoma with metastases in the liver, urinary bladder, ovaries and mesentery
No.472	Pituitary	Adenoma
No.475	Mammary gland	(Fibro)adenoma
No.476	Uterus	Endometrial stromal tumour (polyp, b)
No.477	Uterus	Endometrial stromal tumour (polyp, b)
No.478	Uterus	Endometrial stromal tumour (polyp, b)
No.479	Uterus	Endometrial stromal tumour (polyp, b)
No.480	Pituitary	Adenoma

Histopathological findings on the lower jaw in the region of the gnawing teeth

Dose group 0 ppm, sex ♂

Animal No. 33 Nothing abnormal detected (N.A.D.)

No. 36 N.A.D.

No. 41 Small focal necrosis in the hard substance of the crown of the teeth (caries)

No. 47 Slight to moderate round-cell infiltration in the connective tissue of the gums and muscles

No. 51 N.A.D.

Dose group 1800 ppm, sex ♂

Animal No.390 N.A.D.

No.393 N.A.D.

No.407 Moderate acute, focal gingivitis in the crown-root region

No.411 Moderate acute, focal gingivitis in the crown-root region

No.413 N.A.D.

Dose group 0 ppm, sex ♀

Animal No. 90 Very slight acute, focal gingivitis in the crown-root region

No. 94 N.A.D.

No.114 N.A.D.

No.118 Very slight acute, focal gingivitis in the crown-root region
Small focus of necrosis in the hard substance of the teeth and the pulp.

No.119 N.A.D.

Dose group 1800 ppm, sex ♀

Animal No.341 Moderate subchronic myositis. The epithelium over this is hyperkeratotic. It is assumed that the changes are the result of an external injury.

No.443 Very slight acute, focal gingivitis in the crown-root region

No.457 Slight acute, necrotic focal gingivitis in the crown-root region.
Small focus of necrosis in the hard substance of the crown of the teeth (caries).

No.461 Slight necrotic focal gingivitis in the crown-root region.
Focal oedema in the connective tissue of the gums.

No.466 Slight acute, focal gingivitis in the crown-root region.

ATTACHMENT

Histopathology Incidence

Twenty-One Month Mouse Study

A Brief Coversheet for Carcinogenesis Study Review

NDA 20-356
Drug: Nisoldipine
Sponsor: Miles Inc. Pharmaceutical Division

1. Species & Strain	Mouse, Bor:NMRI (SPF HAN)
2. Name of Laboratory	
3. No./sex/group	50 (additional 20 mice/sex/group were included for interim sacrifice at 12 months)
4. Doses (C, L, M & H)	0, 100, 300 and 900 ppm in diet
5. Basis for dose selection stated: yes (x) no ( )	28-day dietary dose rangefinding study in mice
6. Interim sacrifice	yes
7. Total duration	21 months
8. No. alive at termination (C, L, M & H)	Males: 36, 35, 21 & 10 Females: 22, 16, 16 & 18
9. Statistical methods used	Two-tailed U test, generalized Wilcoxon test & Peto analysis

10. Tumor and non-tumor data for each tissue attached.

The following findings were observed in control and dose animals: no test substance effect (group specificity) was detectable (see Tables in Appendix, p. 341 to 381).

1. Non-blastomatous changes: (interim kill)

Liver : Slight to moderate lipid storage in hepatocytes, isolated round cell infiltrates and slight intracytoplasmic vacuoles were seen in several animals.

Kidneys : Tubular dilations (partially with epithelial desquamation and/or plasma basophilia), PAS-positive casts, cysts in the papillary area and round cell infiltrates in the area of the venae stellatae and the pelvis were found in most animals.

Bladder : Round cell infiltrates in the submucosa were found in several animals.

Testes : Several animals showed spermiogranuloma, atrophy, some convoluted seminiferous tubules and round cell infiltrates.

Uterus : Several animals showed endometrial cysts.

Salivary glands: Slight round cell infiltrates were found in some animals.

Stomach : In several animals, the glandular mucosa showed retention cysts in the fundal area, to some extent with squamous metaplasia and epithelial proliferation. Some animals had round cell infiltrates in the area of the submucosa.

Intestine : Animals 215, 216 (both died) and 503 (all from the 900 ppm dose group) showed a tightly filled, dilated colon.

Eyes : Some animals showed slight round cell infiltrates in the episcleral area.

Thyroid : Several animals showed small cysts (remains of the thyroglossal duct).

Individual findings

No. 14 (0 ppm; ♂), Epididymis: aspermia
18 (0 ppm; ♂), Pancreas: islet cell hyperplasia
Spleen: increased haematopoiesis
Prostate: purulent prostatitis and urethritis
Bladder: purulent cystitis
225 (900 ppm; ♂), Nasopharynx: cellular or inflammatory cellular infiltration, slight
226 (900 ppm; ♂), Subcutis: abscess
284 (0 ppm; ♀), Large intestine: section containing helminthes
286 (0 ppm; ♀), Adrenals: intracytoplasmic vacuoles in adrenal cortex cells
289 (0 ppm; ♀), Kidneys: glomerular atrophy
Ovaries: follicular cysts
291 (0 ppm; ♀), Stomach: erosion with round cell infiltrates in the area of the keratinising squamous epithelium
437 (300 ppm; ♀), Lung: multifocal bronchopneumonia

Heart, brain, seminal vesicle, oesophagus, lymph nodes, pituitary, trachea, skin, bones, bone marrow, skeletal muscles and spinal cord were histopathologically normal.

2. Blastomas

A synoptic comparative review of the tumours is shown on Page 340. All tumours are regarded as having occurred spontaneously and are not attributed to the treatment with the test substance BAY k 5552, as no dose-dependency is recognisable from the number, type or location.

Tumour carriers (interim kill)

Group 0 ppm, ♂

Animal No.	14	Kidney	Malignant lymphoma

Group 900 ppm, ♂

Animal	211	Lung	Alveologenic carcinoma (Kimura Type A)+
	212	Kidney	Malignant lymphoma
	214	Liver	Malignant hepatoma
	224	Lung	Alveologenic carcinoma (Kimura Type AP)

Group 0 ppm, ♀

Animal No.	281	Lung	Alveologenic carcinoma (Kimura Type A)
+	289	Kidney, salivary gland, pancreas, bladder	Malignant lymphoma
	290	Stomach	Keratoacanthoma
	291	Kidney, salivary gland	Malignant lymphoma

Group 900 ppm, ♀

Animal No.	493	Thymus, kidney, serosa	Malignant lymphoma
	499	Kidney	Malignant lymphoma
	501	Lung	Alveologenic carcinoma (Kimura Type A)
		Kidney	Malignant lymphoma
	502	Kidney, bladder	Malignant lymphoma
	503	Lung	Alveologenic carcinoma (Kimura Type A)

Group 300 ppm, ♀

Animal No.	430	Vagina	Keratoacanthoma
	438	Lung	Alveologenic carcinoma (Kimura Type AP)
	439	Thymus	Thymic lymphosarcoma

+ Kimura, J.: Progression of pulmonary tumor in mice. Acta path. jap. 21, 13 ff (1971)

8.5 Autopsy findings

G	died
ZS	sacrificed at interim kill
VE	sacrificed a end of study
MG	killed in extremis

8.6 List of abbreviations for Tables with individual histopathological findings

A	= autolysis
Abd	= abdominal cavity
Abz	= abscess
An	= inflammatory congestion of secretions in the anal gland
Ap	= angiopathy
Asp	= aspermia
At	= atrophy
b	= bilateral
Cy	= cyst(s)
De	= degeneration (for pituitary: cystic degeneration)
Dil	= dilation
e	= unilateral
EPI	= epididymis
Er	= ercsion
F	= hepatocellular fatty infiltration/fat marrow
Fca	= foci of cellular alteration
Gg	= biliary proliferation
H	= pituitary
Hä	= haemorrhage
Hb	= bladder
HD	= Harder's gland
He	= helminthes
Hn	= hydronephrosis
Hp	= increased haematopoiesis
Ht	= skin
Hy	= hyperplasia for the pituitary: diffuse and/or focal hyperplasia for the stomach: adenomatous hyperplasia of the glandular stomach for the uterus: cystic/hyperplastic endometrium for the adrenals: proliferated subcapsular cortical cells for the pancreas: multifocal hyperplasia of the islets of Langerhans for the testes: hyperplasia of the Leydig cells
Iz	= inflammatory infiltrates
Iz Ne	= inflammatory necroses
Kv	= lipid-free, cytoplasmatic vacuoles, lying close to the nucleus. In male mice, the vacuoles are often so paranuclear, that the nucleus is deformed into a crescent shape.

Kz = diffusely proliferated Kupffer's cells
LE = liver
LN = lymph nodes
LU = lung
Ma = mammary gland
Mh = arterial medial hyperplasia
Med = mediastinum
Mes = mesentery
Mi = increased occurrence of mitoses
Min = mineralisation foci in the gastric mucosa
Mp = epithelial metaplasia
Mz = in the liver: megalohepatocytes
in the brain: malacia
Ne = necrosis
Nez = increased occurrence of unicellular necroses
NN = adrenals
NNH = paranasal sinuses
Np = progressive senile nephropathy
O = without histopathological findings: findings within normal variability which correspond particularly to species age of study animals and conventional keeping condition. Normal findings in all animal groups include, amongst others: effects from the kill, preparation artefacts (e.g. different degrees of exsanguination, varying degrees of lung collapse, artificial emphysema, slight autolysis), small round-cell infiltrates e.g. of the head salivary glands, liver, kidneys, bladder; dysontogenetic cysts in the kidneys; individual small endometrium cysts; slight to moderate brown degeneration of the adrenal x-zone (ceroid deposit in the area around the corticomedullary junction, almost only in female animals) and slight subcapsular cell reaction; intranuclear inclusions in the hepatic cells; distended excretory ducts of the mammary glands filled with secretion; slight to moderate progressive senile nephropathy
Oph = ophthalmopathy
Ov = ovary
e = not investigated, no histological sample available
Ö = oedema
P = pelvic serosa
PA = pancreas
PD = hyperplasia of the preputial gland
PDS = secretion congestion in the preputial gland
Pel = peliosis hepatis
Pi = increased occurrence of pigment
PIT = pituitary gland
PRO = prostate gland
PT = organ investigated histopathologically, no parenchyma tumour found
RHS = reticulohistiocytary system
Rz = cellular infiltrates consisting mainly of round cells
Sc = subcutis
SLGL = salivary gland
Spgr = spermiogranuloma
SV = seminal vesicle

T = one tumour
TM = tumor metastasis
bT = benign tumour
mT = malignant tumour
mTL = systemic, malignant tumour, e.g. malignant lymphoma
Ta = telangiectasis
Th = thrombus
Thy = thymus
THYR = thyroid gland
UBL = urinary bladder
Ul = ulcer
Ut = uterus
V = vacuoles

Investigations after 12 months

A = postmortal autolysis
Asp = aspermia (epididymis)
At = atrophy
Cy = cystic changes (e.g. retention cysts in the glandular stomach)
Dil = colon dilated and engorged
emH = extramedullary haematopoiesis
Er = erosion in area of forestomach mucosa
F = Oil Red-O positive substances in hepatocytes
Glo = glomerula atrophy
Ihyp = islet cell hyperplasia (pancreas)
Iz = cellular and/or inflammatory cellular infiltration
Met = squamous epithelium metaplasia in gastric retention cysts
Ne = hepatic cell necrosis
O = findings within normal variability which correspond particularly to species, age of study animals and conventional keeping conditions.
ø = not investigated (not available)
P = cut surface of parasites (intestine)
Pro = proliferation
Sg = spermiogranuloma
T = tumour (blastoma)
Tub = tubular renal changes (dilation, desquamation, epithelial basophilia)
V = intracytoplasmatic vacuoles (liver: lipid-free vacuoles)
Zy = PAS-positive cylinder in renal tubule

Degrees of intensity

\+ = very slight
1 = slight
2 = moderate
3 = severe

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FINDINGS	INCIDENCES OF NON-NEOPLASTIC LESIONS *)							
DOSE (ppm)	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
heart								
n	50	48			50	50		
A2	3	0			5	1		
Ap1	0	1			0	0		
Ap2	1	0			0	2		
De1	1	0			4	0		
De2	1	0			1	0		
Fi1	0	2			1	0		
Fi2	1	0			0	0		
Iz1	1	0			0	1		
Iz3	1	0			0	0		
Rz1	1	0			2	1		
Th1	0	2			0	0		
Th2	0	1			0	0		
trachea								
n	50	44			50	50		
A2	3	0			4	1		
Mp1	0	0			1	0		
D3	0	1			0	0		
Rz2	0	0			1	0		
lung								
n	50	48			50	50		
A2	2	0			5	2		
Iz2	1	0			0	0		
Iz3	1	0			1	0		
D2	2	1			1	0		
D3	0	0			0	1		
Rz2	1	1			0	0		
Th1	0	0			0	1		
Th2	1	0			0	0		
head salivary glands								
n	49	48			50	50		
A2	5	0			3	0		
A3	0	0			3	0		
Ap1	0	3			0	0		
Ap2	0	1			0	0		
At2	0	0			1	0		
D2	0	1			0	1		
Rz2	2	8			1	1		

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FINDINGS	INCIDENCES OF NON-NEOPLASTIC LESIONS +)							
DOSE (ppm)	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
liver								
n	50	47			50	50		
A2	3	0	2	4	2	1	3	3
A3	0	0	0	1	0	0	0	0
Ap2	1	0	0	0	0	0	0	0
clear cell foci	0	0	0	0	0	0	0	1
F2	9	16	11	0	7	6	18	4
F3	0	0	0	0	1	1	2	0
Fca	0	0	0	0	2	0	2	0
Fca1	0	0	0	0	0	1	0	0
Fi1	1	0	0	0	0	1	0	1
Gp3	0	0	0	0	0	1	0	0
Hb2	0	0	0	0	0	1	0	0
Hp1	1	1	3	0	1	1	0	0
Hp2	1	2	0	0	1	1	0	1
Hp3	0	1	0	0	0	0	0	0
Kv1	4	6	7	9	9	6	6	4
Kv2	1	2	1	7	3	8	4	10
Kv3	0	3	1	4	4	8	6	16
Kz2	1	0	1	0	0	0	0	0
Mi	2	1	0	0	0	0	0	0
Mi1	1	0	2	0	0	0	1	0
Mh1	2	0	2	0	1	0	0	0
Mz1	1	0	0	0	0	0	0	0
Mz3	0	0	0	0	0	1	0	0
Ne1	2	1	1	2	4	1	2	0
Ne2	1	1	0	2	1	1	0	0
Ne3	1	0	1	0	0	1	0	0
Nez	0	0	0	0	0	0	0	1
Nez1	3	1	0	0	1	0	0	0
Pi1	0	0	0	0	1	0	0	0
Pi2	1	0	0	0	0	0	0	1
Rz1	6	1	3	0	2	0	1	0
Rz2	0	1	0	0	0	1	0	0
Te2	0	0	0	0	0	1	0	0
Th2	0	1	0	0	0	0	0	0
V1	1	0	0	0	0	0	0	0
pancreas								
n	49	45			50	49		
A2	2	0			5	0		
A3	0	0			0	1		
Ap1	1	0			0	0		
Ap2	0	1			1	0		
Ap3	0	1			0	0		
At1	0	2			0	0		
At2	0	1			0	1		
He	0	0			0	1		
Hy3	1	0			0	0		
D1	0	0			0	2		
D2	0	2			0	3		
Rz2	0	1			0	2		
Rz3	0	1			0	0		

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FINDINGS — INCIDENCES OF NON-NEOPLASTIC LESIONS *)

DOSE (ppm)	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
oesophagus								
n	50	45			50	50		
AZ	1	0			0	0		
Iz Ne3	0	0			1	0		
stomach								
n	49	48	49	49	50	50	49	49
AZ	2	0	4	6	6	6	14	6
A3	1	1	0	3	0	1	2	0
Ap1	1	1	1	0	1	0	0	0
Ap2	0	0	1	0	0	0	0	0
Er1	0	0	0	0	1	0	0	0
My	0	0	0	0	1	0	0	0
My1	5	1	5	4	7	1	3	6
My2	3	2	6	3	5	3	6	1
My3	1	0	4	1	6	1	3	1
Iz2	0	0	0	0	0	0	1	0
Min2	0	0	0	0	0	1	0	0
D2	0	2	1	0	0	0	0	2
Ul1	0	0	0	0	0	2	3	0
Ul2	0	0	0	0	0	1	0	0
small intestine								
n	49	48			50	50	49	49
A2	4	1			5	10	15	6
A3	0	2			1	3	2	1
Ap1	0	2			0	0	0	0
D1	0	1			0	1	0	0
large intestine								
n	49	48			50	50	49	49
AZ	5	1			5	11	15	7
A3	1	2			1	3	2	0
Ap1	1	2			0	0	0	0
Ap2	0	0			0	1	0	0
Ap3	0	1			0	0	0	0
Ne	4	0			5	1	10	1
D2	0	3			0	2	1	4
Ul2	0	0			0	0	0	1

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FINDINGS — INCIDENCES OF NON-NEOPLASTIC LESIONS +)

DOSE (ppm)	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
spleen								
n	49	48			50	49		
A2	3	0			6	1		
At2	0	0			1	0		
At3	0	1			0	0		
Hp1	5	1			4	0		
Hp2	2	6			4	3		
Hp3	0	4			0	3		
Hy2	1	5			3	9		
Hy3	0	0			2	2		
lymph nodes								
n	42	47			48	36		
A2	1	0			5	0		
Ap1	0	1			0	0		
Ap3	0	2			0	0		
Hp2	0	1			0	0		
Hp3	0	1			0	0		
Hy2	0	2			0	5		
Hy3	0	1			0	1		
kidneys								
n	96	96			100	100		
A2	5	0			6	4		
Ap1	2	3			1	2		
Ap2	5	3			3	0		
Ap3	0	0			0	0		
Hn	0	0			1	0		
Hp3	0	6			0	6		
Rz2	10	11			6	11		
Rz3	0	1			0	0		
Th1	0	1			0	1		
testes								
n	98	-			100	-		
A2	2	-			5	-		
Ap1	7	-			9	-		
Ap2	3	-			5	-		
At1b	2	-			0	-		
At1e	6	-			1	-		
At2b	7	-			4	-		
At2e	7	-			8	-		
At3e	0	-			1	-		
Hy1	0	-			2	-		
Hy2	0	-			1	-		

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FINDINGS	INCIDENCES OF NON-NEOPLASTIC LESIONS +)							
DOSE (ppm)	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
epididymis								
n	95	-			95	-		
A2	2	-			5	-		
Asp	18	-			14	-		
Spgr	1	-			0	-		
prostate								
n	45	-			48	-		
A2	2	-			5	-		
Ap1	1	-			0	-		
Iz2	0	-			2	-		
Iz3	2	-			0	-		
seminal vesicle								
n	49	-			50	-		
A2	1	-			6	-		
Ap1	2	-			1	-		
Ap2	0	-			2	-		
Ha2	0	-			1	-		
Iz1	9	-			8	-		
Iz2	20	-			8	-		
urinary bladder								
n	48	67			50	48		
A2	2	2			5	6		
A3	1	1			1	3		
Ha3	0	0			1	0		
Iz1	1	0			0	0		
Rz2	0	7			0	2		
ovaries								
n	-	92			-	96		
Ap1	-	1			-	2		
Ap2	-	2			-	3		
Ap3	-	8			-	3		
Cy3	-	4			-	2		
Th3	-	0			-	1		
Amyloid 1	-	1			-	0		

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FINDINGS

INCIDENCES OF NON-NEOPLASTIC LESIONS +)

DOSE (ppm)	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
uterus								
n	-	46	-	47	-	49	-	46
A3	-	1	-	0	-	0	-	0
Ap1	-	0	-	0	-	2	-	0
Ap2	-	1	-	0	-	0	-	0
Ap3	-	1	-	0	-	2	-	0
Dil1e	-	0	-	0	-	0	-	5
Dil2	-	1	-	0	-	0	-	0
Dil2e	-	0	-	1	-	0	-	0
Dil2b	-	0	-	0	-	0	-	1
Dil3	-	0	-	0	-	2	-	0
focal pelIosis	-	0	-	0	-	0	-	1
granular cell focus	-	0	-	1	-	0	-	0
Hy2	-	3	-	11	-	12	-	8
Hy3	-	3	-	5	-	4	-	5
He2	-	0	-	0	-	1	-	0
Th3	-	0	-	1	-	1	-	0
mamma								
n	-	40			-	33		
Ö3	-	0			-	1		
Rz2	-	0			-	1		
skin								
n	49	47			50	50		
Abz	0	0			1	0		
Iz1	0	0			0	2		
Iz2	0	0			1	2		
Iz3	1	3			0	3		
Iz He3	0	0			4	0		
He	0	1			0	4		
Ö3	0	1			0	1		
pituitary gland								
n	40	35	-	38	46	41	-	38
A1	0	0	-	4	0	0	-	0
A2	1	0	-	0	5	0	-	0
Del	0	1	-	0	0	0	-	0
Dil1	0	1	-	2	0	0	-	0
Dil2	0	1	-	4	0	0	-	0
Hy1	0	2	-	1	0	2	-	8
Hy2	0	2	-	3	0	3	-	5
Hy3	0	2	-	1	0	4	-	3
thyroid gland								
n	45	40			48	47		
A2	1	0			3	1		
Ap1	0	1			0	0		

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FINDINGS	INCIDENCES OF NON-NEOPLASTIC LESIONS *)							
DOSE (ppm)	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
adrenals								
n	95	88			99	99		
A2	3	0			6	1		
Ap2	2	1			0	0		
Cy3	0	0			1	0		
Ma1	0	1			0	0		
My2	12	2			12	0		
My3	5	0			1	0		
Iz1	0	0			0	1		
Pi3	0	23			0	10		
Rz3	0	0			1	0		
Th1	0	0			0	1		
Th3	0	0			1	0		
V2	0	1			0	1		
V3	0	0			0	1		
brain								
n	49	48			50	50		
A2	5	1			6	1		
A3	0	0			0	1		
Cy2	0	0			1	0		
Ma3	0	1			0	0		
Mz1	0	0			1	0		
Rz1	0	0			0	2		
Rz2	0	4			0	1		
spinal cord								
n	49	48			50	50		
A2	3	0			6	1		
A3	1	0			0	0		
Cy1	0	0			1	0		
Rz1	1	2			0	1		
Rz2	0	2			0	1		
eye								
n	98	96			99	98		
A2	5	3			2	9		
A3	2	1			4	3		
Iz1	0	0			4	0		
Iz2	0	0			1	1		
Iz Me2	0	0			2	0		
Iz Me3	0	0			1	0		
Oph2e	1	0			1	0		

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FINDINGS	INCIDENCES OF NON-NEOPLASTIC LESIONS *)							
DOSE (ppm)	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
muscle								
n	50	48			50	50		
A2	5	0			6	1		
Ap1	1	1			0	0		
Ap2	0	1			0	0		
Ap3	0	1			0	0		
D3	0	0			0	1		
Rz1	0	0			1	0		
Rz2	0	2			0	2		
bone, bone marrow								
n	50	48			50	50		
A2	3	0			6	0		
F2	1	0			0	0		
Fi1	2	3			0	11		
Fi2	0	20			0	21		
Hy1	1	0			0	0		
others								
MESENTERIUM (Mes:)								
n #)								
Ap1	0	0			0	1		
Ap2	2	0			1	0		
Iz3	0	0			1	0		
Melz3	0	0			0	1		
Rz2	0	4			3	7		
(PD:)								
(preputial gland)								
n #)								
PDS2	1	0			0	0		
PD3A2	0	0			1	0		
PD3	4	0			1	0		
SUBCUTIS (Sc:)								
n #)								
D2	0	1			0	1		
D3	0	1			0	2		
Iz Me2	1	0			0	0		
Iz3	1	0			0	0		
Abz	1	0			0	0		
An2	0	1			0	2		
Ap3	0	1			0	0		

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FINDINGS	INCIDENCES OF NON-NEOPLASTIC LESIONS *)							
DOSE (ppm)	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
perinasal sinuses n #)								
Iz2	1	0			0	0		
Harder's gland		47				49		
A3		2				1		

*) /Animals sacrificed at interim kill were not considered.

*) In this group, only stomach, uterus, pituitary and liver were examined.

**) In this group, stomach, uterus, pituitary, liver, large and small intestine were examined completely. Organs (extent see group 0 ppm and 300 ppm) were examined in respect to tumors.

#) Only macroscopically changed organs were examined.

Table 17: Summary of number of male and female mice with benign and/or malignant tumours, as well as frequency of benign and malignant tumours encountered

Sex	♂			♀		
Dose ppm	0	300	900	0	300	900
No. of animals investigated	50	50	49	48	50	50
No. of animals with tumours	27	28	24	34	31	30
No. of animals with only benign tumours	7	6	8	6	6	6
No. of animals with only malignant tumours	12	15	14	21	18	17
No. of animals with benign and malignant tumours	8	7	2	7	7	7
No. of animals with more than one primary tumour	15	11	5	13	9	8

Table 18: Comparative summary of tumours occurring according to location, type, number and dignity $ (animals scheduled for terminal kill)

Sex	♂				♀			
Dose ppm	0	100	300	900	0	100	300	900
Lung:		-				-		
bronchiolo-alveolar adenoma	2		3	4	2		1	3
bronchiolo-alveolar carcinoma (malig.)	13		12	5	8		6	5
Stomach:								
papilloma	0	0	0	2	0	0	0	0
sarcoma (malig.)	0	0	2	1	0	0	0	0
Liver:								
hepatocellular adenoma	2	2	2	3	0	0	0	1
hepatocellular carcinoma (malig.)	3	4	5	8	0	0	0	1
haemangiosarcoma (malignant)	1	0	0	0	0	0	0	0
Kidneys:		-				-		
tubular carcinoma (malignant)	0		1	0	0		0	0
haemangiosarcoma (malignant)	0		1	0	0		0	0
Bladder:		-				-		
stromal tumour (benign)	1		1	2	0		0	0
stromal tumour (malignant)	0		1	1	0		0	0
Ovary:	-	-	-	-		-		
granulosa-theca cell tumour (ben.)					5		5	3
granulosa-theca cell tumour (malig.)					1		0	0
luteoma (benign)					2		2	0
tubular adeno-carcinoma (malig.)					1		0	0
Sertoli cell tumour (benign)					0		0	1
Uterus:	-	-	-	-				
adenoma					0	0	1	0
carcinoma (malig.)					1	0	0	0
fibroma					0	0	1	0
myoma					0	0	1	2
myosarcoma (malig.)					0	0	0	1
stromal tumour (benign)					3	0	2	2
stromal sarcoma (malignant)					1	3	2	2

Table 18 (continued):

Sex	♂				♀			
Dose ppm	0	100	300	900	0	100	300	900
Testes:		-				-	-	-
Leydig cell tumour (benign)	2		2	0				
adenoma of rete testis	1		0	0				
Pituitary:		-						
adenoma	2		0	0	0	3	3	1
Thyroid:		-				-		
follicle cell adenoma	0		1	0	0		0	0
papillary cyst-adenoma	1		0	0	0		0	0
Adrenals:		-				-		
cortical adenoma	3		3	1	2		0	0
phaeochromocytoma (benign)	1		0	0	1		0	2
phaeochromocytoma (malignant)	0		0	1	0		0	0
RH system:		-				-		
lymphoma (malig.)	7		3	1	18		12	14
lymph node sarcoma (malignant)	1		0	0	1		0	0
Skin/subcutis:		-				-		
epithelioma (malignant)	0		0	1	0		0	0
sarcoma (malig.)	1		0	0	0		3	0
Mammary gland:	-	-	-	-		-		
carcinoma (malig.)					3		0	0
adeno-ancanthoma (malignant)					0		1	1
Harder's gland:		-				-		
papillary adenoma	3		2	0	1		1	1
Spinal marrow:		-				-		
schwannoma (malig.)	0		0	0	0		1	0
Bones:		-				-		
osteosarcoma (malignant)	0		0	0	0		1	0
Abdomen:		-				-		
haemangiosarcoma (malignant)	0		0	0	0		1	0
Pelvic serosa:		-	-	-	-	-	-	-
sarcoma (malig.)	1							

- Organ not investigated $ Bilateral tumours counted twice

List of blastomas occurring in the histopathologically investigated mice from the terminal kill

Dose group: 0 ppm, male

Animal No.	21	Lung	Bronchiolo-alveolar carcinoma
	22	Liver	Hepatocellular carcinoma
		Adrenal	Phaeochromocytoma, benign
	23	Lung	Bronchiolo-alveolar carcinoma
	28	Testes	Leydig cell tumour, benign
	30	Lung	Bronchiolo-alveolar carcinoma
	32	Adrenal	Cortical adenoma
	36	Lung	Bronchiolo-alveolar carcinoma
	42	Lung	Bronchiolo-alveolar adenoma
	44	Lung	Bronchiolo-alveolar carcinoma
		Liver	Haemangiosarcoma
	46	Harder's gland	Papillary adenoma
	47	Lung	Bronchiolo-alveolar carcinoma
		RHS	Malignant lymphoma of spleen, lymph nodes, liver, kidney, mesentery
	48	Lung	Bronchiolo-alveolar carcinoma
		RHS	Malignant lymphoma of spleen, lymph nodes, heart, lung, head salivary glands, liver, pancreas, stomach, intestine, kidney, bladder, prostate, skin, skeletal muscles, bone marrow, mesentery, urethra
	50	Lung	Bronchiolo-alveolar carcinoma
		RHS	Malignant lymphoma of spleen, lymph nodes
	51	Pituitary	Adenoma
		Subcutis (ear)	Sarcoma
	52	Harder's gland	Papillary cystadenoma
	54	Lymph nodes	Sarcoma with metastases in the pelvic serosa

Animal No. 55	Testes	Leydig cell tumour, benign
58	Testes	Papillary adenoma of the rete testis
	Adrenals	Cortical adenoma
59	RHS	Malignant lymphoma of spleen, heart, lung, liver, pancreas, prostate, skin, skeletal muscles
63	Lung	Bronchiolo-alveolar carcinoma
	Liver*	Hepatocellular adenoma
64	Lung	Bronchiolo-alveolar carcinoma
	Thyroid gland	Papillary cystadenoma
65	Liver	Hepatocellular carcinoma
	RHS	Malignant lymphoma of mesentery
66	Liver*	Hepatocellular adenoma
	RHS	Malignant lymphoma of spleen, lymph nodes, heart, lung, pancreas, oesophagus, stomach, kidneys, bladder, prostate, seminal vesicle, skin, adrenals, skeletal muscles, preputial gland, mesentery
67	Lung	Bronchiolo-alveolar carcinoma
	RHS	Malignant lymphoma of lymph nodes, mesentery
	Pituitary	Adenoma
68	Lung	Bronchiolo-alveolar carcinoma
	Bladder	Polypous stromal tumour with adenomatous parts. The tumour contains foci of polymorphous, partially epithelioid cells with central necrosis and localised argentophilic fibrous structures (benign)
	Adrenals	Cortical adenoma
	Harder's gland	Papillary adenoma
69	Lung	Bronchiolo-alveolar carcinoma
	Pelvic serosa	Sarcoma, infiltrates the neighbouring skeletal muscles

* = The tumour was found in one of the additionally prepared Paraplast blocks

Animal No. 70	Lung	Bronchiolo-alveolar adenoma
	Liver	Hepatocellular carcinoma

Dose group 100 ppm, male

Only the hepatocellular neoplasias found are reported here.

Animal No. 98 Liver: Hepatocellular adenoma
No. 101 Liver: Hepatocellular adenoma
No. 110 Liver: Hepatocellular carcinoma
No. 115 Liver: Hepatocellular carcinoma
No. 125 Liver: Hepatocellular carcinoma
No. 129* Liver: Hepatocellular carcinoma

Dose group 300 ppm, male

Animal No. 161	Liver	Hepatocellular carcinoma
163	Lung	Bronchiolo-alveolar carcinoma
164	Lung	Bronchiolo-alveolar carcinoma
	Testes	Leydig cell tumour, benign
167	Lung	Bronchiolo-alveolar carcinoma
	Kidneys	Haemangiosarcoma
168	Liver	Hepatocellular carcinoma
	Harder's gland	Papillary adenoma
172	Testes	Leydig cell tumour, benign
173	Stomach	Sarcoma
	Kidney	Tubular renal epithelial carcinoma
175	Lung	Bronchiolo-alveolar carcinoma
176	Liver*	Hepatocellular adenoma
	RHS	Malignant lymphoma of spleen, lymph nodes, kidneys, mesentery
177	Lung	Bronchiolo-alveolar adenoma
180	Lung	Bronchiolo-alveolar carcinoma
	Liver*	Hepatocellular carcinoma
181	Lung	Bronchiolo-alveolar carcinoma
182	Lung	Bronchiolo-alveolar carcinoma
	Thyroid gland	Follicle cell adenoma
183	Lung	Bronchiolo-alveolar adenoma
184	Lung	Bronchiolo-alveolar carcinoma
	Harder's gland	Papillary adenoma
188	RHS	Malignant lymphoma of lymph nodes and kidneys
189	Liver	Hepatocellular carcinoma
	Bladder	Small, polypous, benign stromal tumour covered with hyperplastic urothelium, in the centre of which lies a nest of large, epithelioid, polymorphous cells

Animal No. 190	RHS	Malignant lymphoma of spleen, lymph nodes, head salivary glands, liver, pancreas, stomach, kidneys, bladder, epididymis, seminal vesicle
191	Adrenals	Cortical adenoma
195	Stomach	Sarcoma
	Adrenals	Cortical adenoma
196	Liver	Hepatocellular carcinoma
199	Lung	Bronchiolo-alveolar carcinoma
201	Liver*	Hepatocellular adenoma
204	Lung	Bronchiolo-alveolar carcinoma
205	Lung	Bronchiolo-alveolar carcinoma
206	Adrenals	Cortical adenoma
	Lung	Bronchiolo-alveolar adenoma
207	Bladder	Malignant stromal tumour in the subepithelium, consisting of spindle-shaped to epithelioid cells and interspersed with fine argentophilic fibres. The tumour infiltrates the muscular layer of the urinary bladder
210	Lung	Bronchiolo-alveolar carcinoma

Dose group 900 ppm, male

Animal No. 234	Bladder	Stromal tumour (benign)
	Lung	Bronchiolo-alveolar adenoma
238	Lung	Bronchiolo-alveolar adenoma
239	Liver	Hepatocellular carcinoma
240	Liver	Hepatocellular carcinoma
242	RHS	Malignant lymphoma
	Bladder	Benign stromal tumour: a small node in the propria, infiltrated by leucocytes, which consists of spindle-shaped to epithelioid cells. On the lumen side, the propria is oedematous. Overpigmented macrophages are present at the periphery of the node. The urothelium above the tumour is hyperplastic.
	Stomach	Inverted papilloma of pars cutanea
244	Liver	Hepatocellular carcinoma
248	Liver	Hepatocellular carcinoma
	Lung	Bronchiolo-alveolar carcinoma
244	Liver	Hepatocellular carcinoma
250	Lung	Bronchiolo-alveolar carcinoma
251	Liver	Hepatocellular adenoma
252	Lung	Bronchiolo-alveolar carcinoma
255	Lung	Bronchiolo-alveolar carcinoma
257	Liver	Hepatocellular adenoma (frozen section)
258	Liver	Hepatocellular adenoma
260	Skin (ear)	Epithelioma

Animal No. 264	Lung	Bronchiolo-alveolar carcinoma
	Liver	Hepatocellular carcinoma
268	Adrenals	Malignant phaeochromocytoma; the voluminous tumour has numerous mitoses and multi-focal necroses
270	Stomach	Inverted papilloma of pars cutanea
271	Lung	Bronchiolo-alveolar adenoma
273	Adrenals	Cortical adenoma
275	Lung	Bronchiolo-alveolar adenoma
	Liver	Hepatocellular carcinoma
277	Stomach	Subserous sarcoma
279	Stomach	Metastases of an osteosarcoma
280	Liver	Hepatocellular carcinoma

Dose group 0 ppm, female

Animal No. 302	RHS	Malignant lymphoma of spleen, lymph nodes, heart, lung, head salivary glands, liver, stomach, kidneys, bladder, ovaries, uterus, thyroid, adrenals, brain, spinal cord, skeletal muscles, bone marrow, subcutis
303	Mammary gland	Carcinoma with focal necroses
	Lymph nodes	Sarcoma
305	Lung	Bronchiolo-alveolar carcinoma
306	RHS	Malignant lymphoma of mesentery
307	Mammary gland	Carcinoma with focal necroses
308	RHS	Malignant lymphoma of spleen, lymph nodes, head salivary glands, pancreas, stomach, kidneys, bladder, ovaries, skeletal muscles, mesentery
309	Adrenals	Cortical adenoma
310	Lung	Bronchiolo-alveolar carcinoma
313	RHS	Malignant lymphoma of lymph nodes, pancreas, kidneys, thymus, mesentery
314	Adrenals	Cortical adenoma
315	Lung	Bronchiolo-alveolar carcinoma
	RHS	Malignant lymphoma of spleen, lymph nodes, lung, liver, kidneys, brain, bone marrow, skeletal muscles, mammary gland, thyroid

Animal No. 315	Uterus	Stromal tumour (benign)
316	Lung	Bronchiolo-alveolar adenoma
317	RHS	Malignant lymphoma of lymph nodes, liver, kidneys, bladder, skeletal muscles
	Ovary	Granulosa theca cell tumour (benign)
319	Lung	Bronchiolo-alveolar carcinoma
	Ovary	Granulosa theca cell tumour, which infiltrates neighbouring fatty tissue (malignant)
320	RHS	Malignant lymphoma of lymph nodes, heart, lung, pancreas, stomach, kidneys, uterus, mammary gland, pituitary, skeletal muscles, mesentery
	Ovary	Luteoma (benign)
321	Ovary	Granulosa theca cell tumour (benign)
322	RHS	Malignant lymphoma of lymph nodes, lung, head salivary glands, pancreas, kidneys, mammary gland, skin, eye, skeletal muscles, mesentery
323	Uterus	Stromal sarcoma, sarcomatous metastases of lymph nodes, lung, liver, (mes)ovary
324	RHS	Malignant lymphoma of lymph nodes, pancreas, kidneys, mesentery
325	Lung	Bronchiolo-alveolar adenoma
	Uterus	Stromal tumour (benign)

Animal No. 329	RHS	Malignant lymphoma of spleen, lymph nodes, heart, lung, head salivary glands, liver, stomach, kidneys, ovaries, mammary gland, skeletal muscles, bone marrow, mesentery
332	Lung	Bronchiolo-alveolar carcinoma
	RHS	Malignant lymphoma of spleen, lymph nodes, heart, lung, liver, pancreas, stomach, brain, skeletal muscles, mesentery
334	RHS	Malignant lymphoma of lymph nodes, heart, pancreas, kidneys, bladder, ovaries, uterus, mesentery
	Uterus	Stromal tumour (benign)
335	RHS	Malignant lymphoma of lymph nodes, lung, skeletal muscles, mesentery
337	RHS	Malignant lymphoma of lymph nodes, spleen, heart, trachea, lung, head salivary glands, liver, pancreas, stomach, kidneys, bladder, ovaries, uterus, mammary gland, skin, adrenals, brain, spinal cord, eyes, skeletal muscles, bone marrow, mesentery
338	Ovary	Luteoma
	RHS	Malignant lymphoma of thymus
339	Ovary	Granulosa theca cell tumour (benign)
	Adrenals	Phaeochromocytoma (benign)
340	RHS	Malignant lymphoma of spleen, lymph nodes, heart, trachea, lung, head salivary glands, pancreas, kidneys, ovaries, brain, spinal cord, skeletal muscles

Animal No. 341	RHS	Malignant lymphoma of heart, lung, liver, ovaries, skeletal muscles
	Uterus	Carcinoma
342	Ovary	Tubular adenocarcinoma
344	Lung	Bronchiolo-alveolar carcinoma
	Mammary gland	(Adeno)carcinoma
	Harder's gland	Papillary cystadenoma
345	Lung	Bronchiolo-alveolar carcinoma
346	Lung	Bronchiolo-alveolar carcinoma
	Ovaries	Granulosa theca cell tumour, bilateral (benign)
350	RHS	Malignant lymphoma of lymph nodes, pancreas, bladder, brain, skeletal muscles, subcutis, mesentery

Dose group 100 ppm, female

Animal No.	402	Pituitary	Adenoma
	404	Pituitary	Adenoma
	414	Pituitary	Adenoma

Dose group 300 ppm, female

Animal No. 442	RHS	Malignant lymphoma of thymus
444	RHS	Malignant lymphoma of lymph nodes, lung, liver, kidneys, mesentery, skeletal muscles
445	Bone system	Osteosarcomatous metastases of lung and liver
446	Ovary	Granulosa theca cell tumour (benign)
448	Uterus	Polypous stromal sarcoma
449	Spinal marrow	Schwannoma (malignant)
450	Lung	Bronchiolo-alveolar carcinoma
	RHS	Malignant lymphoma of spleen, lymph nodes, lung, mesentery, skeletal muscles
	Ovary	Granulosa theca cell tumour (benign)
451	Abdomen	Haemangiosarcoma, which infiltrates the cortex of one kidney
452	RHS	Malignant lymphoma of lymph nodes, kidney, mesentery, skeletal muscles
	Ovary	Granulosa theca cell tumour (benign)
	Pituitary	Adenoma of the pars intermedia
453	Lung	Bronchiolo-alveolar carcinoma
	Ovary	Luteoma (benign)
	Uterus	Stromal tumour (benign)

Animal No. 454	RHS	Malignant lymphoma of lymph nodes, heart, lung, liver, kidneys, ovaries, uterus, mesentery
455	Uterus	Polypous stromal tumour (benign)
	Subcutis (ear)	Sarcoma (schwannoma)
457	Ovary	Bilateral granulosa theca cell tumour (benign)
458	Lung	Bronchiolo-alveolar carcinoma
	Uterus	Myoma
459	RHS	Malignant lymphoma of pancreas, mesentery, mediastinum, skeletal muscles
460	Lung	Bronchiolo-alveolar carcinoma
	Subcutis (ear)	Sarcoma (schwannoma)
461	Uterus	Fibroma (benign)
464	Subcutis (ear)	Sarcoma (schwannoma) with necroses
465	Uterus	Adenoma
466	RHS	Malignant lymphoma of lymph nodes, heart, lung, pancreas, stomach, kidneys, skeletal muscles, mesentery, mediastinum
471	Lung	Bronchiolo-alveolar carcinoma
	Pituitary	Adenoma
472	Uterus	Stromal sarcoma

Animal No.	473	Lung	Bronchiolo-alveolar carcinoma
	475	Ovary	Luteoma
	478	RHS	Malignant lymphoma of spleen, lymph nodes, pancreas, stomach, large intestine, bladder, skin, thyroid, skeletal muscles, mesentery
	479	Pituitary	Adenoma
		Harder's gland	Papillary adenoma
	482	RHS	Malignant lymphoma of spleen, lymph nodes, head salivary glands, kidneys, bladder
	483	RHS	Malignant lymphoma of pancreas, skeletal muscles, mesentery
	484	Mammary gland	Adenoacanthoma (malignant)
	485	Lung	Bronchiolo-alveolar adenoma
		RHS	Malignant lymphoma of spleen, lymph nodes, heart, lung, head salivary glands, liver, pancreas, bladder, ovaries, mammary gland, skin, skeletal muscles, bone marrow, mesentery
	486	RHS	Malignant lymphoma of pancreas, skeletal muscles, mesentery

Dose group 900 ppm, female

Animal No. 511	RHS	Malignant lymphoma
512	RHS	Malignant lymphoma
515	RHS	Malignant lymphoma, stomach
517	Uterus	Stromal sarcomatous metastases of liver and mesentery
519	Adrenals	Phaeochromocytoma (benign)
	Uterus	Myosarcoma
520	RHS	Malignant lymphoma
521	Adrenals	Phaeochromocytoma (benign)
523	Ovary	Granulosa theca cell tumour (benign)
524	Ovary	Granulosa theca cell tumour (benign)
	Lung	Bronchiolo-alveolar carcinoma
525	RHS	Malignant lymphoma
526	Mammary gland	Adenoacanthoma (malignant)
527	RHS	Malignant lymphoma
528	Liver	Hepatocellular carcinoma
	Uterus	Myoma (benign)
530	Lung	Bronchiolo-alveolar carcinoma

Animal No. 531	Lung	Bronchiolo-alveolar adenoma
535	Lung	Bronchiolo-alveolar carcinoma
537	RHS	Malignant lymphoma
538	RHS	Malignant lymphoma
540	RHS	Malignant lymphoma
541	Lung	Bronchiolo-alveolar carcinoma
	Harder's gland	Papillary cystadenoma
542	RHS	Malignant lymphoma
545	Lung	Bronchiolo-alveolar carcinoma
546	RHS	Malignant lymphoma
	Lung	Bronchiolo-alveolar adenoma
547	Uterus	Angiomatous stromal sarcoma
	Pituitary	Adenoma
551	RHS	Malignant lymphoma
553	Ovary	Granulosa theca cell tumour (benign)
	Lung	Bronchiolo-alveolar adenoma
556	RHS	Malignant lymphoma
557	Uterus	Polypous stromal tumour (benign)
559	Uterus	Stromal tumour (benign)
560	RHS	Malignant lymphoma
	Uterus	Myoma (benign)
	Liver	Hepatocellular adenoma

STATISTICAL REVIEW AND EVALUATION

NDA #: 20-356 Drug Class: Date: JAN 4 1994

Applicant: Miles Pharmaceutical Division

Name of Drug: Nisoldipine (Bay k 5552) Coat-core Tablets, 10, 20, 30, and 40 mg, q.d.

Indication: 1) Hypertension, alone or in combination with other antihypertensive agents; and 2) chronic stable angina (classical effort-associated angina). This review addresses the Hypertension claim.

Documents Reviewed: Volumes 1-5, 379, 388, and 400 of the NDA submission dated March 31, 1993. Also the data for the primary efficacy variable, supine diastolic blood pressure, was submitted on diskette for the double-blind portions of Studies D90-019, D90-029, and D90-039.

Medical Officer: The medical officer for this review is Dr. Cristobal Duarte.

I. INTRODUCTION

Nisoldipine is a dihydropyridine calcium channel blocker derived from nifedipine. The product is currently approved in 23 countries, although clinical development was discontinued in the United States because the brief duration of effect required multiple daily dosing. This application is for an extended-release formulation of nisoldipine, and studies once daily dosing for both hypertension and for angina. The coat-core tablet consists of an inner core containing 20% of the nisoldipine dose in an immediate-release form, surrounded by an outer coat containing 80% of the nisoldipine dose in a slow-release form.

This submission consisted of the results of two Phase II and three Phase III trials for hypertension which were carried out in the United States, and one South African hypertension study. The submission also contained two Phase III trials for angina which were carried out in the United States, and two foreign angina trials, one multi-national and one carried out in Israel. The angina claims will be addressed in another review.

II. CONTROLLED CLINICAL TRIALS

II.A. PROTOCOL NO. D90-019

II.A.1 Study Description

Study D89-019 was a sixteen-center dose-response study designed to compare three fixed doses of diltiazem with placebo in patients with mild to moderate hypertension.

The plan called for patients to be randomized to one of four parallel groups, receiving either placebo, nisoldipine 30 mg qd, nisoldipine 60 mg qd, or nisoldipine 90 mg qd, over an six week double-blind period. The 90 mg nisoldipine arm was deleted from the protocol by amendment before any patients were randomized.

After a four week washout period, patients who had a supine diastolic blood pressure (DBP) between 100 and 114 mmHg on each of the last two pre-randomization visits were eligible for randomization to double-blind treatment. The two supine DBP readings were required to be within 7 mmHg of each other. Blood pressure measurements were taken at trough (24 hr ± 30 minutes post-dose).

A total of 309 patients were enrolled in the placebo run-in period, and 221 were randomized to treatment group, with 72 assigned to placebo, 76 to nisoldipine 30 mg qd, and 73 to nisoldipine 60 mg qd. Nine placebo patients dropped out of the study, as did three low dose and 12 higher dose nisoldipine patients, while 197 patients completed the study A total of 213 were considered valid for efficacy analyses.

Patients were instructed to take three tablets each morning prior to 11 a.m. All patients randomized to nisoldipine began at 30 mg once daily, and those randomized to the 60 mg dose were titrated after one week. The relationship of drug administration to meals was not specified. The double-blind treatment phase lasted a total of six weeks, with patients evaluated weekly for the first four weeks and then again at the end of the study (week 6).

The primary efficacy variable was change from baseline (mean of weeks 3 and 4 of the placebo run-in) to endpoint in supine diastolic blood pressure at trough, 24 hours post-dose. Secondary efficacy variables included change from baseline in trough standing DBP and supine and standing systolic blood pressure (SBP). Response r... at trough were also analyzed, with response define in four different ways, (1) supine DBP no more than 90 mmHg; (2) a fall in supine DBP of at least 10 mmHg, (3) supine DBP≤ 90 mmHg or a fall in supine DBP ≥ 10 mmHg; and (4) supine DBP≤ 90 mmHg **and** a fall in supine DBP ≥ 10 mmHg.

At eight of the 16 centers 24-hour ambulatory blood pressure monitoring (ABPM) was performed at week 3 of the placebo run-in and at week 5 of the double-blind treatment period. The sponsor used the ABPM data to analyze the peak/trough ratio. A total of 117 patients had 24-hour ABPM monitoring. In-clinic blood pressure measurements were also taken at for 12 hours post-dose at seven centers after 4 weeks of placebo run-in and after six weeks of double-blind treatment.

II.A.2 Sponsor's Analysis

The sponsor performed both an evaluable patient analysis, including those patients with at leas two post-baseline valuations who were not protocol violators, and an intent-to-treat analysis including all patients at least one post-baseline evaluation. A last-observation-carried-forward approach was used to include the available data from patients who dropped out of the study. The initial analysis of variance model for the

primary and secondary change from baseline endpoints included treatment group, investigator, and treatment by investigator interaction terms. The interaction term was dropped if it was not significant at the .05 level. The initial comparison was the average of the nisoldipine groups versus placebo. If this was significant then pairwise comparisons were done to identify which nisoldipine doses were favored over placebo.

The sample size for this study was selected to give 90% power to detect a difference of at least 5 mmHg between the nisoldipine groups and the placebo group. This was calculated using an estimated standard deviation of 8 mmHg. The sponsor compared the demographics of the four groups at baseline using a Mantel-Haenszel test on sex, race, smoking status, and use of previous antihypertensive medications, and found no significant differences. The groups were also similar with respect to age, weight, height, and years of hypertension, which were compared using an analysis of variance model.

Change from Baseline Blood Pressure Measurements at Endpoint (LOCF) (Intent-to-Treat Data Set)

	Supine DBP	Supine SBP	Standing DBP	Standing SBP
Placebo (N=71)				
Baseline mean	103.64	155.31	102.91	151.22
Change from baseline	-4.68	-1.88	-2.60	-1.94
Nisoldipine 30 mg (N=76)				
Baseline mean	104.41	157.29	103.82	153.57
Change from baseline	-11.58	-12.10	-9.59	-13.36
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Nisoldipine 60 mg (N=73)				
Baseline mean	104.76	158.43	103.74	154.12
Change from baseline	-14.46	-16.67	-12.41	-15.94
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Interaction p-value	0.4675	0.1074	0.2651	0.4209

The results of the sponsor's analysis of their evaluable patient data set were similar, with all of the active drug groups demonstrating a highly statistically significant difference from the placebo group. The same was true of the analysis at each of the various time points after at least two weeks of double-blind therapy. Each active treatment group tended to reach a plateau in blood pressure response after two or three weeks of double-blind therapy, and the results for the group were very consistent beyond that point. The treatment by center interaction term was not significant at the .05 level.

The response variables also demonstrated results similar to the change from baseline blood pressure values, as can be seen below.

Response Rates

	Placebo N=71	Nisoldipine 30 mg qd N=76	Nisoldipine 60 mg qd N=66
A) DBP ≤ 90 mmHg	16 (23%)	26 (34%)	37 (56%)
B) DBP decrease ≥ 10 mmHg	22 (31%)	37 (49%)	50 (76%)
C) A) or B)	22 (31%)	37 (49%)	50 (76%)
D) A) and B)	16 (23%)	26 (34%)	37 (56%)

The ambulatory blood pressure data was analyzed by smoothing the hourly means from the eight centers using a Fourier transform. The mean trough and peak values and trough to peak ratios were obtained from the smoothed data. Peak effect for each nisoldipine group was defined as the greatest difference between the nisoldipine group and placebo in mean change from baseline. Trough effect was defined as the 24-hour post-dose difference from placebo

The peak diastolic blood pressure response occurred at 13 hours post-dose for the nisoldipine 30 mg group and at 4 hours post-dose for the nisoldipine 60 mg group. The peak/trough ratios for the placebo subtracted ambulatory measurements were -12.1/-9.5 = 78% and -15.2/-14.2 = 93%, respectively. The corresponding systolic peak/trough ratios were 83% and 76%.

The number of adverse events experienced in the placebo group was statistically significantly different from both of the nisoldipine groups. The most frequently reported adverse events included headache and peripheral edema in the placebo group and both nisoldipine groups. Adverse events were most likely to occur early in the first two weeks of double-blind therapy, although they continued to occur at a reduced level throughout the study. One patient experienced a cardiac arrest and died while receiving Placebo during the double-blind portion of the study. No other patients died during the study.

Adverse Events

	Placebo N=72	Nisoldipine 30 mg qd N=76	Nisoldipine 60 mg qd N=73
Patients with ≥ one event	32 (44%)	47 (62%)	54 (74%)
Possibly drug related	19 (26%)	23 (30%)	33 (45%)
Serious adverse events	2 (3%)	4 (5%)	10 (14%)
Withdrew due to a. e.'s	3 (4%)	1 (1%)	11 (15%)

II.A.3. Reviewer's Comments

This study gives substantial evidence that nisoldipine, in doses of 30 and 60 mg qd, reduces blood pressure when compared to placebo. After establishing a drug effect by comparing the mean of the combined nisoldipine groups with placebo and reaching statistical significance, the sponsor compared each dose group directly with placebo, and both were highly statistically significant. The protocol and the study report do not mention any adjustments for multiple comparisons. However, the results of this study were so significant that even using the conservative Bonforonni adjustment for the multiple comparisons, they remain highly statistically significant for the primary and secondary blood pressure endpoints.

This reviewer performed several additional analyses on the data including analysis of covariance using baseline as a covariate for both change from baseline and for endpoint supine DBP. The results of these additional analyses did not differ substantially from the results submitted by the sponsor, and demonstrated the robustness of the efficacy results.

The results of this study demonstrate a dose-response relationship for the 30 mg qd and 60 mg qd doses of nisoldipine in both efficacy and safety. The higher dose group consistently showed a greater reduction in all four blood pressure measurements. This group also had more adverse events and more serious adverse events. This study involved a forced titration, and many of the patients in the high dose group possibly had an adequate response at the lower dose, and did not need the additional risk of adverse events which came with the additional blood pressure reduction. This reviewer feels that doses of nisoldipine lower than those studied here might be adequate for many patients.

The protocol stated that the original model for the analysis of the blood pressure variables would include an interaction term, but that the interaction term would be dropped if it was not significant at the .05 level. The test for interaction is a test with very low power and therefore interaction is usually tested at the .15 level. Using this level, one of the secondary endpoints, supine SBP, demonstrated a significant

treatment by center interaction in the endpoint analysis (p= 0.1074). The primary endpoint also had interaction terms with p-values which were greater than .05 but less than .15 at several time points, but not in the endpoint analysis. The inclusion of the interaction term in the analysis of variance model did not change the statistical significance of any of the primary or secondary endpoints. This reviewer calculated the results by center and found that while the response at the various centers was different in magnitude, they trended in the same direction in almost all centers. The interaction appears to be quantitative in nature, and probably a result of the variability of the blood pressure responses.

II.B. PROTOCOL NO. D89-029

II.B.1. Study Description

D89-029 was a sixteen-center dose-response parallel study comparing placebo with three once daily doses of nisoldipine on a background of atenolol 50 mg qd in patients with mild to moderate hypertension. Patients were randomized to one of four parallel groups, receiving either placebo or nisoldipine 20 mg qd, nisoldipine 40 mg qd, or nisoldipine 60 mg qd, over an six week double-blind period.

Following a two-week placebo run-in period, patients with supine diastolic blood pressure between 100 and 119 mmHg entered a single-blind four week run-in period where all received atenolol 50 mg qd, but no other hypertensive therapy. Patients who had a supine diastolic blood pressure between 95 and 114 mmHg after the four weeks of atenolol therapy were eligible for randomization to one of the four double-blind treatments, while continuing their atenolol therapy.

A total of 418 patients were enrolled in the placebo run-in, and 313 continued [illegible] the atenolol run-in period. Most of the patients who did not continue were not eligible because their blood pressure had dropped too much while receiving atenolol. A total of 251 patients were randomized to double-blind treatment group; 62 to placebo, 62 to nisoldipine 20 mg qd, 63 to nisoldipine 40 mg qd, and 64 to nisoldipine 60 mg qd. Three placebo patients dropped out of the study, as did one low dose, five mid-dose, and seven higher dose nisoldipine patients. A total of 238 patients were considered valid for the efficacy analyses.

Patients were instructed to take two tablets and one capsule each morning prior to 11 a.m. All patients randomized to nisoldipine began at 20 mg once daily, and those randomized to the higher dose groups were titrated weekly. The relationship of drug administration to meals was not specified. The double-blind treatment phase lasted a total of six weeks, with patients evaluated at weeks 1, 2, 4, and 6. The primary efficacy variable was change from baseline (mean supine DBP at week 4 of the single-blind atenolol phase) to endpoint (the last double-blind visit) supine diastolic blood pressure (DBP) at trough, 24 hours post-dose. Secondary efficacy variables included change from baseline in standing DBP and supine and standing SBP. The change

from baseline during the atenolol phase was also evaluated. Response rates at trough were also analyzed, with response define in four different ways, (1) supine DBP no more than 90 mmHg; (2) a fall in supine DBP of at least 10 mmHg, (3) supine DBP≤ 90 mmHg or a fall in supine DBP ≥ 10 mmHg; and (4) supine DBP≤ 90 mmHg and a fall in supine DBP ≥ 10 mmHg.

At eight of the 16 centers 24-hour ambulatory blood pressure monitoring (ABPM) was performed at week 3 of the atenolol run-in and at week 5 of the double-blind treatment period. The sponsor used the ABPM data to analyze the peak/trough ratio. A total of 141 patients (centers 01 through 08) had 24-hour ABPM monitoring, including 33 randomized to placebo, 34 to nisoldipine 20 mg, 35 to nisoldipine 40 mg, and 34 to nisoldipine 60 mg.

II.B.2. Sponsor's Analysis

The sample size for this study was selected to give 90% power to detect a difference of at least 5 mmHg between the nisoldipine 40 mg group and the placebo group. This was calculated using an estimated standard deviation of 8 mmHg. The sponsor compared the demographics of the four groups at baseline using the Cochran-Mantel-Haenszel test (adjusting for center) on sex, race, smoking status, and several other factors. The analysis of the previous use of antihypertensive medications was marginally significant with p=0.065. Only three patients had previously been treated, and two of those three were randomized to the placebo group. The groups were also similar with respect to age, weight, height, and years of hypertension, which were compared using an analysis of variance model.

The sponsor performed both an evaluable patient analysis, including patients with at least 19 days of double-blind therapy, and an intent-to-treat analysis including all patients who had at least one post-baseline evaluation. A last-observation-carried-forward approach was used to include the available data from patients who dropped out of the study. The initial analysis of variance model for the primary and secondary change from baseline endpoints included treatment group, investigator, and treatment by investigator interaction terms. The interaction term was dropped if it was not significant at the .05 level. The initial comparison was the average of the nisoldipine groups versus placebo. If this was significant then pairwise comparisons were done to identify which nisoldipine doses were favored over placebo.

Change from Baseline Blood Pressure Measurements at Endpoint (LOCF) Intent-to-Treat Data Set

	Supine DBP	Supine SBP	Standing DBP	Standing SBP
Placebo + Atenolol (N=62)				
Baseline mean	100.74	158.30	102.10	154.20
Change from baseline	-3.90	-0.12	-1.66	+2.30
Nisoldipine 20 mg + Atenolol (N= 62)				
Baseline mean	100.58	158.19	102.13	153.81
Change from baseline	-10.00	-12.51	-8.85	-10.14
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Nisoldipine 40 mg + Atenolol (N= 62)				
Baseline mean	100.97	159.32	103.46	157.22
Change from baseline	-12.01	-19.03	-12.39	-21.75
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Nisoldipine 60 mg + Atenolol (N= 64)				
Baseline mean	100.81	160.90	102.32	155.72
Change from baseline	-13.73	-22.38	-14.30	-21.89
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Nisoldipine versus placebo	0.0001	0.0001	0.0001	0.0001
Interaction p-value	0.1107	0.3041	0.5361	0.4636

The results of the sponsor's analysis of their evaluable patient data set were similar, with all of the active drug groups demonstrating a highly statistically significant difference from the placebo group. The same was true of the analysis at each of the various time points after the first week of double-blind therapy. Each active treatment group tended to reach a plateau in blood pressure response after two or three weeks of double-blind therapy, and the results were very consistent beyond that point. The treatment by center interaction term was significant at the .05 level for the analysis of supine DBP at the first double-blind visit, but not for the secondary blood pressure variables at that visit, nor for any blood pressure variables at later visits or at endpoint.

The response variables also demonstrated results similar to the change from baseline blood pressure values, as can be seen below.

Response Rates

	Placebo N=59	Nisoldipine 20 mg qd N=61	Nisoldipine 40 mg qd N= 59	Nisoldipine 60 mg qd N= 59
A) DBP ≤ 90 mmHg	19 (32%)	34 (56%)	40 (68%)	39 (66%)
B) DBP decrease ≥ 10 mmHg	14 (24%)	31 (51%)	40 (68%)	44 (75%)

The ambulatory blood pressure data was analyzed by smoothing the hourly means from the eight centers using a Fourier transform. The mean trough and peak values and trough to peak ratios were obtained from the smoothed data. Peak effect for each nisoldipine group was defined as the greatest difference between the nisoldipine group and placebo in mean change from baseline. Trough effect was defined as the 24-hour post-dose difference from placebo.

The peak diastolic blood pressure response occurred at 3 hours post-dose for the nisoldipine 20 mg group, at 23 hours post-dose for the nisoldipine 40 mg group, and at one hour post-dose for the nisoldipine 60 mg group. The peak/trough ratios were -5.0/-9.4 = 53%, -12.8/-13.1= 97%, and -12.9/-13.0 = 99%, respectively. The corresponding systolic peak/trough ratios were 86%, 100%, and 94%.

Adverse Events

	Placebo N=62	Nisoldipine 20 mg qd N= 62	Nisoldipine 40 mg qd N= 63	Nisoldipine 60 mg qd N= 64
Patients with ≥ one event	28 (62%)	37 (60%)	44 (70%)	42 (66%)
Possibly drug related	14 (23%)	16 (26%)	28 (44%)	28 (44%)
Serious adverse events	2 (3%)	5 (8%)	2 (3%)	5 (8%)
Withdrew due to a. e.'s	1 (2%)	1 (2%)	4 (6%)	4 (6%)

The most frequently reported adverse events included headache and peripheral edema in the nisoldipine groups. The most frequently reported adverse events in the placebo group included rhinitis, peripheral edema, and headache. Adverse events were most likely to occur early in the study (prior to week 4), although they continued

to occur at a reduced level throughout the study. There were no deaths reported during this study.

II.B.3. Reviewer's Comments

This study demonstrated that nisoldipine treatment resulted in additional blood pressure reduction when used in the presence of atenolol. The primary and secondary endpoints all demonstrated statistically significant reductions in blood pressure during the six week study. This reviewer again performed several alternative analyses and found that the results were robust.

The results also demonstrate a dose-response trend for the 20 mg qd through 60 mg qd doses of nisoldipine in both efficacy and safety. The higher dose groups consistently showed a greater reduction in each of the blood pressure measurements, and also an increasing number of adverse events. This study again involved a forced titration, and many of the patients in the higher dose groups possibly had an adequate blood pressure response at lower doses and did not need the additional risk of adverse events which came with the additional blood pressure reduction. This reviewer feels that doses of nisoldipine lower than those studied here might be adequate for many patients.

The protocol stated that the original model for the analysis of the blood pressure variables would include an interaction term, but that the interaction term would be dropped if it was not significant at the .05 level. The test for interaction is a test with very low power and therefore interaction is usually tested at the .15 level. Using this level, the primary endpoint, supine DBP, demonstrated a significant treatment by center interaction in the endpoint analysis for both the evaluable patient data set ($p=0.1478$) and for the intent-to-treat data set ($p=0.1107$). This reviewer calculated the results by center and compared them. The centers vary substantially in the response of the various nisoldipine groups, but in no case did the placebo group have a greater response to therapy than did the treated groups.

II.C. PROTOCOL NO. D89-039

II.C.1. Study Description

D89-039 was a four-arm parallel study comparing placebo, two once daily doses of nisoldipine, 20 mg. and 40 mg, and verapamil 240 mg bid, in patients with mild to moderate hypertension. The protocol for the sixteen center study included a fifth group randomized to nisoldipine. 80 mg qd, but this group was dropped shortly after the beginning of the study (not prior to randomization) after the sponsor received information from another study that nisoldipine doses in excess of 60 mg qd were not well-tolerated..

After a four week washout period, patients who had an average supine DBP between 95 and 114 mmHg on each of the last two pre-randomization visits were eligible f

randomization to double-blind treatment. Blood pressure measurements were taken at trough (24 hr ± 30 minutes post-dose). The double-blind portion of the study lasted 12 weeks, but patients randomized to placebo were switched to verapamil 240 mg qd for the final four weeks of the study.

A total of 413 patients were enrolled in the placebo run-in period, and 320 were randomized to treatment group; 75 to placebo, 78 to verapamil, 76 to nisoldipine 20 mg qd, 76 to nisoldipine 40 mg qd, and 15 to nisoldipine 60 mg qd. Eleven patients randomized to the placebo group dropped out before the end of the study, as did five verapamil patients, 12 low dose nisoldipine patients, and 12 medium dose nisoldipine patients. The 15 patients who had been randomized to high dose nisoldipine were dropped from the study when that arm was deleted, so 265 patients completed the study A total of 290 patients were considered valid for efficacy analyses.

Patients were instructed to take two tablets and one capsule each morning prior to 11 a.m. and another capsule 12 hours later. All patients randomized to nisoldipine began at 20 mg once daily, and those randomized to the 40 mg dose were titrated after one week. The relationship of drug administration to meals was not specified. The double-blind treatment phase lasted a total of eight weeks at the original randomized dose groups, followed by four weeks where the placebo group received verapamil 240 mg qd and the other three groups remained on their randomized therapy. Patients were evaluated weekly for the first four weeks, and then biweekly for the rest of the study.

The primary efficacy variable was change from baseline (defined as the mean of six readings, three taken at week 3 and three at week 4 of the placebo run-in period) to endpoint (defined as week 8 of the double-blind portion of the study) in supine diastolic blood pressure (DBP) at trough, 24 hours post-dose. The primary efficacy comparison was between the 40 mg qd nisoldipine group and the placebo group. The comparison of the 20 mg qd nisoldipine group and the placebo group was of secondary importance. Secondary efficacy variables included trough standing DBP and supine and standing systolic blood pressure (SBP). Response rates at trough were also analyzed, with response define in four different ways, (1) supine DBP no more than 90 mmHg; (2) a fall in supine DBP of at least 10 mmHg, (3) supine DBP≤ 90 mmHg or a fall in supine DBP ≥ 10 mmHg; and (4) supine DBP≤ 90 mmHg and a fall in supine DBP ≥ 10 mmHg.

At eight of the 16 centers 24-hour ambulatory blood pressure monitoring (ABPM) was performed at week 3 of the placebo run-in and at week 7 of the double-blind treatment period. The sponsor used the ABPM data to analyze the peak/trough ratio.
A total of 141 patients (centers 01 through 08) had 24-hour ABPM monitoring, including 34 randomized to placebo, 36 to verapamil, 35 to nisoldipine 20 mg, and 36 to nisoldipine 40 mg. A total of 163 patients (centers 06 through 13) had 12-hour post-dose in-house blood pressure readings (every two hours) at week 4 of the placebo run-in and at week 8 of the double-blind treatment period.

II.C.2. Sponsor's Analysis

The sample size for this study was selected to give 90% power to detect a difference of at least 5 mmHg between the nisoldipine 40 mg group and the placebo group. This was calculated using an estimated standard deviation of 8 mmHg. The sponsor compared the demographics of the four groups at baseline using the Cochran-Mantel-Haenszel test (adjusting for center) on sex, race, smoking status, and use of previous antihypertensive medications, and no significant differences were found. The groups were also similar with respect to age, weight, height, and years of hypertension, which were compared using an analysis of variance model.

Change from Baseline Blood Pressure Measurements at Endpoint (LOCF) Intent-to-Treat Data Set

	Supine DBP	Supine SBP	Standing DBP	Standing SBP
Placebo (N=75)				
Baseline mean	99.76	154.69	100.58	151.18
Change from baseline	-4.30	-1.93	-2.09	-2.40
Nisoldipine 20 mg qd (N=75)				
Baseline mean	99.99	152.67	100.80	[illegible]
Change from baseline	-7.97	-10.17	-6.99	-11.42
p-value vs placebo	0.0004	0.0001	0.0001	0.0001
Nisoldipine 40 mg qd (N=76)				
Baseline mean	100.35	154.22	101.27	150.20
Change from baseline	-11.22	-15.50	-11.34	-14.90
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Verapamil 240 mg bid (N=78)				
Baseline mean	99.95	151.67	100.66	148.62
Change from baseline	-14.48	-14.79	-13.21	-14.95
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Interaction p-value	0.6287	0.1693	0.5060	0.6371

The sponsor performed both an evaluable patient analysis, including those patients with at least two post-baseline evaluations who were not protocol violators, and an intent-to-treat analysis including all patients who had at least one post-baseline evaluation. A last-observation-carried-forward approach was used to include the available data from patients who dropped out of the study. The initial analysis of variance model for the primary and secondary change from baseline endpoints included treatment group, investigator, and treatment by investigator interaction terms. The interaction term was dropped if it was not significant at the .05 level.

The results of the sponsor's analysis of their evaluable patient data set were similar, with all of the active drug groups demonstrating a highly statistically significant difference from the placebo group. The same was true of the analysis at each of the various time points after at least two weeks of double-blind therapy. Each active treatment group tended to reach a plateau in blood pressure response after two or three weeks of double-blind therapy, and the results were very consistent beyond that point. The treatment by center interaction term was significant for some visits, but not for the final two visits or for the endpoint values for either data set.

The response variables also demonstrated results similar to the change from baseline blood pressure values, as can be seen below.

Response Rates

	Placebo	Nisoldip ne 20 mg qd	Nisoldipine 40 mg qd	Verapamil 240 mg bid
	N=70	N=72	N=76	N=72
A) DBP ≤ 90 mmHg	19 (26%)	35 (50%)	50 (69%)	62 (82%)
B) DBP decrease ≥ 10 mmHg	10 (14%)	28 (40%)	47 (65%)	59 (78%)
C) A) or B)	20 (28%)	38 (54%)	53 (74%)	67 (88%)
D) A) and B)	9 (13%)	25 (36%)	44 (61%)	54 (71%)

The ambulatory blood pressure data was analyzed by smoothing the hourly means from the eight centers using a Fourier transform. The mean trough and peak values and trough to peak ratios were obtained from the smoothed data. Peak effect for each nisoldipine group was defined as the greatest difference between the nisoldipine group and placebo in mean change from baseline. Trough effect was defined as the 24-hour post-dose difference from placebo.

The peak diastolic blood pressure response occurred at 4 hours post-dose for the nisoldipine 20 mg group, at 24 hours post-dose for the nisoldipine 40 mg group, and at 4 hours after the morning dose for the verapamil 240 mg bid group. The peak/trough ratios were -6.7/-9.7 = 66%, -11.7/-11.7 = 100%, and -11.1/-12.9 = 86%, respectively. The corresponding systolic peak/trough ratios were 66%, 100%, and 78%.

Adverse Events

	Placebo N=75	Nisoldipine 20 mg qd N=76	Nisoldipine 40 mg qd N=76	Verapamil 240 mg bid N=78
Patients with ≥ one event	48 (64%)	49 (64%)	57 (75%)	55 (71%)
Possibly drug related	34 (45%)	33 (43%)	42 (55%)	39 (50%)
Serious adverse events	7 (9%)	9 (12%)	12 (16%)	3 (4%)
Withdrew due to a. e.'s	3 (4%)	10 (13%)	11 (14%)	4 (5%)

The overall incidence of adverse events was not statistically significantly different for the four treatment groups. The most frequently reported adverse events included headache and peripheral edema in the placebo group and both nisoldipine groups. The most frequently reported adverse events in the verapamil group included constipation and headache. Adverse events were most likely to occur early in the study (prior to week 4), although they continued to occur at a reduced level throughout the study. There were no deaths reported during this study.

II.C.3. Reviewer's Comments

This study clearly demonstrates the efficacy of nisoldipine when compared to placebo in the treatment of mild-to-moderate hypertension. A dose-response exists for both diastolic and systolic blood pressure, and for adverse events. The differential between the groups appeared within a few weeks of treatment, and was consistent for the rest of the study. This reviewer again analyzed the data using several other models, and found the results to be consistent.

The group randomized to verapamil consistently demonstrated results which were at least as good as the higher nisoldipine dose, and were often superior (although not often statistically significantly better). The response rates for the verapamil group were also higher than those of the nisoldipine groups. The sponsor stated that verapamil is often used as once-a-day therapy, and the twice-daily dosing used in this study could have given that group an advantage over the once-daily dosing of nisoldipine. Although the verapamil group did not usually achieve statistical significance when compared to the nisoldipine groups, the study was not powered as an equivalence trial and the results should not be interpreted as showing the treatments are the same.

II.D. OTHER HYPERTENSION STUDIES

II.D.1. Study Descriptions

The sponsor submitted the results of three additional placebo-controlled clinical trials, two which were carried out in the United States. These studies lend supportive

evidence of the efficacy and safety of nisoldipine in the treatment of mild to moderate hypertension.

Study D88-054 was a pilot parallel dose-ranging eight-center study comparing placebo with three once daily doses of nisoldipine, 10 mg. 20 mg. and 30 mg over a four week period. This was the first exploratory clinical trial carried out in the target population and approximately 30 patients were randomized to each dose. The 20 mg and 30 mg dose groups both achieved statistically significantly greater reductions in supine DBP, supine SBP, and standing SBP than did the placebo group.

Study D89-026 was a pilot parallel dose titration study comparing placebo with nisoldipine, 10 - 40 mg once daily over a nine week period. Patients randomized to nisoldipine received 10 mg qd for the first week and were titrated upward an additional 10 mg on a bi-weekly basis if their supine DBP remained ≥ 85 mmHg at trough. A total of 72 patients were randomized to nisoldipine and 34 to placebo, which was also titrated based on blood pressure response. At the end of the nine weeks of treatment 6% of the nisoldipine patients remained at 10 mg qd, 6% were receiving 20 mg qd, 30% were receiving 30 mg qd, and 57% had been titrated to 40 mg qd. The nisoldipine group achieved statistically significantly greater reductions in supine and standing DBP and SBP.

Study D90-006 was a parallel multicenter study comparing placebo with three once daily doses of nisoldipine, 10 mg. 20 mg. and 30 mg over a six week period. This study was carried out in South Africa. All patients randomized to nisoldipine began at 10 mg qd and were titrated to their assigned dose after one week. Approximately 50 patients were randomized to each group. In the endpoint analysis all three nisoldipine groups achieved statistically significantly greater reductions in supine and standing DBP and SBP than did the placebo group.

IV. OVERALL SUMMARY AND CONCLUSIONS

The sponsor submitted the results of six trial involving patients with mild-to-moderate hypertension, including three Phase III studies performed in the United States. Study D90-019 was a dose-response study comparing two doses of nisoldipine (30 mg and 60 mg) with placebo in once-daily dosing. Both nisoldipine dose groups had statistically significantly better reduction in blood pressure than did the placebo group, and the groups demonstrated a dose-response relationship for both efficacy and safety. Study D90-029 compared placebo with three once daily doses of nisoldipine (20 mg, 40 mg, and 60 mg) on a background of atenolol 50 mg qd. All three nisoldipine/atenolol groups had statistically significantly better reduction in blood pressure than did the placebo/atenolol group. The responses trended in a dose-response order for both blood pressure reduction and for adverse experiences. Study D90-039 was a four-arm placebo and active-controlled study comparing two doses of nisoldipine (20 mg and 40 mg) with verapamil 240 mg bid and placebo. The nisoldipine groups had statistically significantly better reduction in blood pressure than the placebo group. The verapamil group had a somewhat better response than

did the nisoldipine groups, although the results were not often statistically significant. However, this trial was not designed as an equivalence trial, and was under-powered to detect the differences seen in the study. The study could not detect a difference, but that does not imply that the treatments are the same.

Clearly the nisoldipine doses studied in this trial (20 mg to 60 mg, qd) are effective at lowering blood pressure. What is not clear is that the dose range has been adequately examined, especially at the lower end. The maximal dose appears to be limited by adverse reactions. This reviewer feels that doses of nisoldipine lower than those studied here might be adequate for many patients and should be examined.

There were two potential problems in the design of these studies. The original model for the primary analysis of variance included a treatment by center interaction term which was dropped if the p-value for interaction was less than .05. The test for interaction is a very low powered test, and interaction is usually tested at the .15 level. The results of these studies were robust whether or not an interaction term was included in the model, and the interactions which were statistically significant appeared to be qualitative rather than quantitative. These studies each involved multiple doses of nisoldipine, and none of the analysis plans included adjustments for multiple comparisons. The p-values that resulted from the analyses, however, were all less than 0.001, and thus could stand up to a Bonforonni adjustment.

The overall summary and conclusions section may be conveyed to the sponsor.

Nancy D Smith

Nancy D. Smith, Ph.D.
Mathematical Statistician

Concur: Dr. Chi Chi 1/4/94

for Dr. Dubey 1-4-94

B10 REVIEWS

NDA No: 20-356
Date of Document: November 14, 1994
Generic Name: Nisoldipine
Brand Name: NISOCOR
Formulation: Nisoldipine CC (Extended Release Tablets)
Sponsor: Miles Incorporated
Type of Submission: NDA Amendment
Reviewers: Olof Borga, Ph.D. and Alfreda Burnett, Ph.D.

BACKGROUND

Nisoldipine is a dihydropyridine calcium antagonist, similar to felodipine. NISOCOR tablet contains either 10, 20, 30, or 40 mg of nisoldipine as a Coat-Core (CC) formulation: the coat is a slow release formulation while the core is a fast release formulation. Previous studies with felodipine (1), nifedipine (1) and nitrendipine (2), all dihydropyridines (DHPs), have shown that simultaneous intake of grapefruit juice increases the bioavailability of the DHP drug. These drugs are all subject to first-pass metabolism, and the mechanism of interaction is believed to be inhibition of cytochrome P450 3A4 responsible for this step. Grapefruit juice is known to contain high amounts of flavonoids, mainly present as glycosides, which probably undergo hydrolysis in the intestine to the corresponding aglycones and sugars (3). In vitro studies in rat and human liver microsomes have shown that the aglycones, mainly narengenin, quercitin, and kaempferol all have inhibitory potency with regard to the metabolism of DHPs (4).

The sponsor has conducted a clinical study to investigate the possibility of an interaction between grapefruit juice and NISOCOR (nisoldipine cc) with respect to the pharmacokinetics and pharmacodynamics of nisoldipine and its major metabolites in plasma. An account of the study has also been published (5).

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5. Bailey, D.G., et al. Effect of grapefruit juice and naringin on nisoldipine pharmacokinetics. Clin Pharmacol Ther 1993; 54(6): 589-94.

SYNOPSIS

The sponsor has submitted a food drug interaction study. The primary objective was to investigate a possible interaction between grapefruit juice and nisoldipine cc with respect to the pharmacokinetics of nisoldipine (BAY k 5552) and its major plasma metabolites (the hydroxylated metabolite BAY r 9425 and the pyridine metabolite BAY 0 3199). The secondary objective was to investigate the hypothesis that one bioflavonoid, naringin, in grapefruit juice is responsible for the proposed interaction. The study was conducted in 12 normotensive male subjects. Subjects randomly received each of 3 treatments on 3 separate study days, separated by a washout period of at least 7 days. Blood

pressure and heart rate assessments and determination of plasma concentrations of nisoldipine and its two major plasma metabolites were carried out for 48 hours after each treatment. Also, included in this submission is the proposed annotated package insert for NISOCOR (nisoldipine).

STUDY SUMMARY

See Appendix.

CONCLUSION:

The sponsor has demonstrated that
a) grapefruit juice alters the pharmacokinetics of nisoldipine ce in healthy subjects
b) naringin does not appear to be the bioflavonoid in grapefruit juice responsible for the interaction
c) all treatments produced minor effects on supine blood pressure and heart rate, probably because subjects were normotensive

LABELING COMMENTS: The following wording is suggested by the firm:"In a study of twelve healthy male subjects, the bioavailability of nisoldipine was increased by as much as 3-fold when taken with grapefruit juice, compared to when taken with water. A similar finding has been seen with some other dihydropyridine calcium antagonists, but to a somewhat lesser extent than seen with nisoldipine." We suggest the following wording:"**In a study in twelve healthy male subjects, the AUC of nisoldipine was increased up to 4-fold (mean 75%) and C_{max} increased up to 7-fold (mean 350%) when taken with grapefruit juice, compared to when taken with water. This is probably caused by inhibition of first-pass elimination of the drug. A similar interaction has been seen with other dihydropyridine calcium antagonists, but to a somewhat lesser extent.**"

RECOMMENDATION:

The Division of Biopharmaceutics recommends that the label be amended to reflect that after intake of grapefruit juice:
a) In 12 healthy males the C_{max} of nisoldipine increased up to 7 fold. The mean C_{max} increased 350%.
b) The AUC_{0-48} increased up to 4-fold. The mean AUC_{0-48} increased approximately 75%.

Olof Borga, Ph.D. Alfreda Barnett, Ph.D. Date
Pharmacokinetics Review Branch

FT Initialed by Ameeta Parekh,Ph.D.

cc: NDA 20-356, HFD-110, HFD-42o (Fleischer, Parekh, Drug, FOI (HFD-19), Chron, HFD-540 (Vishwanathan), F

NOV 21 1994

NDA: 20-356
Nisoldipine Coat-Core tablets
10, 20, 30 and 40 mg
Sustained release tablets
Miles Pharmaceutical Division
Priority: 1S

Submission Date: March 31, 1993
August 2, 1993.
September 24, 1993.
October 14, 1993.
November 04, 1993.
November 23, 1993.
December 29, 1993.
March 14, 1994.
March 18, 1994.
June 27, 1994.
July 18, 1994.
July 29, 1994.

Type of submission: new molecular entity. Reviewer: Patrick J. Marroum.

Synopsis:

-The sponsor has adequately studied the pharmacokinetics (single and multiple dose) of nisoldipine coat-core tablet.
-Dosage form proportionality has been established between the 20 and 30 mg tablets only but not between the 20 and 40 mg strengths.
-Dose proportionality was established for AUC and CMAX in the dosing range of 20 to 60 mg. The 10 mg tablet strength gave more than dose proportional plasma levels when compared to the higher strengths.
-The effect of food on the rate and extent of nisoldipine absorption was investigated in 3 separate studies. Food increased CMAX by up to 300 % while decreasing AUC by up to 26 %.
-Adequate studies have been performed in elderly normal and hypertensive subjects.
-The effect of liver and renal disease has been adequately characterized in this NDA.
-Drug interaction studies between nisoldipine and warfarin, cimetidine, ranitidine, digoxin, quinidine, propranolol and atenolol have been performed.
-The sponsor failed to characterize the effect of gender on the pharmacokinetics of nisoldipine.
-The sponsor has adequately validated the gas chromatographic assay used in these studies.
-The sponsor attempted to correlate the in vitro dissolution with the in vivo performance of 3 different formulations of nisoldipine but failed to establish any relationship due to the fact that nisoldipine undergoes site specific gut wall metabolism.
-The dissolution method proposed by the sponsor for nisoldipine C.C. seems to be acceptable with the specifications recommended by the Division of Biopharmaceutics.

RECOMMENDATION:

The sponsor's NDA 20-356 appears to be acceptable for meeting the biopharmaceutics requirements provided that the comments on Pages 13 to 15 are adequately addressed by the sponsor.

TABLE OF CONTENTS:

The following studies were not reviewed because they either pertain to the immediate release formulation which is not subject for approval under this NDA or were not deemed pertinent for the approval of nisoldipine C.C.

Study 339	Investigation of the relation between the systemic bioavailability and the dose of BAY K5552 in healthy subjects.
Study 115	Plasma concentrations after oral administration of capsules and tablets (micronized substance and coprecipitate) to healthy volunteers.
Study 297	Investigation into the bioequivalence of various oral formulations of nisoldipine in healthy volunteers by a crossover trial.

Study D85-038	Bioequivalence study of formulations of nisoldipine 2.5 mg, 5 mg and 10 mg tablets in normal subjects.
Study D85-037	Bioequivalence study of formulations of nisoldipine 20 mg tablets in normal subjects.
Study 116	Plasma concentrations after the oral administration of 5 and 10 mg tablets. Comparison of micronized and ground active substance.
Study 330	Steady-state pharmacokinetics of nisoldipine in healthy male volunteers.
Study 102-106	Plasma concentrations after oral administration of different doses (6 to 20 μg/kg) of BAY K5552 to healthy test subjects.
Study 125	Plasma levels in volunteers after oral administration of 10 and 20 mg of BAY K5552 in the form of tablets.
Study 294	Plasma concentrations during treatment with BAY K5552 of hypertensive patients with impaired liver function.
Study 452	Pharmacokinetics and hemodynamic effects of nisoldipine in patients with liver cirrhosis after PO and IV administration.
Study 364	Nisoldipine pK in renal dysfunction.
Study 399	Pharmacokinetics and hemodynamic effects of nisoldipine and its interaction with cimetidine in healthy volunteers.
Study D88-054	Comparative double blind pilot study of the safety and efficacy of once daily doses of nisoldipine 10, 20, 30 mg CC tablets vs placebo in hypertensive patients.
Study D89-029	Double blind randomized study of safety and efficacy of once daily doses of nisoldipine 20, 40 and 60 mg (2x30 mg) CC tablets vs placebo in combination with atenolol 50 mg in hypertensive patients.
Study D89-039	Comparative double blind study of safety and efficacy of once daily doses of nisoldipine 20, 40 and 80 mg CC tablets vs a twice daily doses of verapamil SR 240 mg caplets vs placebo in hypertensive patients.
Study	A double blind randomized study of the safety and efficacy

D90-019 [redacted] of once daily doses of nisoldipine CC tablets 10, 20 (2x10), 30 (3x10) mg. vs placebo in hypertensive patients.

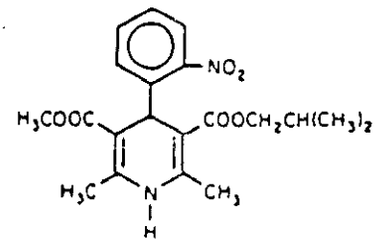
Study D88-060 Efficacy and safety of CC nisoldipine 10, 20 and 30 mg qd vs placebo in patients with stable exertional angina pectoris.

Study 0417 Acute pK/pD interaction of nisoldipine and propranolol.

Appendix II:

Background:

Nisoldipine is a dihydropyridine calcium channel blocker. It is 3,5-pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-methyl 2-methylpropyl ester. It has the following structural formula:



Nisoldipine is a yellow crystalline substance, practically insoluble in water but soluble in ethanol. No information about the octanol/water partition coefficient was submitted in this NDA. It has a molecular weight of 388.4. Nisoldipine coat core tablets consist of an external coat and internal core. Both contain nisoldipine, the coat in a slow-release formulation and the core in a fast-release formulation. Nisoldipine CC tablets contain either 10, 20, 30 or 40 mg of nisoldipine for once a day oral administration.

Nisoldipine C.C. is indicated for the treatment of hypertension and may be used alone or in combination with other antihypertensive agents. In general, therapy should be initiated with 10 mg orally once daily. The usual maintenance dosage is 20 or 30 mg once daily. Doses beyond 40 mg are not recommended.

It is to be noted that this NDA was first submitted on March 31, 1993. An approvable letter was issued on March 25, 1994 based on deficiencies in both the Chemistry and the Clinical sections of the application. It was resubmitted to the Agency on August 3, 1994. The user fee goal for this application is February 3, 1995.

SUMMARY OF BIOAVAILABILITY/PHARMACOKINETICS/PHARMACODYNAMICS:

I-BIOAVAILABILITY/BIOEQUIVALENCE:

A-Absolute Bioavailability:

The absolute bioavailability of 20 mg nisoldipine coat core compared to the dose corrected IV dose infused over 1 hour was 5.5 % with a 95 % CI ranging from 4.8 % to 6.4 %. (Study 0637).

B-Food Effects:

The effect of food on the pharmacokinetics of nisoldipine C.C. was investigated in 2 separate studies. Study 666 where a 20 mg nisoldipine C.C. tablet was given fasted, together with a high fat breakfast, 1 hour after a high fat breakfast and together with dinner showed that the coadministration with meals remarkably increased CMAX from 26 %(with dinner) up to 48% (together with breakfast) with a corresponding shortening of TMAX by about 2 to 3 hours. However food did not seem to have any effect on the extent of bioavailability of nisoldipine since there was no difference between the AUCs in fed and fasted states.

Study D92-045-02 showed that the effect of food was even more pronounced on the 30 and 40 mg C.C. tablets. Food increased the CMAX for nisoldipine on the average by 250 to 300 % (CMAX increased from 1.9 to 4.5 ng/ml for the 30 mg and from 2.7 to 7.5 ng/ml for the 40 mg strength) while decreasing the AUC by 26 % in the fed state as compared to the fasted state (AUC decreased from 49.2 to 35.4 ng*hr/ml for the 30 mg and from 70.4 to 53 ng*hr/ml for the 40 mg strength).

Similar results as far as CMAX is concerned were observed with the 20 mg IR tablet of nisoldipine. Food increased the AUC by 28 % and CMAX by 31 % compared to the fasting state (Study 323).

C-Bioequivalence:

Study 5678 showed that 1x40 mg was bioequivalent to 2x20 mg C.C. tablets as far as AUC was concerned since the 90 % CI of the log transformed AUC was 85.26 to 112.24 %. The same could not be said for CMAX because the 90 % CI of the log transformed CMAX was 94.62 to 140.03 %. Thus, 1x40 mg C.C. tablet is considered not bioequivalent to 2x20 mg tablets.

On the other hand Study D90 020 01 showed that 2x10 mg C.C. tablets were bioequivalent to 3x20 mg tablets. The AUC ratio (2x10/3x20) was 1.0352 with a log transformed 90 % CI of 95 to 115 while the CMAX ratio was 0.9587 with a corresponding log transformed 90 % CI of 83.64 to 109.87 %.

Study KF 715 established the bioequivalence between the old 20 mg and the new 20 mg C.C. formulation as far as AUC was concerned. As for CMAX, none of the three strengths of the new formulation were bioequivalent to the old 20 mg formulation. The 5, 10 and 20 mg strengths were higher on the upper limit of the 90 % CI compared to the old 20 mg strength.

II.PHARMACOKINETICS:

The terminal half-life of nisoldipine was estimated to be about 7 hours after an infusion of 0.08 mg/kg for 20 hours. This corresponded to a systemic clearance of 544 to 768 ml/hr/kg. The volume of distribution was estimated to be between 2.3 and 3.4 l/kg (Study 330).
Following the administration of nisoldipine C.C. 20 mg once a day for 7 days, the CMAX was 0.84 ng/ml on day 1 and 1.09 ng/ml on day 7. The AUCnorm was 40.3 g*hr/l on day 1 and 58.9 g*hr/l for day 7 giving an accumulation ratio based on AUC of 1.46. CMAX following administration of an immediate release 10 mg tablet CMAX was 2.18 ng/ml on day 1 and 1.95 ng/ml on day 7 indicating that there is no accumulation of nisoldipine with the immediate release formulation. The fluctuation index for the IR tablet given bid was 439 % as compared to 113 % following the controlled release formulation (Study 645).
Study 606/618 showed that after IV administration similar plasma concentrations were obtained for both enantiomers. However, after oral administration the concentration of the (+) nisoldipine (which is pharmacodynamically more active than the (-)) was about 6 times higher than the (-) nisoldipine. The CMAX for the (+) hydroxylated dihydropyridine (M9) was 7.6 times higher than the (-) enantiomer.

III-METABOLISM:

In man, hydroxylation of the isobutyl ester appears to be the major biotransformation pathway. 70 to 80 % of the urinary metabolites in the first 12 hours after administration are the metabolites M4, M5 and M12 (Study 400),(see metabolic scheme in Appendix II). Metabolites M1 and M2 represent about 10 % of the urine metabolites in man (Study 16626). Only metabolite M12 was hydrolyzable with beta glucuronidase yielding M5 as the aglycon. The only metabolite with known activity is BAY r9425 (M9) with 10 % of the activity exhibited by nisoldipine and is present in approximately equal concentrations in the plasma as nisoldipine. Study 600 provided some evidence that nisoldipine undergoes some degree of gut wall metabolism which is decreasing from the proximal to the distal parts of the intestine with no metabolism occurring in the colon.
It is to be noted that even though nisoldipine seems to be extensively metabolized, the sponsor did not identify the enzymes responsible for its biotransformation.

IV-DOSE PROPORTIONALITY:

The dose proportionality of immediate release nisoldipine was established between the doses of [redacted] 2.6 and 20 mg since both AUC and CMAX increased in a dose proportional manner (Study D85-024-01).

As for the coat core formulation, the dose proportionality was established for doses in the range of 20 to 60 mg (using the 20 mg tablet except for the 10 mg dose where the 10 mg tablet was used). However, the dose normalized values for CMAX and AUC for the 10 mg dose were somewhat higher and statistically different as compared to the values for the 20, 40 and 60 mg doses (Study D91-035). These differences in results were not explained by the sponsor.

V-SPECIAL POPULATIONS:

A.Renal Impairment:

In patients with severe renal impairment (creatinine clearance less than 30 ml/min), nisoldipine plasma concentrations on day 1 were higher by as much as 2 fold compared to subjects with normal renal function. However, this difference seemed to have subsided by day 7. Even though renal impairment does not seem to alter significantly the pharmacokinetics of nisoldipine C.C. and its metabolites. Caution should be exercised in dosing and titrating these patients. (Study D92-001).

B.Hepatic Impairment:

Study D90-026-01 shows that liver impairment has a pronounced effect on the pharmacokinetics of nisoldipine C.C. since both AUC and CMAX were increased four fold as compared to normal volunteers. Therefore extra care should be exercised when giving this drug to patients with impaired liver function.

C.Elderly:

Study 563 shows that the plasma concentrations of nisoldipine following the administration of 10 mg IR for 8 days tended to be higher in the elderly as compared to the young. CMAX was 1.76 (+/-0.84)ng/ml compared to 4.96 (+/-3.22) ng/ml in the elderly. AUC in the young was 7 (+/-3.12) ng/hr/ml compared to 15.04 (+/-9.33) ng/hr/ml in the elderly. However, there was no difference in the single dose and multiple dose of nisoldipine in both the young and the elderly and there was no accumulation upon multiple dose administration.
On the other hand, study 712 where 20 mg nisoldipine C.C. was administered for 7 days showed that after single dose the elderly normal and hypertensive patients tended to have about 50 % higher AUCs than young healthy subjects. CMAX in the elderly hypertensives was also about 50 % higher than either the healthy young or elderly volunteers. Moreover, upon multiple administration, there was a greater tendency for increase in AUC and CMAX for both the elderly healthy and hypertensive subjects compared to the young. It is to note that there was essentially no accumulation in the young in this study but in the elderly healthy and hypertensives, the accumulation ratio was around 2.

D-Gender:

The effect of gender on the pharmacokinetics of nisoldipine has not been investigated by the sponsor. An attempt by this reviewer to correlate gender with AUC and CMAX from data obtained from Study 712 was inconclusive due to insufficient data.

V-DISEASE STATES:

A-Hypertension:

Studies D90-022 and D88-059 showed that hypertension does not have any effects on the pharmacokinetics of nisoldipine C.C. since the plasma levels obtained from these studies were similar to those obtained in healthy subjects.

VI-DRUG INTERACTIONS:

A-Ranitidine:

Coadministration of ranitidine with nisoldipine C.C. did not have any effect on the pharmacokinetics of nisoldipine (Study 738).

B-Cimetidine:

Cimetidine seems to have a pronounced effect on nisoldipine C.C. pharmacokinetic parameters since there was more than 50 % increase in some parameters of interest. Multiple dose administration of 400 mg of cimetidine increase nisoldipine CMAX from 1.05 to 1.74 ng/ml and its AUC from 14.97 to 23.2 mcg*hr/l. Therefore, great caution should be exercised when both these drugs are administered concomitantly, the patients should be monitored and dose adjustments made as necessary (Study 738).

C-Warfarin:

Study 349 showed that coadministration of steady state doses between 3 and 10 mg of warfarin with 10 mg IR nisoldipine did not have any effect on the pharmacokinetics of nisoldipine. Moreover, nisoldipine did not affect the prothrombin times of patients that were on warfarin.

D-Quinidine:

Coadministration of twice a day of 10 mg of nisoldipine IR with 500 mg of quinidine bid increased quinidine's AUC by 25 % (Study 84). The effect of quinidine on nisoldipine pharmacokinetics could not be assessed since no nisoldipine plasma concentrations were measured in this study.

E-Propranolol:

Coadministration of 20 mg nisoldipine capsules either acutely or chronically caused a significant increase in both AUC and CMAX for propranolol. The propranolol AUC increased from 1556 (+/-) 1135) to 2098 (+/- 1501) with single dose nisoldipine and up to 2482 (+/- 2099) ng.hr/ml with multiple dosing of nisoldipine. CMAX increased from 143 (+/- 44) ng/ml to 222 (+/- 67) ng/ml with either single dose or multiple dose administration of the calcium channel blocker (Study 3982).
However Study 704 showed that coadministration of 20 mg nisoldipine C.C. with 40 mg propranolol tid did not have any significant effects on the plasma concentrations of either drugs.

F-Atenolol:

Coadministration of 20 mg nisoldipine capsules either acutely or chronically caused a significant increase in both AUC and CMAX of atenolol. The CMAX for atenolol increased from 455 (+/- 135) ng/ml to 540 (+/- 146) ng/ml while the AUC for the beta blocker increased from 5854 (+/- 2291) ng*hr/ml to 6987 (+/- 2269) ng*hr/ml. These results are very similar to what was seen when nisoldipine was coadministered with propranolol (Study 3982).

G-Beta Acetyl Digoxin:

Coadministration of 10 mg of nisoldipine IR tablets bid with 0.6 mg/day of beta acetyl digoxin did not seem to have any effect on the pharmacokinetics of beta acetyl digoxin (Study 413).
(Note: In the labelling, the results of this study appear as lack of interaction with Digoxin and not acetyldigoxin. This issue was discussed with Dr Chen supervisory Medical Officer HFD 110 who thought that labelling the results of this study as no interaction with digoxin was appropriate.)

VII-PHARMACOKINETIC/PHARMACODYNAMIC RELATIONSHIPS:

The sponsor attempted to establish a pharmacokinetic/pharmacodynamic model in hypertensive patients using the results of study D90-022. This pharmacodynamic model was established using the program Attract which is based on linear system analysis and methods utilizing hysterisis minimization. The sponsor reported that the pharmacodynamic responses (mainly drop in blood pressure) follow a sigmoid EMAX model.
The modelling method used by the sponsor is not valid due to the fact that it is very difficult to see hysterisis with this formulation of this drug (see also the comments following Study D90-022).
The Division of Biopharmaceutics attempted to model the pharmacodynamics with the

pharmacokinetics of Nisoldipine C.C. using a population approach. The data from study D90-022 and study D88-059 were used in this attempt. However due to the fact that both studies for the same 30 mg dose gave totally different plasma concentrations (study D90-022 gave almost double the plasma concentrations of study D88-059), these studies could not be combined and the final model was established using data from the same study that the sponsor model. The best model that describes this set of data was found to be an EMAX model with a maximal reduction of diastolic blood pressure of -23.9 mm of Hg. The EC50 was estimated to be 3.94 ng/ml.

VIII-FORMULATION:

The coat-core tablet consists of a slowly dissolving coat surrounding a more rapidly dissolving core. Within the coat the active ingredient is finely distributed in a matrix of a hydrophilic gel-forming polymer. On contact with water a swelling process begins at the tablet surface and the soft material formed is continuously eroded. The active ingredient contained in the eroded material can then be dissolved and absorbed. Initially, the diameter of the tablet and thus its surface area change very little resulting in a constant release of active ingredient over a period of about 6 to 8 hours (i.e. zero order dissolution kinetics). When the erosion of the coat has advanced, the dissolution of the fast-release core causes an increase in the release rate over a period of about 2 hours. Thus, the decreasing release rate of drug from the tablet coat (due to diminishing tablet surface area) is countered by the rapid dissolution of the core.
The composition of the different strengths tablets of nisoldipine Coat Core is summarized in Appendix II.
It is to be noted that all the pivotal clinical trials were done using the to be marketed formulation.

IX-PROTEIN BINDING:

The plasma protein binding of nisoldipine is very high since less than 1 % is unbound at a concentration range between 100 ng/ml and 10 mcg/ml. The binding is primarily to albumin. There was no stereoselectivity in binding since both enantiomers had similar degree of binding as observed with the racemate. (study 19611)

X-RED BLOOD CELL PARTITIONING:

The erythrocyte/plasma partition coefficients are about 0.3 mostly independent of the concentration in the range studied 0.1 to 10 mcg/ml. However, the partition between erythrocytes and plasma water is very high in the order of 56 indicating a high affinity of the blood cells and other tissues to nisoldipine. There was no difference in partitioning between the racemate and its enantiomers. (Study 19611).

XI-DISSOLUTION:

The proposed dissolution method for the coat-core tablet formulation was the USP method II (paddle method) at a paddle speed of 50 rpm. The medium was 900 ml of phosphate buffer with

1% sodium lauryl sulfate. The proposed dissolution specifications were:
-3 hours
-6 hours
-12 hours: Not less than
*For the evaluation after stage 2 of the USP acceptance table, single tablets and mean value are specified by the limits % respectively.
Based on the performance of the bio lots submitted in this NDA, the following dissolution specifications are recommended:
-3 hours:
-6 hours:
-12 hours: not less than .

XII-IN VIVO IN VITRO CORRELATION:

An attempt was made by the sponsor to correlate the in vitro and in vivo performance of the nisoldipine coat-core tablets. However, the sponsor could not establish any level of correlation (A,B or C) due to the fact that nisoldipine undergoes variable gut wall metabolism which is dependent on the site in the gastro-intestinal tract.

XIII-ASSAY:

Concentrations of nisoldipine and its metabolites from biological fluids were determined using capillary gas chromatography with electron capture as a detection mode. Overall, the assay methodology as well as its validation were satisfactory.

Comments to be Sent to the Firm:

1-Nisoldipine appears to exhibit stereospecific first pass metabolism. After P.O. administration the (+) nisoldipine plasma levels are 6 times higher. Since the (+) enantiomer of nisoldipine is responsible for most if not all the activity of this drug, the sponsor should have used a stereospecific assay for all pk studies.

2-The sponsor did not determine the ratio of the two enantiomers in special populations. The sponsor is asked to submit any available data on the ratio of the enantiomers of the parent compound as well as any relevant metabolites in dose proportionality, drug interaction studies and special populations (such as liver impairment patients, elderly etc...) as compared to healthy volunteers.

3-The sponsor failed to identify the specific enzymes that are responsible for the metabolism of nisoldipine. The sponsor is requested to identify the enzyme systems responsible for the metabolism of nisoldipine even though the sponsor believes that there is no need to conduct such studies because it is believed that the same enzyme that is responsible for the metabolism of nifedipine (i.e. 3A4) is also responsible for the metabolism of nisoldipine. Nevertheless, it is necessary to confirm this by conducting an in vitro metabolic study.

4-The dissolution data submitted in this NDA showed a great deal of variability specially around the 9 hour time point. Some of the higher strengths lots i.e. the 20 and 30 mg tablet strength, seem to give different results when the same lot studied under the same conditions were tested within the same day and also on different days. The firm is requested to give an explanation for this variability.

5-Based on the performance of the bio lots submitted in this NDA, the following dissolution test and specifications are recommended:
USP paddle at a speed of 50 rpm in 900 ml of phosphate buffer pH 6.8 with 1 % sodium lauryl sulfate
-3 hours:
-6 hours:
-12 hours: not less than

6-Studies D90-022 and D88-059 do not give the same plasma levels for the 30 mg dose. In the first study (D90-022) the plasma levels obtained with the 30 mg dose are almost double than what was obtained with the same dose in Study D88-059. AUC was 74.28 +/- 7.96 compared to 33.097 +/- 6.077 ng*hr/ml while CMAX was 4.79 +/- 0.68 compared to 2.473 +/- 0.458 ng/ml. The sponsor is asked to explain the observed discrepancy between the 2 studies.

7-In several of the studies, the sponsor did not include any assay validation. It is the Division of Biopharmaceutics policy to recommend that a full description of the analytical assay be included in the study reports. Data on the linearity, specificity, sensitivity and on the accuracy and precision of the analytical methodology should be included in each study report.

8-The modelling approach taken by the sponsor to analyze the relationship between the pK of nisoldipine and its pd was found to be inadequate by the reviewer due to the reasons outlined in the Comments following Study D90-022 on page 189.

9-In Studies D90-022 and D88-059, CMAX and AUC (only in Study D90-022) increased in a less than dose proportional manner with dose. Yet, the results of Study D91-035 indicate that the pharmacokinetics of nisoldipine from the coat-core formulation should be linear between 20 and 60 mg. The sponsor is asked to explain the discrepency in these results.

10-Quinidine is a known inhibitor of the cytochrome P450 isoenzyme DII6. In the drug interaction study between quinidine and IR nisoldipine, the sponsor only measured the plasma concentrations of quinidine. The sponsor should have also measured the nisoldipine plasma concentrations to see whether quinidine had any effect on the metabolic pathways responsible for the metabolism of this dihydropyridine compound.

11-In the renal impairment study, the sponsor should have determined the protein binding of nisoldipine. It is not uncommon to see significant protein binding changes which will affect the plasma levels of the drug under study.

12-The sponsor did not investigate either the effect of gender or race on the pharmacokinetics of nisoldipine. The sponsor is asked to submit any additional data that might be available that would address this issue.

13-The Pharmacokinetics and metabolism section of the package insert should be rewritten as follows:

Pharmacokinetics:

Nisoldipine activity is primarily due to the (+) enantiomer. Studies with radiolabelled drug have demonstrated that administered nisoldipine is relatively well absorbed into the systemic circulation with 87 % of the radiolabel recovered in urine and faeces. Elimination of nisoldipine is exclusively by metabolism with no unchanged nisoldipine recovered in the urine. Nisoldipine pharmacokinetics are independent of dose in the range of 20 to 60 mg. Upon multiple dosing, nisoldipine accumulation is predictable from a single dose. The bioequivalency between 2x30 mg and 3x20 mg nisoldipine has been established. However 1x20 vs 2x10 and 2x20 mg vs 1x40 mg tablets were inequivalent with respect to CMAX.

Absorption: The absolute bioavailability of nisoldipine was found to be 5.5 %. Nisoldipine's low bioavailability is due to presystemic metabolism with evidence of gut wall metabolism which decreases from the proximal to the distal parts of the intestine with no metabolism occurring in the colon.

Food has a pronounced effect on the release of nisoldipine from the coat-core formulation. CMAX increased by up to 300 % and AUC decreased by up to 26 %. However, the food effect was not as pronounced on the immediate release capsule since AUC increased by 28% and CMAX by 31 %. Concomitant intake of food with nisoldipine Coat-Core is contraindicated.

The volume of distribution of nisoldipine after IV administration was estimated to be between 2.3 and 3.4 l/kg. The plasma protein binding is very high since less than 1 % is unbound over the plasma concentrations of 100 ng/ml to 10 mcg/ml. Nisoldipine poorly penetrates into red blood cells with a blood/plasma ratio of 0.3 mostly independent of concentration over the range of 0.1 to 10 mcg/ml.

Maximal plasma concentrations of nisoldipine are reached 6 to 12 hours after dosing. The terminal elimination half-life ranges from 7 to 12 hours. With a 40 mg tablet of nisoldipine Coat-Core, CMAX was 3.1 ng/ml and the $AUC_{0,8}$ was 54.3 ng*hr/ml. After oral administration, the concentration of the (+) nisoldipine was about 6 times higher than the (-) isomer.

Metabolism: 11 metabolites have been identified in the urine. In man the major biotransformation pathway appears to be the hydroxylation of the isobutyl ester. Metabolite M9, which is the hydroxylated derivative of the side chain of nisoldipine, is the only one that appears to have any activity (10 % of the parent compound) and is present in equal amounts as nisoldipine in plasma. Cytochrome P450 is believed to play a major role in the metabolism of nisoldipine, however, the particular isoenzyme system that is responsible for its metabolism has not been identified.

Excretion: No unchanged nisoldipine is eliminated in the urine.

Special Populations:

Geriatric: Elderly patients have been found to have 2 to 3 fold higher plasma concentrations than young subjects.

Renal dysfunction: Because renal elimination is not a significant pathway, dosing adjustments in patients with mild to moderate renal impairment is not necessary.

Hepatic Insufficiency: In patients with liver cirrhosis given 10 mg nisoldipine C.C., plasma concentrations of the parent compound were found to be 4 to 5 times higher than healthy young subjects. Thus lower maintenance doses may be required in both cirrhotic patients and in the elderly.

Gender and Race: The effect of gender or race on the pharmacokinetics of nisoldipine has not been investigated.

Disease States: Neither hypertension nor stable exertional angina pectoris alter the pharmacokinetics of nisoldipine.

Drug-Drug Interactions: No significant interactions were found between nisoldipine immediate release (IR) and warfarin or beta acetyl-digoxin. However, IR nisoldipine increased plasma quinidine concentrations by about 20 %.
A 30 to 40 % increase in AUC and CMAX of nisoldipine was observed with concomitant administration of 400 mg cimetidine twice daily. There was no interaction with ranitidine 150 mg twice daily..
Coadministration of 20 mg nisoldipine IR with 160 mg propranolol once daily caused a 35 % increase in propranolol AUC and 55 % increase in CMAX. The interaction with nisoldipine C.C. was negligible. Atenolol's AUC and CMAX were increased by 20 % when coadministered either acutely or chronically with 20 mg IR nisoldipine capsules.

9-In the Dosage and Administration section of the package insert, a statement should be included that nisoldipine coat-core should be taken on an empty stomach.

P Marroum 11/18/1994
Patrick J. Marroum Ph.D.

Biopharm Day October 4 1994 (Collins, Ludden, Malinowski, Fleischer, Chen, Gillespie, Parekh, Marroum).
RD/ FT initialed by A Parekh Ameeta Parekh 11/21/94

cc: NDA 20-356, HFD 110, HFD 426 (Marroum, Fleischer), Chron, Drug, FOI (HFD 19), HFD 340 (Vishwanathan), F, CR, A, DI, Pk/PD, RI, A, CD.

END

peripheral vascular resistance, careful monitoring of blood pressure during the initial administration and titration of NISOCOR is recommended. Close observation is especially important for patients already taking medications that are known to lower blood pressure. Although in most patients the hypotensive effect of NISOCOR is modest and well tolerated, occasional patients have had excessive and poorly tolerated hypotension. These responses have usually occurred during initial titration or at the time of subsequent upward dosage adjustment.

Congestive Heart Failure: Although acute hemodynamic studies of nisoldipine in patients with NYHA Class II-IV heart failure have not demonstrated negative inotropic effects, safety of NISOCOR in patients with heart failure has not been established. Caution therefore should be exercised when using NISOCOR in patients with heart failure or compromised ventricular function, particularly in combination with a beta-blocker.

Patients with Hepatic Impairment: Because nisoldipine is extensively metabolized by the liver and, in patients with cirrhosis, it reaches blood concentrations about 5 times those in normals, NISOCOR should be administered cautiously in patients with severe hepatic dysfunction (See Dosage and Administration)

Information for Patients: NISOCOR is an extended release tablet and should be swallowed whole. Tablets should not be chewed, divided or crushed. NISOCOR should not be administered with a high fat meal. Grapefruit juice, which has been shown to increase significantly the bioavailability of nisoldipine and other dihydropyridine type calcium channel

Summary Basis of Approval
Cover Form

Appl #: 020356
Firm: ZENECA PHARMS
Reviewing Div: 110
Trade Name: SULAR (NISOLDIPINE) E R TABLETS
Generic Name:
NISOLDIPINE

Approval Letter:	Y	Statistician Review:	Y
SBA Form:	N	Bio/Dissolution Review:	Y
Final Printed Labeling:	N	Microbiologist Review:	N
Medical Officer Review:	Y	NAS/NRC Review:	N
Chemist Review:	Y	Pharmacologist Review:	Y
Federal Register Notice:	N	Completion Date:	24-MAR 97

Approval Letter And Related Correspondence

Kordon

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 20-356

FEB 2 1995

Miles Inc.
Pharmaceutical Division
Attention: Nancy Motola, Ph.D.
400 Morgan Lane
West Haven, CT 06516-4175

Dear Dr. Motola:

Please refer to your March 31, 1993 new drug application resubmitted on August 3, 1994 under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Nisocor (nisoldipine) Tablets, 10, 20, 30 and 40 mg.

We acknowledge receipt of your amendments and correspondence dated May 31, June 20 and 27, July 18 and 20 (two), September 8 and 16, October 19, November 8, 9, 17, 18 and 21, and December 16, 20 (two), 22 (three) and 28, 1994; and January 20, 23, and 27, 1995.

This new drug application provides for the use of Nisocor in the treatment of hypertension.

We have completed the review of this application including the submitted draft labeling and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the enclosed marked-up draft labeling. Accordingly, the application is approved effective on the date of this letter.

The final printed labeling (FPL) must be identical to the enclosed marked-up draft labeling. Marketing the product with FPL that is not identical to this draft labeling may render the product misbranded and an unapproved new drug

The approved dissolution specifications are as follows:

3 hours
6 hours
12 hours NLT

Please submit fifteen copies of the FPL as soon as it is available, in no case more than 30 days after it is printed. Please individually mount ten of the copies on heavy weight paper or similar material. For administrative purposes this submission should be designated "FINAL PRINTED LABELING" for approved NDA 20-356. Approval of this labeling by FDA is not required before it is used.

Should additional information relating to the safety and effectiveness of the drug become available, revision of that labeling may be required.

In addition, please submit three copies of the introductory promotional material that you propose to use for this product. All proposed materials should be submitted in draft or mock-up form, not final print. Please send one copy to the Division of Cardio-Renal Drug Products and two copies of both the promotional material and the package insert directly to:

Food and Drug Administration
Division of Drug Marketing, Advertising and Communications, HFD-240
5600 Fishers Lane
Rockville, Maryland 20857

Validation of the regulatory methods is ongoing. At the present time, it is the policy of the Office not to withhold approval because the methods are being validated. Nevertheless, we expect your continued cooperation to resolve any deficiencies that may occur.

Please submit one market package of the drug when it is available.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any questions, please contact:

Mr. David Roeder
Consumer Safety Officer
(301) 594-5300

Sincerely yours,

Robert Temple, M.D.
Director
Office of Drug Evaluation I
Center for Drug Evaluation and Research

Enclosure

NISOCOR

(nisoldipine)

Extended Release Tablets

For Oral Use

DESCRIPTION

NISOCOR (nisoldipine) is an extended release tablet dosage form of the dihydropyridine calcium channel blocker nisoldipine. Nisoldipine is 3,5-pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, methyl 2-methylpropyl ester, $C_{20}H_{24}N_2O_6$, and has the structural formula:

supply formula →

Nisoldipine is a yellow crystalline substance, practically insoluble in water but soluble in ethanol. It has a molecular weight of 388.4. NISOCOR tablets consist of an external coat and an internal core. Both coat and core contain nisoldipine, the coat as a slow release formulation and the core as a fast release formulation. NISOCOR tablets contain either 10, 20, 30, or 40 mg of nisoldipine for once-a-day oral administration.

Inert ingredients in the formulation are: hydroxypropylcellulose, lactose, corn starch, crospovidone, microcrystalline cellulose, sodium lauryl sulfate, povidone and magnesium stearate. The inert ingredients in the film coating

are: hydroxypropylmethylcellulose, polyethylene glycol, ferric oxide, and titanium dioxide.

CLINICAL PHARMACOLOGY

Mechanism of Action

Nisoldipine is a member of the dihydropyridine class of calcium channel antagonists (calcium ion antagonists or slow channel blockers) that inhibit the transmembrane influx of calcium into vascular smooth muscle and cardiac muscle. It reversibly competes with other dihydropyridines for binding to the calcium channel. Because the contractile process of vascular smooth muscle is dependent upon the movement of extracellular calcium into the muscle through specific ion channels, inhibition of the calcium channel results in dilation of the arterioles. *In vitro* studies show that the effects of nisoldipine on contractile processes are selective, with greater potency on vascular smooth muscle than on cardiac muscle. Although, like other dihydropyridine calcium channel blockers, nisoldipine has negative inotropic effects *in vitro*, studies conducted in intact anesthetized animals have shown that the vasodilating effect occurs at doses lower than those that affect cardiac contractility.

The effect of nisoldipine on blood pressure is principally a consequence of a dose-related decrease of peripheral vascular resistance. While nisoldipine, like other dihydropyridines, exhibits a mild diuretic effect, most of the antihypertensive activity is attributed to its effect on peripheral vascular resistance.

Nisoldipine pharmacokinetics are independent of the dose in the range of 20 to 90 mg, with plasma concentrations proportional to dose. Nisoldipine accumulation, during multiple dosing, is predictable from a single dose.

Nisoldipine is relatively well absorbed into the systemic circulation with 87% of the radiolabeled drug recovered in urine and feces. The absolute bioavailability of nisoldipine is about 5%. Nisoldipine's low bioavailability is due, in part, to pre-systemic metabolism in the gut wall and this metabolism decreases from the proximal to the distal parts of the intestine. Food with a high fat content has a pronounced effect on the release of nisoldipine from the coat-core formulation and results in a significant increase in peak concentration (C_{max}) by up to 300% Total exposure, however, is decreased about 25%, presumably because more of the drug is released proximally. This effect appears to be specific for nisoldipine in the controlled release formulation, as a less pronounced food effect was seen with the immediate release tablet. Concomitant intake of a high fat meal with NISOCOR should be avoided.

Maximal plasma concentrations of nisoldipine are reached 6 to 12 hours after dosing. The terminal elimination half-life (reflecting post absorption clearance of nisoldipine) ranges from 7 to 12 hours. C_{max} and AUC increase by factors of approximately 1.3 and 1.5 respectively from first dose to steady state. After oral administration, the concentration of (+) nisoldipine, the active enantiomer, is about 6 times higher than the (-) inactive enantiomer. The

plasma protein binding of nisoldipine is very high, with less than 1% unbound over the plasma concentration range of 100 ng/mL to 10 mcg/mL. Nisoldipine is highly metabolized; 5 major urinary metabolites have been identified. Although 60 - 80% of an oral dose undergoes urinary excretion, only traces of unchanged nisoldipine are found in urine. The major biotransformation pathway appears to be the hydroxylation of the isobutyl ester. A hydroxylated derivative of the side chain, present in plasma at concentrations approximately equal to the parent compound, appears to be the only active metabolite, and has about 10% of the activity of the parent compound. Cytochrome P_{450} enzymes are believed to play a major role in the metabolism of nisoldipine. The particular isoenzyme system responsible for its metabolism has not been identified, but other dihydropyridines are metabolized by cytochrome P_{450} IIIA4. Nisoldipine should not be administered with grapefruit juice as this has been shown, in a study of 12 subjects, to interfere with nisoldipine metabolism, resulting in a mean increase in C_{max} of about 3-fold (ranging up to about 7-fold) and AUC of almost 2-fold (ranging up to about 5-fold). A similar phenomenon has been seen with several other dihydropyridine calcium channel blockers.

Special Populations:

Renal dysfunction: Because renal elimination is not an important pathway, bioavailability and pharmacokinetics of NISOCOR were not significantly different in patients with various degrees of renal impairment. Dosing adjustments in patients with mild to moderate renal impairment are not necessary.

Geriatric: Elderly patients have been found to have 2 to 3 fold higher plasma

concentrations (C_{max} and AUC) than young subjects. This should be reflected in more cautious dosing (See Dosage and Administration).

Hepatic Insufficiency: In patients with liver cirrhosis given 10 mg NISOCOR, plasma concentrations of the parent compound were 4 to 5 times higher than those in healthy young subjects. Lower starting and maintenance doses should be used in cirrhotic patients (See Dosage and Administration).

Gender and Race: The effect of gender or race on the pharmacokinetics of nisoldipine has not been investigated.

Disease States: Hypertension does not significantly alter the pharmacokinetics of nisoldipine.

Pharmacodynamics

Hemodynamic Effects

Administration of a single dose of nisoldipine leads to decreased systemic vascular resistance and blood pressure with a transient increase in heart rate. The change in heart rate is greater with immediate release nisoldipine preparations. The effect on blood pressure is directly related to the initial degree of elevation above normal. Chronic administration of nisoldipine results in a sustained decrease in vascular resistance and small increases in stroke index and left ventricular ejection fraction. A study of the immediate release formulation showed no effect of nisoldipine on the renin-angiotensin-aldosterone system or on plasma norepinephrine concentration in normals. Changes in blood pressure in hypertensive patients given NISOCOR were dose related over the range of 10 - 60 mg/day.

Nisoldipine does not appear to have significant negative inotropic activity in intact animals or humans, and did not lead to worsening of clinical heart

failure in three small studies of patients with asymptomatic and symptomatic left ventricular dysfunction. There is little information, however, in patients with severe congestive heart failure and all calcium channel blockers should be used with caution in any patient with heart failure.

Electropyhsiologic Effects

Nisoldipine has no clinically important chronotropic effects. Except for mild shortening of sinus cycle, SA conduction time and AH intervals, single oral doses up to 20 mg of immediate release nisoldipine did not significantly change other conduction parameters. Similar electrophysiologic effects were seen with single iv doses, which could be blunted in patients pre-treated with beta-blockers. Dose and plasma level related flattening or inversion of T-waves have been observed in a few small studies. Such reports were concentrated in patients receiving rapidly increased high doses in one study; the phenomenon has not been a cause of safety concern in large clinical trials.

Clinical Studies in Hypertension

The antihypertensive efficacy of NISOCOR was studied in 5 double-blind, placebo-controlled, randomized studies, in which over 600 patients were treated with NISOCOR as monotherapy and about 300 with placebo; 4 of the five studies compared 2 or 3 fixed doses while the fifth allowed titration from 10 - 40 mg. Once daily administration of NISOCOR produced sustained reductions in systolic and diastolic blood pressures over the 24 hour dosing interval in both supine and standing positions. The mean placebo-subtracted reductions in supine systolic and diastolic blood pressure at trough, 24 hours post-dose, in these studies, are shown below. Changes in standing blood

pressure were similar:

MEAN SUPINE TROUGH SYSTOLIC AND DIASTOLIC

BLOOD PRESSURE CHANGES (mm Hg)

NISOCOR Dose (mg/day)	10 mg	20 mg	30 mg	40 mg	60 mg	10-40mg titrated
Systolic:	8	11	11	14	15	15
Diastolic	3	5	7	7	10	8

In patients receiving atenolol, supine blood pressure reductions with NISOCOR at 20, 40 and 60 mg once daily were 12/6, 19/8 and 22/10 mm Hg, respectively. The sustained antihypertensive effect of NISOCOR was demonstrated by 24 hour blood pressure monitoring and examination of peak and trough effects. The trough/peak ratios ranged from 70 to 100% for diastolic and systolic blood pressure. The mean change in heart rate in these studies was less than one beat per minute. In 4 of the 5 studies, patients received initial doses of 20 - 30 mg NISOCOR without incident (excessive effects on blood pressure or heart rate). The fifth study started patients on lower doses of NISOCOR

Patient race and gender did not influence the blood pressure lowering effect of NISOCOR. Despite the higher plasma concentration of nisoldipine in the elderly, there was no consistent difference in their blood pressure response except that the 10 mg dose was somewhat more effective than in non-elderly patients. No postural effect on blood pressure was apparent and there was no

evidence of tolerance to the antihypertensive effect of NISOCOR in patients treated for up to one year.

INDICATIONS AND USAGE

NISOCOR is indicated for the treatment of hypertension. It may be used alone or in combination with other antihypertensive agents.

CONTRAINDICATIONS

NISOCOR is contraindicated in patients with known hypersensitivity to dihydropyridine calcium channel blockers.

WARNINGS

Increased angina and/or myocardial infarction in patients with coronary artery disease: Rarely, patients, particularly those with severe obstructive coronary artery disease, have developed increased frequency, duration and/or severity of angina, or acute myocardial infarction on starting calcium channel blocker therapy or at the time of dosage increase. The mechanism of this effect has not been established. In controlled studies of NISOCOR in patients with angina this was seen about 1.5% of the time in patients given nisoldipine, compared with 0.9% in patients given placebo.

PRECAUTIONS

General:

Hypotension: Because nisoldipine, like other vasodilators, decreases

peripheral vascular resistance, careful monitoring of blood pressure during the initial administration and titration of NISOCOR is recommended. Close observation is especially important for patients already taking medications that are known to lower blood pressure. Although in most patients the hypotensive effect of NISOCOR is modest and well tolerated, occasional patients have had excessive and poorly tolerated hypotension. These responses have usually occurred during initial titration or at the time of subsequent upward dosage adjustment.

Congestive Heart Failure: Although acute hemodynamic studies of nisoldipine in patients with NYHA Class II-IV heart failure have not demonstrated negative inotropic effects, safety of NISOCOR in patients with heart failure has not been established. Caution therefore should be exercised when using NISOCOR in patients with heart failure or compromised ventricular function, particularly in combination with a beta-blocker.

Patients with Hepatic Impairment: Because nisoldipine is extensively metabolized by the liver and, in patients with cirrhosis, it reaches blood concentrations about 5 times those in normals, NISOCOR should be administered cautiously in patients with severe hepatic dysfunction (See Dosage and Administration)

Information for Patients: NISOCOR is an extended release tablet and should be swallowed whole. Tablets should not be chewed, divided or crushed. NISOCOR should not be administered with a high fat meal. Grapefruit juice, which has been shown to increase significantly the bioavailability of nisoldipine and other dihydropyridine type calcium channel

blockers, should not be taken with NISOCOR

Laboratory Tests: NISOCOR is not known to interfere with the interpretation of laboratory tests.

Drug Interactions: A 30 to 45% increase in AUC and C_{max} of nisoldipine was observed with concomitant administration of cimetidine 400 mg twice daily. Ranitidine 150 mg twice daily did not interact significantly with nisoldipine (AUC was decreased by 15 - 20 %). No pharmacodynamic effects of either H_2 antihistamine were observed.

Pharmacokinetic interactions between nisoldipine and beta-blockers (atenolol, propranolol) were variable and not significant. Propranolol attenuated the heart rate increase following administration of immediate release nisoldipine. The blood pressure effect of NISOCOR tended to be greater in patients on atenolol than in patients on no other antihypertensive therapy.

Quinidine at 648 mg bid ~~increased~~ **decreased** the bioavailability (AUC) of nisoldipine **by 26%**, but not the peak concentration. The immediate release, but not the coat-core, formulation of nisoldipine increased plasma quinidine concentrations by about 20 %. This interaction was not accompanied by ECG changes and its clinical significance is not known.

No significant interactions were found between nisoldipine and warfarin or digoxin.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Dietary administration of nisoldipine to male and female rats for up to 24 months

(mean doses up to 82 and 111 mg/kg/day, [redacted] and [redacted] times the maximum recommended human dose (MRHD) on a mg/m² basis, respectively) and female mice for up to 21 months (mean doses of up to 217 mg/kg/day, [redacted] times the MRHD on a mg/m² basis) revealed no evidence of tumorigenic effect of nisoldipine. In male mice receiving a mean dose of 163 mg nisoldipine/kg/day ([redacted] times the MRHD of 60 mg/day on a mg/m² basis), an increased frequency of stomach papilloma, but still within the historical range, was observed. No evidence of stomach neoplasia was observed at lower doses (up to 58 mg/kg/day). Nisoldipine was when tested negative in a battery of genotoxicity assays, including the Ames test and the CHO/HGRPT assay for mutagenicity mutagenicity and clastogenicity tests, and the in vivo mouse micronucleus test and in vitro CHO cell test for clastogenicity. When administered to male and female rats at doses of up to 30 mg/kg/day (about 5 times the MRHD on a mg/m² basis) nisoldipine had no effect on fertility.

Pregnancy Category C: Nisoldipine was neither teratogenic nor fetotoxic at doses that were not maternally toxic. Nisoldipine was fetotoxic but not teratogenic in rats and rabbits at doses resulting in maternal toxicity (reduced maternal body weight gain). In pregnant rats, increased fetal resorption (post-implantation loss) was observed at 100 mg/kg/day and decreased fetal weight was observed at both 30 and 100 mg/kg/day. These doses are, respectively, about 5 and 16 times the MRHD when compared on a body surface area basis. In pregnant rabbits, decreased fetal and placental weights were observed at a dose of 30 mg/kg/day, about 10 times the MRHD when compared on a body surface area basis. In a study in which pregnant monkeys (both treated and control) had high rates of abortion and mortality, the only surviving fetus from a group exposed to a maternal dose of 100 mg nisoldipine/kg/day (about 30 times the MRHD when compared on a body

surface-area-basis) presented with forelimb and vertebral abnormalities not previously seen in control monkeys of the same strain. There are no adequate and well controlled studies in pregnant women. NISOCOR should be used in pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers: It is not known whether nisoldipine is excreted in human milk. Because many drugs are excreted in human milk, a decision should be made to discontinue nursing, or to discontinue NISOCOR, taking into account the importance of the drug to the mother.

ADVERSE EXPERIENCES

More than 6000 patients world-wide have received nisoldipine in clinical trials for the treatment of hypertension, either as the immediate release or the NISOCOR extended release formulation. Of about 1,500 patients who received NISOCOR in hypertension studies, about 55% were exposed for at least 2 months and about one third were exposed for over 6 months, the great majority at doses of 20 to 60 mg daily.

NISOCOR is generally well-tolerated. In the U.S. clinical trials of NISOCOR in hypertension, 10.9% of the 921 NISOCOR patients discontinued treatment due to adverse events compared with 2.9% of 280 placebo patients. The frequency of discontinuations due to adverse experiences was related to dose, with a 5.4% discontinuation rate at 10 mg daily and a 10.9% discontinuation rate at 60 mg daily.

The most frequently occurring adverse experiences with NISOCOR are those related to its vasodilator properties; these are generally mild and only

occasionally lead to patient withdrawal from treatment. The table below, from U.S. placebo-controlled parallel dose response trials of NISOCOR using doses from 10 - 60 mg once daily in patients with hypertension, lists all of the adverse events, regardless of the causal relationship to NISOCOR, for which the overall incidence on NISOCOR was both >1% and greater with NISOCOR than with placebo.

Adverse Event	Nisoldipine (%) (n=663)	Placebo (%) (n=280)
Peripheral Edema	22	10
Headache	22	15
Dizziness	5	4
Pharyngitis	5	4
~~Asthenia~~	~~4~~	~~4~~
Vasodilation	4	2
Sinusitis	3	2
Palpitation	3	1
Chest Pain	2	1
Nausea	2	1
Rash	2	1

not greater on Nisocor

Only peripheral edema and possibly dizziness appear to be dose related.

Adverse Event	Placebo	NISOCOR 10 mg	NISOCOR 20 mg	NISOCOR 30 mg	NISOCOR 40 mg	NISOCOR 60 mg
(Rates in %)	N=280	N=30	N=170	N=105	N=139	N=137

Peripheral Edema	10	7	16	20	27	29
Dizziness	4	7	3	3	4	10

The common adverse events occurred at about the same rate in men as in women, and at a similar rate in patients over age 65 as in those under that age, ~~with the exception~~ except that headache was much less common in older patients. Except for peripheral edema and vasodilation, which were more common in whites, adverse event rates were similar in blacks and whites.

The following adverse events occurred in ≤ 1 % of all patients treated for hypertension in U.S. and foreign clinical trials, or with unspecified incidence in other studies. Although a causal relationship of NISOCOR to these events cannot be established, they are listed to alert the physician to a possible relationship with NISOCOR treatment.

Body As A Whole: cellulitis, chills, facial edema, fever, flu syndrome, malaise,

Cardiovascular: atrial fibrillation, cerebrovascular accident, congestive heart failure, first degree AV block, hypertension, hypotension, jugular venous distension, migraine, myocardial infarction, postural hypotension, ventricular extrasystoles, supraventricular tachycardia, syncope, systolic ejection murmur, T wave abnormalities on ECG (flattening, inversion, nonspecific changes), venous insufficiency,

Digestive: abnormal liver function tests, anorexia, colitis, diarrhea, dry mouth, dyspepsia, dysphagia, flatulence, gastritis, gastrointestinal

hemorrhage, gingival hyperplasia, glossitis, hepatomegaly, increased appetite, melena, mouth ulceration,

Endocrine: diabetes mellitus, thyroiditis,

Hemic and Lymphatic: anemia, ecchymoses, leukopenia, petechiae,

Metabolic and Nutritional: gout, hypokalemia, increased serum creatine kinase, increased nonprotein nitrogen, weight gain, weight loss,

Musculoskeletal: arthralgia, arthritis, leg cramps, myalgia, myasthenia, myositis, tenosynovitis,

Nervous: abnormal dreams, abnormal thinking and confusion, amnesia, anxiety, ataxia, cerebral ischemia, decreased libido, depression, hypesthesia, hypertonia, insomnia, nervousness, paresthesia, somnolence, tremor, vertigo,

Respiratory: asthma, dyspnea, end inspiratory wheeze and fine rales, epistaxis, increased cough, laryngitis, pharyngitis, pleural effusion, rhinitis, sinusitis,

Skin and Appendages: acne, alopecia, dry skin, exfoliative dermatitis, fungal dermatitis, herpes simplex, herpes zoster, maculopapular rash, pruritus, pustular rash, skin discoloration, skin ulcer, sweating, urticaria,

Special senses: abnormal vision, amblyopia, blepharitis, conjunctivitis, ear pain, glaucoma, itchy eyes, keratoconjunctivitis, otitis media, retinal detachment, tinnitus, watery eyes, taste disturbance, temporary unilateral loss of vision, vitreous floater, watery eyes,

Urogenital: dysuria, hematuria, impotence, nocturia, urinary frequency, increased BUN and serum creatinine, vaginal hemorrhage, vaginitis.

In addition to experience with NISOCOR, there is extensive experience with the immediate release formulation of nisoldipine. Adverse events were generally similar to

those seen with NISOCOR. Unusual events observed with immediate release nisoldipine but not observed with NISOCOR, were one case each of angioedema and photosensitivity. Spontaneous reports from postmarketing experience with the immediate release formulation of nisoldipine have not revealed any additional adverse events not identified in the above listings.

OVERDOSAGE

There is no experience with nisoldipine overdosage. Generally, overdosage with other dihydropyridines leading to pronounced hypotension calls for active cardiovascular support including monitoring of cardiovascular and respiratory function, elevation of extremities, judicious use of calcium infusion, pressor agents and fluids. Clearance of nisoldipine would be expected to be slowed in patients with impaired liver function. Since nisoldipine is highly protein bound, dialysis is not likely to be of any benefit; however, plasmapheresis may be beneficial.

DOSAGE AND ADMINISTRATION

The dosage of NISOCOR must be adjusted to each patient's needs. Therapy usually should be initiated with 20 mg orally once daily, then increased by 10 mg per week or longer intervals, to attain adequate control of blood pressure. Usual maintenance dosage is 20 to 40 mg once daily. Blood pressure response increases over the 10 - 60 mg daily dose range but adverse event rates also increase. Doses beyond 60 mg once daily are not recommended. NISOCOR has been used safely with diuretics, ACE inhibitors, and beta-

blocking agents.

Patients over age 65, or patients with impaired liver function are expected to develop higher plasma concentrations of nisoldipine. Their blood pressure should be monitored closely during any dosage adjustment. A starting dose not exceeding 10 mg daily is recommended in these patient groups

NISOCOR tablets should be administered orally once daily. Administration with a high fat meal can lead to excessive peak drug concentration and should be avoided. Grapefruit products should be avoided before and after dosing. NISOCOR is an extended release dosage form and tablets should be swallowed whole, not bitten or divided.

HOW SUPPLIED

NISOCOR extended release tablets are supplied as 10 mg, 20 mg, 30 mg, and 40 mg round film coated tablets. The different strengths can be identified as follows:

Strength	Color	Markings
10 mg	Oyster	**891** on one side and **MILES 10** on the other side.
20 mg	Yellow Cream	**892** on one side and **MILES 20** on the other side.
30 mg	Mustard	**893** on one side and **MILES 30** on the other side.
40 mg	Burnt Orange	**894** on one side and **MILES 40** on the other side.

NISOCOR Tablets are supplied in:

	Strength	NDC Code
Bottles of 30	10 mg	0026-8911-30
	20 mg	0026-8921-30
	30 mg	0026-8931-30
	40 mg	0026-8941-30
Bottles of 100	10 mg	0026-8911-51
	20 mg	0026-8921-51
	30 mg	0026-8931-51
	40 mg	0026-8941-51
Unit Dose Packages of 100	10 mg	0026-8911-48
	20 mg	0026-8921-48
	30 mg	0026-8931-48
	40 mg	0026-8941-48

The tablets should be protected from light and moisture and stored below 86°F (30°C). Dispense in tight, light-resistant containers.

Distributed by:

Miles Inc.

Pharmaceutical Division

400 Morgan Lane

West Haven, CT 06516 USA

Made in Germany

(Revise throughout labeling)

Nisocor

891130 NDC 0026-8911-48

[redacted]

(nisoldipine)
Extended Release Tablets
10 mg
100 Tablets Unit Dose

891130 NDC 0026-8911-48

NIS(01) CC

(nisoldipine) Extended Release Tablet

10 mg
100 Tablets
Unit Dose

For institutional use only.
RECOMMENDED STORAGE:
STORE BELOW 86°F (30°C).
Each tablet contains 10 mg nisoldipine.
Tablets should be swallowed whole, not bitten or divide
Dosage: See accompanying prescribing information.

MILES

Distributed By
Miles Inc.
Pharmaceutical Division
400 Morgan Lane
West Haven, CT 06516
Made in Germany

W 74 mm x H 106 mm x D 72.5 mm
Panel opposite the glue flap is 1/8" shorter

Filename: NIS CC 10 mg Carton/PN101072
Job #: 18356
Control #: 2836
Date: 3/4/93

04 03 0000019

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

CSO

Food and Drug Administration
Rockville MD 20857

MAR 25 1994

NDA 20-356

Miles Inc.
Pharmaceutical Division
Attention: Nancy C. Motola, Ph.D.
400 Morgan Lane
West Haven, CT 06516-4175

Dear Dr. Motola:

Please refer to your March 31, 1993 new drug application submitted under section 505(b)(1) of the Federal Food, Drug and Cosmetic Act for nisoldipine coat core tablets.

We also acknowledge receipt of your amendments and correspondence dated May 24 (two), June 3 and 8, July 15, 16, and 26 (two), August 2 and 17, September 2, 13 (two), 16, 24, 27, and 28, October 7 (two) and 14, November 4 (two), 10, 16, and 23 (two), and December 6, 9, and 29, 1993; and January 28, February 7, 8, and 24 (two), and March 15, 1994.

We have completed our review and find the information presented is inadequate and the application is not approvable. Under section 505(d) of the Act and 21 CFR 314.125(b) the data and information submitted do not establish safety and efficacy of nisoldipine (BAY k 5552) coat core tablets. The deficiencies may be summarized as follows:

1. Your proposed expiry date cannot be properly evaluated with the data submitted. Therefore, please submit additional stability data, especially in support of the shelf life specification limits proposed for the nitropyridine compound.

2. Before the application can be approved, must include a stereochemical identity test to demonstrate that the new drug substance is a racemic mixture as described in the enclosed FDA policy statement for new stereoisomeric drugs.

3. You have not responded to our July 1, 1993 request (item #6) for the maximum time that will be allowed between manufacture and packaging of the tablets.

4. Please submit the updated version of the procedure that will include the system suitability information

5. Please submit labeling for the unit dose blisters.

6. We remind you that any reprocessing procedures must be approved, and you must submit a proprietary name as soon as possible so that we will have sufficient time to review it.

Although the clinical data appear to provide adequate support for the hypertension indication, Although nisoldipine significantly improved exercise tolerance at trough in two of the four major clinical trials, this was accompanied by an effect on ischemia in only one and on time to angina in only one. Two other studies of good size showed no significant effect on any measure. Although the peak effects were slightly more prominent, especially at 40-60 mg, an effective angina drug should work throughout the dosing interval. In the largest and longest trial (D89-042), the only controlled trial lasting more than two weeks, nisoldipine CC was less effective than in the other major studies in its effect even on peak measurements.

As noted, nisoldipine was not shown to be

As noted in the February 28 and March 2, 1994 telephone discussions between Dr. Charles Resnick of this Agency and Drs. Fred Sundermann and Michael Porter of Miles, we are concerned about the occurrence of brain tumors in nisoldipine-treated male rats and stomach tumors in nisoldipine-treated male mice (specifically, granular cell tumors of the rat brain and papillomas of the mouse stomach).

The tumor findings could possibly have an impact on the approvability of your application. We are awaiting your submission of historical control data on the these tumors as well as other information requested by Dr. Resnick. The nisoldipine carcinogenicity studies will likely be considered by our Carcinogenicity Assessment Committee (CAC) and, should this occur, Miles will be invited to make a presentation.

Within 10 days after the date of this letter, you are required to amend the application, notify us of your intent to file an amendment, or follow one of your other options under 21 CFR 314.120. In the absence of any such action FDA may proceed to withdraw the application. Any amendment should respond to all the deficiencies listed. We will not process a partial reply as a major amendment nor will the review clock be reactivated until all deficiencies have been addressed.

Should you have any questions, please contact:

Mr. David Roeder
Consumer Safety Officer
Telephone: (301) 443-4730

Sincerely yours,

Robert Temple, M.D.
Director
Office of Drug Evaluation I
Center for Drug Evaluation and Research

Enclosure

FDA'S POLICY STATEMENT FOR THE DEVELOPMENT OF NEW STEREOISOMERIC DRUGS

I. INTRODUCTION AND BACKGROUND

Stereoisomers are molecules that are identical in atomic constitution and bonding, but differ in the three-dimensional arrangement of the atoms. For the purpose of this document, the stereoisomeric pairs of greatest interest are those with one or more asymmetric (chiral) centers whose enantiomers (individual stereoisomers) are mirror images. They have essentially identical physical (except for optical rotatory) and chemical (except in a chiral environment) properties.

This document focuses on issues relating to the study and pharmaceutical development of individual enantiomers and racemates. Such stereoisomers usually require specialized chiral techniques for their correct identification, characterization, separation and measurement. They are often readily distinguished by biological systems, however, and may have different pharmacokinetic properties (absorption, distribution, biotransformation, and excretion) and quantitatively or qualitatively different pharmacologic or toxicologic effects.

When stereoisomers are biologically distinguishable, they might seem to be different drugs, yet it has been past practice to develop racemates (i.e., compound with 50:50 proportion of enantiomers). The properties of the individual enantiomers have not generally been well studied or characterized. Whether separated enantiomers should be developed was largely an academic question because commercial separation of racemates was difficult. Now that technological advances (large scale chiral separation procedures or asymmetric syntheses) permit production of many single enantiomers on a commercial scale, it is appropriate to consider what FDA's policy with respect to stereoisomeric mixtures should be. Development of racemates raises issues of acceptable manufacturing control of synthesis and impurities, adequate pharmacologic and toxicologic assessment, proper characterization of metabolism and distribution, and appropriate clinical evaluation.

It should be noted that the term "stereoisomers" is a general one for all isomers that differ only in the orientation of the atoms in space. Stereoisomers include not only the mirror image enantiomers, but also geometric (cis/trans) isomers and diastereoisomers (isomers of drugs with more than one chiral center that are not mirror images of one another). Diastereoisomers and geometric isomers are both chemically distinct and pharmacologically different (unless they are interconverted *in vivo*) and are generally readily separated without chiral techniques. Geometric isomers and diastereoisomers therefore should, with the rare exception of cases where *in vivo* interconversion occurs, be treated as separate drugs and developed accordingly. There is no reason to consider developing mixtures of geometric isomers or diastereoisomers unless they

fortuitously represent a reasonable fixed dose combination (see 21 CFR 300.50). Even in that case, whether the optimal ratio of the two isomers is the ratio produced by an undirected or unmodified synthesis should be critically examined. In general, geometric isomers have been developed as single isomers. Practice with respect to diastereoisomers has been variable. These categories of stereoisomers will not be considered further in this document.

Examination of cases in which the properties of enantiomers have been evaluated reveals 1) instances in which both members had similar desirable activities (both enantiomers of dobutamine are positive inotropes; both ibuprofen enantiomers are anti-inflammatory agents; both enantiomers of warfarin and phenprocoumon are anticoagulants; the enantiomers of bupivicaine both produce local anesthesia, the enantiomers of the quinolones and the β-lactam antibiotics are all antibacterial), 2) instances in which one member of a pair was pharmacologically active and the other inactive (l-propranolol is a β-blocker; d-propranolol is not), and 3) cases in which the enantiomers had completely different activities (d-sotalol is a type 3 antiarrhythmic while l-sotalol is a β-blocker) or h different concentration-response relationships for a given property. While inactivity of one member of a pair might be considered trivial, there are instances in which toxicity has been linked to one member of a pair of stereoisomers, not necessarily the active isomer (granulocytopenia is related to the d-isomer of levodopa; vomiting is caused by the d-isomer of levamisole; and myasthenia gravis symptoms were no longer observed when the d-isomer was removed from d,l-carnitine), and there are examples of an effect on the disposition of one member of a pair by the other. In addition, there are many cases in which enantiomers have been shown to have different pharmacokinetic behavior. Differences in pharmacokinetic behavior may not pose a major therapeutic problem although it can make non-chiral blood level assays difficult to interpret with respect to activity and confuse interpretation of non-clinical data if the pharmacokinetic properties of the isomers in animals differ from those in humans.

While some enantiomeric pairs have had interesting and useful therapeutic properties (e.g., dl-sotalol, dl-dobutamine), there is no reason to expect the optimum ratio of the components to be the 1:1 ratio of a racemate (i.e., the dose-response curves would not usually be expected to be congruent).

Despite the problems identified with some racemates, the common practice of developing racemates has resulted in few recognized adverse consequences. Although it is now technologically feasible to prepare purified enantiomers, development of racemates may continue to be appropriate. However, currently available information suggests that the following should be conside.ed in product development:

1. Appropriate manufacturing and control procedures should be used to assure stereoisomeric composition of a product with respect to identity, strength, quality and purity. Manufacturers should

notify compendia of these specifications and tests.

2. Pharmacokinetic evaluations that do not use a chiral assay will be misleading if the disposition of the enantiomers is different. Therefore, techniques to quantify individual stereoisomers in pharmacokinetic samples should be available early. If the pharmacokinetics of the enantiomers are demonstrated to be the same or to exist as a fixed-ratio in the target population, an achiral assay or an assay that monitors one of the enantiomers may be used, subsequently.

II. POLICY

General

The stereoisomeric composition of a drug with a chiral center should be known and the quantitative isomeric composition of the material used in pharmacologic, toxicologic, and clinical studies known. Specifications for the final product should assure identity, strength, quality, and purity from a stereochemical viewpoint.

To evaluate the pharmacokinetics of a single enantiomer or mixture of enantiomers, manufacturers should develop quantitative assays for individual enantiomers in *in vivo* samples early in drug development. This will allow assessment of the potential for interconversion and the absorption, distribution, biotransformation, and excretion (ADME) profile of the individual isomers. When the drug product is a racemate and the pharmacokinetic profiles of the isomers are different, manufacturers should monitor the enantiomers individually to determine such properties as dose linearity and the effects of altered metabolic or excretory function and drug-drug interactions. If the pharmacokinetic profile is the same for both isomers or a fixed ratio between the plasma levels of enantiomers is demonstrated in the target population, an achiral assay or an assay that monitors one of the stereoisomers should suffice for later evaluation. *In vivo* measurement of individual enantiomers should be available to help assess toxicologic findings, but if this cannot be achieved, it would be sufficient in some cases to establish the kinetics of the isomers in humans.

Unless it proves particularly difficult, the main pharmacologic activities of the isomers should be compared in *in vitro* systems, in animals and/or in humans.

A relatively benign toxicologic profile using the racemate would ordinarily support further development without separate toxicologic evaluation of the individual enantiomers. If, however, there are toxic findings other than those that are natural extensions of the pharmacologic effects of the drug, and especially if they are unusual or occur near the effective dose in

animals or near the planned human exposure, toxicologic evaluation of the individual isomers in the study where the toxicity was detected should be undertaken.

FDA invites discussion with sponsors concerning whether to pursue development of the racemate or the individual enantiomer. All information developed by the sponsor or available from the literature that is relevant to the chemistry, pharmacology, toxicology, or clinical actions of the stereoisomers should be included in the IND and NDA submissions.

Specific

Chemistry:

The chemistry section of the application should contain the requisite information to assure the identity, quality, purity and strength of the drug substance and drug product. In addition, the following considerations should be taken into account when dealing with chiral drug substances and drug products.

Methods and Specifications

Drug Substance

Applications for enantiomeric and racemic drug substances should include a stereochemically specific identity test and/or a stereochemically selective assay method. The choice of the controls should be based upon the substance's method of manufacture and stability characteristics.

Drug Product

Applications for drug products that contain an enantiomeric or racemic drug substance should include a stereochemically specific identity test and/or a stereochemically selective assay method. The choice of the controls should be based upon the product's composition, method of manufacture and stability characteristics.

Stability

The stability protocol for enantiomeric drug substances and drug products should include a method or methods capable of assessing the stereochemical integrity of the drug substance and drug product. However, once it has been demonstrated that stereochemical conversion does not occur, stereoselective tests might not be needed.

Labeling

The labeling should include a unique established name and a chemical name with the appropriate stereochemical descriptors.

Pharmacology/Toxicology:

Pharmacology

The pharmacologic activity of the individual enantiomers should be characterized for the principal pharmacologic effect and any other important pharmacological effect, with respect to potency, specificity, maximum effect, etc.

Pharmacokinetic Profile

To monitor *in vivo* interconversion and disposition, the pharmacokinetic profile of each isomer should be characterized in animals and later compared to the clinical pharmacokinetic profile obtained in phase 1.

Toxicology

It is ordinarily sufficient to carry out toxicity studies on the racemate. If toxicity other than that predicted from the pharmacologic properties of the drug occurs at relatively low multiples of the exposure planned for clinical trials, the toxicity study where the unexpected toxicity occurred should be repeated with the individual isomers to ascertain whether only one enantiomer was responsible for the toxicity. If toxicity of significant concern can be eliminated by development of a single isomer with the desired pharmacologic effect, it would in general be desirable to do so. The Agency would be pleased to discuss any cases where questions exist regarding the definition of "significant toxicity".

Impurity Limits

It is essential to determine the concentration of each isomer and define limits for all isomeric components, impurities, and contaminants on the compound tested preclinically that is intended for use in clinical trials. The maximum allowable level of impurity in a stereoisomeric product employed in clinical trials should not exceed that present in the material evaluated in nonclinical toxicity studies.

Developing a Single Stereoisomer After the Racemate is Studied

To develop a single stereoisomer from a mixture that has already been studied non-clinically, an abbreviated, appropriate pharmacology/toxicology evaluation could be conducted to allow the existing knowledge of the racemate available to the sponsor to be applied to the pure stereoisomer. Bridging studies would usually include the longest repeat-

dose toxicity study conducted (up to 3 months), and the reproductive toxicity segment II study in the most sensitive species, using the single enantiomer. These studies should include a positive control group consisting of the racemate. If there is no difference between the toxicological profile of the single stereoisomeric product and the racemate, no further studies would be needed. If the single enantiomer is more toxic, the explanation should be sought and the implications for human dosing considered.

Clinical and Biopharmaceutical:

Where little difference is observed in activity and disposition of the enantiomers, racemates may be developed.

In some situations, development of a single enantiomer is particularly desirable (e.g., where one enantiomer has a toxic or undesirable pharmacologic effect and the other does not). A signal that should trigger further investigation of the properties of the individual enantiomers and their active metabolites is the occurrence at clinical doses of toxicity with the racemate that is not clearly expected from the pharmacology of the drug or the occurrence of any other unexpected pharmacologic effect with the racemate. These signals might be explored in animals but human testing may be essential. It should be appreciated that toxicity or unusual pharmacologic properties might reside not in the parent isomer, but in an isomer-specific metabolite.

In general, it is more important to evaluate both enantiomers clinically and consider developing only one when both enantiomers are pharmacologically active but differ significantly in potency, specificity, or maximum effect, than when one isomer is essentially inert. Where both enantiomers are fortuitously found to carry desirable but different properties, development of a mixture of the two, not necessarily the racemate, as a fixed combination might be reasonable.

If a racemate is studied, the pharmacokinetics of the two isomers should be studied in Phase 1. Potential interconversion should also be examined. Based on Phase 1 or 2 pharmacokinetic data in the target population, it should be possible to determine whether an achiral assay or monitoring of just one enantiomer where a fixed ratio is confirmed will be sufficient for pharmacokinetic evaluation.

If a racemate has been marketed and the sponsor wishes to develop the single enantiomer, evaluation should include determination of whether there is significant conversion to the other isomer, and whether the pharmacokinetics of the single isomer are the same as they were for that isomer as part of the racemate.

FEB 2 1995

CSO Application Overview

Application: NDA 20-356
Nisoldipine Coat Core Tablets

Sponsor: Miles Pharmaceuticals

NDA Receipt Date: April 1, 1993

NDA Resubmission Date: August 3, 1994

User Fee Goal Date: February 3, 1995

Date of Overview: November 28, 1994

Background

NDA 20-356 provides for the use of a sustained release (once-daily) formulation of nisoldipine in the treatment of hypertension. No formulation of nisoldipine is currently approved in the U.S.

A non-approval letter was issued on March 25, 1994, that listed deficiencies in the Chemistry, Pharmacology, and Clinical sections. The firm responded fully to this letter on August 3, 1994. In resubmitting the NDA, they

Review

Chemistry

Reviewer: Danute Cunningham

Reviews: 6/7/93 11/29/93 2/4/94 9/16/94

Ms. Cunningham's review of the application has been completed.

The trade name "Nisocor" has been approved by the Nomenclature Committee.

The facility inspection has been completed and was found to be satisfactory. We received a satisfactory response to our FUR on November 22, 1994.

The deficiencies outlined in the environmental assessment review were sent to the firm on October 27, 1994. The response from the firm has not been received yet.

Pharmacology

Reviewers: Xavier Joseph, D.V.M.
Sidney Stolzenberg, Ph.D.

Review: September 2, 1994

The reviewers comments have been incorporated into the draft labeling. The application went before the CAC, and the recommendations from that committee have also been incorporated into the labeling. The minutes of the CAC meeting have not been completed yet.

Biopharmaceutics

Reviewer: Patrick Marroum, Ph.D.

The Biopharm Day was held for this application, revisions have been made, and the draft is now under supervisory review.

Dr. Marroum has made a number of comments including extensive revisions of the labeling (see pp 13-16 of Dr. Marroum's review). The labeling recommendations have been incorporated into the draft package insert. He has also recommended that the dissolution specifications be revised as follows:

from:		to:	
	3 hours 15 to		3 hours
	6 hours		6 hours
	12 hours NLT		12 hours NLT

Statistical

Reviewer: Nancy Smith

Review (hypertension): 1/4/94

Dr. Smith has reviewed only the hypertension indication. There were no serious problems identified in the review.

Clinical

Reviewers: Shaw Chen, M.D., Ph.D. (Clinical Pharmacology): 2/16/94
Phil Dern, M.D. (Safety): 9/27/93
Cristobal Duarte, M.D. (hypertension): 8/4/93
Norman Stockbridge, M.D., Ph.D. (angina): 8/4/93

The reviewers recommend approval for the hypertension indication.

The final safety update is under review.

DSI Audits

Four of the seven requested DSI audits are completed. There have been no problems so far.

Labeling

Dr. Chen has provided a marked up copy of the package insert.

David Roeder
Consumer Safety Officer

dr/9-4-94/9-27-94/10-28-94/11-23-94/11-28-94

cc: NDA 20-356
HFD-110
HFD-111/DRoeder

Roeder

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

Date: 10/25/94

From: Shaw T. Chen, M.D., Ph.D., Medical Group Leader, HFD-110
Through: Director, Division of Cardiorenal Drug Products, HFD-110
To: Director, Office of Drug Evaluation I, HFD-100

NOV 21 1994

SUBJECT: NDA 20-356, Nisoldipine Core-Coat for Hypertension, Approvability

OVERVIEW

This memorandum and the attached material constitute the Division's recommendation that NDA 20-356, Nisoldipine Core Coat (referred to as CC formulation) Tablets be approved for treatment of hypertension.

This package is being transmitted with a draft Summary Basis of Approval (SBA) prepared by the sponsor, which has not been edited by the Division but appears to be accurate in its contents to serve as one of the references for secondary/tertiary reviews of the application. In the draft SBA, any description or interpretation of the data different from that of this memo should be disregarded.

As one of the new team approaches, the primary medical review of the NDA were conducted in parallel by the following medical officers:

Clinical Pharmacology:	Dr. Chen
Hypertension -Efficacy:	Dr. Duarte
Hypertension -Safety:	Dr. Dern

Pharmacology sections of the application were also reviewed concurrently by two reviewers (Drs Joseph and Stolzenberg); a synoptic summary of all pharmacologic issues has been prepared by Dr. Joseph. As of the date of this memo, the chemistry, biopharmaceutical, pharmacological and statistical reviews have been completed. There are no major, unresolved preclinical issues which may affect the action recommended. Related labeling have been suitably edited.

Nisoldipine is a new calcium channel blocker of the dihydropyridine type and structurally related to nifedipine. It appears to be a less active inotrope than nifedipine *in vitro* but the two were not distinguishable in intact animals. There are no major efficacy or safety issues that should preclude the approvability of this drug for the hypertension.

The adverse experiences in the NDA have been amended with the First Safety Updates of 08/17/93. Selected major trials should be inspected before final approval of the application.

PRECLINICAL EVALUATIONS

Chemistry

There are no outstanding issues regarding the manufacturing and analytical controls. Final inspection will be scheduled.

Preclinical Pharmacology

Nisoldipine has been adequately characterized with respect to its preclinical pharmacokinetic and pharmacodynamic properties. There are no outstanding issues related to animal toxicity or carcinogenicity which may affect approvability of the drug.

Changes in proposed labeling, as recommended by the pharmacology reviewers, are summarized and commented below. They have been adopted with minor modification.

- Negative findings in carcinogenic studies should be qualified with the dosages studied, comparison with human dose should be based on both body weight and surface area calculations.
- Fetotoxicities in animals are suggestive, not conclusive. However, detailed description of the problematic monkey studies is not necessary. Again, basis of safety margin (toxic animal dose vs maximal human dose) should be specified (body weight and surface area).
- The pharmacology reviewers do not think malformation is increased in rabbits. Other recommendations related to fetotoxicity in rats/rabbits, sections of *Labor and Delivery*, and *Nursing Mothers* are all appropriate (Pharmacology Review, p 145).

CLINICAL PHARMACOLOGY

Pharmacokinetics/Pharmacodynamics

At the proposed dosages of 10-40 mgs, the pharmacokinetic profile of nisoldipine CC formulation supports a once-daily regimen. Compared with the immediate release (IR) form, availability of nisoldipine from the CC tablets was prolonged with lower C_{max} and higher AUC over 24 hrs. Bioavailability of nisoldipine CC was low for the unchanged drug but linear and dose-proportional over the range of 10-60 mg; it accumulates moderately after multiple oral dosing (7 days). While nisoldipine is extensively metabolized, the only active metabolite contributes about 10% of the pharmacologic effects.

The states of both hepatic and renal functions are potentially important for pharmacokinetics of the active drug, since nisoldipine is extensively metabolized and excretion of the metabolites is predominantly renal. Bioavailability of the parent drug was indeed increased by 4-5 fold in patients with hepatic failure, but changes in AUC and C_{max} due to various degree of renal impairment were only transient and diminished with multiple dosing. Plasma levels of nisoldipine were also higher in the elderly but dosage adjustment may not be required (see Efficacy -Hypertension). Nisoldipine metabolism probably involves P_{450} cytochrome system (as nifedipine), but no attempt to identify isozyme has been documented. Modest changes in bioavailability of CC nisoldipine were observed with

concomitant use of ranitidine (decreased 15-20%), cimetidine (increased 30-45%), quinidine (reduced by 25%), and propranolol ($t_{1/2}$ shorter by 20%). These interactions are probably of no significant clinical consequences, but labeling has been edited accordingly.

As noted in the Clinical Pharmacology and Biopharmaceutical Reviews, the problem of dose-dumping when nisoldipine CC is administered in a non-fasted state or with grapefruit juice (see biopharm review of Study 770) can not be ignored. Nisoldipine CC should not be administered concomitantly with meal or grapefruit juice, but instead after overnight fast and 1-2 hours before breakfast. Appropriate instructions to avoid dose administration in such settings have been included in the labeling.

Nisoldipine is a vasodilating antihypertensive with pharmacodynamic activities similar to other approved calcium channel blockers. Cardiovascular and hemodynamic effects of nisoldipine have been fairly well-established. Correlation between nisoldipine dose, plasma level and blood pressure reduction was good over the recommended dosage range.

Nisoldipine has no appreciable inotropic effects, but its clinical advantages over nifedipine has not been documented. Except for T-wave changes mostly at high doses (see Safety), nisoldipine had minimal electrophysiology activities. There is some evidence that iv nisoldipine improves coronary blood flow, but its anti-ischemic effect was not established in clinical pharmacology studies.

Nisoldipine did not affect regional blood flow in kidney or liver, and has no significant pharmacologic activities on non-cardiovascular systems.

Biopharmaceutics

Issues raised in the Bio-pharmaceutical Review are commented as follows.

- The ratio of two enantiomers (and other metabolites) in special patient groups were not determined. Since no surprising clinical effects were observed in these patients which may required explanation, such data are not relevant for approval or prescription instruction.
- Nisoldipine is metabolized by the P-450 enzyme system, but the specific iso-enzyme involved has not been identified. While metabolism of nisoldipine is not expected to be significantly different from that of nifedipine, the study should be done post-approval, however.
- Inconsistent C_{max} (by 2-folds) obtained after a 30 mg dose in two small pharmacology studies may be related to the variability in dissolution and need further clarification, as different blood pressure reductions from placebo were also noted in the efficacy trials (see below). Since no efficacy/safety problems were attributed to this variation, approval is not affected.
- Assay validation was described in most of the studies, but missing in a few reports. It is reasonable to assume that same assay was used in all trials.
- Variations in dose-proportionality in two Phase II studies were small and of no clinical significance.
- Pharmacokinetics and metabolism sections of the labeling have been edited to accommodate the recommendations of Biopharmaceutic Review.

CLINICAL: EFFICACY

Major Trials Supporting Approval

Nisoldipine has been evaluated as an antihypertensive treatment in 1,914 patients at dosages up to 80 mg/day. The efficacy data supporting approval were derived from the results of 7 double-blind, parallel placebo controlled studies in 1,360 patients with hypertension, 886 of whom received nisoldipine. Long-term efficacy was supported by five open-label, 6-12 month follow-up of 554 patients (Studies X89-039, X90-019, X90-006, 675, 690).

The primary efficacy endpoint in each of these studies was the change in supine diastolic blood pressure (SDBP) from baseline at the end of dosing interval (trough effect) after 4-9 weeks of therapy. Data from five of the seven studies should be considered for major evidence of efficacy:

Study	Doses, mg/day	Duration		Remarks
D88-054	10, 20, 30	QD	4 wks	fixed dose
D89-026	10-40	QD	9 wks	dose titrated per response
D90-019	30, 60	QD	6 wks	fixed dose (after week 1)
D89-039	20, 40	QD	8 wks	fixed dose (after week 1)
D90-006	10, 20, 30	QD	6 wks	fixed dose (after week 1)

In the last three trials listed above, high doses were phased in after one week of low dose treatment. It should be noted that in Study D89-039, another group of 15 patients were randomized to receive nisoldipine CC 80 mg qd, but the arm was terminated due to safety concerns before collection of efficacy data. Nisoldipine was also compared with verapamil 240 mg (additional group of 78 patients) in this parallel placebo controlled study.

The remaining two controlled studies may provide instructions on how to use nisoldipine in a practical setting, but are not very useful as primary evidence for assessing efficacy of nisoldipine (vs placebo in general population who are not treated with other concurrent antihypertensive agents). Nisoldipine was evaluated in patients all receiving atenolol 50 mg qd as background therapy in one study (D89-029), and in the other (Study D90-029), lisinopril, hydrochlorothiazide (HCTZ) and placebo were compared in the presence of nisoldipine in all groups. Results of these studies will be commented in the Section of "Comparison/Combination with other Antihypertensives".

Overall Treatment Effects vs Placebo

The primary efficacy data in Table 1 on Page 6 demonstrate that nisoldipine, at 20-60 mg qd, is a consistently and significantly more effective antihypertensive agent than placebo with adequate duration of activity for once daily treatment. At this dose range, the placebo-subtracted net decreases in SDBP at trough ranged from 3.6 to 9.9 mmHg after 4-9 weeks of therapy. Treatment effects were less consistent for the 10 mg dose, but was superior to placebo in the larger trial (D90-006) with a decent drop in SDBP. Similar results were obtained for supine systolic blood pressures (SSBP) (same Table) and standing blood pressures (excluding the smallest trial, D88-054, results not shown in this memo).

The **percentages of responders** (SDBP reduction of ≥ 10 mmHg at trough or to ≤ 90 mmHg) are also summarized in Table 2 on next page. In the major trials, the response rates for 20-60 mg/day were in the range of 17-45% more than that of placebo. Again, less patients (around 17% over placebo) responded to 10 mg dose.

With respect to blood pressure changes and response rate, there were no significant differences between various statistical analyses, i.e. per-protocol or intent-to-treat (final visit).

Dose Response

The **dose-response** relationship, at trough, has been examined within the range of 10-60 mg once daily (Tables 1 & 2, in the associated Figure 1, % response curve was shifted on the dose axis for clarification). While dose of 10 mg/day was not consistently better than placebo as monotherapy, it is appears that blood pressure reduction may increase further at doses above 60 mg (but not for % responders). Although there are evidence from small pilot studies of hypertensive patients that dose-response for 30-90 mg/day was rather flat (Study D90-022, see Clinical Pharmacology Review), the finding should be accepted with reservation because dosages were forced-escalated rapidly in that study. There was a concern, also in the same early phase study, of asymptomatic T-wave changes at high doses (see Safety below). However, when doses were increased slowly as in efficacy trials, less patients reported the same abnormality. Besides, such ECG changes were common in hypertensive patients and their clinical meaning are not yet clear. Thus, effective doses of nisoldipine CC range from 20 to 60 mg once daily, with a weak support for the high-end limit.

Correlation between blood nisoldipine level and blood pressure reduction has been demonstrated at trough in several clinical trials.

Time-Effect Relationship

While only once-daily regimen was used in clinical trials and no direct comparison with other dosing schedule was performed, appropriate **dosing interval** for nisoldipine was established in the following studies:

	Studies	
Peak/Trough Effect	D88-054, D90-019, D89-039, D90-006	10-60 mg/day, QD
24-hour BPs	D88-054, D90-019, D89-039, D90-006	10-60 mg/day, QD

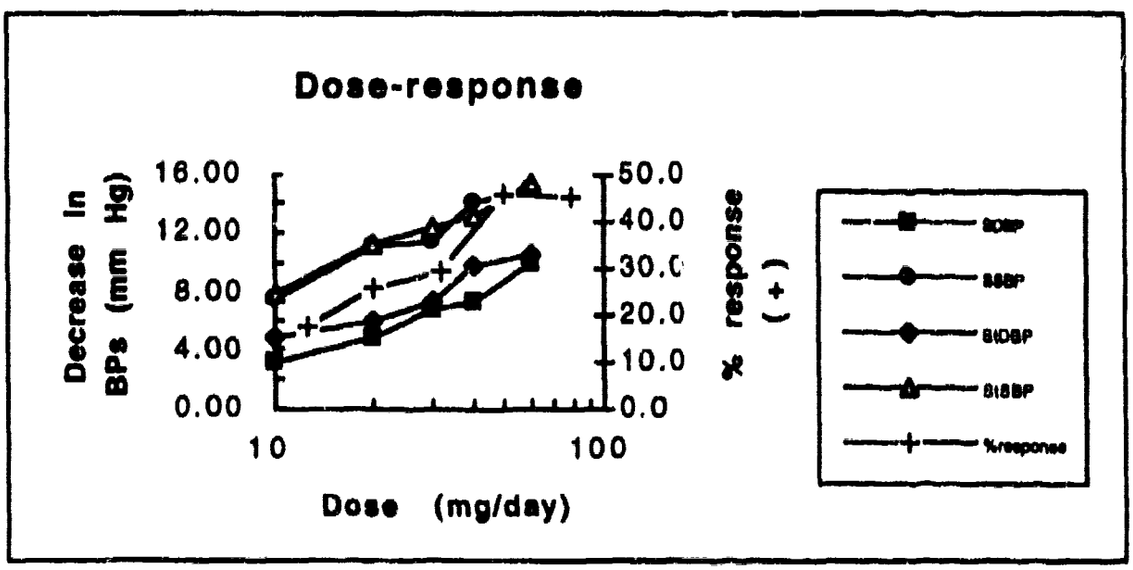
For the doses studied, the placebo-subtracted **trough-to-peak** ratios appeared to be acceptable, ranging from 70 to 100% for SDBP and SSBP.

NISOLDIPINE Hypertension Efficacy							
Final visit, Placebo subtracted,			[redacted]	are significantly different from placebo			

TABLE 1: Change in supine BPs from baseline at trough									
		Last		regimen:					
Analy-		Visit	mg/day:	10	20	30	40	60	10-40
sis	protocol	Wks	N/arm						titrated
SDBP	D88-054	1-4	30	2.96	[redacted]				
ITT	D89-026	1-9	72						[redacted]
	D90-019	1-6	74			[redacted]		[redacted]	
	D89-039	1-8	76		[redacted]		[redacted]		
	D90-006	1-6	52	[redacted]	[redacted]				
	wtd avg		SDBP	3.12	4.81	6.70	7.33	9.91	
SSBP	D88-054	1-4	30	5.31	[redacted]				
ITT	D89-026	1-9	72						[redacted]
	D90-019	1-6	74			[redacted]		[redacted]	
	D89-039	1-8	76		[redacted]		[redacted]		
	D90-006	1-6	52	[redacted]	[redacted]				
	wtd avg		SSBP	7.55	10.98	11.44	13.96	14.90	

TABLE 2: Response Rate (trough SDBP < 90 or drop by > 10)									
		Last		regimen:					
Analy-		Visit	mg/day:	10	20	30	40	60	10-40
sis	protocol	Wks	N/arm						titrated
ITT	D88-054	1-4	30	16.7	16.7	[redacted]			
	D89-026	1-9	72						[redacted]
	D90-019	1-6	74			[redacted]		[redacted]	
	D89-039	1-8	76		[redacted]		[redacted]		
	D90-006	1-6	52	17.5	[redacted]	[redacted]			
	wtd avg		%respons	17.2	25.8	29.0	45.8	44.8	

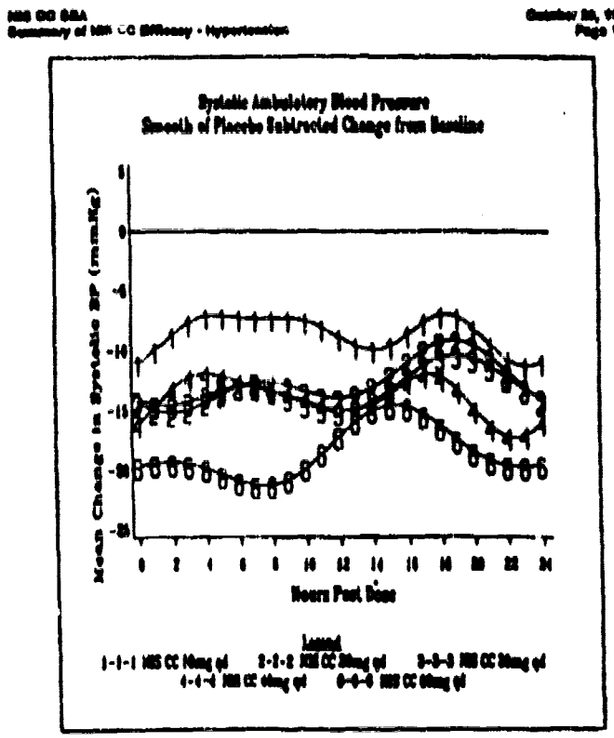
Figure 1

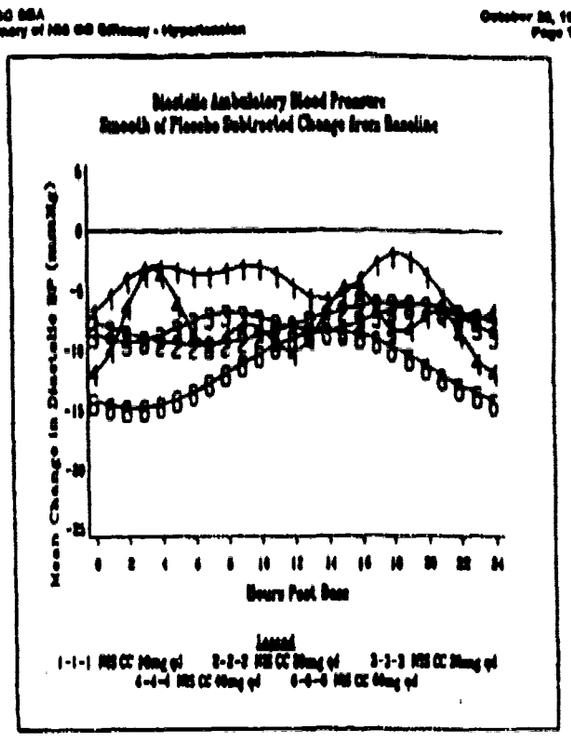


Total of 359 patients from the listed studies were pooled for analysis of 24 hours ambulatory blood pressure change, the majority were white (65%) and male (60%). As shown by the 24-hour blood pressure curves, treatment effects of at least 5 mmHg reduction in SDBP over placebo were maintained during 24 hours for doses 20 mg and above (see Figure 2 below).

It is concluded that although other dosing schedules have not been evaluated, once-daily treatment with nisoldipine CC 20-60 mg per day appeared to be adequate to cover the dosing period.

Figure 2





Responses in Demographic Groups

In post hoc analyses (see draft SBA), nisoldipine appeared to be equally effective in male/female, with a slightly more pronounced dose-response relationship in the male patients. Despite increased bioavailability in the elderly, dose-response was less evident in such patients and there were no significant differences in blood pressure reduction between groups of age below and above 65 years. While blood pressure responses to nisoldipine were numerically greater in black than in white patients, such retrospective finding should not be described in the labeling or used in promotion. Not surprisingly, response to nisoldipine was greater in patients with higher baseline blood pressure, with a more significant dose-response relation.

Comparison/Combination with other Antihypertensives

Nisoldipine was compared or combined with the following antihypertensive agents in 3 controlled trials.

Comparison groups			Studies
nisoldipine+atenolol	vs	placebo+atenolol	D89-029
nisoldipine+lisinopril	vs	nisoldipine+placebo	D90-029
nisoldipine+HCTZ	vs	nisoldipine+placebo	D90-029

While results of the first study listed above indicated that concomitant atenolol did not affect the efficacy of nisoldipine CC, the second study suggested that some patients may have further response when a diuretic or ACE inhibitor is added. Up to one third of all patients received additional antihypertensive therapies in long-term, uncontrolled, follow-up studies. Overall, not much weight can be placed on these active controlled data for the efficacy claim.

Long-Term Efficacy

Long-term effectiveness of nisoldipine was evaluated in five open-label studies up to one year. Without a placebo control, reductions in supine blood pressures from baseline appeared to be sustained in more than 80% of 554 patients treated with nisoldipine for 6 months to one year

CLINICAL: SAFETY

Database

The database appeared to be adequate for analysis of the safety of nisoldipine, which includes cumulative experiences of nearly 4,200 hypertensive patients as of 10/29/93. Of 1,466 patients# (921 in US trials) who were treated with nisoldipine Coat-Care formulation, about 55% were exposed for at least 2 months (approx. 33% over 6 months) and a great majority were on doses of 20 to 60 mg.

The majority of comparative experience was based on the results of 6 randomized, double-blind, parallel group, placebo-controlled trials of 4-9 week duration (all U.S. double-blind controlled trials, see list in Efficacy Section)*, which included 678 patients on nisoldipine and 280 patients on placebo.

Data from the first 120-day Safety Update were not incorporated into the following summary, however, the numbers added were small and did not change the safety profile of the drug (see Reviews of Safety Update by Dr. Dern).

Comparative Experiences

There were no surprising findings in the safety profile of nisoldipine CC used in hypertensive patients. Overall frequency and rates of some specific adverse clinical experiences and abnormal laboratory findings were more common in nisoldipine than placebo treated patients, but none were serious or unexpectedly frequent.

The percentage of nisoldipine-treated patients reporting an **adverse event** in controlled trials (68%, N=678) was higher than that in the placebo group (53%, N=280). Among the adverse experiences, the following were more common for nisoldipine than placebo with incidence of ≥ 3%:

ADE	Nisoldipine(%) N=678	Placebo(%) N=280
peripheral edema	22	10
headache	22	15
dizziness	5	4
asthenia	4	4
vasodilatation	4	2
palpitation	3	1

\# From draft SBA. Different numbers of trials and patients exposed were given in Integrated Summary and Draft SBA, the later is probably more updated. Some calculations shown below were based on data from Integrated Summary of Safety.

* Foreign data also included some placebo-controlled safety experiences (D90-006). However, a great majority of the non-U.S. studies were not controlled and thus were not considered in comparative experiences. Nisoldipine was used in all treatment groups in one U.S. study (D90-029), but results of that study were not excluded from the comparative analysis.

As expected, the most commonly reported adverse events were related to nisoldipine's vasodilating effects. Most were mild and infrequently leading to withdraw.

While the overall frequency of adverse experiences was not affected significantly by patient age, sex, race, or body weight in the controlled trials, some minor differences in the incidence of a few adverse events may change the tolerability of nisoldipine in demographic subgroups. Headache appeared to be less common in the elderly, which would be surprising if the adverse event is pharmacokinetics-related (see Clinical Pharmacology). Peripheral edema was more frequent in female (as suspected with other dihydropyridine agent) and heavier patients (>185 lbs), but the sex difference was only seen in foreign studies, not in the U.S. controlled trials. Compared to blacks, incidences of headache and edema were slightly higher in whites.

The percentage of nisoldipine-treated patients withdrawn due to adverse clinical experiences was higher than that of placebo (7.8 vs 3.2%, U.S. controlled trials only), and dose-related (up from 5.4% at 10 mg to 10.9% at 60mg). The reasons for withdrawal were mostly related to nisoldipine's pharmacologic activities and within the scope of common adverse experiences:

Reasons for withdrawal	Nisoldipine(%) N=678	Placebo(%) N=280
headache	3.8	0.4
peripheral edema	2.9	0.4
vasodilatation	1.5	0.0
nausea	0.9	0.0
palpitation	0.9	0.0
dizziness	0.7	0.4

There were 2 deaths (car accident and metastatic prostate cancer) in nisoldipine-treated patients in controlled trials (non-U.S. studies only), compared with two deaths in the placebo groups. None were considered drug-related. Other serious events occurred with similar frequencies in nisoldipine (2.0%) and placebo group (1.5%). However, they are dose-related (increased from 0.2% at 20 mg to 4.5% at 60 mg) and half of these serious events led to withdrawal.

Abnormal laboratory findings in controlled trials were both rare and no different between nisoldipine and placebo groups. In U.S. controlled trials, incidences of such reports were in the range of 0-4% for hematology, 0-2% for hepatic functions, 0-1% for creatinine/BUN and 0-6% for lipid profile. While there were more reports of increased fasting blood glucose in nisoldipine than in placebo group from non-U.S. controlled trial, the phenomenon was not dose-related, not seen in the U.S., and cases of increases to above 140 mg/dl were not more frequent (than placebo).

Overall Exposures

In general, the overall safety experiences in all patients treated with nisoldipine in all clinical trials were not unexpectedly different from those described above for controlled trials.

Approximately 62% of all patients reported one or more **adverse events**, while the incidence was lower in European trials (43% vs 75% in U.S.). Prominent complaints were similar to those in controlled trials (e.g., 18% headache, 15% edema in U.S. Trial).

In all clinical trials, about 9% were **withdrawn** due to adverse experiences (10% in U.S. studies), not too different from that of comparative experiences. There was no additional death other than those noted above in the comparative experiences. Accumulative experiences of abnormal **laboratory** findings in all U.S. studies were also similar to that in the controlled trials.

Class Specific Safety Issues

As noted above, adverse experiences relatively specific to calcium channel blockers were also reported in nisoldipine-treated patients. They were not more severe or frequent than in other members of the class; however, the database may not be large enough for detecting some of the rare events.

Clinically significant **hypotension** and other related adverse experiences in nisoldipine treated patients were not common and rarely resulted in withdrawal. As described earlier in time-effect relationship, at doses that produced adequate trough blood pressure reduction, average peak response was not excessive. In all U.S. and non-U.S. trials, symptomatic hypotension occurred in about 0.2% and syncope was reported in 0.1% of patients. **Orthostatic** hypotension and related symptoms were slightly more common, reported in approx. 0.4% of patients on nisoldipine monotherapy, but very few were considered serious and required intervention. Overall, hypotensive reactions to nisoldipine treatment did not appear to be more frequent or severe than those with other calcium channel blockers. Appropriate warning related to hypotensive reaction is included in the draft labeling.

Like other dihydropyridines, nisoldipine has no significant effects on electrophysiology or **cardiac rhythms**. Tachycardia was reported in about 1% of all nisoldipine-treated patients, with a small mean changes in heart rate (< 1bpm, placebo-adjusted). It is most likely due to hypotensive reflex, rarely led to withdrawal, and occurred equally frequently in placebo groups. Some minor changes in ECG (QRS) were noted more frequently than that in placebo group, especially in patients receiving concomitant atenolol, but the magnitudes were of no clinical meaning. While dose (plasma level) and magnitude of BP reduction-related **T-wave flattening/inversions** were observed in a small phase II study (D90-022) with rapid dose escalation, such ECG finding was less clearly related to dose and not as frequent (similar to that in placebo groups) in a retrospective but blinded analysis of data from three efficacy trials. It is somewhat re-assuring that no angina or thallium test-documented ischemia were reported in any of the patients with T-wave changes in Study D90-022.

Limited experiences with concomitant use of nisoldipine and atenolol, lisinopril or HCTZ have not identified any unexpected safety or tolerability issue. Combination of nisoldipine with HCTZ or lisinopril may increase slightly the incidences of asymptomatic hypotension, tachycardia, palpitation and dizziness. Rebound hypertension after withdrawal has not been a problem with other dihydropyridines and was not significant in a small pharmacodynamic study for nisoldipine.

PEDIATRIC/GERIATRIC USE

There are no clinical trials assessing the efficacy or safety of nisoldipine in pediatric patients, either completed or in progress. The sponsor claimed that the drug has little potential for use in children and thus did not commit to any study in hypertensive children.

Efficacy and safety of nisoldipine as treatment for hypertension in the elderly (65 year and older) are not significantly different from that of general patient population.

DRAFT LABELING

The draft labeling submitted by the sponsor has been edited.

CONCLUSIONS

Nisoldipine appeared to be an effective and safe treatment for hypertension.

While there is little doubt that nisoldipine at 20-60 mg/day is an antihypertensive more effective than placebo, it is not certain if the entire useful dose range has been fully explored. Nisoldipine should be started at 10 mg once daily and titrated slowly (e.g. every few weeks) to 60 mg according to blood pressure response.

It is recommended that nisoldipine be approved with the edited draft labeling.

Shaw T. Chen, M.D.,Ph.D.

cc:
ORIG: NDA- 20-356
HFD-110
HFD-110/CSO
HFD-110/SChen/10/26/94

Roeder

DEC 19 1994

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

Date: 12/15/94

From: Shaw T. Chen, M.D., Ph.D., Medical Group Leader, HFD-110
Through: Director, Division of Cardiorenal Drug Products, HFD-110 Lipicky
To: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: NDA 20-356, Nisoldipine Core-Coat for Hypertension, Approvability

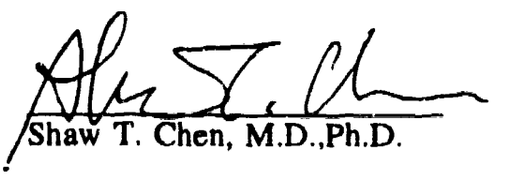
This is in response to the comments and questions raised in your draft memo of 12/13/94 regarding some labeling issues for the above application.

1. As stated in the Secondary Review (Dose Response), we also think that the effective dosage range is 20-60 mg/day and the recommended doses should include 60 mg. Usual maintenance doses in the labeling were changed to 20-40 mg in the Division's draft, which were concurred in your memo. We agree with your reasoning that dose titration should start at 20 mg.

2. With respect to worsening of angina, we totally agree that nisoldipine is no different from other dihydropyridine type calcium channel blockers and it is neither a safety nor an approvability issue. In the controlled angina trials, there was no significant increase in angina attack rate and worsening of angina was not a frequently reported event in hypertension studies. As you noted, the open label, non-controlled data in angina studies were not much useful for us to draw any conclusion. This was not discussed in details in the secondary review because the sponsor had withdrawn the angina claim after the primary review was completed and it was not clear then to me that we could describe the angina data for review of hypertension indication.

3. We understand that the effects of food and grapefruit on the kinetics of nisoldipine CC are different. They were put together only in "Information for Patients", as they are both "food". The wording in your marked-up draft certainly described the problem much clearer.

4. A cleaned-up draft of package insert with your mark-ups has been prepared.



Shaw T. Chen, M.D.,Ph.D.

cc:
ORIG: NDA- 20-356
HFD-110
HFD-110/CSO
HFD-110/SChen/12/15/94

Koeder

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

Date: 12/22/94

From: Shaw T. Chen, M.D., Ph.D., Medical Group Leader, HFD-110
Through: Director, Division of Cardiorenal Drug Products, HFD-110 Lipicky
To: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: NDA 20-356, Nisoldipine Core-Coat for Hypertension, Labeling

We did not get to see your memo in final prior to responding to your memo. There was one question that you asked that was not answered in our response of 12/17/94.

There were 2 "food studies" conducted by Miles. One of them was not the FDA "high fat" and studied only the 20 mg tablet (Study 666, it was more than average but not high) and in that study there was no evidence of "dose-dumping'. The other study was conducted using the FDA "high fat" meal and in that study there was dose dumping. However, in this study (D92-045-02), 30 and 40 mg tabs were used and the 20 mg dose was not repeated. The food effect on the C_{max} was average 3 fold, and 5 of the 28 subjects had 5-11 fold changes. Thus either the fat content in food is important or dose-dumping by food may be dose-related.

Miles did conduct a pharmacokinetic/pharmacodynamic study, a review of that study was done by Dr. Marroum, in which they found that the pharmacodynamic effects (lowering of blood pressure) was a function of the log of the plasma concentration. So ten-fold changes in plasma concentration make a sizable difference, three-fold changes do not make a big difference. The slope of the concentration-response curve goes over 2 order of magnitude from beginning of effect to definitely over the maximum effect.

Although Dr. Marroum's review criticized the analysis of the study, the qualitative statements above are not materially affected by the quantitative problems that Dr. Marroum found.

So, it seems to us that there is considerable latitude that can be given with respect whether nisoldipine **must** be taken fasting. We do not think it must be taken fasting. To be silent about fasting or fed in the Dosage and Administration section is reasonable but since there are more than 5-fold changes in C_{Max} in 18% of subjects, the Dosage and Administration should probably say "..., preferably in a fasted state (see Clinical Pharmacology)". In Clinical Pharmacology the 11-fold increase in C_{Max} should be stated to be an upper limit when a High fat meal is ingested.

or even other alternatives as we discussed in the FM of 12/22/94. SC

Shaw T. Chen, M.D.,Ph.D.

cc:
ORIG: NDA- 20-356
HFD-110
HFD-110/CSO
HFD-110/SChen/12/22/94

M E M O R A N D U M

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: DEC 16 1994

FROM: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: Nisoldipine Core Coat (NISOCOR, NDA 20-356, Miles)

TO: Dr. Raymond Lipicky, HFD-110

Nisoldipine appears to be a typical dihydropyridine CCB, developed sensibly as a controlled release product. It is possible to argue that doses above 60 mg/day could have larger effects, but the ADE rate seems already to be rising at that level (see, e.g., MOR angina p. 15-16) and I doubt higher doses would be appealing or useful. I do believe the 60 mg dose is well-enough tolerated to be included in the recommended dose range. I have a few questions in addition to comments/questions on labeling.

1. Dose

The proposed D&A was for a starting dose of 10 mg, with titration up to 40. As indicated, I believe 60 mg should be included. Where this dose was studied (90-019, 89-029) it had a numerically larger effect and was adequately tolerated.

The basis for starting at 10 mg is not clear to me. In study 89-026, few patients (5/79) stayed at 10 mg, and not many stayed at 20 (5/79). Starting doses of at least 20 mg were used in studies 88-054, 90-019 (30 mg), 89-039, and 89-029. Only in studies 89-026 (titration) and 90-006 did all patients get 10 mg to start. As 1) the overall response rate at 10 mg is low, 2) there seemed to be no adverse consequence of starting at 20-30, alone or added to a beta-blocker, and 3) extra titration steps are costly and can discourage therapy, I suggest a recommended starting dose of 20 mg, with reductions in the elderly and patients with hepatic dysfunction.

I believe the usual maintenance dose will not be 20-30 mg as proposed but 30-40. In the titration study almost all patients reached 30-40 mg. I was first inclined to change the expected maintenance to 30-40 mg, but the mean effect (especially added to a beta-blocker) of 20 mg was respectable.

Labeling should clearly show D/R of ADE's.

I note that in all studies the relationship of dose to meals was not specified. Is there really need for taking nisoldipine on an empty stomach an hour form meals, as labeling now requires or could this be at least softened somewhat by reference to the trial results? Note also that the extreme effect of food occurred in only one of two studies.

2. Worsened angina

Dr. Stockbridge noted concern about worsened angina. Certainly, all dihydropyridines have been associated with this, presumably resulting from a combination of increased HR and decreased diastolic (coronary perfusion) pressure. Unless I am missing something, however, nisoldipine does not seem unusual. Acute worsening was infrequent, about 1%. I have tabulated the cases of worsening in the controlled phases of the four angina trials, including AMI, increased chest pressure or angina(A) unstable angina(UA) etc. (I called the event UA only if the specific term was used.) I get this:

Study	P1	N10	N20	N30	N40	N60
88-060	4008(A)	1109(A)*	10002(D AMI)	21013(A)		
89-042			5232 (AMI)			
90-010	403(A) 773(UA) 1103(AMI)		416(A)		307(A) 707(A)	
90-015	6004(A)		6013(A) 16014(A)		2008(A) 9016(UA) 36006(A)	28001(A)

* = this patient also had increased AF, which could account for worse angina

I would say there seems no clear evidence here of stimulated worsening by nisoldipine. I note also that the people studied were fairly ill as judged the frequent history of MI and vascular procedures (see following table) and the poor outcome in study 90-015 during the run-in phase (4 deaths).

	88-060	89-042	90-010	90-015
n-single blind lead-in	390	378	408	483
n-randomized	271	302	303	312
prior MI (%)	44-53%	44-53	52-62	40-49
prior CABG (%)	20-36%	14-17	16-20	24-29
prior angioplasty (%)	11-16%	6-15	6-9	19-27
hypertension (%)	37-48%	--	22-32	42-54
diabetes (%)	11-19%	--	12-20	15-19
deaths in run in (2-3 week)	0	0	0	4/483=0.8%

I read Dr. Dern's review after writing the above. He too (p.20-21), I believe, finds no excess (vs placebo) of discontinuations due to worsening angina or AMI on nisoldipine, although he does not necessarily interpret the results as I have. If we take the combined U.S. and non-U.S. angina trials we have:

	Nisoldipine		Placebo	
	n	rate	n	rate
U.S.	438	--	145	--
worse angina	10	2.3	2	1.4
AMI	2	0.5	0	0
NON-U.S.	447	--	189	--
worse angina	3	0.7	1	0.5
AMI	2	0.4	1	0.5
COMBINED	885	--	334	--
worse angina	13	1.5	3	0.9
AMI	4	0.5	1	0.3
Both	17	1.9	4	1.2

All in all, I find it hard to see evidence of increased AMI rate in this (that would be worrisome) and the worsened angina rates also seem very close. In the U.S. trials, the source of the higher rate on nisoldipine, most of the excess is due to the 40 mg group, with lower rates at 20, 30, and 60 mg. I wrote the angina numbers into the labeling (Warning). Despite the small numbers and small differences, Dr. Dern's observation that many of the worsened angina events occur early in the U.S. trials, is worth noting.

It is only in the open label studies that the frequency of worsened angina or AMI/death climbs (to 17%) and it is hard to know the correct comparison for this. There were 2 open-label deaths, surely not too surprising in light of 4 placebo run-in deaths in study 90015. There were 10 open-label AMI's among 305 patients in long-term trials, or 3%. Dr. Stockbridge notes 10 AMIs among 479 nicardipine patients, obviously in the same range as nisoldipine, and 13 AMI deaths in diltiazem long-term trials of 737 people, a 1.8% rate that does not include non-fatal AMIs. All of these numbers would require substantial refinement (patient characteristics, duration) to be used rigorously, but even the crude comparisons make it seem fairly clear that the nisoldipine long-term results are similar to those of nicardipine (another dihydropyridine) and diltiazem with respect to coronary event rate. I am not sure we have useful data for these drugs with respect to the worsened angina/unstable angina question.

I should note that the exacerbated angina/progression question is not related only to If we really do have evidence that nisoldipine causes "hastened progression of underlying CAD" (MOR p.29), we probably should not be approving it for hypertension either (although Dr. Dern makes the case that the history in hypertensive patients in very benign). I do not, however, see any evidence of such hastened progression. I have no doubt that CCB's, or at least dihydropyridine CCB's, can sometimes exacerbate angina, but that is more a matter of labeling, as it is a reversible phenomenon, known to us from other CCB's, and certainly did not seem prominent in the hypertension trials.

3. The effects of food and grapefruit juice are different. Food increases the Cmax but decreases the AUC, i.e., it causes dose-dumping but no apparent showing of systemic metabolism. Grapefruit juice increases both the Cmax and AUC, as would be expected of a substance that blocks metabolism of nisoldipine (as it blocks metabolism of nifedipine, nitrendipine, felodipine and, probably, all dihydropyridine CCB's).

Robert Temple, M.D.

Medical Officers Review

JUL - 7 1994

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
MEDICAL OFFICER'S NDA REVIEW

NDA: 20- 356

DRUG: Nisoldipine

SPONSOR: Miles

DATE SUBMITTED: 17 August, 1993

DATE REVIEWED: 7 July, 1994

REVIEWER: Philip L. Dern M.D.

RESUME:

This is a 120- day safety review and includes both completed and uncompleted trials, foreign and domestic. For uncompleted trials, data is still blinded and treatment is listed as "either drug 1 or drug 2" if these are the possibilities for a particular patient.

I. Deaths
A. Completed US studies

One death is recorded for this update and was also included in the NDA for an on-going trial.

Study # D90- 029-06; Pt 6004.

This 44- yr- old female with a qualification, single- blind BP of 190/111 and a randomization BP of 141/97 died suddenly at home on day 24 of the study. She was on NIS 40 mg qd and HCTZ 25 mg qd. Serum potassium at baseline and last visit were, respectively, 3.9 and 3.6 mEq/l. Autopsy revealed moderate coronary atherosclerosis without thrombi.

B. Completed Non- US studies

Study 752. No deaths.

C. On-going Studies (all non- US)

Study 764; Pt 128.
This 83-yr- old female was being treated with either NIS or lisinopril (LIS). She died at home of acute pulmonary edema and has an associated abdominal infarction.

Study 769: pt 16002.

This 53 yr- old male was being treated with either NIS or atenelol (ATN). The patient had a TIA on 9/91. Baseline BP was 162/105 after three weeks of placebo. He was admitted to hospital on 5/18/92 after 53 days of treatment. He died the following day of a CVA. BP at last clinic visit was 177/83.

Study 769; pt 17013.

This patient, a male aged 73, was being treated with either NIS or ATN. After 5 days of therapy the patient developed diarrhea, nausea, and vomiting, and, three days later, a fatal MI.

Study 769; pt 54002.

The patient, a male aged 66, was on either NIS or ATN. Baseline BP was 155/93. After 25 days of treatment he developed a CVA and died. BP at last clinic visit was 145/83.

II. Discontinuations due to adverse experiences

A. Completed US studies

REASON WITHDRAWN	DAY	DRUG & DOSE
Edema,erythema	24	NIS40
headache	2	NIS20
headache	1	NIS20
tachycardia,vasodil	1	NIS20
Dizzy, n & v,headach	2	NIS20
Gout	5	NIS20
Faint at phlebotomy	10	NIS40
edema	13	NIS40
edema	19	NIS40
Abn Liver function*	35	NIS40, HCTZ25
Cough	8	NIS40+ LIS20
card. arrest	24	NIS40. HCTZ25
sinus tachycardia	10	NIS40, HCTZ25

* Also abnormal during pre- NIS phase

n.b. There is a notable number of cases withdrawn relatively early in the active dose phase especially for those signs and/or symptoms likely to be due to the vasodilatory action of nisoldipine.

B. Ongoing trials (all non- US):

SELECTED SIGNS/SYMPTOMS	N*
edema	23
migraine	3
headache	28
angina	1
MI	2

* often sign/symptom occurred with others

The above table concentrates on typical findings on nisoldipine therapy (edema, headache) but notes occurrence of migraine. Number of cases of angina and MI is small. Total number of cases withdrawn due to syncope (1), postural hypotension (1), hypotension (1), suggests that excessive BP fall was uncommon. N=1 case was withdrawn for thrombocytopenia.

Among 1370 cases in these foreign studies, 100 were withdrawn due to adverse experiences.

Conclusions:

The withdrawals for adverse effects were often due to the pharmacologic action of nisoldipine and consequent to vasodilation (headache, vasodilation). In this hypertensive population few cases of angina or MI occurred. However, the data base for this report is not cumulative and the number of cases in the completed US trial is too small to provide a good estimate of the risk of angina/MI. The database for the foreign studies is larger and even though treatment assignment is in doubt, the small number of angina/MI cases is notable (See this Reviewer's review of the hypertension safety segment of the NDA for comments on the association of symptoms/signs of vasodilation and angina/MI).

cc: HFD/110
HFD/110 orig
HFD/110 CSO ✓
HFD/110 pld

OCT 2 6 1993

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
MEDICAL OFFICER'S REVIEW

NDA: 20-356 (Nisoldipine Coat-Core tablets for exertional angina; Bay K 5552; IND

Sponsor: Miles Inc. Pharmaceuticals Div.

Submission: NDA 120 day Safety Update

Submission date: 17 August 1993.

Receipt date: 19 August 1993.

Review date: 26 October 1993.

Reviewer: N. Stockbridge, M.D., Ph.D.

1. US trials

There is only 1 ongoing US trial, #X90-015, an open-label, long-term follow-on to Study #90-015 (q.v.). There are no new safety data for this trial. However, two subjects who completed this trial and continued to receive nisoldipine coat-core under an individual investigator's IND experienced serious adverse experiences

Subject was a 69 year old Caucasian female who had generally received 20 mg q.d. The dose was reduced because of dizziness and light-headedness. He experienced chest pain or discomfort for two weeks prior to admission at 734 days for unstable angina. Enzymes did not indicate myocardial infarction and she was discharged after 3 days. She was readmitted with similar history at 770 days, at which time hiatal hernia was diagnosed.

Subject was a 68 year old Caucasian who suffered myocardial infarction while receiving 30 mg q.d. Three months later he was readmitted for severe angina while on 40 mg.

2. Non-US trials

2.1. Study #697

This is a randomized, double-blind, parallel group study being conducted in Germany and Italy. The groups are nisoldipine 40 mg q.d. (n=138) and diltiazem 60 mg t.i.d. (n=136).

Subject #11-009 was a 72 year old female who discontinued after 8 months because of allergic exanthema.

Subject #41-005 was a 55 year old female who discontinued after 3 months because of resting tachycardia.

2.2. Study #701

This is a randomized, double-blind, parallel group study being conducted in Germany. The groups are nisoldipine coat-core 20 mg q.d. (n=70) and nisoldipine immediate release 10 mg b.i.d. (n=72).

Subject 0101 was a 58 year old female with a 2-year history of angina. She discontinued at 5 days with severe burning sensation of the skin, headache, and vasodilation, all of which began with the first dose.

Subject #0115 was a 68 year old female with a 6-month history of angina. She discontinued at 4 months with nausea, inner "trembling", and pressure and heat sensation in hands and feet.

2.3. Study #718

This is an ongoing randomized, double-blind parallel group trial with nisoldipine 20 and 40 mg q.d., and diltiazem 60 and 120 mg b.i.d. and t.i.d.

Subject #303 was a 51 year old male with a 6-year history of angina. He withdrew after

4 days because of chest pressure and palpitations.

Subject #306 was a 54 year old male with an 8-year history of angina. During month 7, he complained of flatulence. After 8 months, he withdrew because of hypotension, malaise, and fatigue.

Study #771

This is an ongoing randomized, double-blind, comparison of nisoldipine 20 to 40 mg q.d. with diltiazem 60 mg t.i.d. or q.i.d.

Subject #3307 was a 55 year old male with a 7 month history of angina and myocardial infarction 12 years previously. He discontinued at 5 months with atrial fibrillation which resulted in prolonged hospitalization; outcome unknown.

Subject #3602 (was a 78 year old male with 2 month history of angina. He suffered cramps from the onset of treatment and discontinued after 4 weeks with the onset of fasciculations.

Subject #3703 was a 63 year old male with a 1-year history of angina. He discontinued after 3 weeks because of vertigo.

2.4. Study #781

This is an ongoing randomized, double-blind trial in Germany comparing 20 and 40 mg q.d. nisoldipine with ISDN 20 and 40 mg b.i.d.

Subject #21. was a 70 year old female with a 3-month history of angina. She discontinued after 3 weeks because of severe headaches and nausea.

Subject #214 (was a 62 year old female with a 1-year history of angina. She discontinued after 6 weeks because of leg edema.

Subject #603 . was a 64 year old female with a 3-year history of angina. She discontinued after 2 weeks because of severe headaches, diarrhea, restlessness, and general malaise.

Subject #906 , was a 69 year old female with a 3-year history of angina. She discontinued after 2 months because of hair loss.

Subject #1004 was a 68 year old male with a 3-year history of angina and myocardial infarction 1 year previously. He was discontinued after 1 week because of non-compliance.

Subject #1016 was a 58 year old male with a 1-year history of angina and possibly 2 previous myocardial infarctions. He was discontinued after 2 months because of non-compliance.

2.5. Study #761

This is an ongoing open-label trial in Israel with nisoldipine 10 to 60 mg q.d.

Subject #122 was a 59 year old Caucasian male who discontinued after 6 months because of unstable angina.

Subject #404 . was a 54 year old Caucasian male who suffered a myocardial infarction at 6 months.

Subject #414 (was a 73 year old Caucasian male who discontinued after 4 months because of unstable angina.

Subject #617 (was a 95 year old (?) Caucasian male who suffered a myocardial infarction after 7 months.

Subject #100. was a 72 year old Caucasian male who discontinued for constipation after 6 months.

Subject #1017 (was a 66 year old Caucasian male who discontinued after 4 months because of intermittent claudication and fissure following prostatectomy.

2.6. Study #762

This is an ongoing randomized, double-blind trial in Italy comparing 20 mg q.d. nisoldipine coat-core with 10 mg b.i.d. nisoldipine immediate release.

Subject #402 (was a 21 year old Caucasian male with a 2-month history of angina who had a myocardial infarction at 1 month.

Subject #1504 was a 61 year old Caucasian male with an 11-year history of angina. He

discontinued after 4 weeks because of unstable angina.

Subject #605 was a 69 year old Caucasian male with an 8-month history of angina. He discontinued after 2 weeks because of unstable angina.

Subject #607 was a 64 year old Caucasian male with a 2-year history of angina. He discontinued after 2 weeks because of rash and hypotension.

Subject #615 was a 58 year old Caucasian male with a 2-year history of angina. He discontinued after 2 weeks with rhinitis, edema of the legs, erythema, and pruritus which began on the second or third day of treatment. Symptoms resolved 2 days after withdrawal.

Subject #810 was a 60 year old Caucasian male with a 1-year history of angina. He dropped out after 3 weeks because of unstable angina.

2.7. Study #10011 (X90-010)

This is an ongoing open-label study in Israel with doses 10 to 60 mg q.d.

Subject #101 was a 56 year old Caucasian male who developed thyroiditis and tonsillitis after 4 months.

Subject #103 was a 63 year old Caucasian male who discontinued after 1 month because of unstable angina.

Subject #207 was a 64 year old Caucasian male who was hospitalized after 5 weeks because of unstable angina.

Subject #421 was a 69 year old Caucasian male who discontinued after 3 months because of pedal edema.

Subject #805 was a 63 year old Caucasian male who experienced severe prolonged angina and tachycardia 9 days after beginning treatment. He was off study drug for a short period and later completed 12 months.

Subject #911 was a 70 year old Caucasian female with a history of ophthalmological disease. She had intraocular hypertension for 9 months during study.

Subject #911 was a 65 year old Caucasian male was hospitalized for severe angina after 2 weeks. He subsequently completed 6 months, with complaints of decreased libido. The reason for discontinuation is not explicitly stated.

Subject #913 was a 61 year old Caucasian male with a 3-year history of angina. He complained of flank pain at week 2; the complaint resolved.

Subject #915 was a 52 year old Caucasian male who discontinued after 3 months because of facial flushing.

Subject #1004 was a 71 year old Caucasian male who was hospitalized for unstable angina at 7 months. He completed 12 months of treatment.

Subject #1018 was a 60 year old Caucasian male. He was twice hospitalized for chest pain, the second was after about 12 months of treatment.

Subject #1107 was a 57 year old Caucasian male who developed severe chest pain and underwent CABG 4 days after completing 12 months treatment.

3. Summary

Headache, vasodilation, and peripheral edema remained the common treatment-related adverse events.

Several of the cases described appear to represent acute worsening of angina during treatment. Subject #1107 in Study #10011 may represent a rebound phenomenon.

The information provided in this 120-day safety update do not materially affect conclusions made with the Medical Officer's review (2 August 1993) of safety and efficacy pertaining to the angina indication.

D. Roeder

DIVISION OF CARDIO-RENAL DRUG PRODUCTS

MEDICAL OFFICER'S REVIEW OF NDA AUG 4 1993

NDA Number : 20-356

Name of Drug : Nisoldipine (NIS CC)

Drug Category : Calcium Channel Blocker

Indication : Hypertension

Sponsor : Miles Inc Pharmaceutical Division

Date of Submission : March 31, 1993

Date Received : April 1, 1993

Date Review Completed : July 30, 1993

Reviewer : Cristobal G. Duarte, MD

Background. NIS CC is an extended release tablet dosage form of the dihydropyridine calcium channel blocker Nisoldipine The sponsor has submitted a NDA for approval of Nisoldipine for the treatments of hypertension This review will be concerned only with the efficacy in the treatment of hypertension.

As pivotal protocols in support of the effectiveness of Nisoldipine in the control of hypertension the sponsor is submitting the following studies : D90-006, D90-019, D89-026, D89-029, and D89-039.

Protocol D89-026

Title of Study : " A Pilot Dose-Titration Study of the Safety and Efficacy of Nisoldipine Coat-Core 10 mg, 20 mg, 30 mg and 40 mg versus Placebo in Patients with Mild to Moderate Hypertension ".

Investigators : Ginsberg D, Flamenbaum W, Canzanello V, Townsend R, Winer N,Schnaper H.

Places of Study. Harleysville, Englewood Cliffs, Winston-Salem, Galveston, Kansas City, Birmingham/USA

Objectives. The objectives of this study were :

1. To determine whether Nisoldipine given once daily lowers the blood pressure significantly more than placebo.

2. To determine the efficacy and safety of Nisoldipine ien titrated from 10 mg to 40 mg qd.

Inclusion Criteria. Ambulatory patients, male and female, 21 years of age or older, with a history of essential hypertension were eligible for the study. Hypertension was defined as mean supine diastolic blood pressure of 95-115 mmHg.

Exclusion Criteria. Patients with the following conditions were excluded from the study :

1. Labile hypertension.
2. Recent myocardial infarction
3. Patients with cerebrovascular accident or signs suggesting impending MI or CVA, heart failure, angina pectoris, intermittent claudication, major arrhythmia or cardiac conduction disturbances.
4. Insulin-dependent diabetes mellitus, failure of a major organ system, impaired renal function (serum creatinine >2 mg/dl), severe infection, malignancy or psychosis.
5. Patients likely to have impaired drug absorption such as with chronic diarrhea, ulcerative colitis, regional enteritis, diverticulitis, partial or complete gastrectomy or small bowel resection.
6. Women of childbearing potential, alcohol or drug abusers, history of allergy to dihydropyridines.
7. Excluded concomitant medications were : antihypertensive drugs, cimetidine, monoamino oxidase inhibitors, sedatives, tranquilizers, tricyclic antidepressants, neuroleptic drugs, anorectics and decongestants.

Qualification for Randomization. Patients with mild or moderate hypertension discontinued previous antihypertensive treatment and were given a single-blind placebo once daily (regimen A) during a three to four-week qualifying run-in period. There was an optional extension of one week if the blood pressure was not in the qualifying range. Those patients with mean supine diastolic pressure ≥ 95 mmHg to ≤ 114 mmHg after three to four weeks of placebo were randomized and were given regimen B (Nisoldipine or placebo).

Drug-Regimen Protocol. At week 0 qualified subjects were given either Nisoldipine 10 mg qd or placebo qd (regimen B) for 2 weeks. On subsequent visits 5 through 7 (scheduled every two weeks) the once daily dose of Nisoldipine was titrated in a stepwise fashion to 20 mg (regimen C), 30 mg (regimen D), or 40 mg (2X20 mg) (regimen E) if mean trough supine diastolic pressure for that visit was ≥ 85 mmHg. Patients randomized to placebo underwent corresponding dummy titration. Two patients were randomized to Nisoldipine for each patient that was randomized to placebo.

Patients took two tablets before 11 am through the study but did not take the medication on the morning of clinical visits until trough blood pressure has been measured. Patients took the medication fasting or with food.

Patients were seen either weekly or biweekly in the morning throughout the study. At each visit supine and standing blood pressures were measured 24 hours ± 30 minutes after the last dose.

The duration of the double-blind phase was 9 weeks.

Expulsion. A subject was to be dropped from the study if the mean supine diastolic blood pressure was greater than 114 mmHg at any visit or if they had significant physical or laboratory abnormalities or a significant concurrent illness.

They also were to be withdrawn for blatant non-compliance, for missing visits or significant adverse experiences.

Assessment. Patients were seen in the morning at weekly or biweekly intervals. A history was taken at the first visit. Complete physical examination and 12-lead electrocardiogram were done at the first visit, at baseline (after 3 to 4 weeks on placebo) and at the last visit (after 9 weeks of double blind drug). Brief physical examinations were done at all other visits. A chest X-ray was done at the first visit unless a report was available within the previous 6 months.

Blood was drawn for the following laboratory tests at the first visit, after 3 to 4 weeks of single-blind placebo, and after 9 weeks of double-blind drug : CBC, differential, and platelet count, serum glucose, uric acid, calcium, phosphate, sodium, potassium, chloride, bicarbonate, creatinine, BUN, total protein, albumin, cholesterol, triglycerides, CPK, SGOT, LDH, alkaline phosphatase and total bilirubin, Complete urinalysis including microscopic and casts.

The primary endpoint of the study was a change in trough diastolic blood pressure (measured 24 hours after dosing) from baseline (mean of diastolic blood pressure after 3 or 4 weeks of single-blind placebo) to endpoint (the last valid visit on double-blind drug for each valid patient) in the Nisoldipine group compared to the placebo group.

Secondary endpoints were supine systolic blood pressure at trough and standing blood pressure at trough.

Statistical Analysis. The primary efficacy analysis was based on change from baseline in trough supine diastolic blood pressure at endpoint. No analysis based on level of titration achieved was done. Responders were considered those who achieved efficacy results according to the following criteria : blood pressure 90 mmHg or less, at least a 10 mmHg fall in blood pressure from baseline, either of the above and both of the above.

All tests were two-sided and based on the least square means estimated by the model.

Data from previous hypertension studies had suggested that the standard deviation of change from baseline in trough supine diastolic blood pressure at endpoint would be 7.5 mmHg. In order to detect a 5 mmHg difference from placebo in an alpha = 0.05, two tailed tests of

significance, and in order to obtain as much data as possible on the Nisoldipine 40 mg qd, it was decided to randomized 72 patients to Nisoldipine and 36 to placebo. Based on this information, the study, as designed, had 80 % power to detect a significant difference of at least 5 mmHg.

Subjects Studied. Of 166 patients enrolled, 43 were disqualified for randomization. The reasons for which patients did not qualify for randomization id given in the following table :

Mean Diastolic blood pressure at visit 4 did not qualify for randomization (95 mmHg to 114 mmHg)	26
Supine diastolic blood pressure >114 mmHg-	
At any time	4
Non compliance	1
Illness not due to medication	3
Other	9
Total	43

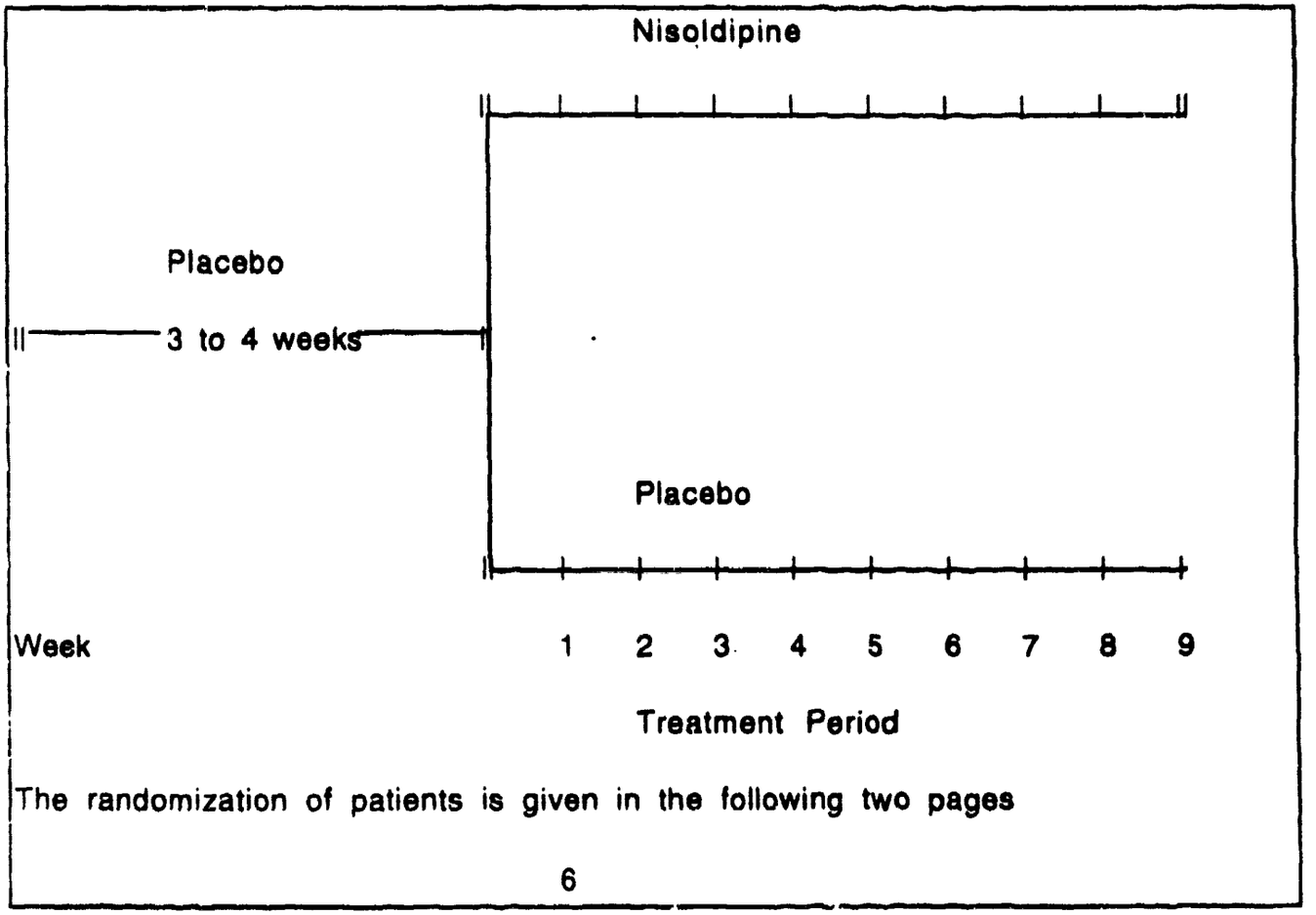
The demography and baseline characteristics of the patients valid for analysis of efficacy is given in the following table :

		Nisoldipine (n=79)	Placebo (n=38)
Sex	Male	46 (58 %)	22 (58 %)
	Female	33	16
Race	Caucasian	53 (67 %)	21 (55 %)
	Black	25	16
	Other	1	1
Age (years)	Mean	53	57
Years of hypertension	Mean	10	14
Baseline blood pressure	Supine	153/100	160/101
	Standing	149/100	156/102

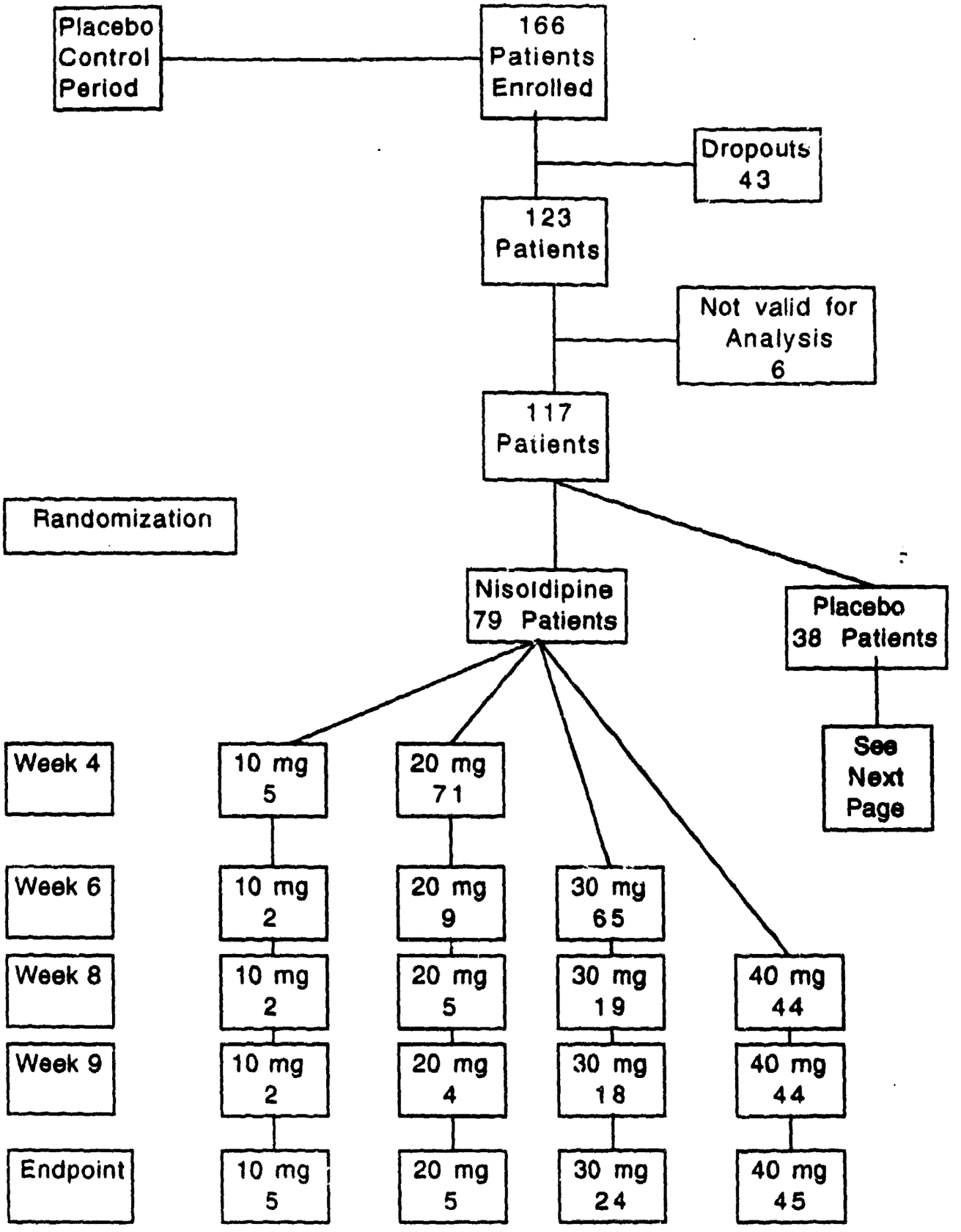
The reasons for discontinuation of double-blind therapy are given in the following table :

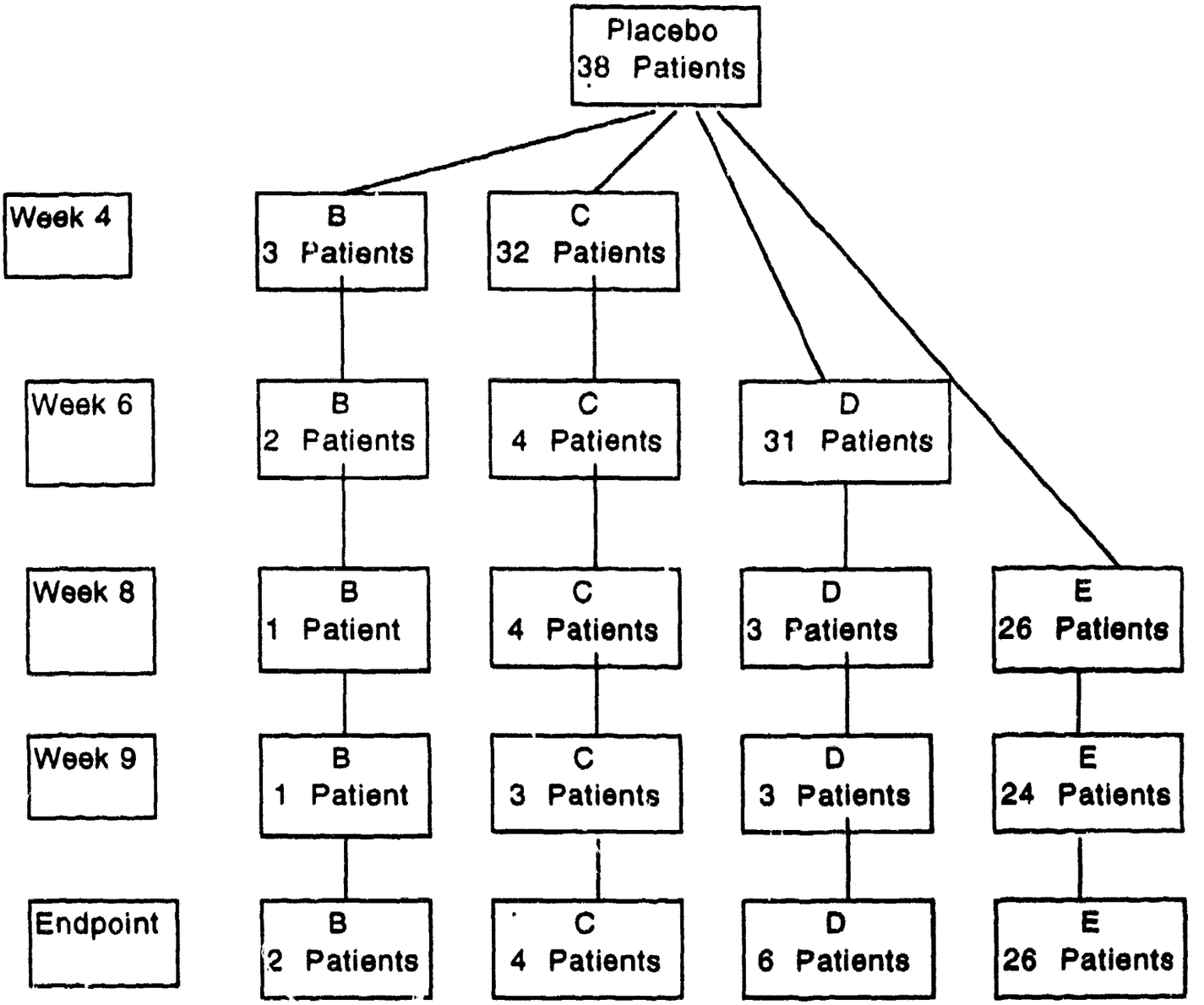
Reason	Nisoldipine n=83	Placebo n=40
Lack of Efficay	0	5
Adverse Event	6	0
Abnormal Laboratory Value	0	1
Lost to Follow-up	3	0
Other	2	9

The protocol that was followed is represented schematically in the following graph



The randomization of patients is given in the following two pages


Placebo Control Period
166 Patients Enrolled
Dropouts 43
123 Patients
Not valid for Analysis 6
117 Patients
Randomization
Nisoldipine 79 Patients
Placebo 38 Patients
See Next Page
Week 4
10 mg 5
20 mg 71
Week 6
10 mg 2
20 mg 9
30 mg 65
Week 8
10 mg 2
20 mg 5
30 mg 19
40 mg 44
Week 9
10 mg 2
20 mg 4
30 mg 18
40 mg 44
Endpoint
10 mg 5
20 mg 5
30 mg 24
40 mg 45


Placebo
38 Patients
Week 4
B
3 Patients
C
32 Patients
Week 6
B
2 Patients
C
4 Patients
D
31 Patients
Week 8
B
1 Patient
C
4 Patients
D
3 Patients
E
26 Patients
Week 9
B
1 Patient
C
3 Patients
D
3 Patients
E
24 Patients
Endpoint
B
2 Patients
C
4 Patients
D
6 Patients
E
26 Patients

Efficacy. Doses of Nisoldipine were titrated from regimen B (10 mg QD) to regimen E (40 mg QD) in 10 mg steps. The following table shows the actual number of patients that were given each dose level and had valid visits at each week for the population of patients valid for efficacy :

			Week of Therapy					
			2	4	6	8	9	End-point
Group	Reg.	Dose	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)
NIS	B	10 mg QD	79 (100)	5(7)	2 (3)	2 (3)	2 (3)	5 (6)
	C	20 mg QD		71 (93)	9 (12)	5 (7)	4 (6)	5 (6)
	D	30 mg QD			65 (86)	19 (27)	18 (27)	24 (30)
	E	40 mg QD				44 (63)	44 (65)	45 (57)
PLA	B		38 (100)	3 (9)	2 (5)	1 (3)	1 (3)	2 (5)
	C			32 (91)	4 (11)	4 (12)	3 (10)	4 (11)
	D				31 (84)	3 (9)	3 (10)	6 (16)
	E					26 (77)	24 (77)	26 (68)

The following table shows trough supine diastolic blood pressure response at different weeks of treatment and at endpoint for both groups for the set of all valid patients.

Trough Supine Diastolic Blood pressure
Mean Change (mmHg) by visit

	Week 2	Week 4	Week 6	Week 8	Week 9	End-point
Nisoldipine						
(n)	(79)	(76)	(76)	(70)	(68)	(79)
Mean Change	-5.7*	-6.5	-10.1*	-10.6*	-10.0*	-9.5*
Placebo						
(n)	38	35	37	34	31	
Mean Change	-3.0	-4.9	-3.4	-4.4	-3.4	-1.2

* Significantly different from placebo

In the following table, changes from baseline at endpoint by treatment regimen at endpoint for all valid patients is demonstrated :

	Nisoldipine			
	Reg B (n=5)	Reg C (n=5)	Reg D (n=24)	Reg E (n=45)
Supine				
Systolic	-8.3	-23.1	-10.9	-16.7
Diastolic	-5.9	-12.4	-9.4	-9.8
Standing				
Systolic	-5.3	-13.5	-10.3	-15.3
Diastolic	-7.2	-9.9	-6.6	-8.6

	Placebo			
	Reg B (n=2)	Reg C (n=4)	Reg D (n=6)	Reg E (n=26)
Supine				
Systolic	-19.7	-8.0	+20.4	-2.2
Diastolic	+ 1.7	-10.0	+6.9	-1.4
Standing				
Systolic	-3.7	-1.3	+13.9	-0.1
Diastolic	-0.7	-7.5	+5.0	-1.6

The responder rates are given in the following table :

Responder Rates
Based on Trough Supine Diastolic Blood Pressure at Endpoint

	Nisoldipine	Placebo
BP<90 mmHg at endpoint	49 %	18 %
At least a 10 mmHg fall at Endpoint	54 %	13 %
Either of the Above	62 %	21 %
Both of the Above	42 %	11 %

The change from baseline in trough blood pressure by treatment for all patients valid for analysis of efficacy is given in the following table :

Supine Diastolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg.B	Reg. B or C	Reg. BC or D	Reg.BCD or E	Reg BCDorE	
Nisoldipine n	79	76	76	70	68	79
Baseline Mean LS	100.36	100.36	100.36	100.14	100.36	100.36
LS Mean Change	-5.65*p	-6.52*	-10.06*p	-10.58*p	-9.98*p	-9.51*p
SE of Change	0.68	0.75	0.88	0.84	0.80	0.89

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg B	Reg B or C	Reg BC or D	Reg BCD or E	Reg BCDorE	
Placebo n	38	35	37	34	31	38
Baseline LSMean	101.45	101.31	101.20	100.52	100.79	101.45
LS Mean Change	-3.04*	-4.89*	-3.39*	-4.42*	-3.36*	-1.16
SE of Change	0.99	1.10	1.26	1.21	1.20	1.29
p Values						
Drug	0.0323	0.2234	0.0001	0.0001	0.0001	0.0001
Drug-Center	0,1582	0.2604	1.381	0.0539	0.0472	0.0332

P Significantly different from placebo
* Significant Change from baseline

Values for supine systolic blood pressure are given in the following tables:

Supine Systolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg B	Reg B or C	Reg BC or D	Reg BDC or E	Reg BCD or E	
Nisoldipine n	79	76	76	70	68	79
Baseline LS Mean	153.11P	153.39	153.41	153,35	153.42	153,11p
LS Mean Change	-8.67*	-10.123*p	-15.04*p	-14.10*p	-15.62*p	-14.73*p
SE of Change	1.33	1.61	1.61	1.61	1.64	1.89
Placebo n	38	35	37	34	31	38
Baseline LS Mean	159.59	158.99	158.23	157.78	159.71	159.59
LS Mean Change	-4.96*	-2.39	-1.90	-4.91*	-5.51*	-0.00
SE of Change	1.92	2.38	2.33	2.31	2.43	2.72

P-Values	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug	0.1140	0.0074	0.0001	0.0015	0.0008	0.0001
Drug Center	0.6059	0.0481	0.0416	0.1372	0.1224	0.0726

p Significantly different from placebo
* Significant change from baseline

Standing Diastolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg.B	Reg.B or C	Reg. BC or D	Reg BDC or E	Reg BCD	or e
Nisoldipine n	79	76	76	70	68	79
Baseline LSMean	100.40	100.31	100.31	100.21	100.27	100.40
LS Mean Change	-5.03*p	-6.24*p	-8.62*p	-10.18*p	-8.29#p	-7.86*p
SE of Change	0.75	0.75	0.85	0.83	0.98	1.00
Placebo n	38	35	37	34	31	38
Baseline SL Mean	102.15	101.85	101.85	101.22	100.89	102.15
LS Mean Change	-2.05	-1.99	-1.87	-4.27*	-3.65*	-1.18
SE of Change	1.08	1.11	1.22	1.20	1.45	1.44
P Values						
Drug	0.0253	0.0021	0.0001	0.0001	0.0095	0.0002
Drug-Center	0.2060	0.0052	0.6446	0.5733	0.4304	0.3160

p Significantly different from placebo
* Significant change from baseline

Standing Systolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg B	Reg B or C	Reg BC or D	Visit BCD	or E Visit	BCD orE
Nisoldipine n	79	76	76	70	68	79
Baseline LS Mean	149.22p	149.54	149.56	149.41	149.57	149.22p
LS Mean Change	-8.37*	-10.78*p	-14.18*p	-14.84*p	-13.76*p	-13.*p
SE of Change	1.34	1.49	1.78	1.73	1.66	1.77
Placebo n	38	35	37	34	31	38
Baseline LS Mean	156.22	155.79	155.30	154.22	155.46	156.22
LS Mean Change	1.94	2.20	2.55	2.49	2.48	2.57
P values						
Drug	0.0832	0.0005	0.0001	0.0002	0.0002	0.0001
Drug-Center	0.1162	0.0979	0.1092	0.2475	0.0099	0.0330

P Significantly different from placebo * Significant change from baseline

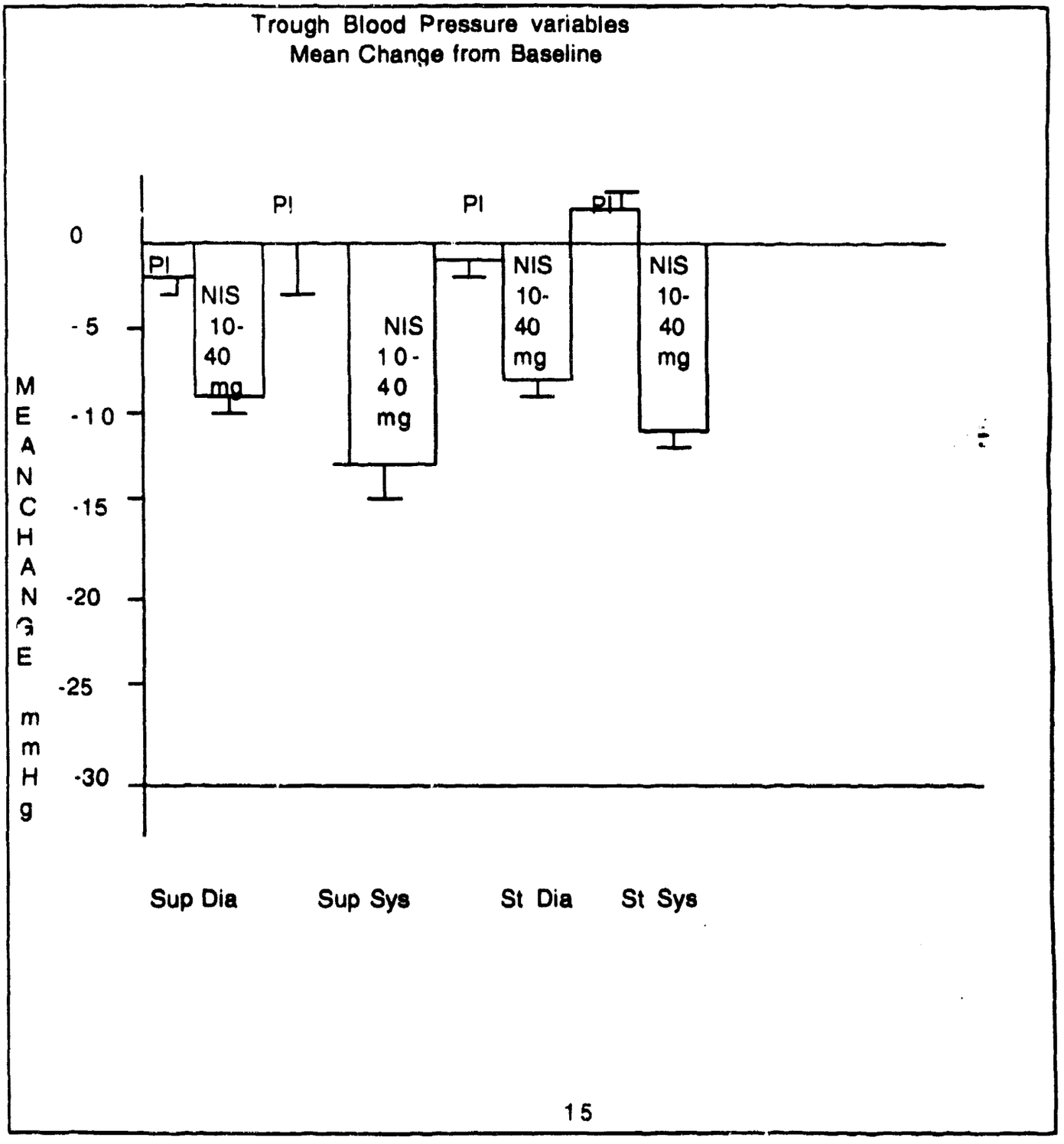
The effect of Nisoldipine on trough supine and standing systolic and diastolic blood pressure at study endpoint in all Nisoldipine treated patients is shown in the following table :

Change from Baseline to Endpoint in Trough Blood Pressures
Mean and SEM in mmHg

	Placebo n=38	Fisoldipine n-79
Supine Diastolic Blood Pressure	-1.16±1.29	-0.51±0.89*
Supine Systolic Blood Pressure	-.000±2.72	-14.73±1.89*
Standing Diastolic Blood Pressure	-1.18±1.44	-7.86±1.00*
Standing Systolic Blood Pressure	+0.89±2.57	-13.00±1.77*

* Significantly different from placebo. p<0.05

The change from baseline to endpoint in the primary efficacy blood pressure parameter supine diastolic blood pressure, as well as the 3 secondary blood pressure parameters is shown for placebo and all Nisoldipine doses in the figure below :



Conclusion. This was a titration study in which doses of Nisoidipine 10 mg, 20 mg, 30 mg, 40 mg and placebo were evaluated. In the course of the study most patient were moved to the higher doses in order to decrease the blood pressure and very few patients remained in the lower doses (see flow sheets pages 7, 8, and 9). Therefore a dose-range study could not be carried and only a global evaluation was possible. Such assessment demonstrated that Nisoldipine was very effective in lowering the blood pressure (pages 10-15).

Protocol D89-029

Title of Study : " Double-Blind Randomized Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine 20, 40 and 60 mg (2X30 mg) Core-Coat Tablets vs Placebo in Combination with Atenolol 50 mg in Hypertensive Patients ".

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Objectives : The objectives of this study were to determine the dose response and safety of 20 mg, 40 mg, and 60 mg Nisoldipine tablets as compared to placebo when administered once daily as additive treatment for hypertensive patients not controlled on once daily Atenolol 50 mg.

Inclusion Criteria. Ambulatory patients, male or female, of age 21 or older, with a history of essential hypertension were eligible for enrollment in the placebo run-in period.

Exclusion Criteria. Criteria foe exclusion were : labile hypertension, renal failure (plasma creatinine > 2.0 mg/dl), significant liver disease, insulin-dependent diabetes mellitus, history or presence of bronchial asthma, obstructive pulmonary disease, significant peripheral vascular disease, recent (3 months) myocardial infraction, cerebrovascular accident, or clinical signs suggesting impending myocardial infarction or cerebrovascular disease. Also excluded were patients with heart failure, major arrhythmias, conduction disturbances greater than first degree block, sinus bradycardia, failure of a major organ system, malignancy, psychosis, impaired absorption (such as chronic diarrhea), pregnancy,

women with childbearing potential, abuse of alcohol or drugs, allergy to dihydroperidines or beta blockers and participation in an investigational drug study within the past 30 days.

Qualifications for Randomization. Patients discontinued all previous antihypertensive medication and were given a single-blind placebo once daily in a 2-week qualifying period (Regimen A). Patients with a mean SUDBP 100-119 mmHg at the end of the placebo run-in period were given 1 capsule containing 50 mg Atenolol and 2 placebo tablets under single-blind conditions for 4 weeks (Regimen B). Patients with mean SUDBP 95-114 mmHg after 4 weeks of single-blind Atenolol were randomly assigned to 1 to 4 treatment groups and given double-blind drug.

Drug-Regimen Protocol (Regimen C). Patients who qualified for randomization received Atenolol 50 mg + Nisoldipine (20 mg, 40 mg, or 60 mg) or Atenolol 50 mg + placebo for 6 weeks.

Drugs for the double-blind period (Regimen C) contained encapsulated Atenolol 50 mg with one of the following :

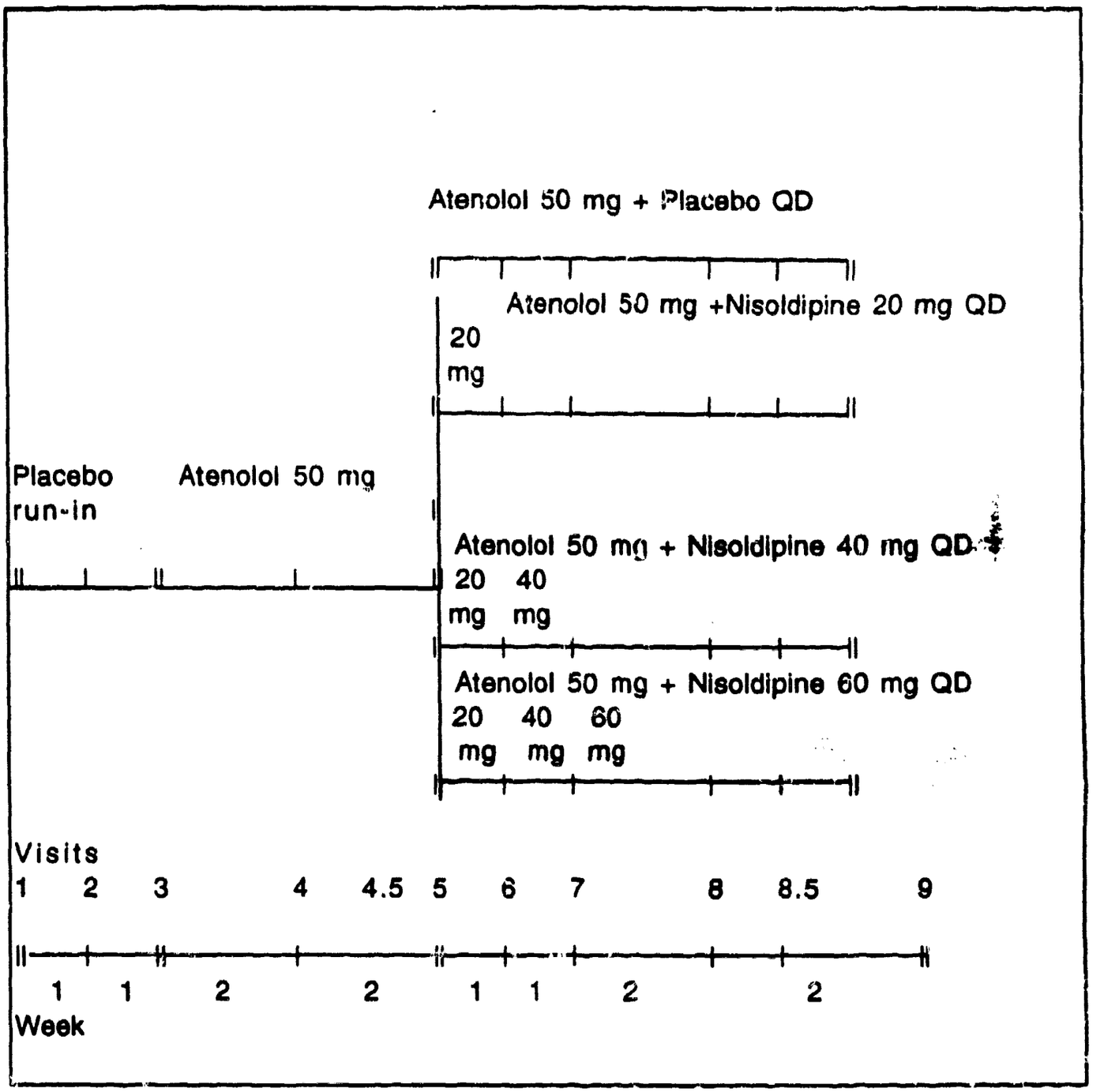
One Nisoldipine 20 mg tablet and
one placebo tablet once daily for 6 weeks

One Nisoldipine 20 mg tablet and
One placebo tablet once daily for 1 week
Forced titrated to
One Nisoldipine 40 mg tablet and
One placebo tablet once daily for 5 weeks

One Nisoldipine 20 mg tablet and
One placebo tablet once daily for 1 week
Forced titrated to
One Nisoldipine 40 mg tablet and
One placebo tablet once daily for 1 week
Forced titrated to
Two Nisoldipine 30 mg tablets once daily for 4 weeks

Two placebo tablets once daily for 6 weeks

The study design is illustrated in the following graph :



Removal of patients from Study or Analysis. Patients could leave the study at any time if they so wished. Patients could be discontinued if they had significant physical or laboratory abnormalities, or if they had significant concurrent illness or deterioration of their condition. Patients could also be withdrawn if they were blatantly non-compliant. Patients with significant adverse events and those patients with elevations in SUDBP > 114 mmHg were also discontinued from the study.

Statistical Methods. All statistical tests were two-tailed and were conducted at a significant level of 0.05. Pairwise comparisons and within-group changes were tested via the least square means estimated by the model.

Results. . Democraphic Characteristics. The demographic characteristics are given in the following table :

	Atenolol+ Nis 20 mg n=61	Atenolol+ Nis 40 mg n=59	Atenolol+ Nis 60 mg n=59	Atenolol+ Placebo n=59
Mean Age (years)	52	54	56	54
Mean wt (lbs)	201	198	198	195
Baseline BP (mmHg)				
Supine	159/101	159/101	162/101	156/110
Standing	154/102	157/103	156/103	152/101
% Male	79	73	70	64
% Caucasian	61	53	58	53
% Diabetic	8	9	14	12
% Mild Hypertensive	60	56	51	59
% Moderate Hypertensives	40	44	49	41

The sponsor states that there were no statistically significant differences between the groups for any of the characteristics examined.

Assessment. Patients were seen in the morning at weekly and biweekly intervals. A history complete physical examination and 12-lead electrocardiogram were taken in the firs visit, at baseline (after 4 weeks of Atenolol) and at the last visit on double-blind drug. Electrocardiograms

were included in visits 7 and 8. Twenty-four hour ambulatory electrocar-cardiograms were taken at some centers. on weeks 4.5 after 3 weeks of single Atenolol and at 8.5 weks of double-blind therapy. Chest X-ray were taken after 2 weeks on placebo.

Laboratory tests performed in the course of the study included blood hematology, serum electrolytes, battery of liver function tests, and urinalysis.

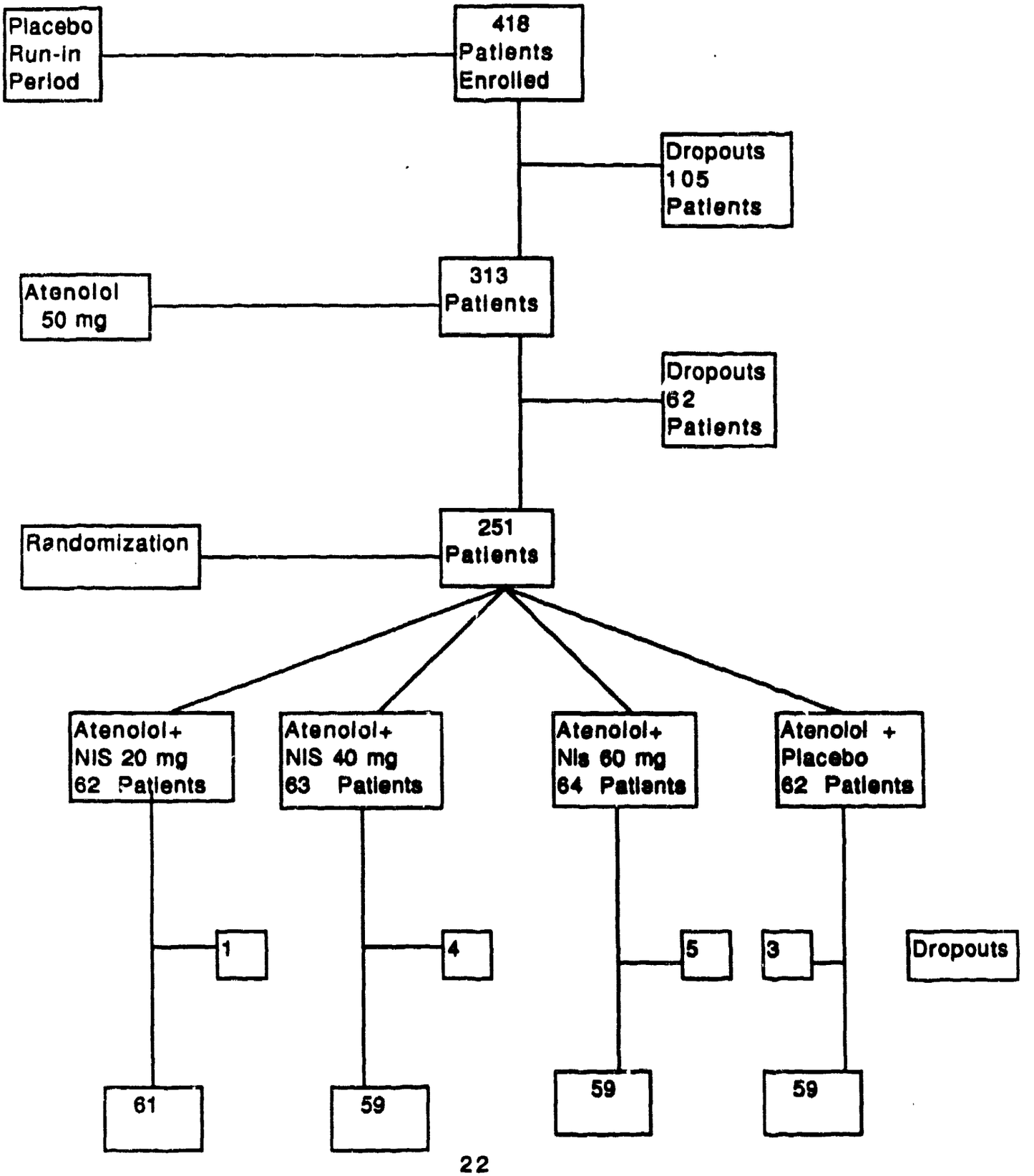
At the end of the single blind Atenolol phase and at the end of the double-blind phase blood was taken at trough for Nisoldipine assay.

At each visit vital signs were taken.

Criteria for Effectiveness. The change from baseline in SUDBP was the primary efficacy variable in this study. The primary time point was the endpoint which was defined as the last double-blind visit for all valid patients. A valid patient was one who had at least 3 weeks of double-blind drug. This criterion was later amended before braking the random code to 19 days. The overall treatment efficacy was determined by the change from baseline in trough SUDBP at endpoint between the average of the three Atenolol-Nisoldipine groups and the Atenolol-Placebo group. Secondary efficacy parameters were supine systolic blood pressure change at trough, standing blood pressure changes at trough, and ambulatory blood pressure trough/peak ratios.

An average decrease in diastolic blood pressure of at least 5 mmHg more than placebo was considered to be clinically meaningful. The actual power for the study was >95 %.

The disposition of the patients is given in the following flow-sheet :


Placebo Run-in Period
418 Patients Enrolled
Dropouts 105 Patients
Atenolol 50 mg
313 Patients
Dropouts 62 Patients
Randomization
251 Patients
Atenolol+ NIS 20 mg 62 Patients
Atenolol+ NIS 40 mg 63 Patients
Atenolol+ Nis 60 mg 64 Patients
Atenolol + Placebo 62 Patients
1
4
5
3
Dropouts
61
59
59
59

The reasons because the patients were withdrawn from the placebo run-in period are given in the following Table :

Mean SUDBP at visit 3 <100 mmHg or > 119 mmHg	=58
Mean SUDBP >119 mmHg during placebo run-in period	= 6
Adverse event during placebo run-in period	=10
Other illness	= 3
Abnormal laboratory value	= 4
Abnormal electrocardiogram	= 2
Noncompliance	= 3
Investigator discretion	= 5
Consent withdrawn	= 9
Lost to follow-up	= 5
Total	105

Patients withdrawn during the single Atenolol period and therefore not randomized :

Mean SUDBP at visit 5 <95 mmHg or >114 mmHg	=37
Mean SUDBP > 114 mmHg on 2 consecutive visits after placebo run-in	= 4
Adverse Event	= 4
Other illness	= 2
Abnormal electrocardiogram	= 1
Noncompliance	= 1
Investigator discretion	= 4
Consent withdrawn	= 4
Lost to follow-up	= 2
Enrolled after enrollment date	= 3
Total	62

The reasons for invalidity for patients that were withdrawn during the treatment period are given in the following table :

Drug Group	Number of Patients	Reasons for Invalidity
Atenolol + NIS 20 mg	1	Less than 19 days on double-blind drug
Atenolol + NIS 40 mg	4	Less than 19 days on double-blind drug
Atenolol + NIS 60 mg	4	Less than 19 days on double-blind drug
	1	Visit 5 diastolic BP not between 95 and 114 mmHg
Atenolol + Placebo	2	Less than 19 days on double-blind drug
	1	Visit 5 diastolic BP not between 95 and 114 mmHg
Total	13	

Effectiveness. The change from baseline in trough blood pressure by treatment for all patients valid for analysisof efficacy are given in the following tables

Supine Diastolic

Drug Group	Visit 6 Week 1	Visit 7 Week 2	Visit 8 Week 4	Visit 9 Week 6	Endpoint
ATN+NIS 20 mg					
N	58	61	61	61	61
Baseline BP	100.56	100.56	100.56	100.57	100.56
Mean Change	-8.53*C	-9.80*C	-9.10*AB	0.88 -10.09*	0.88 -10.09*
SE	0.85	0.78	C 0.89	ABC 0.88	ABC 0.88
ATN+NIS 40 mg					
N	57	58	58	57	58
Baseline BP	100.75	100.75	100.75	100.77	100.75
Mean Change	-8.52*C	-11.26*C	-12.87*C	-12.75*C	-12.69*C
SE	0.86	0.81	0.92	0.92	0.91
ATN+NIS 60 mg					
N	56	59	58	57	59
Baseline BP	101.12	101.11	101.00	101.27	101.11
Mean Change	-9.72*C	-12.15*C	-12.82*C	-14.33*C	-14.24*C
SE	0.87	0.80	0.92	0.91	0.90
ATN+PL					
N	58	59	59	59	59
Baseline BP	99.93	99.93	99.93	99.93	99.93
Mean Change	-4.00*	-4.31*	-4.61*	-4.29*	-4.28*
SE	0.85	0.80	0.91	0.90	0.90

Drug Group	Visit 6 Week 1	Visit 7 Week 2	Visit 8 Week 4	Visit 9 Week 6	Endpoint
ATN+NIS 20 mg					
N	61	61	61	61	61
Baseline BP	158.71	158.70	158.70	158.71	158.70
Mean Change	-10.82*C	-12.96* ABC	-13.30* BC	-13.14* ABC	-13.15* ABC
SE					
ATN+NIS 40 mg					
N	58	58	58	57	58
Baseline BP	158.78	158.77	158.77	158.97	158.77
Mean Change	-12.93*C	-19.04*C	-19.03*C	-20.09*C	-19.83*C
SE	2.01	2.11	2.29	2.05	2.05
ATN+NIS 60 mg					
N	58	59	58	57	59
Baseline BP	161.46	161.58	161.70	162.01	161.58
Mean Change	-12.29*C	-20.38*C	-21.00*C	-23.43*C	-23.09*C
SE	2.00	2.08	2.28	2.03	2.02
ATN+Pla					
N	59	59	59	59	59
Baseline BP Mean	156.30	156.29	156.29	156.28	156.29
Mean Change	-3.78	-2.51	-0.02	-0.87	-0.85
SE	1.99	2.09	2.26	2.01	2.02

Standing Diastolic

ATN+NIS 20 mg					
N	61	61	61	61	61
Baseline BP	102.15	102.16	102.16	102.16	102.16
Mean Change	-7.50*C	-8.46*C	-7.90* ABC	-8.93* ABC	-8.93* ABC
SE	0.87	1.00	0.89	0.89	0.89
ATN+NIS 40 mg					
N	58	58	58	57	58
Baseline BP	103.42C	103.43C	103.43C	103.45C	103.43C
Mean Change	-8.55*C	-12.23*C	-12.85*C	-15.00*C	-14.93*C
SE	0.89	1.02	0.92	0.93	0.91
ATN+NIS 60 mg					
N	58	59	58	57	59
Baseline BP	102.97	102.94	102.97	103.18	102.94
Mean Change	-8.55*C	-12.23*C	-12.85*C	-15.00*C	-14.93*C
SE	0.89	1.02	0.91	0.91	0.91
ATN+Pla					
N	59	59	59	59	59
Baseline BP	101.13	101.13	101.13	101.12	101.13
Mean Change	-3.29*	-4.24*	-3.19*	-1.96*	-1.95*
SE	0.89	1.02	0.91	0.91	0.91

Standing Systolic

Drug Group	Visit 6 Week 1	Visit 7 Week2	Visit 8 Week4	Visit 9 Week 6	Endpoint
ATN+NIS 20 mg					
N	61	61	61	61	61
Baseline BP	152.33	152.23	152.23	152.20	154.37
Mean Change	-10.69*C	-11.17* ABC	-11.20* ABC	-10.97* ABC	-10.97* ABC
SE	2.00	2.10	2.17	2.04	2.00
ATN+NIS 40 mg					
N	58	58	58	57	58
Baseline BP	156.89	156,87	156.87	156.85	156.87
Mean Change	-13.25*C	-17.66*C	-21.52*C	-22.35*	-22.38*C
SE	2.10	2.18	2.25	2.13	2.11
ATN+NIS 60 mg					
N	58	58	58	57	59
Baseline BP	156.17	156.30	156.33	156.73	156.30
Mean Change	-11.16*C	-19.76*C	-20.50*C	-22.36*C	-22.10*C
SE	2.08	2.15	2.24	2.12	2.08
ATN+Pla					
N	59	59	59	59	59
Baseline BP	152.23	152.23	152.23	152.20	152.23
Mean Change	-3.17	-1.42	-0.90	1.88	1.89
SE	2.07	2.15	2.22	2.08	2.09

P-values

	Visit 6 Week1	Visit 7 Week2	Visit 8 Week 4	Visit 9 Week 6	Endpoint
Drug* Center	0.0203	0.6905	0.1363	0.1813	0.1478
NIS vs PLA	0.0001	0.0001	0.0001	0.0001	0.0001
20 mg vs PLA	0.0002	0.0001	0.0004	0.0001	0.0001
40 mg vs Pla	0.0003	0.0001	0.0001	0.0001	0.0001
60 mg vs Pla	0.0001	0.0001	0.0001	0.0001	0.0001

A: Significantly different from ATN+NIS 40 mg QD
B. Significantly different from ATN+NIS 60 mg
C. Significantly different fron ATN+Pla
* Significant change from baseline

The effect of Nisoldipine on trough SUDBP during the course of the double-blind treatment is shown in the following table :

Placebo Substracted Change in SUDBP
Mean in mmHg

NIS Dose	Week 1	Week 2	Week 4	Week 6
20 mg	-4.53*	-5.49*	-4.48*	-5.80*
40 mg	-4.52*	-6.95*	-8.26*	-8.46*
60 mg	-5.72*	-7.84	-8.21*	-10.07*

* Denotes values when Nisoldipine blood pressure responses are significantly different from placebo, <0.05

The changes from baseline to endpoint in trough blood pressure, mean and SEM in mmHg are given in the following table :

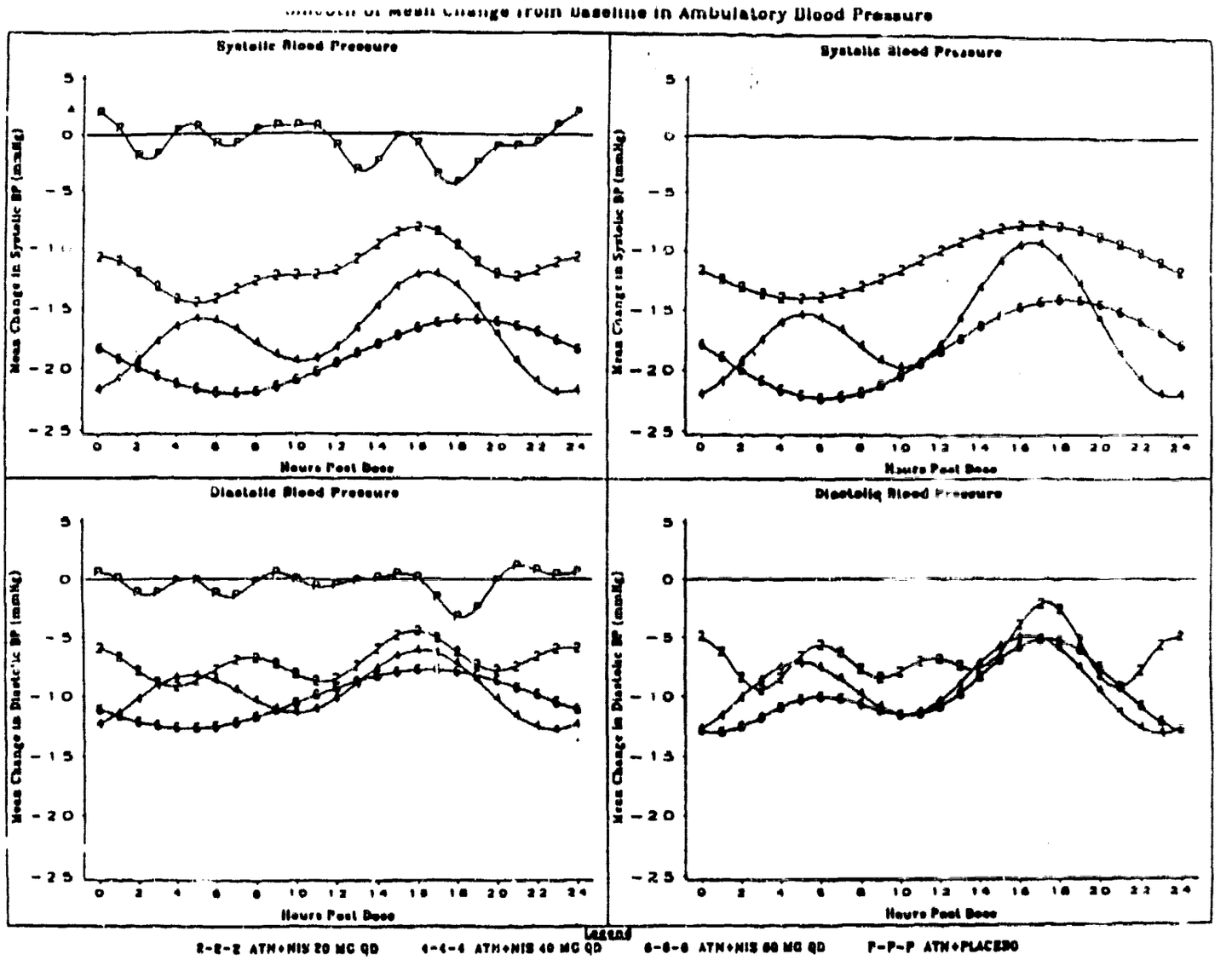
	ATN+PLA n=59	ATN + NIS 20 mg n=61	ATN + NIS 40 mg n=58	ATN + NIS 60 mg n=59
SUDBP	-4.28±0.90	-10.08±0.9[B]	-12.69±0.9*	-14.24±0.9*
SUSBP	-0.85±2	-13.15±2[AB]	-19.83±2*	-23.1±2*
STDBP	-1.95±0.91	-8.93±0.9[AB]	-13±0.92*	15±0.91*
STSBP	+1.89±2.09	-11±2.03*	-22.38±2.1*	-22.10±2.1*

A denotes values NIS 20 mg significantly different from placebo,p<0.05
B denotes values NIS 20 mg significantly different from Nisoldipine 60 mg<0.05.

	ATN+PL:A	ATN+NIS 20 mg	ATN+NIS 40 mg	ATN+NIS 60 mg
Trough response mmHg	% Patients	% Patients	% Patients	% patients
SUDBP ≤90	32.2	55.7*	67.8*	66.1*
Fall in SUDBP ≥10	23.7	50.8*	67.8*	74.6*
SUDBP≤90 or Fall in SUDBP ≥10	39	63.9*	74.6*	78
SUDBP ≤90 and fall in SUDBP ≥ 10	16.9	42.6*	61*	62.7*

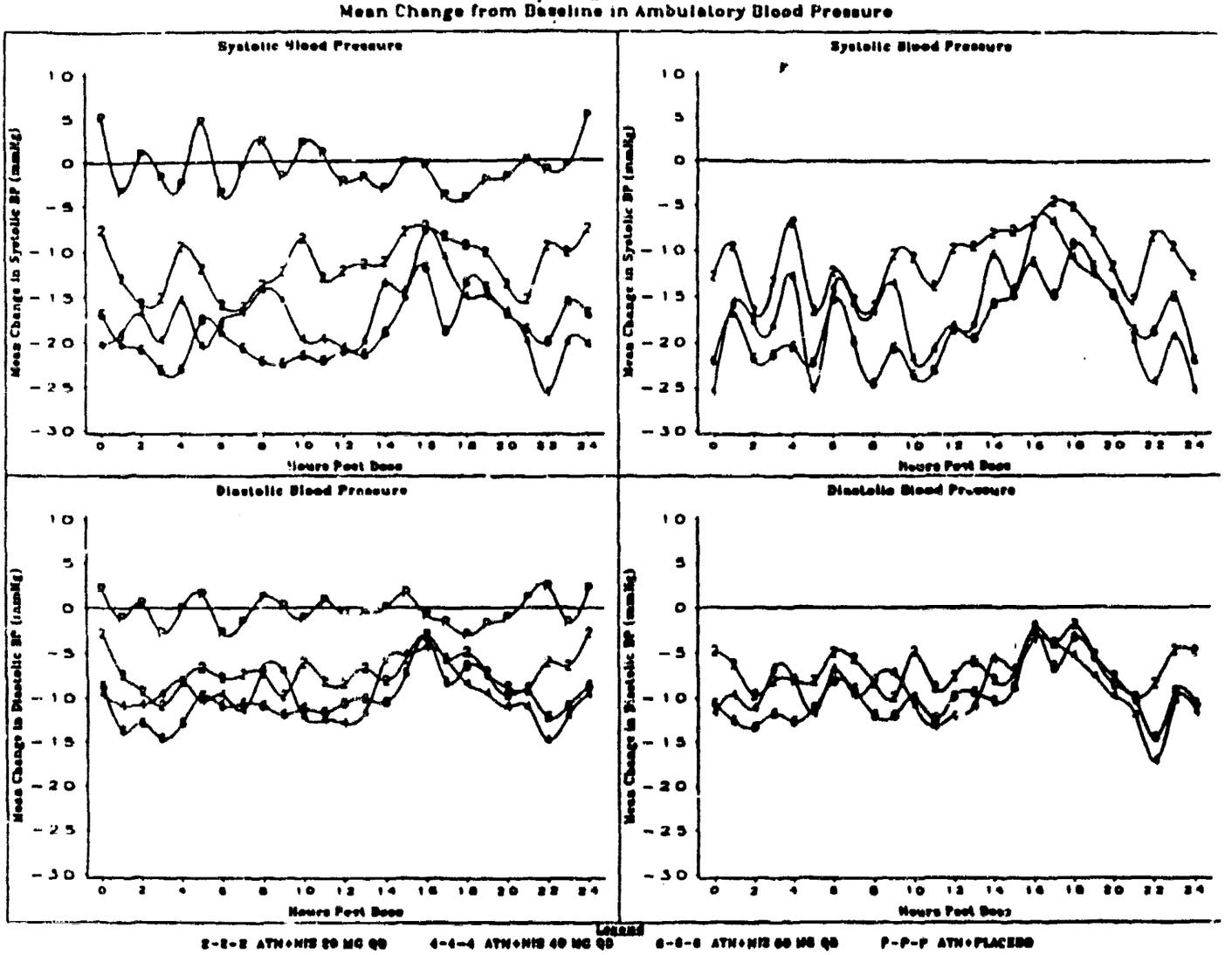
* p <0.01 vs placebo

At 8 centers ambulatory blood pressure monitoring (ABPM) was done after 3 weeks on single blind Atenolol and after 5 weeks of double-blind therapy. Smoothed and unsmoothed means for the ambulatory data are shown in the graphs in the following two pages :



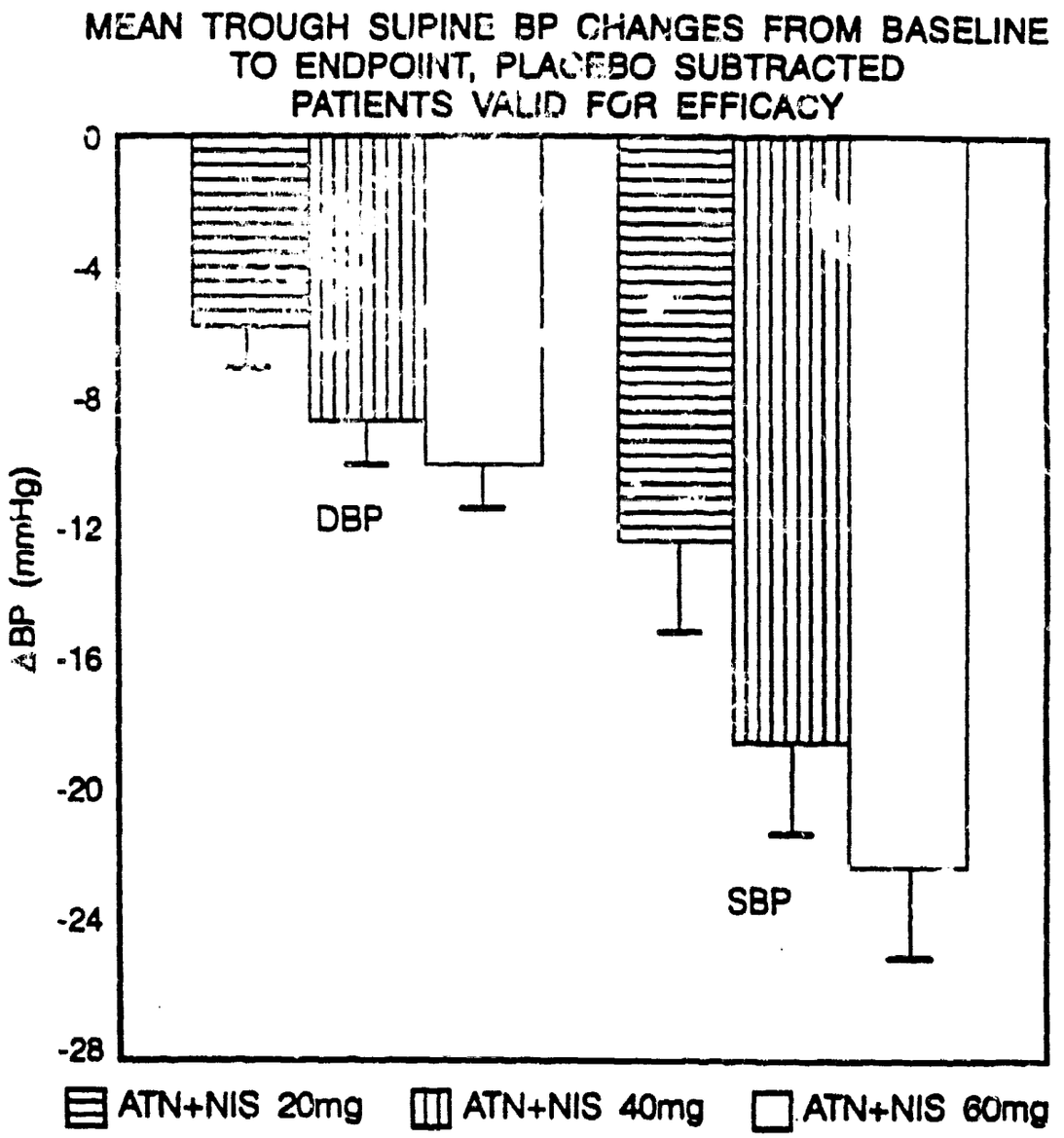
2-2-2 ATN+NIS 20 mg 4-4-4 ATN+NIS40 mg 6-6-6 ATN+NIS 60 mg
P-P-P ATN+Placebo

Figure 10
Mean Change from Baseline in Ambulatory Blood Pressure



Symbols as in previous graph

The placebo-substracted trough SUDBPs are showed in the following graph:



The trough and peak ambulatory blood pressure changes and trough to peak ratios for patients valid for efficacy analysis are given in the following table :

Smoothed Data - Difference from Placebo Group

		Trough mmHg	Peak mmHg	Hours to Peak	Trough to Peak Ratio
Variable	Drug				
Diastolic	ATN+NIS 20 mg QD	-5.0	-9.4	3	53%
	ATN+NIS 40 mg QD	-12.8	-13.1	23	97%
	ATN+NIS 60 mg QD	-12.9	-13	1	99%
Systolic (at corresponding diastolic peak)	ATN+NIS 20 mg QD	-11.7	-13.6	3	86%
	ATN+NIS 40 mg QD	-22.0	-22.0	23	100%
	ATN+NIS 60 mg	-17.9	-19.0	1	94%
Systolic (actual)	ATN+NIS 20 mg QD	-11.7	-14.0	5	83%
	ATN+NIS 40 mg QD	-22.0	-22.0	24	100%
	ATN +NIS 60 mg QD	-17.9	-22.3	6	80%

Pharmacodynamic Results. Trough plasma samples were drawn at visits 5 and 9. Visit 9 samples for all patients were analyzed for Nisoldipine. The results for patients whose treatment regimens did include Nisoldipine are shown in the following table :

	n	Mean Trough Concentration (ng/ml)	Range of Trough Concentrations (ng/ml)
AT+NIS 20 mg	57	1.6	0-7.41
AT+NIS 40 mg	55	2.5	0-12.0
AT+NIS 60 mg	59	3.3	0-10.40

Conclusions. In this protocol Atenolol was used as a positive control and studies were performed with Atenolol in combination with placebo and with NIS in the concentrations of 20 mg, 40 mg and 60 mg. Atenolo with placebo had not significant effect in blood pressure but in combination with NIS demonstrated significant hypotensive effects in relation with the concentration of NIS. Threfore the possibility of drug interaction should be considered. Measurements of NIS in plasma were performed and they increased as would be expected with increasing concentrations of NIS and in relation to their effects on blood blood pressure but unfortunately concentrations of Atenolol in plasma were not measured. In other studies the sponsor did not find clinically relevant drug interaction between Nisoldipine, and the beta blocker Propanol (study 704, PB#21521, Volume 142, pp. 08-17-0010374). However some studies in the literature do not agree with this conclusion.

Elliott et al studying the interactions between Nisoldipine and Atenolol and Propanolol found that Nisoldipine, in single and multiple doses, significantly increased the peak plasma concentration of Propanolol and Atenolol. There was no evidence that either beta blocker influenced the pharmacokinetics of Nisoldipine. (The interactions between Nisoldipine and two beta-adrenoceptor antagonists : atenolol and propanolol. H.L.Elliott et al. Brit J Clin Pharmacol 1991;32(6):379-85).

Levine et al demonstrated pharacodynamic and pharmacokinetic interactions between Nisoldipine and Propanolol (MAH Levine et al. Pharmacokinetic and pharmacodynamic interactions between Nisoldipine and Propanolol. Clin Pharmacol Ther 1988; 43:39-48).

These contradictions could have been clarified had the sponsor included in this protocol a true placebo group and groups of Nisoldipine without Atenolol. Plasma levels of Atenolol should have been also measured.

Protocol D90-019

Title of Study : " A Double-Blind Randomized Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine Coat-Core Tablets 30 mg, 60 mg (2X30) and 90 mg (3X30) vs Placebo in Hypertensive Patients ".

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Objectives. The objectives of this study were :

1. To determine the antihypertensive efficacy and safety of NIS tablets in doses of 30 mg. 60 mg and 90 mg daily in patients with mild to moderate hypertension.

2. To assess the time and magnitude of peak blood pressure response and the ratio of trough to peak antihypertensive effect by 24-hour ambulatory blood pressure monitoring.

Inclusion Criteria. Ambulatory patients, male and female, 21 to 75 years of age, with a history of mild to moderate essential hypertension were eligible for the study.

Exclusion Criteria. Patients with the following conditions were excluded from the study : labile hypertension, renovascular or other secondary forms of hypertension, patients whose SUDBP after 3 and 4 weeks of placebo run-in were not ≥100 or ≤ 114 mmHg, previous myocardial infarction or cerebrovascular accident, heart failure, frequent arrhythmias, conduction disturbances, angina pectoris, use of other antihypertensive drugs, and many other drugs.

Also excluded were patients with failure of a major organ system, liver/ kidney disease, malignancy or psychosis, patients with previous history of gastrointestinal disease which could result in incomplete absorption of the drug, women with childbearing potential, patients with alcohol or drug abuse, or allergy to dihydropyridines

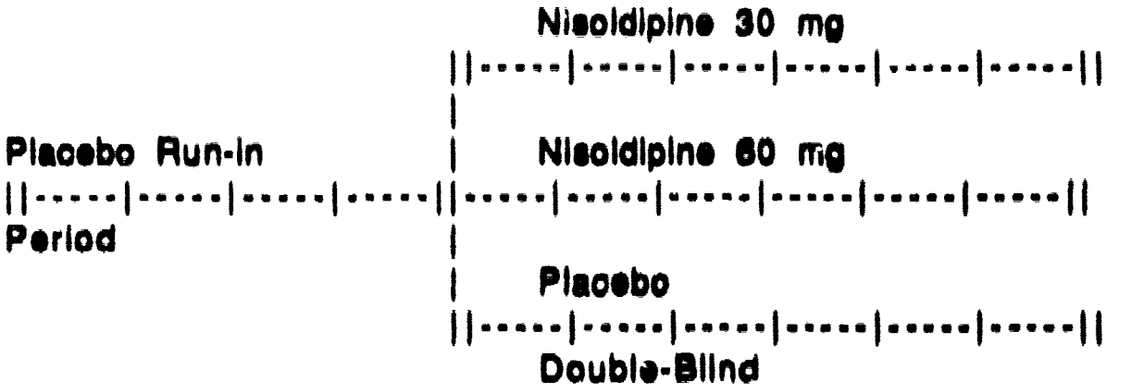
Study Design. . The study consisted of a single-blind placebo run-in period and a treatment period.

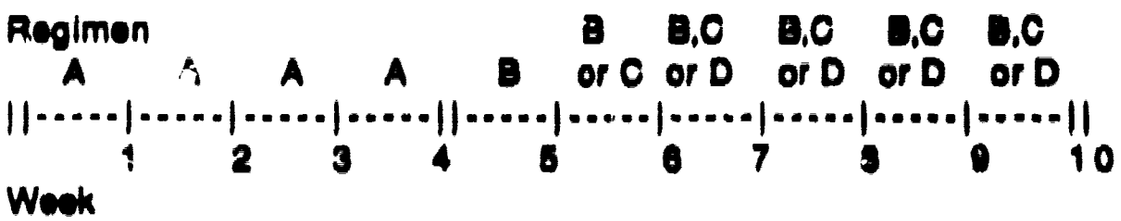
Placebo Run-in Period. During this period of 4 weeks duration pacients discontinued all previous antihypertensive medication and were given a single-blind placebo which consisted of 3 tablets once a day in the morning for a 4-week qualifying run-in period. Then patients with confirmed hypertension, with a trough SUDBP ≥100 to ≤ 114 mmHg after 3 and again after 4 weeks of placebo and whose SUDBP at these 2 visits were within 7 mmHg of each other were admitted into the treatment period.

Treatment Period. After four weeks of single-blind placebo patients with confirmed hypertension were randomized to one of three treatment groups: Placebo, Nisoldipine 30 mg or Nisoldipine 60 mg. Patients randomized to placebo received placebo for the remainder of the study. Placebo randomized to Nisoldipine 30 mg received the same dose for the remainder

of the study. Patients randomized to Nisoldipine 60 mag were given Nisoldipine 30 mg for one or two weeks and the dose was titrated to Nisoldipine 60 mg (2X30) for the final 4 to 5 weeks of the double-blind treatment. A group of patients was to be titrated to 90 mg (3X30) but this arm was discontinued before any patients were randomized because a concurrent high-dose forced-titration clinical pharmacological study showed evidence of symptomatic T wave flattening/or inversion predominantly at doses above Nisoldipine 60 mg.

The study design is shown schematically in the following graph :



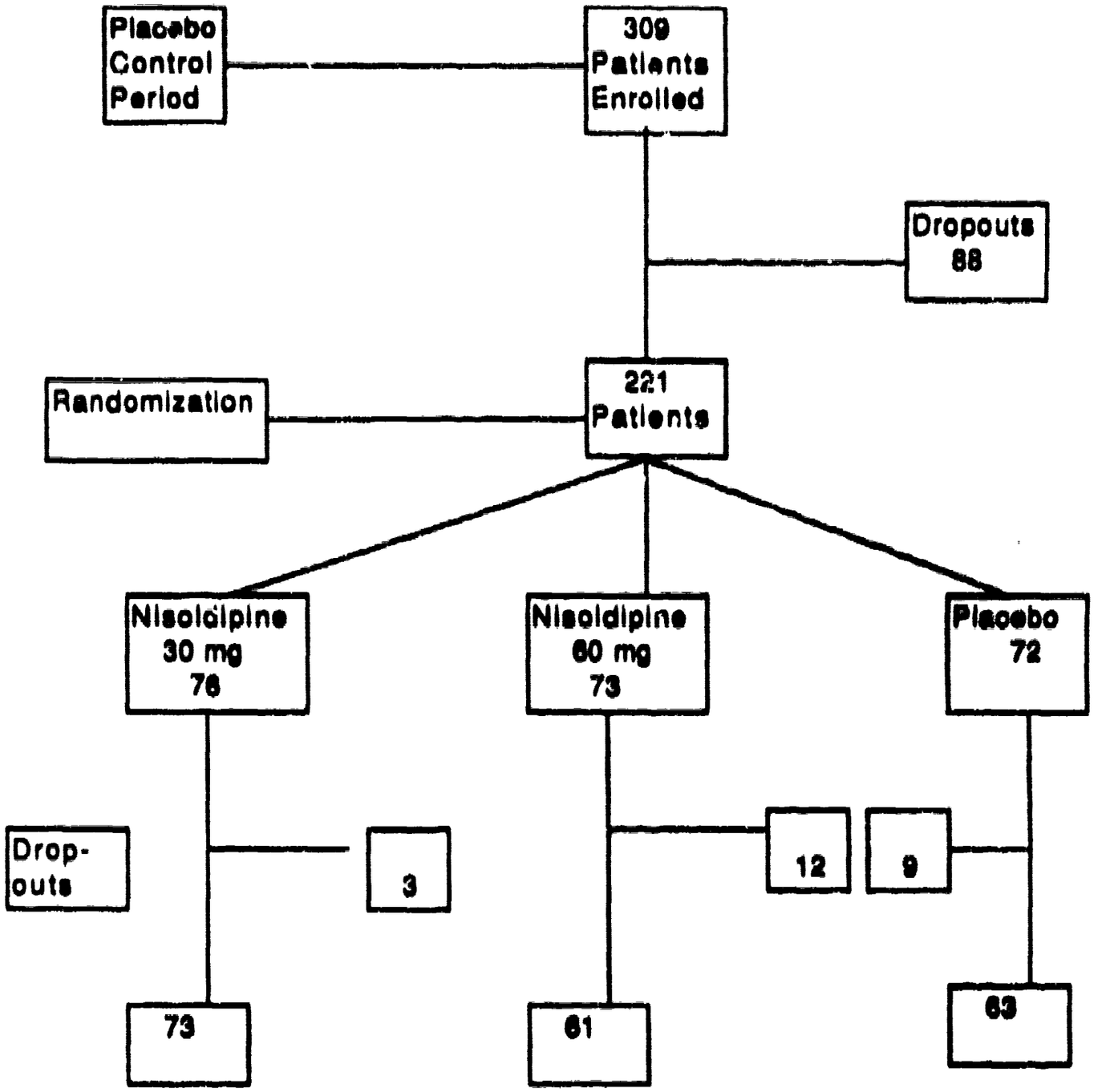


Group	Regimen B	Regimen C	Regimen D
NIS 30 mg	30 mg	30 mg	30 mg
NIS 60 mg	30 mg	60 mg	60 mg
Placebo	Placebo	Placebo	Placebo

Demography. The demography and baseline characteristics in patients valid for analysis of efficacy is given in the following table :

	NIS 30 mg N=76	NIS 60 mg N=66	Placebo N=71
Mean Age (years)	52	52	52
Mean wt (Lbs)	197	197	202
Baseline BP Supine Standing	 157/104 153/104	 158/105 154/104	 155/104 151/103
History of Hypertension (years)	11	9	10
% Male	61	56	52
% Caucasian	58	58	72
% History of Diabetes	9	11	13
% History of Hyperlipidemia	13	12	8
% History of MI	0	2	1
% Mild Hypertensives	62	58	70
% Moderate Hypertensives	38	42	30

The distribution and randomization of patients is seen in the following graph



The listing of patients who did not qualify for randomization is given in the following table :

Mean supine diastolic blood pressure at visit 4 and at visit 5 did not qualify	45
Adverse events	7
Low diastolic blood pressures	6
Patient request	6
Blood pressure too high	5
Elevated serum transaminase levels	5
Childbearing potential	2
Elevated serum lipids	2
Family considerations	2
Illness not due to study medication	2
Intercurrent medical considerations	2
Administrative problems	1
Change in supine diastolic blood pressure ≥ 7 mmHg from visit 4 to visit 5	1
Low hemoglobin/hematocrit	1
Protocol violation	1
Total	88

The reasons for discontinuation of double-blind therapy population in all randomized patients are given in the following table :

Reason	Nisoldipine		Placebo
	30 mg N=76	60 mg N=73	N=72
Adverse Event	1	11	3
Lack of efficacy	1	0	2
Physician dissatisfied with treatment	0	1	2
Patient dissatisfied with treatment	0	0	2
Lost to follow-up	1	0	0

The listing of dropouts due to adverse events for patients valid for safety analysis is given in the following table :

Drug Group	Adverse Experience Causing Patient to Drop	Day of Onset	Dose/ Duration (Days)	Intensity/ Relation-ship to Drug
Placebo	EKG abnormality	-1	Pla/1	Mild/ Remote
	Cardiac arrest	24	Pla/1	Severe/ Remote
	Frontal headaches	28	Pla/>3	Severe/ Possible
Nisoldipine	Edema lower extremity	24	30 mg/ >7	Moderate/ Probable
	Severe headache	3	30 mg/ >11	Severe/ Possible
	Headache	-1	Pla/5	Severe/ Possible
	Severe headache	0	30 mg/7	Severe/Pos
	2+ ankle edema, bilateral	24	60 mg/ 10	Moderate/ Probable
	3+ pretibial edema, bilateral	24	60 mg/ 10	Severe/ Probable
	Trace edema, bilateral	24	60 mg/ 10	Mild/ Probable
	Atypical chest pain	9	60 mg/5	Mod/Poss
	Headache	12	60 mg/ >1	Severe/ Possible
	3 + ankle edema	33	60 mg/ >3	Severe/ Possible
	Headache,vomiting	7	60 mg/ 1	Severe/ Possible
	Headache	12	60 mg/>1	Sev/Rem
	Confusion,	0	30 mg/>1	Sev/Rem
	Nausea,	0	30 mg/3	Mild/Prob
	Headache	6	30 mg/2	Mod/ Probable

Efficacy

Actual Dosage and Duration of Treatment. Patients were to receive Nisoldipine 30 mg, 60 mg or Placebo over a 6 week double-blind treatment period. Upward titration from Nisoldipine 30 mg to Nisoldipine 60 mg was required in the Nisoldipine 60 mg group after one or two weeks of double-blind medication if trough SUDBP was greater than or equal to 80 mmHg. Placebo and Nisoldipine 30 mg underwent sham titration. The following table shows the number of patients that were given each dose level and had valid visits at each week for the population of patients valid for efficacy :

Double-Blind Visit			6	7	8	9	10	
Week of Double-Blind Therapy			1	2	3	4	6	Endpoint
Group	Regimen	Dose	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Nis 30 mg	B (30 mg qd)		75 (100)	10 (13)	4 (5)	3 (4)	3 (4)	3 (4)
	C (30 mg QD)			66 (87)	71 (95)	71 (96)	70 (96)	73 (96)
NIS 60 mg	B (30 mg QD)		65 (100)	7 (11)	5 (8)	5 (8)	4 (6)	5 (8)
	C (60 mg QD)			59 (89)	59 (92)	59 (92)	58 (94)	61 (92)
Placebo	B (Placebo)		71 (100)	6 (8)	2 (3)	2 (3)	2 (3)	2 (3)
	C (Placebo)			65 (92)	65 (97)	64 (92)	62 (97)	69 (97)

Analysis of Effectiveness. Two hundred thirteen of the 221 enrolled patients had at least one valid blood pressure measurement after randomization and were included in the primary efficacy analysis (endpoint) :76 were randomized to the Nisoldipine 30 mg group, 66 were randomized to the Nisoldipine 60 mg group, and 71 were randomized to the placebo group.

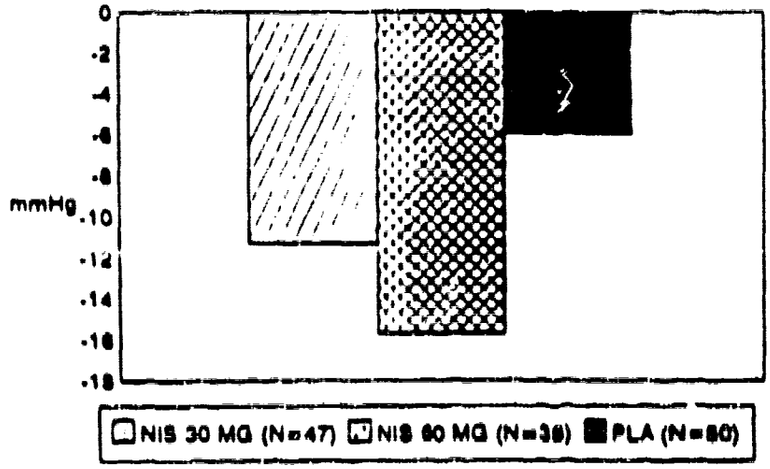
Trough raw means of blood pressure at each visit as well as at endpoint are given in the following table :

	Supine			Standing		
	NIS 30 mg	NIS 60 mg	Placebo	NIS 30 mg	NIS 60 mg	Placebo
Base-line (N)	158/ 104 (76)	158/ 105 (66)	155/ 104 (71)	154/ 104 (76)	155/ 104 (66)	152/ 103 (71)
Week 1 (N)	148/95 (75)	146/94 (65)	152/98 (71)	144/96 (75)	142/94 (65)	149/ 100 (71)
Week 2	147/93 (76)	141/90 (66)	152/98 (71)	143/95 (76)	139/91 (66)	149/99 (71)
Week 3 (N)	144/92 (75)	139/89 (64)	149/97 (67)	140/93 (75)	136/90 (64)	148/98 (67)
Week 4 (N)	142/92 (75)	139/87 (64)	152/98 (66)	140./93 (75)	135/87 (64)	150/99 (66)
Week 6 (N)	145/92 (73)	140/89 (62)	152/98 (64)	140/94 (73)	136/90 (62)	149/ 100 (64)
End-point (N)	146/93 (76)	141/90 (66)	154/99 (71)	141/94 (76)	137/91 (66)	150/ 101 (71)

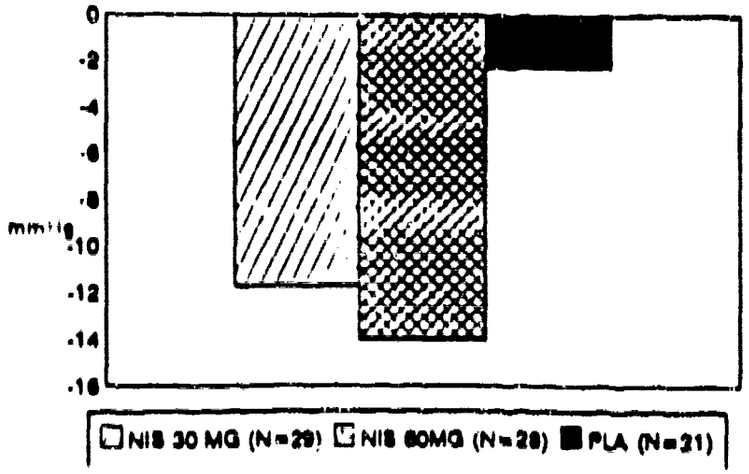
Mean changes (mmHg) in SUDBP at endpoint for patients wit mild (baseline SUDBP ≥ 100 to ≤ 104 mmHg)and moderate (baseline SUDBP ≥ 105 to ≤ 114 mmHg) hypertension are shown in the followings table and figure:

	Nisoldipine 30 mg		Nisoldipine 60 mg		Placebo	
	(N)	Change	(N)	Change	(N)	Change
Mild	47	-11.3	38	-15.7	50	-6.0
Moderate	29	-11.7	28	-14.0	21	-2.3

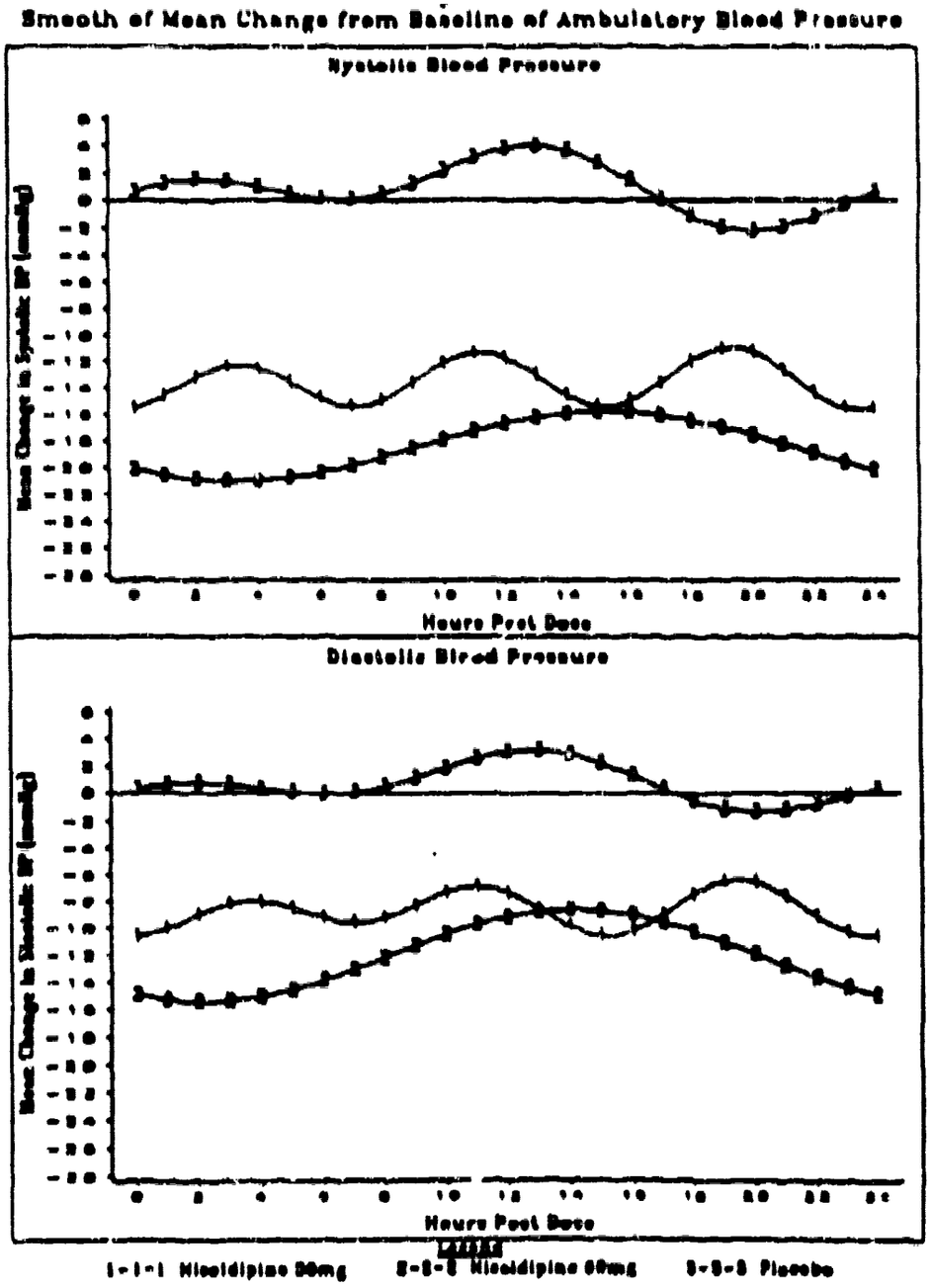
MEAN CHANGE FROM BASELINE (mmHg)
FOR SUPINE DIASTOLIC BP AT ENDPOINT
PATIENTS WITH MILD HYPERTENSION



MEAN CHANGE FROM BASELINE (mmHg)
FOR SUPINE DIASTOLIC BP AT ENDPOINT
PATIENTS WITH MODERATE HYPERTENSION



The 24-hour ambulatory blood pressure profile of mean systolic and diastolic blood pressure responses for the three treatment groups are shown in the following graph :



The ambulatory blood pressure falls and trough to peak ratios change from placebo for patients valid for efficacy analysis are given in the following table :

	N	Peak Hour	Peak value * (mmHg)	Trough* (mmHg)	Trough to Peak ratio
Diastolic BP					
NIS 30 mg	39	13	12.13	9.52	78 %
NIS 60 mg	29	4	15.24	14,22	93 %
Systolic BP (at corresponding time of diastolic peak)					
NIS 30 mg	39	13	17.46	14.52	83 %
NIS 60 mg	29	4	23.13	17.66	76 %
Systolic Blood Pressure (actual)					
NIS 30 mg	39	13	17.46	14.52	83 %
NIS 60 mg	29	5	23.71	17.66	75 %

*Change from placebo, baseline corrected

The mean changes from baseline in diastolic blood pressure over the 24-hour period of ambulatory blood pressure were -8.6 mmHg for Nisoldipine 30 mg, -12.0 mmHg for Nisoldipine 60 mg and +0.7 mmHg for placebo.

Pharmacokinetics Results. Trough blood samples were drawn at all 16 centers at visits 5 an 10. Seven centers also drew blood samples at visit 10.1 at 2 and 12 hours post-dosing. Samples were assayed for Nisoldipine blood levels. Results are presented in the following table :

	N	Mean Concentration (SD) ng/ml	Change in SUBP (Systolic/Diastolic) in mmHg
NIS 30 mg :			
Trough	68	1.5 (1.3)	-12/-11
2 hours post dosing*	27	2.3 (1.9)	-17/-15
12 hours post dosing	27	2.1 (1.2)	-19/-17
NIS 60 mg:			
Trough	55	3.2 (2.8)	-17/-16
2 hours post dosing	27	6.0 (5.2)	-20/-19
12 hours post dosing	26	4.9 (2.8)	-21/-22

There was a statistically significant correlation between plasma concentration and change from baseline to endpoint in supine diastolic blood pressure at trough. The greater the correlation, the greater was the decrease in supine diastolic blood pressure. Twenty percent of the variability in the observed change in supine diastolic blood pressure was explained by plasma concentration.

Assessment. The results of this study indicate that Nisoldipine, at the dose of 30 mg and 60 mg daily once daily, is effective in reducing systolic and diastolic blood pressure at trough in patients with mild to moderate hypertension. The reductions in systolic and diastolic blood pressure were greater than 50 percent of peak effect at trough. Furthermore, ambulatory measurements of blood pressure for 24-hours demonstrated that reductions in blood pressure in Nisoldipine-treated patient was maintained through the hours of observation. The effect was more effective with the 60 mg of Nisoldipine than with the 30 mg dose and in the latter more effective than placebo. Pharmacokinetic studies demonstrated that effect on diastolic blood pressure was proportional to the concentration of Nisoldipine in blood.

Side effects were significantly increased by drug administration as compared to control and were greater with the 60 mg Nisoldipine dose than with the 30 mg. Adverse events are to be discussed by another reviewer.

Protocol D89-039

Title of Study : " Comparative Double-Blind Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine 20 mg, 40 mg, 80 mg Coat Core (CC) Tablets vs a Twice Daily Dose of Verapamil SR 240 mg caplets vs Placebo in Hypertensive Patients ".

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Objectives. The objective of this study was to determine the efficacy and safety of once daily doses of Nisoldipine 20 mh, 40 mg and 80 mg to a twice daily dose of Verapamil 240 mg and to Placebo in patients with mild to moderate hypertension.

Inclusion and Exclusion Criteria. Ambulatory male and female patients, 21 years of age or older, with history of mild to moderate hypertension, were eligible for enrollment in this study.

Patients with the following conditions were excluded from this study : recent myocardial infarction or cerebral vascular accident ; herat failure, major arrhythmias, conduction distrurbances, angina pectoris, sinus bradycardia or severe left ventricular dysfunction ; patients with impaired absorption of the drug ; females pregnant or with childbearing potential ; patients with failure of a major organ system such as liver renal disease, malignancy or psychosis ; alcohol abuse or drug intake ; allergy to dihydropyridines, verapamil or other antagonists ; also excluded were patients who participated in another investigational drug study within the previous 30 days.

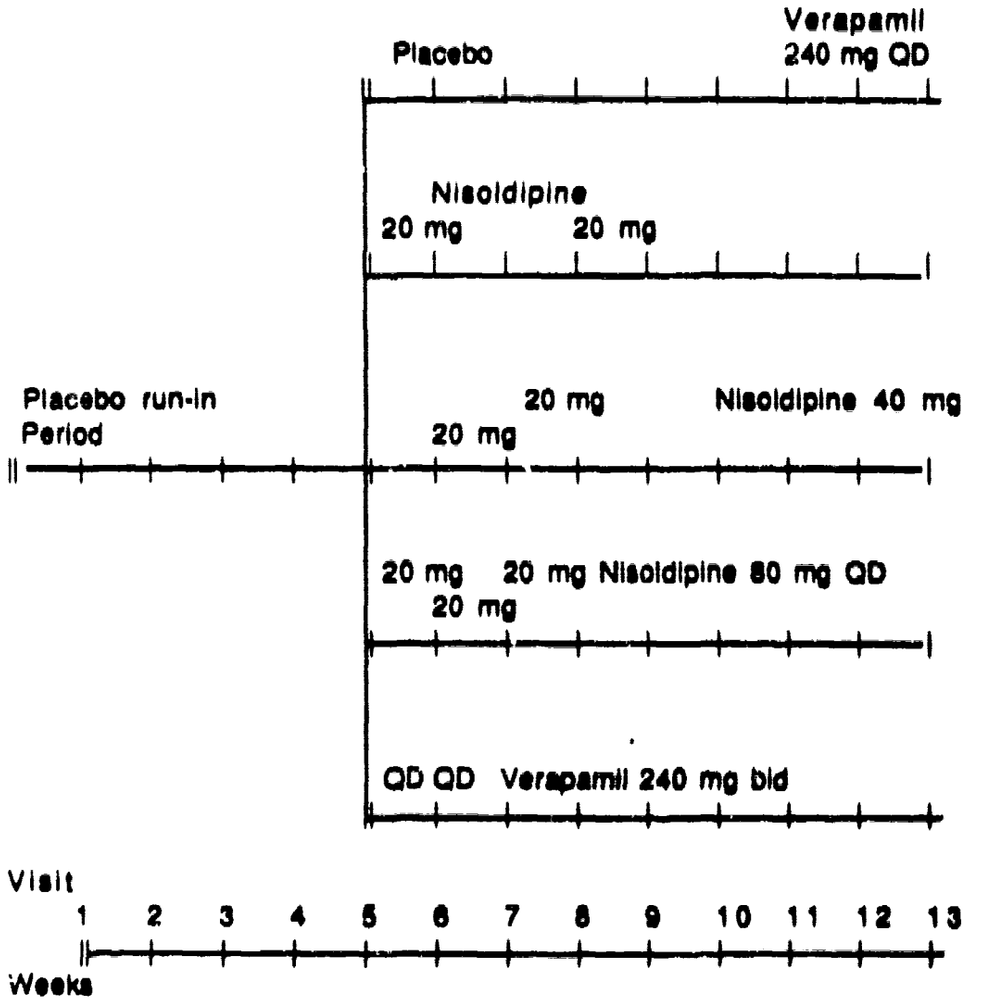
Study Design. The study consisted of a single-blind run-in period and a treatment period.

Single-Blind Run-In Period. Patients were given two placebo tablets and one placebo capsule in the morning and anothe placebo-capsule in the evening each day during a 4-week single-blind-run-in period. Drug for the single-blind placebo run-in period was labeled as Regimen A.

Qualification for Randomization. Patients whose mean SUDBP (the average of 3 readings over a five minute period in the supine position) were 95-114 mmHg after 3 and after 4 weeks on placebo and whose SUDBP after 3 and 4 weeks on placebo were within 7 mmHg of each other were eligible for randomization.

Double-Blind Treatment Period. After the placebo run-in period, a forced titration was designed as follows : Regimen B : Nisoldipine 20 mg, Verapamil 240 mg qd or Placebo which patients took for one week; Regimen C : Nisoldipine 20 mg, Nisoldipine 40 mg, Verapamil 240 mg twice daily or Placebo which patients took for one week ; Regimen D : Nisoldipine 20 mg, Nisoldipine 40 mg, Nisoldipine 80 mg (2 X 40), Verapamil 240 mg twice daily or Placebo which patients took for 6 weeks. After 8 weeks of double-blind drug, patients given Nisoldipine or Verapamil continued on the same drug regimen while patients given Placebo were switched to Verapamil 240 mg qd for the remaining of the 4 weeks of study.

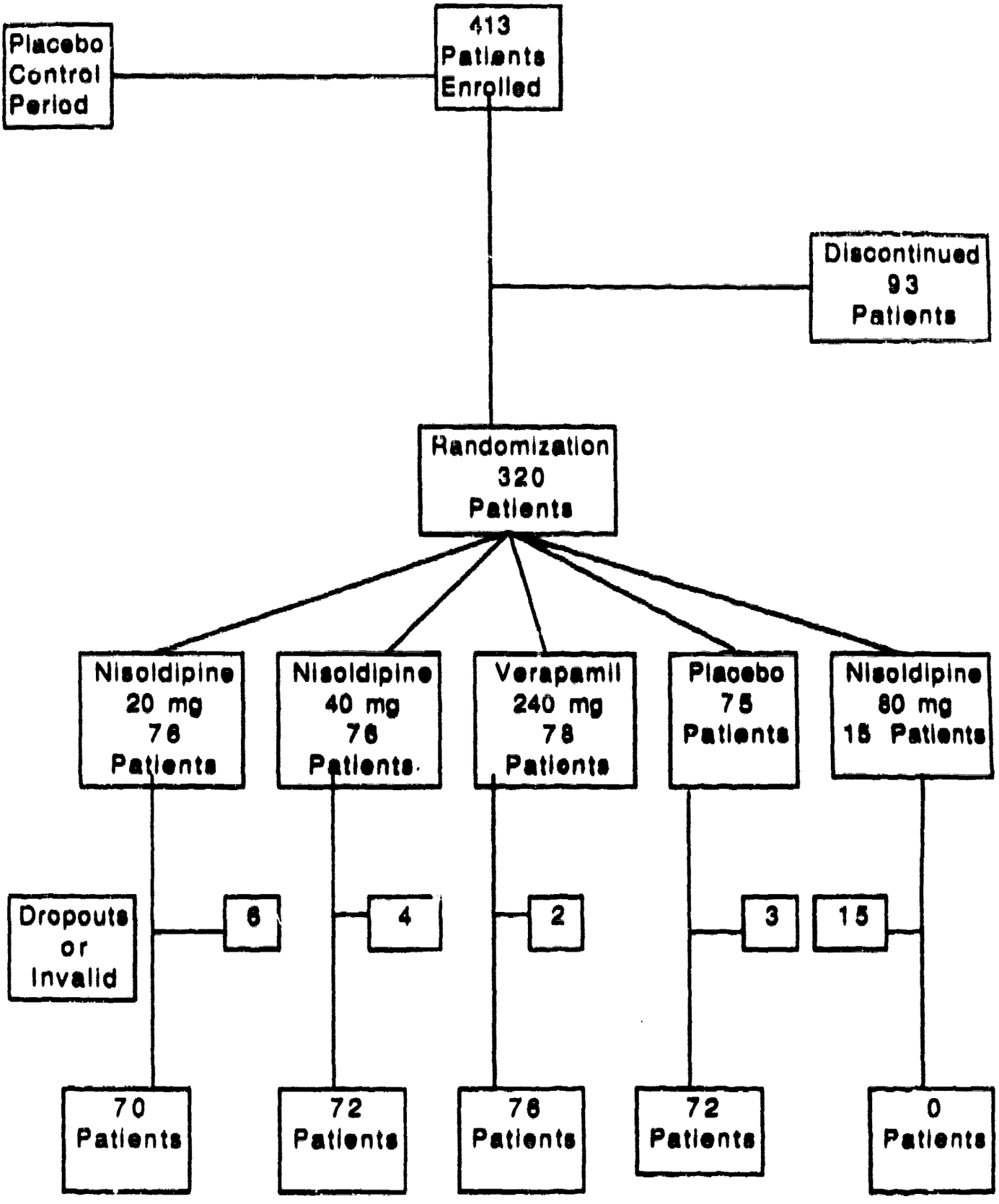
The study design is demonstrated schematically in the following graph :



Demographics. The demographic characteristics are shown in the following table :

	Nisoldipine 20 mg n=70	Nisoldipine 40 mg n=72	Verapamil n=76	Placebo n=72
Mean age (years)	53	54	52	55
Mean wt (lbs)	202	197	198	196
Baseline BP (mmHg)				
Supine	153/100	155/100	151/100	154/100
Standing	151/101	151/101	148/101	151/100
Male	57 %	61 %	55 %	67 %
Black	31 %	24 %	28 %	21 %
History of Diabetes	9 %	1 %	11 %	8 %
History of Hyperlipidemia	3 %	1 %	3 %	10 %
History of MI	3 %	1 %	0 %	1 %
Hypertensives				
Mild	84 %	86 %	83 %	89 %
Moderate	16 %	14 %	17 %	11 %

The distribution of patients and randomization are given in the following graph:



The reasons that disqualified enrolled patients for randomization are given in the following table :

Mean Supine Diastolic Blood Pressure at visit 4 or visit 5 did not qualify for randomization (95 mmHg to 11 mmHg)	47
Adverse events	13
Patient chose to withdraw	9
Other Illness/Surgery/Screening abnormality	5
Blood pressure too high off medications for patient's safety	5
Lost to follow-up	4
Elevated transaminases at screening	3
Noncompliance	3
Called to military service	2
Blood pressure too low after in-clinic	1
Inadequate quality control during ambulatory blood pressure	1
Total	93

The number of dropouts during the treatment period and the reasons for elimination from the study are given in the following table :

Nisoldipine 20 mg. N=76

Event	Days on Drug
Palpitations, depression, headache, emesis	2
Headache, shortness of breath, fatigue	3
Headache, flashing, head congestion	4
Headache, flushing, palpitations	6
Peripheral edema	12
Headache, nausea	12
Peripheral edema	24
Peripheral edema	73
Pleural effusion	77
Myocardial infarction	89
Noncompliance	7
Chose to withdraw	54

Nisoldipine 40 mg. N=76

Event	Days on Drug
Headache, rash	1
Headache, nausea	2
Headache, nausea	2
Peripheral edema	10
Peripheral edema	12
Myocardial infarction	13
Headache, tremor, flushing, palpitations hypesthesia, asthenia	14
Peripheral edema	16
Peripheral edema	22
Peripheral edema	40
CVA	41
Chose to withdraw	16
Chose to withdraw	32

Nisoldipine 80 mg. N=15

Headache, flushing, palpitations, chest pain	1
Flushing, palpitation	14
Deep T wave inversion	15
Discontinued	3
Discontinued	4
Discontinued	6
Discontinued	12
Discontinued	13
Discontinued	14
Discontinued	17
Discontinued	20
Discontinued	20
Discontinued	24
Discontinued	28
Discontinued	31

Verapamil 240 mg. N=78

Event	Days on Drug
Headache, dizziness, tachycardia, leg pain, tinnitus	0
Peripheral edema	17
Hypotension	22
Headache, chills, peripheral edema	36
Cholecystitis	7

Placebo. N=75

CVA	6
Fatigue, edema	14
Peripheral edema	32
Lack of efficacy	5
Lack of efficacy	48
Lost to follow-up	21
Lost to follow-up	69
Chose to withdraw	47
Chose to withdraw	62
DBP> 114 mmHg	27
Retinal disorder	55

Efficacy

Criteria for Effectiveness. The change from baseline to endpoint in trough SUDBP (blood pressure measured 24 hours after the previous day's morning dose and 12 hours after the previous day's evening dose) in the Nisoldipine 40 mg group compared to the placebo group was the primary criterion used to determine the effectiveness of the drug.The comparison of Nisoldipine 20 mg to Placebo was of secondary importance.

Secondary efficacy parameters included standing diastolic blood pressure and both standing and supine systolic blood pressure. In addition in eight centers ambulatory blood pressure changes (the difference between measurements made over the 24 hours after 3 weeks of placebo run-in and the 24 hours after 7 weeks of double-blind therapy) were compared among

groups. The 12-hour in-clinic monitoring data were also compared among groups. The peak effect and the time to peak effect were calculated for both the ambulatory and 12-hour in-clinic monitoring. In addition the trough to peak ratio was calculated for ambulatory blood pressure. Plasma samples were drawn at baseline (visit 5) and at visit 11 for analysis of Nisoldipine plasma concentrations.

Statistical Methods. All statistical methods were two-tailed and were conducted at a significance level of 0.05. Pairwise comparisons and within group changes were tested via the least squares means estimated by the model.

Analysis of Effectiveness. The mean blood pressure changes at endpoint (mmHg) for patients valid for efficacy analysis are given in the following table :

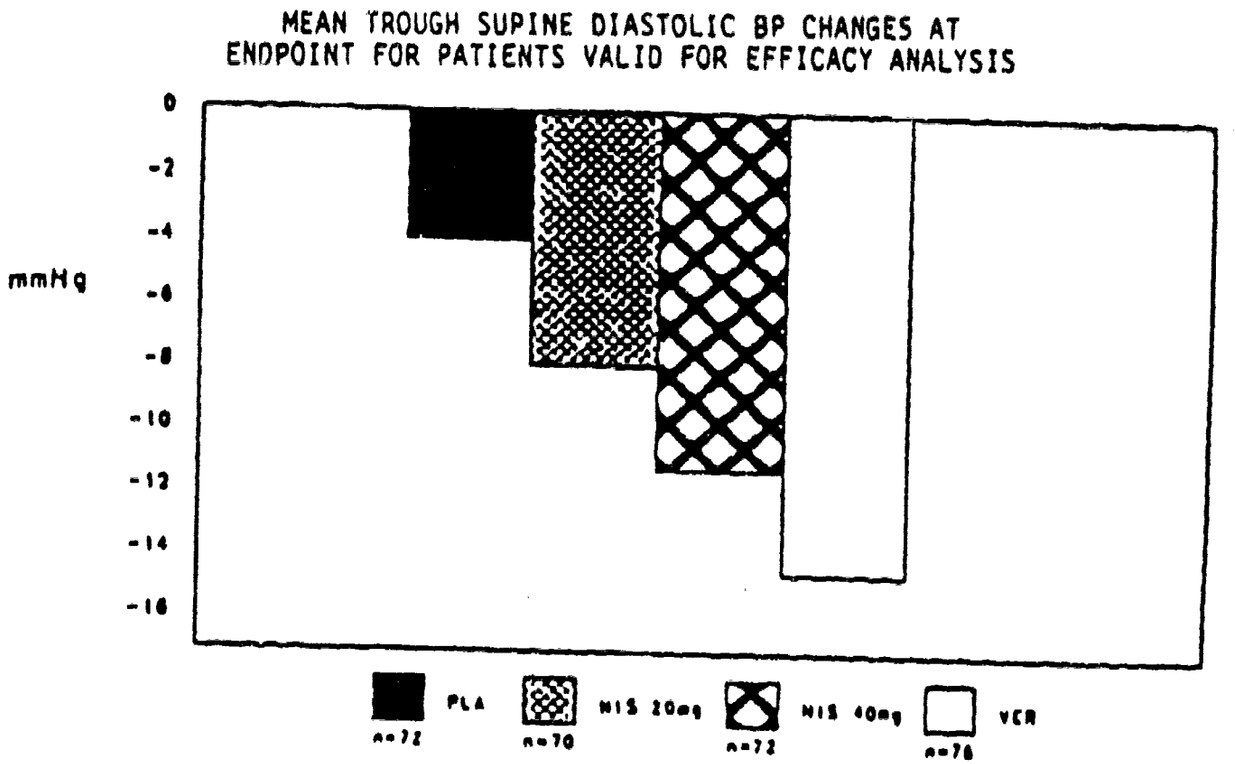
	Nisoldipine 20 mg N=70	Nisoldipine 40 mg N=72	Verapamil N=76	Placebo N=72
Supine				
Diastolic	-8.1 ABP	-11.4 BP	-14.7 P	-4.0
Systolic	-9.6 ABP	-16.2 P	-16.0 P	-2.2
Standing				
Diastolic	-7.1 ABP	-11.8 BP	-13.9 P	-2.0
Systolic	-11.6 BP	-15.4 P	-16.4 P	-2.4

A Significantly different from Nisoldipine 40 mg
B Significantly different from Verapamil
P Significantly different from Placebo

Mean changes (mmHg) in SUDBP at endpoint for patients with mild (baseline SUDBP 95-104 mmHg) and moderate (Baseline SUDBP 105-114 mmHg) are shown in the following table :

	Nisoldipine 20 mg		Nisoldipine 40 mg		Verapamil		Placebo	
	n	Change	n	Change	n	Change	n	Change
Mild	59	-8.4	62	-11.3	63	-14.1	64	-4.1
Moderate	11	-6.5	10	-13.0	13	-18.0	8	-3.5

The effect on SUDBP at endpoint in the Nisoldipine (24 hours after dose), Verapamil group (12 hours after dose) and Placebo group is shown in the figure below :



The results by visit for SUDBP are shown in the following graph :

	Week 1	Week 2	Week 3	Week 4	Week 6	Week 8
NIS 20 mg						
n	69	70	66	65	68	68
Mean Change	-7.8	-7.2	-8.6	-7.5	-7.9	-8.0
NIS 40 mg						
n	72	72	66	65	65	63
Mean Change	-7.4	-9.8	-9.9	-11.2	-11.0	-11.8
Ver						
n	76	75	73	69	73	71
Mean Change	-5.9	-11.0	-12.8	-13.1	-12.6	-14.6
Placebo						
n	72	72	68	67	69	67
Mean Change	-4.0	-4.7	-5.1	-4.4	-5.7	-4.1

During the secon phase of the double-blind period, the differences between the active drugs decreased, while the Placebo group experienced the expected further decrease in blood pressure after switching to Verapamil. The changes from baseline in trough SUDBP at the two visits in thus phase are presented below :

	Week 10	Week 12
NIS 20 mg	-8.8	-10.1
NIS 40 mg	-12.2	-10.3
Verapamil	-13.1	-11.5
Placebo	-7.3	-7.0

Various demographic variables were examined including sex, weight, age, smoking status, race and baseline blood pressure. Of these only age exhibited a marked difference in blood pressure response. Mean changes from baseline in supine blood pressures for each drug group for patients at least 60 years old vs patients younger than 60 years old are provided in the table below :

	NIS 20 mg		NIS 40 mg		Verapamil		Placebo	
	n	Mean	n	Mean	n	Mean	n	Mean
Diastolic								
Age ≥ 60	28	-10.4	26	-13.6	24	-16.2	23	-4.0
Age < 60	42	- 6.6	46	-10.4	52	-14.1	49	-4.0
Systolic								
Age ≥ 60	28	-14.0	26	-21.2	24	-19.3	23	-2.3
Age < 60	42	-6.8	46	-13.6	52	-14.5	49	-2.2

Responders rate based on trough SUDBP are presented in the following table :

	NIS 20 mg N=70	NIS 40 mg N=69	Verapamil N=76	Placebo N=72
DBP ≤ 90 mmHg	35 (50 %)	50 (69%)	62 (82 %)	19 (26 %)
DBP decrease ≥ 10 mmHg	28 (40 %)	47 (65 %)	59 (78 %)	10 (14 %)

In clinic monitoring was done for 12 hours and 24-hour ambulatory blood pressure monitoring for 24 hours.

The in clinic monitoring, that covered only half of the dosing interval yielded the following results :

Dose	Mean Change Range mmHg/	Hour
Nisoldipine 20 mg	-9.8	12
	-13.5	8
Nisoldipine 40 mg	-10.8	12
	-15.5	4
Verapamil	-11.8	
	-15.1	
Placebo	-2.5	
	-5.5	

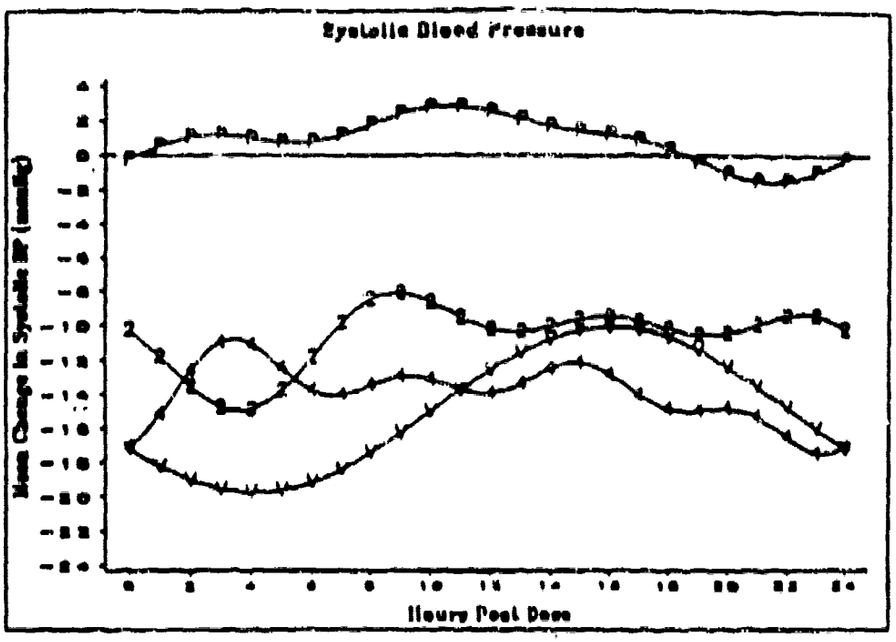
On ambulatory blood pressure monitoring response after 7 weeks of therapy was observed for 24 hours after Nisoldipine 40 mg therapy, 4 hours after Nisoldipine 20 mg therapy, and 4 hours after the morning dose of Verapamil. with blood pressure changes (systolic/diastolic)of -17.5/ -10.7 mmHg, -15.1/10.2 mmHg, and -19.7/-14.3 mmHg respectively. The mean 24-hour systolic and diastolic blood pressure changes during ambulatory blood pressure monitoring were :

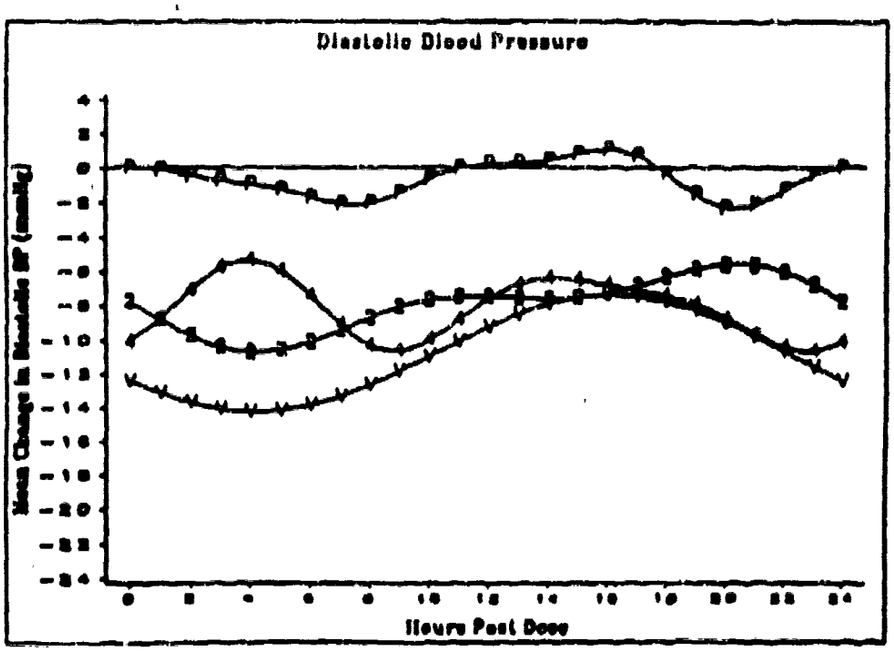
Nisoldipine 40 mg	-13.6/-8.0
Nisoldipine 20 mg	-11.1/-7.9
Verapamil	-14.8/10.8

Based on smoothed ambulatory blood pressure data, the trough/peal ratios for the treatment groups are summarized in the following table :

	Trough mmHg	Peak mmHg	Trough to peak ratio
Diastolic BP			
NIS 20 mg	-6.7	-9.7	69 %
NIS 40 mg	-11.7	-11.7	100 %
Verapamil	-11.1	-12.9	86 %
Systolic BP			
NIS 20 mg	-9.9	-15	66 %
NIS 40 mg	-14.3	-14.3	100 %
Verapamil	-15.9	-20.9	78 %

The unsmoothed change from baseline ambulatory data in systolic and diastolic blood pressure are shown below

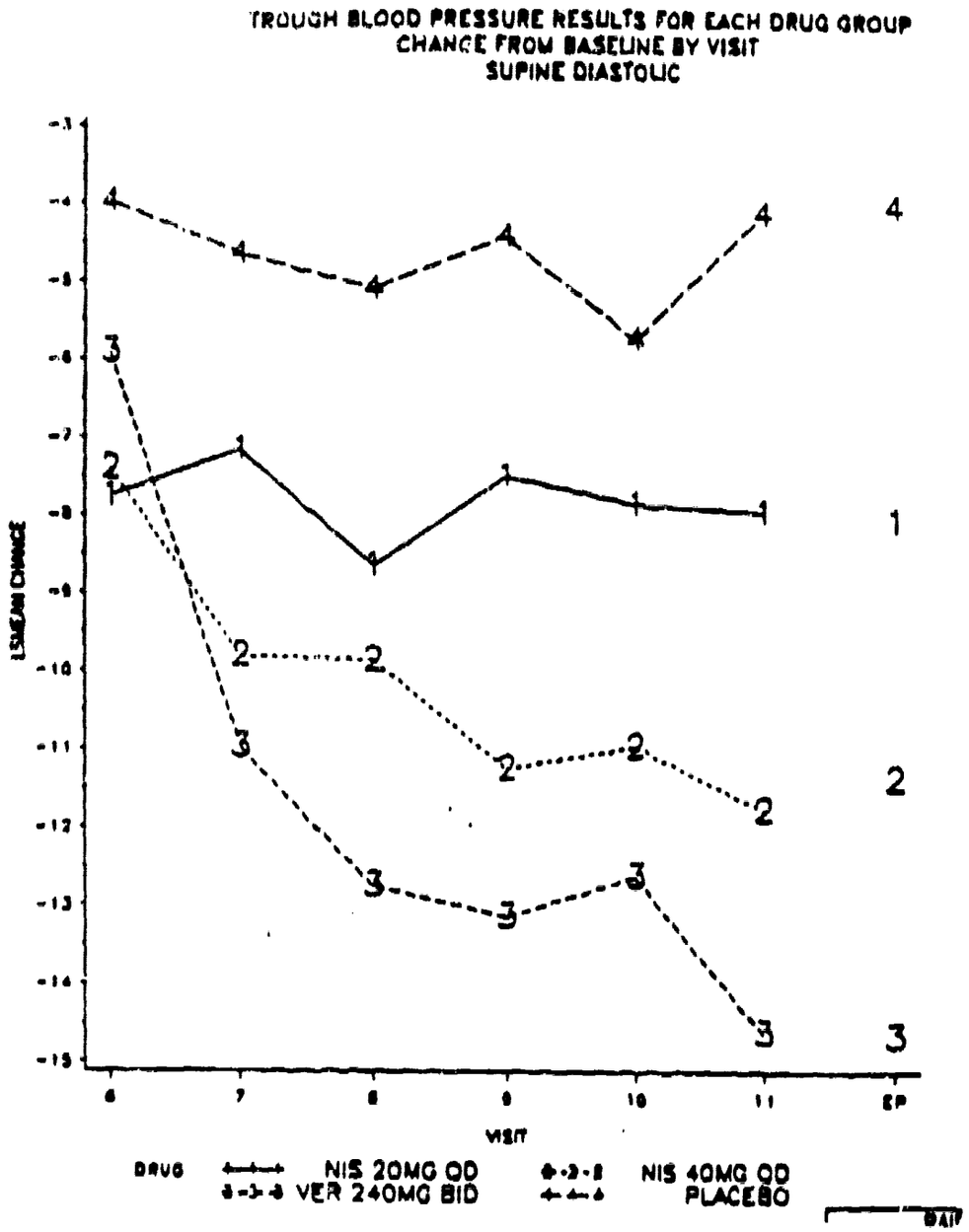




Legend

8-8-8 Nis 80mg 4-4-4 Nis 40mg V-V-V Verapamil P-P-P Placebo

The trough blood pressure results for each drug group change from baseline by visist supine diastolic is given in the following graph :



Pharmacokinetic Results. Trough blood samples were drawn at visits 5 and 11. Visit 11 samples were analyzed for Nisoldipine and results are summarized below :

	n	Range of Concentrations (ng/ml)	Mean Concentrations (ng/ml)
NIS 20 mg	66	0-3.19	1.0
NIS 40 mg	61	0-6.83	2.2
NIS 80 mg	3	0-5.24	2.3

Assessment. The study was initially designed to determine the effectiveness of Nisoldipine at doses of 20, 40, 80 mg, Verapamil and placebo. The 80 mg dose of Nisoldipine was dropped when in another study of a high-dose forced-titration study of Nisoldipine 120 mg daily showed asymptomatic T waves flattening and/or inversion on electrocardiogram predominantly at doses above 60 mg daily.

The 20 and 40 concentrations of Nisoldipine demonstrated to be more effective in lowering the blood pressure than placebo, and the 40 mg more effective than the 20 mg. Also the effectiveness was greater in subjects older than 60 years especially in lowering the systolic blood pressure. Verapamil bid was more effective in lowering blood pressure than any of the concentrations of Nisoldipine.

Peak and trough values were determined by ambulatory blood pressure monitoring and the antihypertensive effect was well sustained at 24 hours after dose administration in all concentrations of Nisoldipine evaluated in this study.

By pharmacokinetic studies the concentration of Nisoldipine in blood was determined and was found to be more elevated after the 40 mg administrations of Nisoldipine than after the 20 mg concentration. There was no major difference between the 40 mg and 80 mg dose of Nisoldipine.

Protocol D90-006

Title of Study : " South-African Multicentre Study to Investigate the Anti-Hypertensive Effect of Three Single Oral Daily Doses of Nisoldipine Administered as a Long Acting "Coat-Core" Tablet Formulation."

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Objectives. The objectives of this study were :

1. To compare the anti-hypertensive efficacy and safety of three daily doses of Nisoldipine coat-core formulation, namely 10 mg, 20 mg and 30 mg with placebo.

2. To study a dose-response relationship for Nisoldipine coat-core.

3. To assess the consistency of anti-hypertensive response over 6 weeks.

Additional objectives were :

1. To describe the blood pressure profile of the last day of therapy by continuous automated ambulatory blood pressure monitoring in a group of patients, and hence :

2. To quantify the trough/peak blood pressure relationship for this therapy.

Inclusion Criteria. Patients with newly diagnosed mild to moderate hypertension were eligible to enter the study. In addition patients with mild to moderate hypertension being treated who, in the opinion of the investigator, were no significantly placed at risk by withdrawal of previous anti-hypertensive medication during the 4-week placebo run-in period could also be enrolled in the study.

Exclusion Criteria. Patients were not eligible if they had labile hypertension, clinical evidence of major arrhythmias, angina pectoris, conductio disturbances or heart failure, or recent or impending myocardial infarction, or a cerebral vascular accident in the previous 3 months, history of allergy to dihydropyridines, type 1 diabetes mellitus, impaired renal function, liver disease, elevated transaminases, treatment with antihypertensives or any other drug that may affect the blood pressure or may interact with the effects of calcium antagonists.

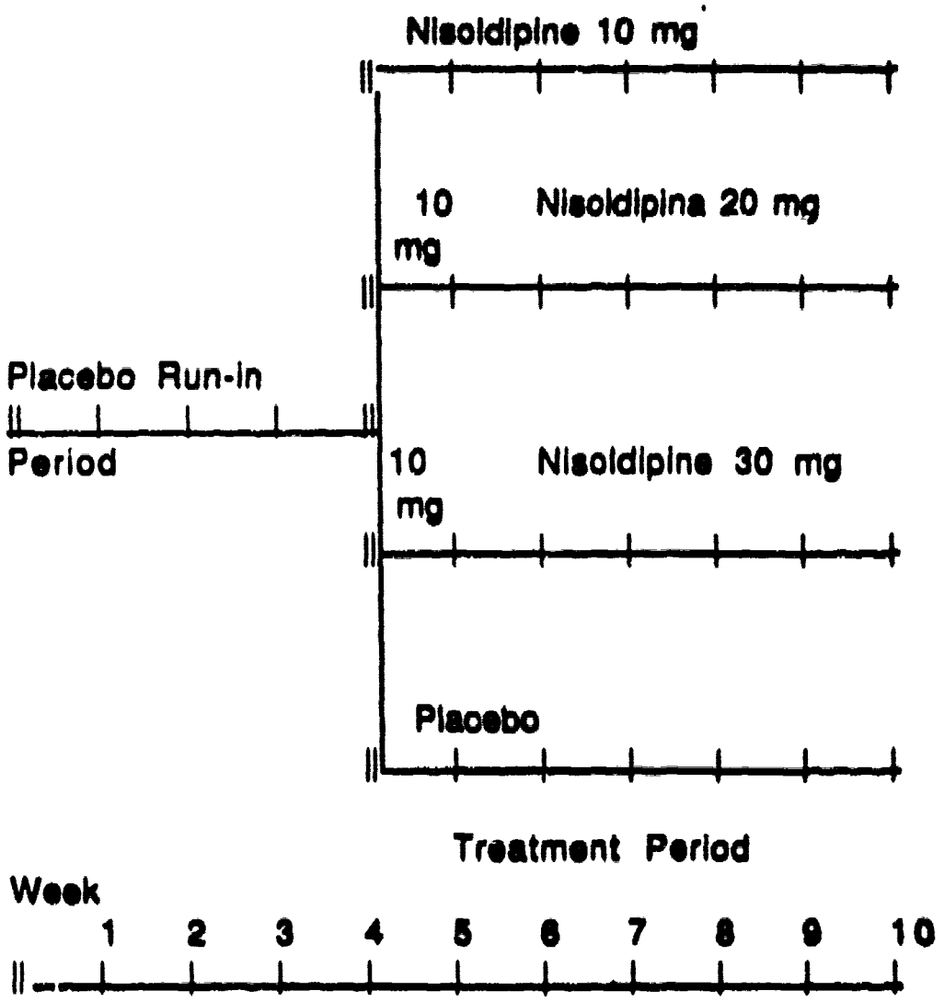
Study Design. This was a 10 week, multi-centre, randomized, placebo controlled, parallel group comparison of Nisoldipine coat-core 10 mg, 20 mg, 30 mg versus placebo. The study consisted of two periods : a single-blind placebo run-in period and a double-blind, randomized, placebo-controlled, group comparison (treatment period.

Placebo run-in Period. During this period of 4 weeks duration all antihypertensive medication was discontinued and one placebo tablet was given to be taken in the morning before breakfast. Patients whose SUDBP was ≥ 95 mmHg and ≤ 114 mmHg at visits 2 and 3 were eligible for enrollment in the active treatment phase.

Treatment Period. Eligible patients were randomized to one of four arms : placebo, 10 mg Nisoldipine, 20 mg Nisoldipine and 30 mg Nisoldipine.

Patients randomized to placebo or 10 mg Nisoldpine were to receive their treatment for 6 weeks. Patients in the two higher dose groups (Nisoldipine 20 mg or Nisoldipine 30 mg) were to receive 10 mg for the first week following by 5 weeks of their randomized treatment in order to avoid rapid exposure to the higher doses.

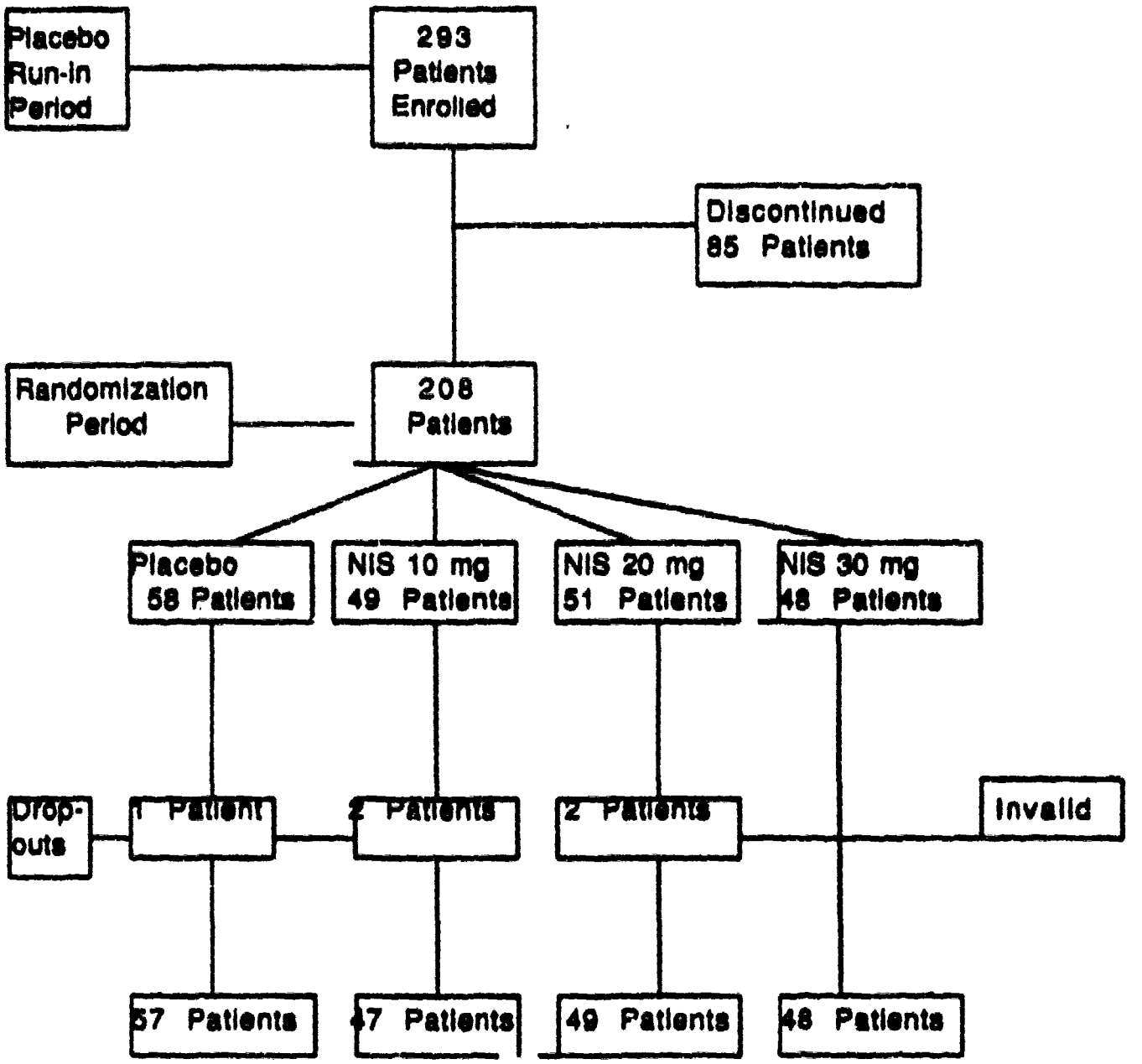
The study design is demonstrated schematically in the following graph :



The demographic information is given in the following table :

		Placebo n=58	NIS 10 mg n=49	NIS 20 mg n=51	NIS 30 mg n=48
Sex (p=0.78)	Male Female	27 (47 %) 31 (53 %)	24 (49 %) 25 (51 %)	20 (39 %) 31 (61 %0	21 (44 %) 27 (56 %)
Race (p=0.98)	Caucasian Black Asian Other	30 (52 %) 27 (29 %) 6 (20 %) 5 (9 %)	24 (53 %) 16 (33 %) 2 (4 %) 5 (10 %)	25 (49 %) 18 (35 %) 6 (12 %0 2 (4 %)	26 (54 %) 14 (29 %) 5 (11 %) 3 (6 %)
Age (years) Mean	Mean Mean=0.2	53	50	55	50
Weight (kg)	Mean (p=0.69)	80.8	77.3	79.7	80.3
Baseline Means BP Supine Systolic Diastolic Standing Systolic Diastolic	 (p=0.17) (p=0.65) (p=0.69) (p=0.09	 163.8 103.5 160.5 105.1	 161.2 104.7 159.7 107.9	 167.2 104.8 163.8 107.4	 164.3 104.4 161.7 107.4
Mild Hypert. n Baseline SDBP		 33 99.7	 25 99.3	 25 99.2	 27 100.1
Moder. Hypert. N Baseline SDBP		 25 108.5	 24 110.2	 26 110.1	 21 110.1

The distribution and randomization of patients is illustrated in the following graph :



The reasons for patients who did not enter the double-blind treatment period is given in the following table :

Reason	Number of Patients
Supine diastolic blood pressure < 95 mmHg	58
Supine diastolic blood pressure > 114 mmHg	13
Unwilling to continue	5
Patient had raised serum calcium levels	1
Uncontrolled non-insulin dependent diabetes mellitus	1
Raised liver enzymes	3
Left ventricular failure	1
Right ventricular failure when taken off diuretic	1
Major arrhythmias	2
Total	85

Invalid Results and Drop-outs During the Treatment Period. Three patients dropped-out during the treatment period. One patient in the placebo group died after experiencing cerebral hemorrhage 33 days after entering the double-blind treatment period. One patients in the Nisoldipine 10 mg experienced severe tinnitus 30 days after entering the double-blind treatment period. Another patient in the 10 mg Nisoldipine group had a severe headache and dropped 17 days after entering the double-blind treatment period.

Efficacy.

Criteria for Efficacy. The primary variable for assessing efficacy was the trough 24-hour supine diastolic blood pressure (SUDBP), and especifically the change in suDBP from baseline to endpoint (visit 6, week 6 or the last valid visit). The change from baseline in each of the three Nisoldipine treatment groups was compared to the Placebo group. Secondary efficacy variables were supine systolic BP and standing diastolic and systolic blood pressure.

Statistical Analysis. Two types of analysis were followed. The first and primary analysis was the standard endpoint analysis, also referred as the main efficacy analysis. The second was the intent-to treat analysis (ITT).

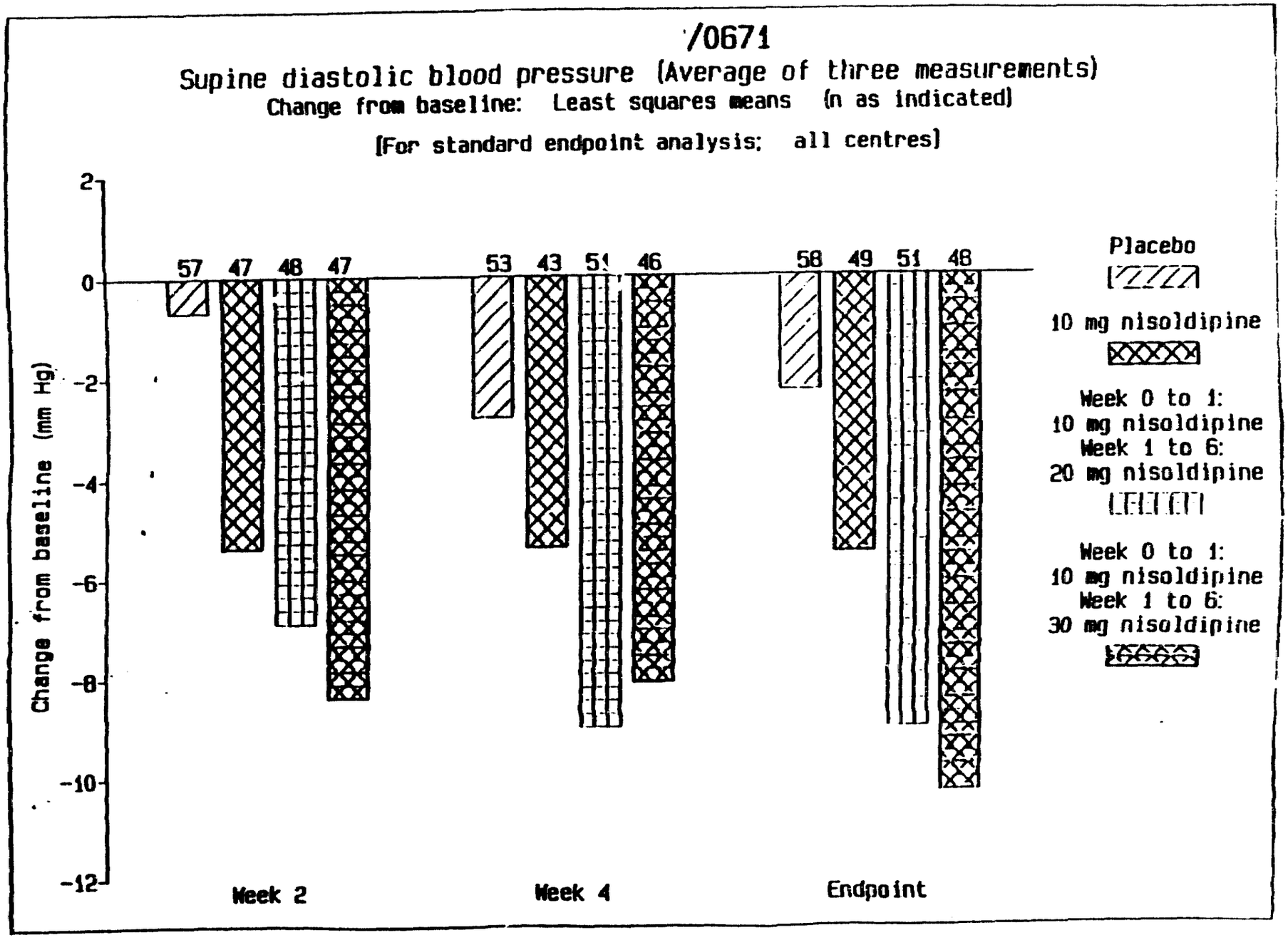
All patients adherent to the protocol with a valid treatment duration of at least 2 weeks on double-blind treatment were included in the main efficacy analysis. These patients completed at least a two-week double-blind treatment period during which they were compliant. and after which the blood pressure was taken between 22.5 h and 25.5 h after the last tablet intake. Patients who discontinued treatment because of lack of efficacy or adverse events were also included. Only 2 patients who received double-blind treatment were considered invalid for the main efficacy

analysis. They were included in the intent to treat analysis. In one patient the baseline measurements were lost
and in another patient only 10 tablets instead of 20 tablets were dispensed.

The results of change from baseline at endpoint in trough blood pressure in all patients valid for the main efficacy analysis (n=206) are given in the following table :

	Placebo n=58	NIS 10 mg n=49	NIS 20 mg n=51	NIS 30 mg n=48
Supine DBP				
Baseline	103.5	104.7	104.8	104.4
Endpoint	101.1	99.3	95.7	94.3
Difference (NIS-Placebo)		-3.2	-6.7	-8.0
Supine SBP				
Baseline	163.8	167.2	167.2	164.3
Endpoint	163.3	149.8	149.8	148.8
Difference (NIS-Placebo)		-8.9	-17.8	-15.9
Standing DBP				
Baseline	105.1	107.9	107.4	107.4
Endpoint	104.6	101.1	98.1	96.9
Difference (NIS-Placebo)		-6.9	-9.2	-10.6
Standing SBP				
Baseline	160.5	159.7	163.8	161.7
Endpoint	160.5	150.6	147.5	145.7
Difference (NIS-Placebo)		-9.5	-15.9	-16.2

The results on SDBP are demonstrated in the following graph :


/0671
Supine diastolic blood pressure (Average of three measurements)
Change from baseline: Least squares means (n as indicated)
[For standard endpoint analysis: all centres]
Change from baseline (mm Hg)
2
0
-2
-4
-6
-8
-10
-12
57 47 48 47
53 43 51 46
58 49 51 48
Week 2
Week 4
Endpoint
Placebo
10 mg nisoldipine
Week 0 to 1:
10 mg nisoldipine
Week 1 to 6:
20 mg nisoldipine
Week 0 to 1:
10 mg nisoldipine
Week 1 to 6:
30 mg nisoldipine

The mean change from baseline in supine diastolic pressure (mmHg) for each treatment group after stratification for age is shown in the following table : (Main efficacy analysis = 206).

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg	Overall Least Square Means
Age < 45 years	-6.5 (12)*	-4.1 (14)	-10.6 (11)	-8.9 (15)	-7.5
Age≥ 45 and < 65 years	-1.4 (38)	-6.9 (28)	-8.3 (31)	-10.7 (27)	-6.8
Age ≥ 65 years	-1.3 (8)	-2.0 (7)	-10.1 (9)	-10.7 (6)	-6.0

* The number of patients used for calculating the mean values are given in brackets.

Results from ANOVA

Age effect : p=0.62
Treatment Effect : p=0.0001
Treatment by age interaction effect : p=0.12.

These results indicate that there is no association between age and the diastolic blood pressure response.

The mean change from baseline in supine diastolic blood pressure (mmHg) for each treatment group after statification for race is given in the following table : (Main efficacy analysis = 206).

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg	Overall Least Square Means
Caucasian	-1.2 (30)*	-4.6 (26)	-7.5 (25)	-9.2 (26)	-5.6
Black	-2.3 (17)	-5.9 (16)	-10.3 (18)	-10.1 (14)	-7.2
Other	-6.3 (11)	-7.0 (7)	-11.5 (8)	-13.1 (8)	-9.5

Analysis of Response and Normalization Rates. Responders were defined as patients who had SDBP of less than or equal to 90 mmHg or patients who had a drop in SDBP of at least 10 mmHg at endpoint. A patient's blood pressure was said to be normalized when satisfied these two conditions, namely, a drop in supine DBP to 90 mmHg or below, and a drop of at least 10 mmHg.

The following table shows the response rates for each treatment group, odds ratio and relative efficacy of each Nisoldipine treatment relative to Placebo from the main efficacy analysis of the last visit:

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg
Total number of Patients Responders Response Rate	58 10 17 %	49 17 35 %	51 24 47 %	48 30 63 %
Odds Ratio (OR) NIS relative to Placebo 95 % CI for OR		2.4 1.0 ; 5..5	4.6 1.8 ; 12	8.8 3.8 ; 22

Relative Efficacy (RE) NIS relative to Placebo 95 % CI for RE		2.0 1.0 ; 3.9	2.6 1.5 ; 4.8	3.7 2.1 ; 6.2

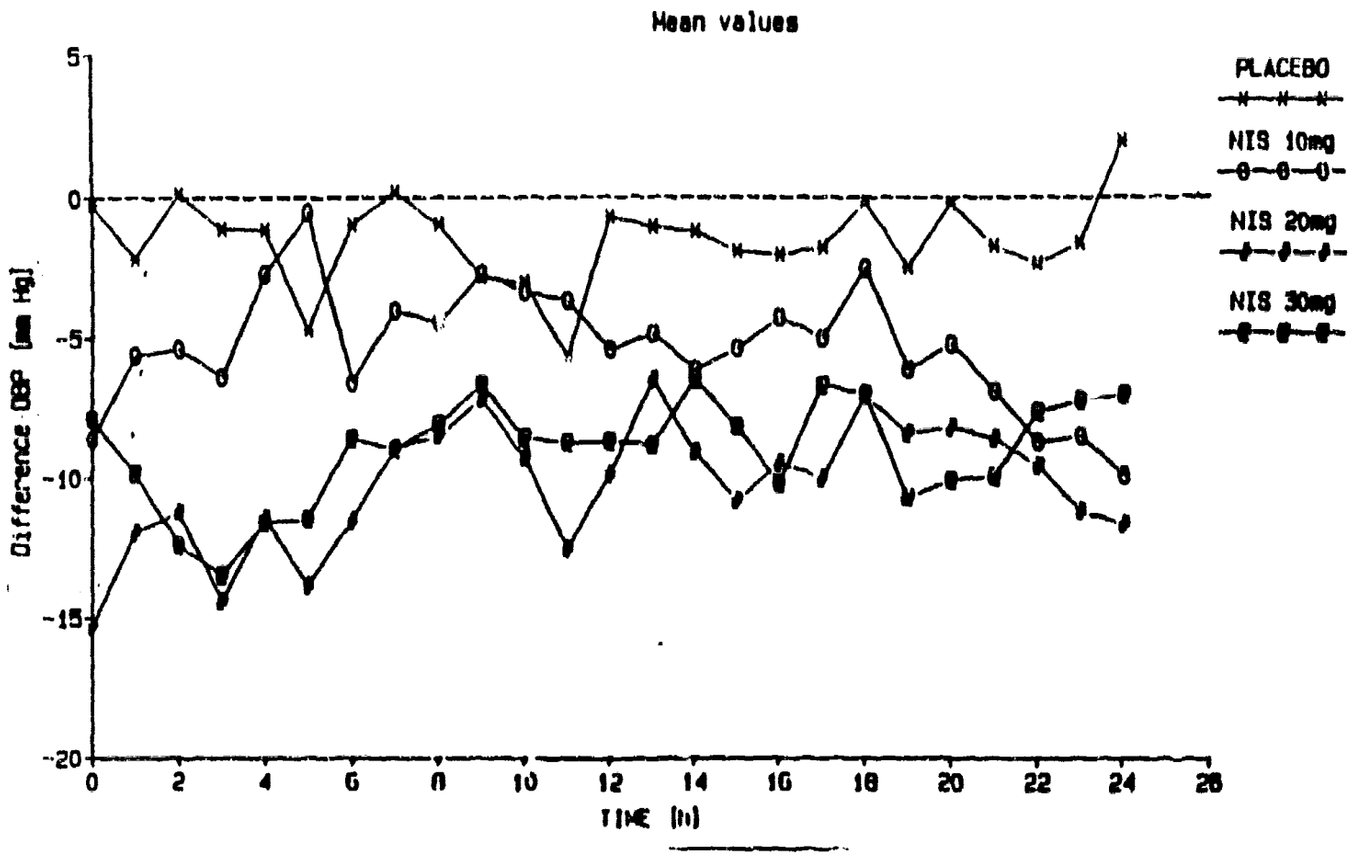
These results can be interpreted as indicating that the response rate for placebo was 17 %, Nisoldipine 10 mg 35 %, Nisoldipine 20 mg 47 % and Nisoldipine 30 mg 63 %. A relat'ye efficacy of 2.6 of Nisoldipine 20 mg vs Placebo means that a positive treatment response is 2.6 times more likely to occur under Nisoldipine 20 mg than placebo. The confidence interval of 1.5 to 4.8 indicates that the true relative efficacy is likely (95% confidence limits) to be at least 1.5 and at most 4.8.

The following table shows the normalization rates for each treatment group, odds ratio, and relative efficacy of each Nisoldipine treatment relative to Placebo from the main efficacy analysis of the last visit :

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg
Total Number of patients	58	49	51	48
Number of Patients	5	5	13	13
Normalization Rate	8.6 %	10 %	25 %	27 %
Odds Ratio (OR) NIS relative to Placebo 95% CI for OR		1.2 0.31 ; 4.8	4.3 1.4 ; 1.3	4.3 1.4 ; 13
Relative Efficacy (RE) NIS relative to Placebo 95 % CI for RE		1.2 0.37 ; 3.9	3.1 1.3 ; 7.3	3.3 1.3 ; 8.1

These results can be interpreted in the same manner as described for the response rates.

Analysis of Ambulatory Blood Pressure Monitoring. Of the 165 patients who entered the ambulatory blood pressure monitoring phase of the study 137 patients were evaluable. The means across patients (change from baseline in diastolic blood pressure) are graphically presented in the following figure



Various clinically meaningful variables could be calculated from the hourly mean diastolic blood pressure profiles. The following table shows results of trough/peak ratios calculated from hourly means of ambulatory monitoring data :

	Trough (mmHg)	Peak (mmHg)	Hour of Peak	Trough to peak Ratio
Diastolic BP				
NIS 10 mg.	-11.95	-11.95	24	100 %
NIS 20 mg	-13.70	-13.70	24	100 %
NIS 30 mg	-9.03	-12.57	2	72 %
Systolic BP *				
NIS 10 mg	-15.70	-15.70	24	100 %
NIS 20 mg	-20.72	-20.72	24	100 %
NIS 30 mg	-18.31	-18.31	24	100%
Systolic BP#				
NIS 10 mg	-15.70	-15.70	24	100 %
NIS 20 mg	-20.72	-20.72	24	100%
NIS 30 mg	-18.31	-10.64	2	172%

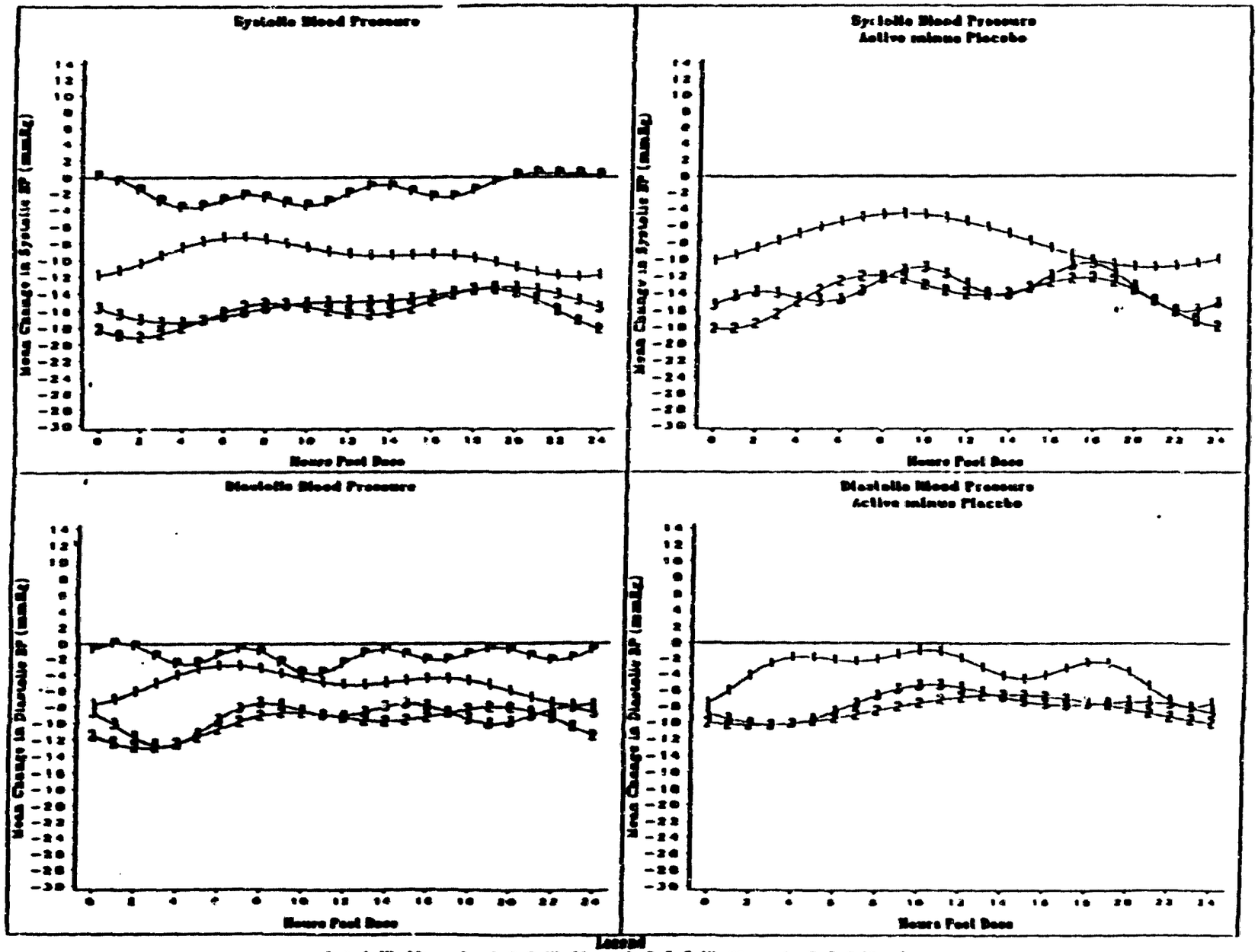
* Using timepoint of systolic peak.
# Using timepoint of diastolic peak

The results indicate that there was a good dose-response pattern in both systolic and diastolic blood pressure falls from baseline for placebo Nisoldipine 10 and 20 mg while the fall of Nisoldipine 30 mg was very similar to that in the 20 mg group. The effect of the 3 Nisoldipine group was maintained over the entire dosing period. This is also in evidence by observing the following graph of hourly means in a smoothed curve :

D90-008

9JAN92

Smooth of Mean ange from Baseline of Ambulatory Blood P sure



Assessment. This study demonstrated that Nisoldipine, at concentrations of 10 mg, 20 mg and 30 mg, was more effective than placebo in lowering the blood pressure, but this effect was not potentiated when the dose was increased from 20 to 30 mg. Ambulatory blood pressure measurements were done which demonstrated that the effectiveness of Nisoldipine extended throughout the 24 hours after administration , trough values frequently being equal to peak values.

It is interesting that in this study this calcium channel blocker demonstrated to have a greater effectiveness in blacks, a patients population usually more refractory to antihypertensive treatment, than in caucasians.

In reference to age, this study concluded that Nisoldipine was more effective in individuals 65 years of age or older. (table page 73). This finding is consistent with those of protocol D89-039 in which Nisoldipine was more effective in this age range especially in lowering systolic blood pressure. (table page 60).

Protocol D88-054

Title of Study : " Comparative Double-Blind Pilot Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine 10, 20, 30 mg Core-Coat Tablets vs Placebo in Hypertensive Patients ".

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Objectives. The objectives of this study were :

1. To test whether Nisoldipine core-coat given 10 mg, 20 mg, 30 mg once daily lowers the blood pressure significantly more than placebo at the end of 24-hour dosing interval (trough).

2. To record blood pressure and pulse rates for four hours after the first dose of double-blind drug to monitor patient response to acute administration of the drug.

3. To determine peak response and calculate ratios of trough to peak effect by 24-hour ambulatory blood pressure monitoring.

Inclusion and Exclusion Criteria. Male or female patients, 21 to 70 years of age, with a history of mild to moderate essential hypertension and a mean supine diastolic blood pressure of 95 to 114 mmHg after three and four weeks of placebo were eligible for the study.

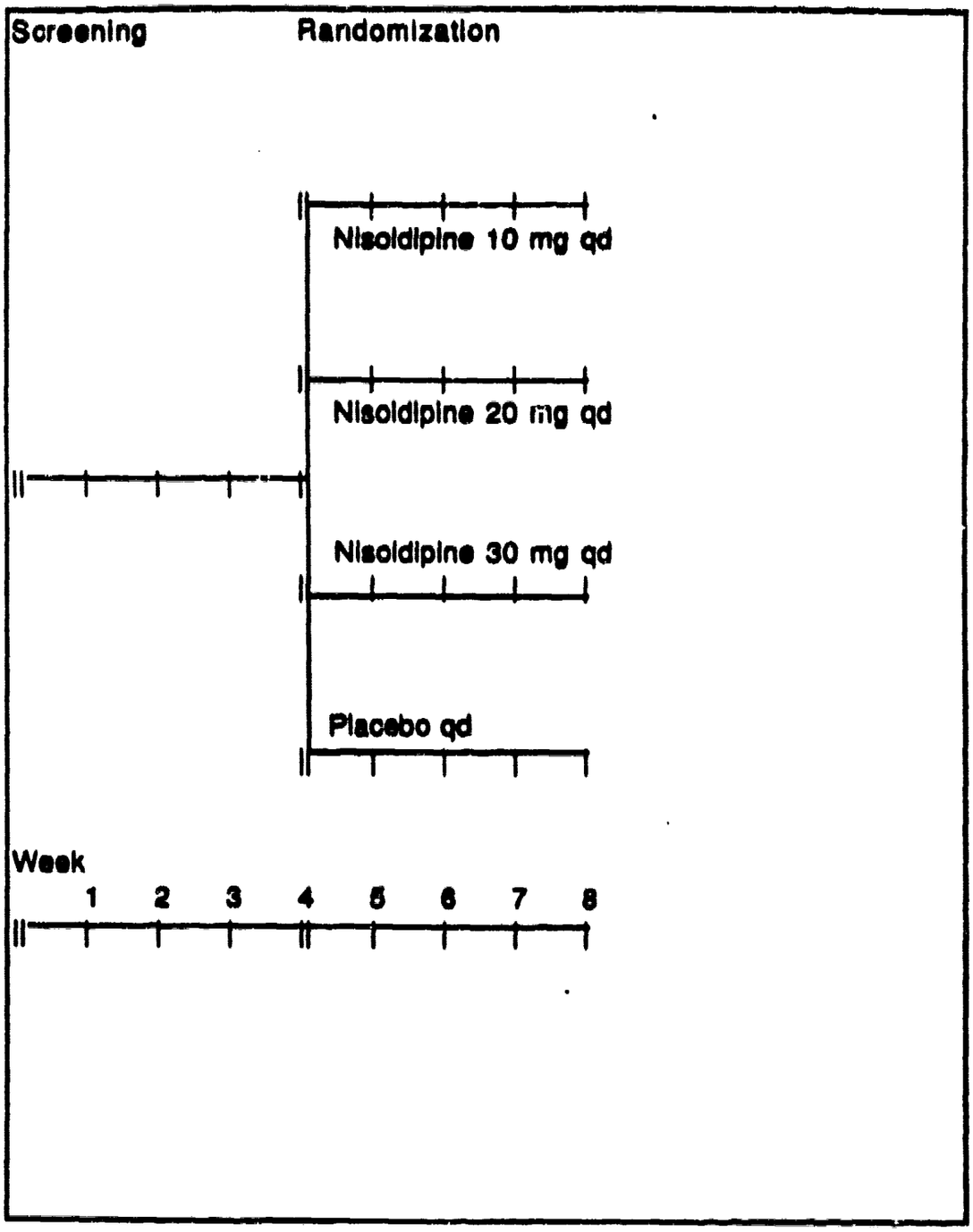
Excluded from the study were patients with labile hypertension, a change in supine diastolic blood pressure greater than 7 mmHg between the last 2 placebo run-in visits, impaired renal or liver function, recent or impending myocardial infarction, or cerebral vascular accident, angina pectoris or intermittent claudication, heart failure, major arrhythmias, conduction disturbance, failure of a major organ system, severe infection, malignancy, psychosis, chronic diarrhea, ulcerative colitis, regional enteritis, diverticulitis, partial or complete gastrectomy or small bowel resection, history of allergy to dihydropiridines, pregnant women or those with childbearing potential and patients known to abuse alcohol or drugs.

Study Design. This was a randomized, double-blind, parallel group, placebo controlled study of eight weeks duration consisting of a screening period and a randomization treatment period.

Screening Period. During this period of 4 weeks duration patients discontinued all previous antihypertensive medication and were given a single-blind placebo once daily. Those patients with a mean supine diastolic pressure ≥ 95 mmHg to ≤ 114 mmHg after three to four weeks of placebo and within 7 mmHg at both visits were transferred to the treatment period.

Randomization Period. Patients were randomized to receive either Nisoldipine 10 mg qd, Nisoldipine 20 mg qd, Nisoldipine 30 mg qd or Placebo qd for four weeks.

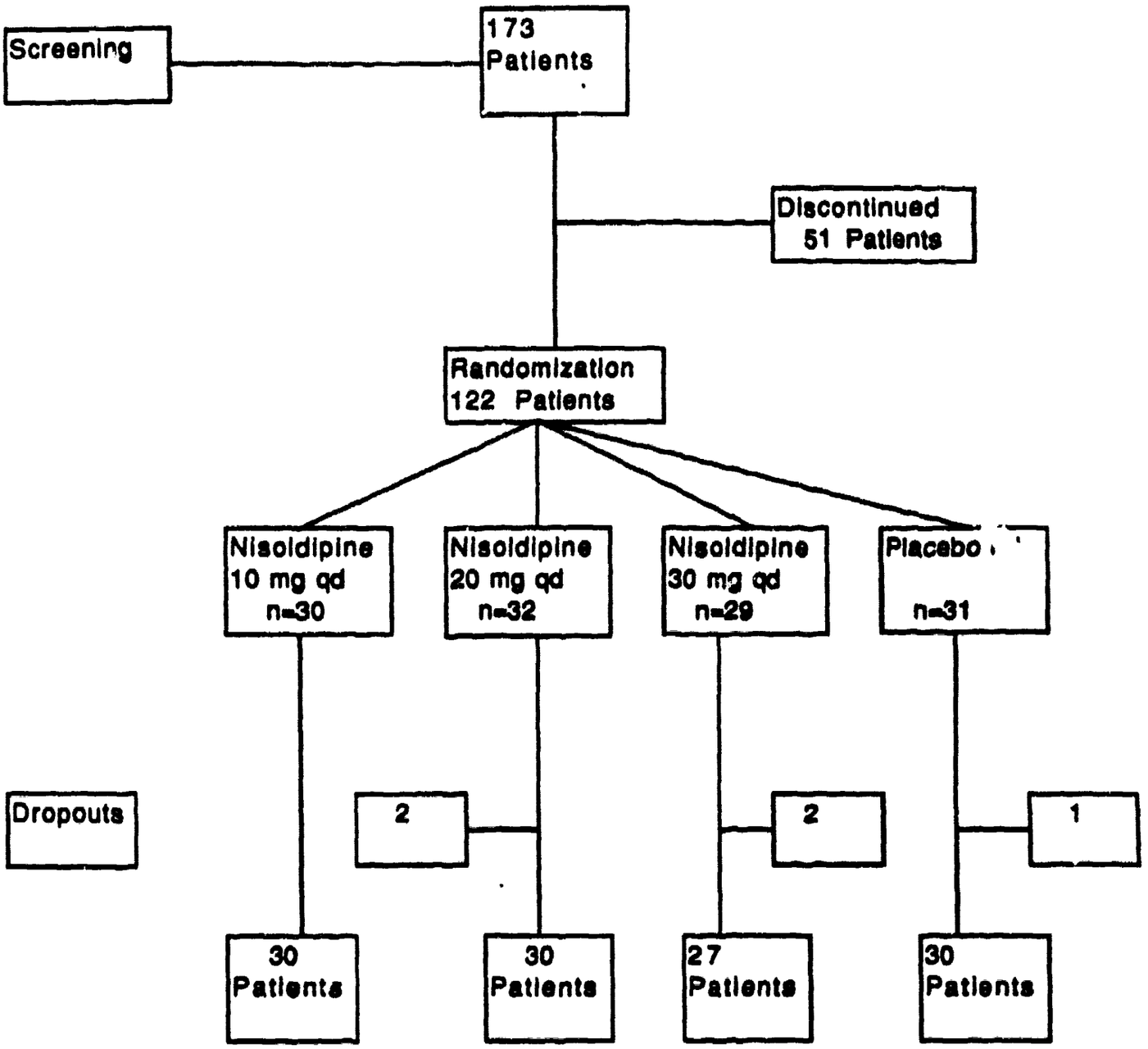
The study design is demonstrated schematically in the following graph :


Screening
Randomization
Nisoldipine 10 mg qd
Nisoldipine 20 mg qd
Nisoldipine 30 mg qd
Placebo qd
Week
1
2
3
4
5
6
7
8

Demography. The demography and baseline characterisitics are given in the following table :

		Nisoldipine 10 mg n=30	Nisoldipine 20 mg n=30	Nisoldipine 30 mg n=29	Placebo n=30
Sex	Male Female	20 (67 %) 10	19 (63 %) 11	19 (66 %) 10	21 (70 %) 9
Race	Caucasian Black Hispanic	23 (77 %) 4 3	23 (77 %) 7 0	20 (69 %) 9 0	23 (77 %) 7 0
Age (years)		56	53	52	51
Weight (lbs)		186	200	207	190
Baseline Blood Pressure mmHg	Supine Standing	146/99 144/100	147/99 144/100	145/99 144/100	148/100 145/101

The distribution of patients and randomization are given in the following graph :


Screening
173 Patients
Discontinued 51 Patients
Randomization 122 Patients
Nisoldipine 10 mg qd n=30
Nisoldipine 20 mg qd n=32
Nisoldipine 30 mg qd n=29
Placebo n=31
Dropouts
2
2
1
30 Patients
30 Patients
27 Patients
30 Patients

The reasons that disqualified enrolled patients for randomization are given in the following table :

Reasons for disqualification	Patients
Supine diastolic blood pressure < 95 mmHg	21
+ 7 mmHg difference in supine diastolic blood pressure (visits 4 and 5)	3
Supine diastolic blood pressure >114 mmHg	4
Unable to make scheduled visits	3
Illness not due to study medication	4
Lost to follow-up	2
Abnormal laboratory values	3
Non-compliance	2
Systolic blood pressure above acceptable limit	1
High blood pressure readings during ambulatory monitoring	1
Chest pain at visit 1	1
Chose to withdraw	1
Total	46

The reasons for dropping out during the double-blind randomization period are given in the following table :

Drug Group	Final visit	Days on Drug	Reasons for dropping-out- Severity Drug Relationship
Placebo	7.0	11	Dizziness-Moderate Probable
Nisoldipine 20 mg	6.0	5	Intolerance to all-night visits
	5.5	5	Shortness of breath-Cough Mild-Probable
Nisoldipine 30 mg	8.0	23?	Noncompliance
	6.0	7	Flushing-Severe-Probable

Efficacy

Criteria for Effectiveness. The change in trough supine diastolic blood pressure from baseline to endpoint compared among the groups was the primary efficacy criterion. Endpoint was defined as the last valid visit on the double-blind drug for each valid patient. Patients were to be included in this efficacy analysis if trough blood pressure data was available after at least 7 days on double-blind drug.

Statistical Methods. All tests of significance were performed as two-tailed tests with alpha + 0.05 unless stated otherwise.

Analysis of Efficacy. The mean blood pressures by visit for all patients valid for analysis of efficacy are given in the following table :

Week Post Randomization — Supine Blood Pressure

	Nisoldipine 10 mg Sys/Dia (n)	Nisoldipine 20 mg Sys/Dia (n)	Nisoldipine 30 mg Sys/Dia (n)	Placebo Sys/Dia (n)
Week-1	143/99 (30)	145/99 (30)	144/99 (29)	145/99 (30)
Week 0	145/99 (30)	146/98 (30)	144/98 (29)	147/100 (30)
Week 1	136/91 (30)	136/90 (30)	136/91 (29)	141/95 (30)
Week 2	140/91 (30)	139/92 (29)	133/90 (28)	140/94 (30)
Week 3	138/91 (30)	136/91 (30)	134/89 (28)	142/95 (29)

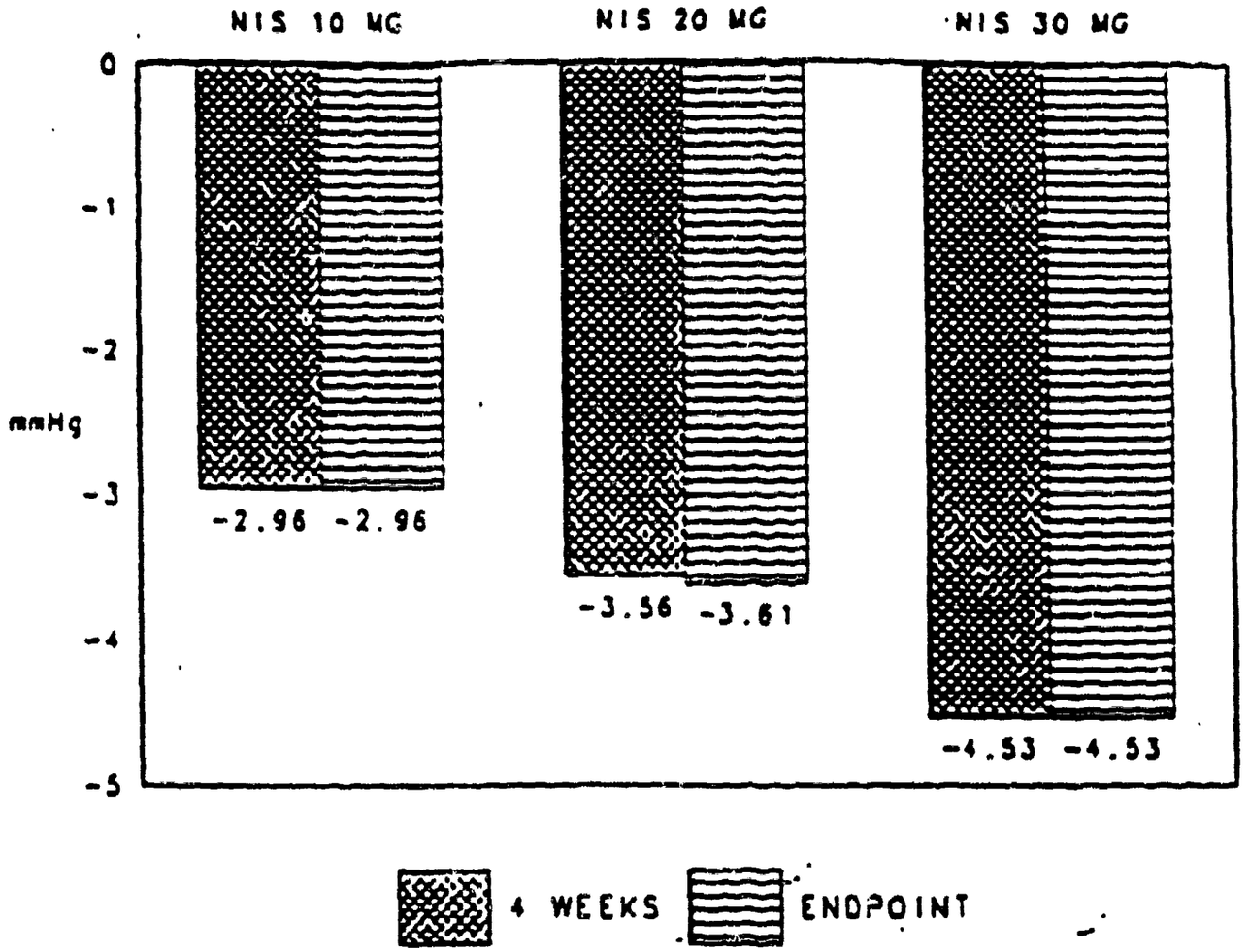
Week 4	137/90 (30)	134/90 (30)	133/89 (27)	144/94 (30)
Endpoint	137/90 (30)	134/90 (30)	133/89 (30)	144/94 (30)

In the following table, the results of the analysis at endpoint are summarized :

	Nisoldipine 10 mg n=30	Nisoldipine 20 mg n=30	Nisoldipine 30 mg n=29	Placebo n=30
Supine				
Systolic	8.4	11.5*	10.7*	3.0
Diastolic	8.3	8.9*	9.9*	5.3
Standing				
Systolic	8.3	11.8*	10.7*	3.4
Diastolic	6.2	7.3	7.0	5.1

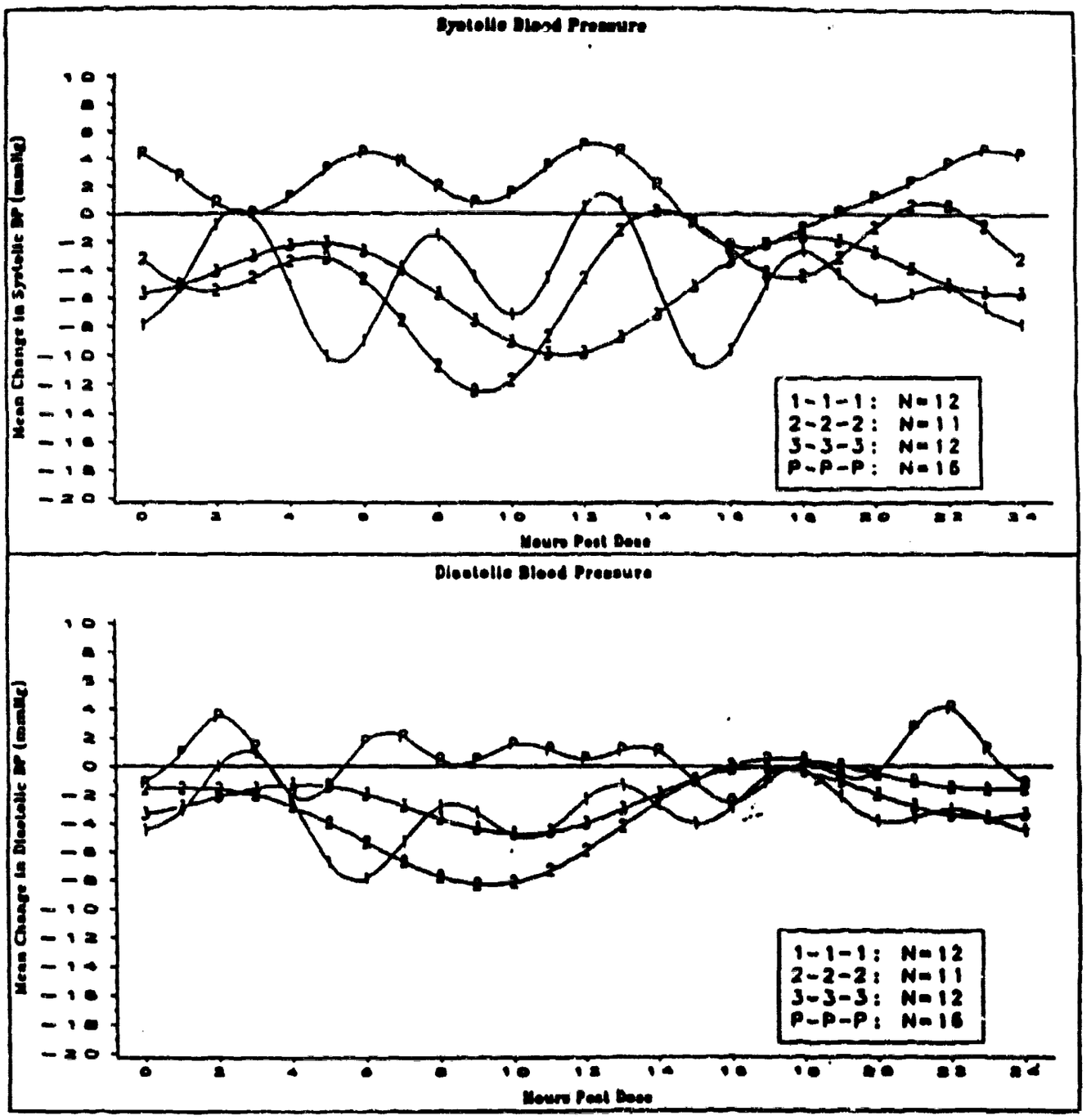
*Significant difference from the placebo group $p<0.05$

The change in trough supine diastolic blood pressure at 4 weeks and endpoint, placebo subtracted, are shown in the following graph :



Ambulatory monitoring and supine in-clinic blood pressures were smoothed and results are demonstrated in the following graph :

SMOOTH OF MEAN CHANGE FROM BASELINE OF AMBULATORY BLOOD PRESSURE



Legend

1-1-1 NIS 10mg
2-2-2 NIS 20mg
3-3-3 NIS 30mg
P-P-P Placebo

The Trough/Peak ratios from smoothed ambulatory monitoring data for valid patients are given in the following table :

	Nisoldipine		
	10 mg (n=12)	20 mg (n=11)	30 mg (n=12)
Diastolic	7 %	35 %	68 %
Systolic	92 %	43 %	108 %
Peak hour Post-dose	6	9	8
Nisoldipine levels at trough ng/ml	0.82	1.04	1.49

Assessment. This is a small pilot studied carried in a relatively small number of subjects consisting mostly of middle-age caucasian male obese patients. Although the results on blood pressure with the 10 mg dose of Nisoldipine was not significantly different from placebo the 20 and 30 mg doses were but the effect of both did not seem to be very different from each other.

Other Studies. Other studies were performed in which Nisoldipine was administered to patients with renal disease, to cirrhotic, elderly and young people. The effect of food on drug absorption was also investigated. The effects of combination with other antihypertensive agents was studied in long term extension studies.

Study in Cirrhosis. Protocol M.M.R.R. # 1118

Title of Study. "The Effect of Cirrhosis on the Steady-State Pharmacokinetics of Nisoldipine Coat-Core Sustained-Release Tablets ".

This was a single center, non-randomized, non-blinded, comparison of single dose and steady-state pharmacokinetics of Nisoldipine coat-core tablets in cirrhotic and healthy subjects.

Sixteen subjects participated in the study : 8 cirrhotic and 8 healthy subjects. There were 4 males and 4 females in each group. In stage 1 a

single 10 mg dose of Nisoldipine was administered and in stage 11 10 mg of Nisoldipine was administered qd for 7 days.

Results. Administration of Nisoldipine to patients with cirrhosis resulted in a 3 to 4-fold increase in peak plasma concentration and $AUC_{(024)}$. Nisoldipine had little effect on blood pressure in either group.

Assessment. These results are indicative of possibility that the dose of Nisoldipine may need to be adjusted in patients with cirrhosis.

Study in Renal Disease. Report 5837 (R).

Title of Study. "Influence of Renal Function on the Pharmacokinetics of Nisoldipine CC Tablets After Single and Multiple Dosing ".

This was a multicenter, non-blinded, non-randomized, comparative study among 4 groups to compare the effects of renal function on the pharmacokinetics of Nisoldipine CC after a single dose as well as after achievement of a steady state.

A total of 40 patients were enrolled in 3 centers.. The following groups of patients were enrolled :

1. Control. Nine subjects with creatinine clearance > 90 ml/min/1.73 m^2

2. Mild Renal Failure. Twenty subjects with creatinine clearance 61 ≤ 90 ml/min/1.73 m^2

3. Moderate Renal Failure. Nine subjects with creatinine clearance 30 to ≤ 60 ml/min/1,73 m^2

4. Severe Renal Failure. Seven subjects with creatinine clearance < 30 ml/min/1.73 m^2.

Results. Although there was not a statistically significant difference in the Nisoldipine AUC_{norm} between the groups with impaired renal function and the normal control, in the former an increase in plasma Nisoldipine of approximately 2-fold could not be excluded.

Assessment. An increase in plasma levels of Nisoldipine in patients with impaired renal function may require the adjustment of the dose. There were only modest effects on blood pressure across all groups.

The Factor Age . Report 5857 (P).

Title of Study : "A Study to Determine the Single Dose and Steady-State Pharmacokinetic Profile of Nisoldipine Coat-Core (CC) Tablet 20 mg in Elderly and Young Volunteers and in Elderly Hypertensive ".

This was an open, multiple-dose, non-randomized study. Nisoldipine CC was administered at the dose of 20 mg qd for 7 days. Plasma samples were collected and blood pressure and heart rate were measured.

The following groups of patients were studied :

Young Volunteers. Twenty healthy young volunteers, 18 to 23 years of age, completed the study.

Elderly Volunteers. Twenty healthy elderly volunteers, 65 to 84 years of age, completed the study.

Hypertensive Elderly. Eleven hypertensive patients, 66 to 77 years of age, completed the study.

Results. The plasma concentrations of Nisoldipine were higher in elderly volunteers and hypertensive patients than in young volunteers. After multiple dose administration the supine diastolic blood pressure remained essentially unchanged in normal young healthy volunteers but a moderate decrease in elderly healthy volunteers and a significant decrease in elderly hypertensive patients was observed.

The Effect of Diet. Study Number D92-045-02.

Title of Study : " The Effect of Food on the Pharmacokinetics of 30 mg and 40 mg Nisoldipine CC Tablets in Healthy Male Volunteers ".

This study was an open-label, randomized, two-way cross over evaluation of the effect of food on the pharmacokinetics of 30 and 40 mg

Nisoldipine. Subjects were randomized to receive a single 30 mg or 40 mg dose of Nisoldipine either in a fasted or a fed state. After one week washout period there was a crossover to the opposite state.

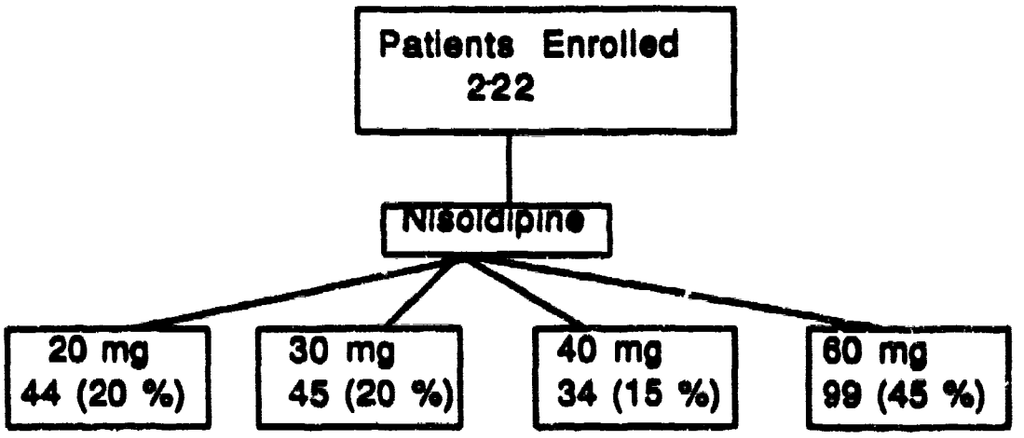
Twenty-eight healthy male subjects between the ages of 18 and 45 years completed the study. There were no significant effects on mean sitting diastolic blood pressures in the fed or fasted states at the 30 or 40 mg. doses.

Long Term Extension Studies. Drug Combination. Protocols X89-039 and X90-019.

These were long term extension studies of the 6-month efficacy studies and safety of Nisoldipine CC in the treatment of mild to moderate hypertension. Patients completing studies D89-039 and D90-019 were given the option of immediately entering an open-label extension protocol.

Patients were initially given Nisoldipine CC 20 mg or 30 mg tablets once a day as initial therapy. Then the dose of Nisoldipine was to be increased sequentially every one or two weeks as tolerated, to 40 mg qd, 60 mg qd and 80 mg qd. or 60 mg qd and 90 mg qd until SUDBP was ≤ 90 mmHg. However the maximum dose of Nisoldipine was in fact limited to 60 mg qd before any patient enrolled. Atenolol 50 mg to 100 mg qd and/or Hydrochlorothiazide 24 to 50 mg qd could be added at the investigator's discretion at any time. Thus tablets used were Nisoldipine CC 20, 40, 2X30 mg for monotherapy with the addition of Atenolol 50 and 100 mg and.or Hydrochlorothiazide 25 and 50 mg for combination therapy.

The distribution of patients is shown in the following graph :



With Atenolol
20 Patients (9 %)
1 Patient 25 mg
15 Patients 50 mg
4 Patients 100 mg

With Hydrochlorothiazide
78 Patients (35 %)
44 Patients 25 mg
34 Patients 50 mg

The results are summarized below :

	Supine		Standing	
	SBP mmHg	DBP mmHg	SBP mmHg	DBP mmHg
Baseline	154.0	101.1	149.7	100.3
Endpoint	135.7	86.0	132,4	86.5
Mean Dif	-18.3	-15.2	-17.3	-13.6

Assessment. These were open-label uncontrolled studies in which results were all pooled together and therefore they should not be valid for evaluation of combined therapy.

Total Assessment of Efficacy

Peak Drug Effect on Blood Pressure. The effect of Nisoldipine on blood pressure at the approximate time of peak drug plasma concentration (i.e. the maximal response between 6-10 hours post-dose) in the supine and standing position is shown below for the systolic and diastolic blood pressure.

	Placebo Substracted Change in Peak Blood Pressure Dose Nisoldipine				
	10 mg	20 mg	30 mg	40 mg	60 mg
Study			SUDBP		
D88-054	-11.6	-9.5	-14.1	NA	NA
D89-039	NA	-8.0	NA	-8.3	NA
D90-019	NA	NA	-6.3	NA	-10.6
			SUSBP		
D88-054	-8.6	-7.6	-12.8	NA	NA
D89-039	NA	-15.2	NA	-15.3	NA
D90-019	NA	NA	-13.0	NA	-11.1
			STDBP		
D88-054	-9.3	-7.8	-11.5	NA	NA
D89-039	NA	-7.6	NA	-8.5	NA
D90-019	NA	NA	-6.6	NA	-13.4
			STSBP		
D88-054	-4.7	-11.6	-11.0	NA	NA
D89-039	NA	-14.4	NA	-17.6	NA
D90-019	NA	NA	-15.5	NA	-19.1

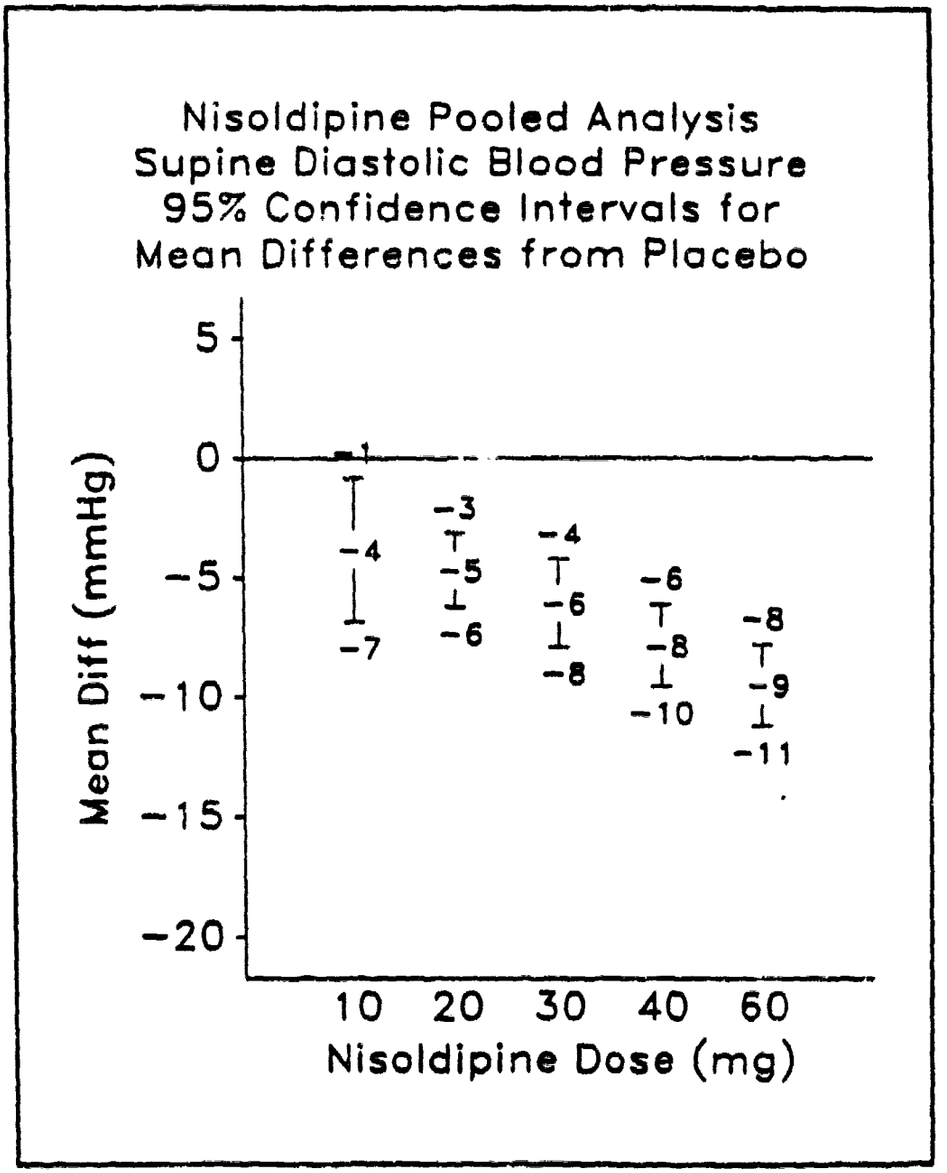
Twenty Four Hour Mean BP Reduction. Ambulatory blood pressure was used in a majority of the clinical trials of Nisoldipine in hypertension. In addition to characterizing the temporal profile of its effect on blood pressure, these data provide an estimate of the time-average reduction in blood pressure for each dosage of the drug. The pooled results of several studies are shown in the following table :

Nisoldipine Dosage (mg)	24 Hour AVG BP Reduction, Mean ± SEM Systolic	Diastolic
Placebo	-0.7±8.7	-0.9±6.3
10	-8.4±11.8	-4.6±7.5
20	-12.7±11.5	-8.4±7.1
30	-12.7±10.8	-7.9±6.9
40	-13.6±12.1	-8.0±6.8
60	-18.4±9.9	-12.0±7.2

The change in trough blood pressure from baseline to endpoint (Mean±SEM in mmHg) is given in the following table :

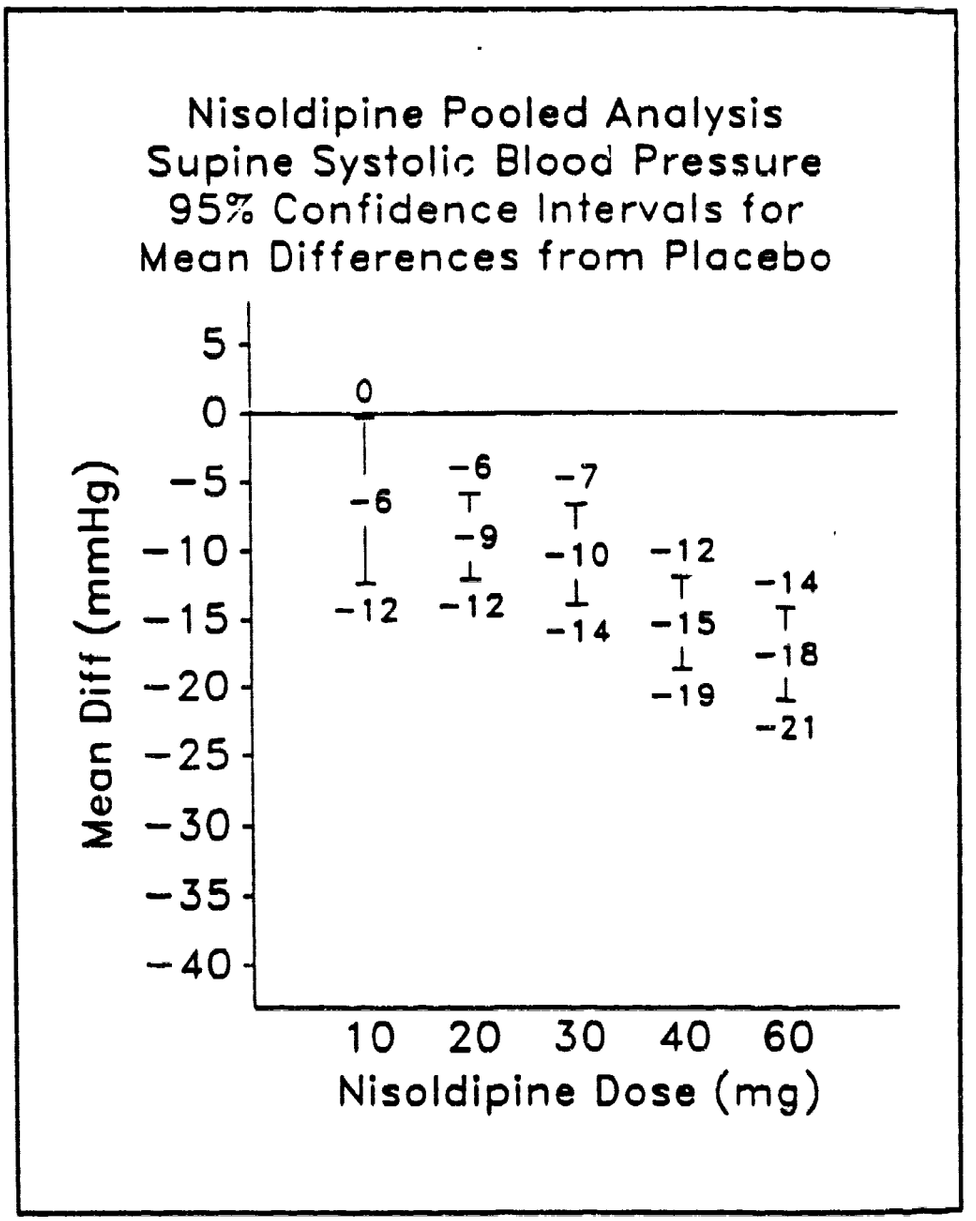
Pooled Dosage	Placebo N=232	Nisoldipine 10 mg N=30	20 mg N=161	30 mg N=105	40 mg N=131	60 mg N=125
SUDBP	-4±0.5	-8.4±1.4	-9.2±0.6	-10.6±0.8	-12.4±0.7	-14.0±0.7
SUSBP	-2.0±0.5	-8.3±2.9	-10.9±1.2	-12.2±1.6	-17.2±1.4	-19.5±1.4
STDBP	-2.7±0.5	-7.0±1.5	-7.9±0.7	-9.0±0.8	-12.6±0.8	-13.6±0.8
STSBP	-1.5±1.0	-8.2±3.0	-11.4±1.3	-12.9±1.6	-18.7±1.5	-19.2±1.5

In the following graph, pooled results of placebo subtracted values for trough SUDBP reduction by dose are demonstrated :



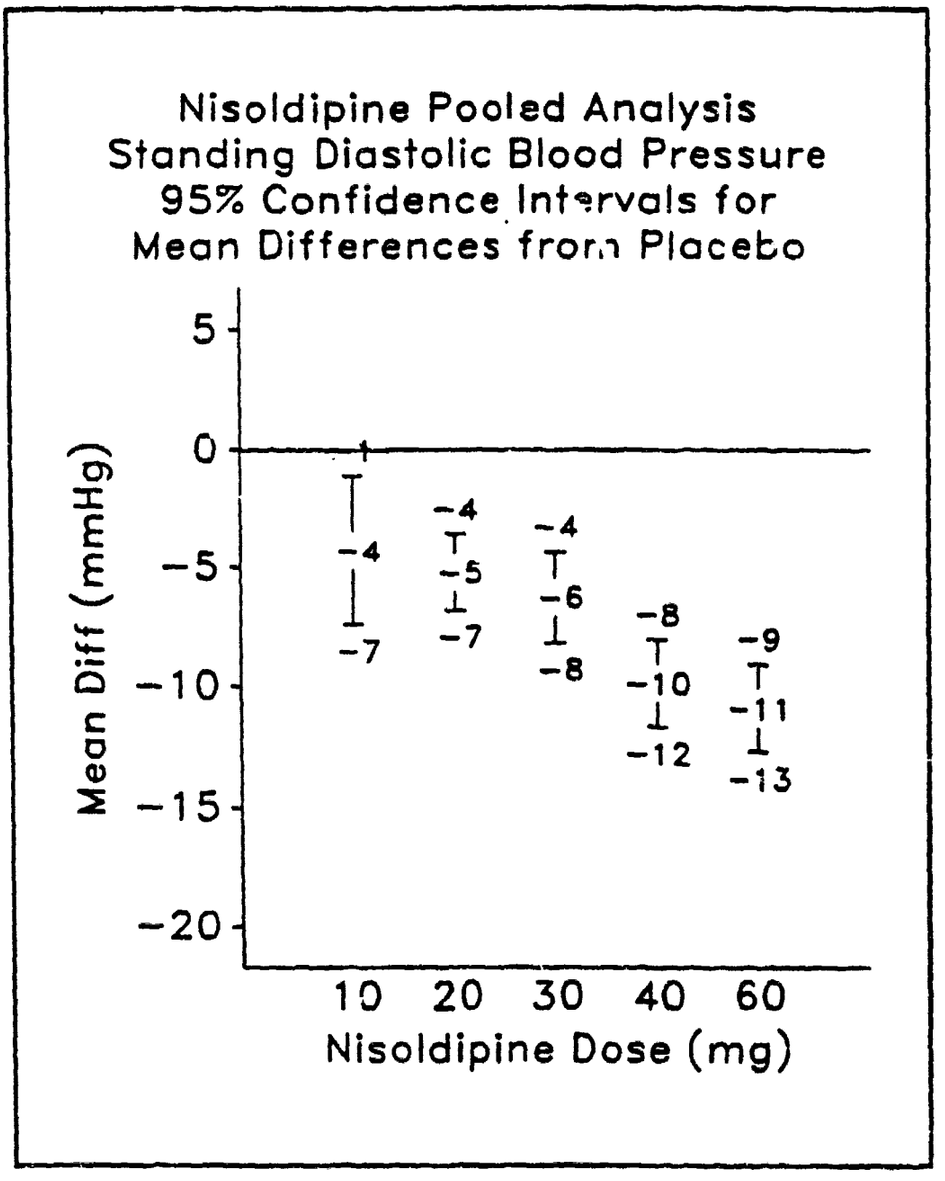
A linear relationship of blood pressure reduction by Nisoldipine in dosages between 10 and 60 mg is apparent without evidence of a plateau.

Similar results for SUSBP are shown in the following figure :



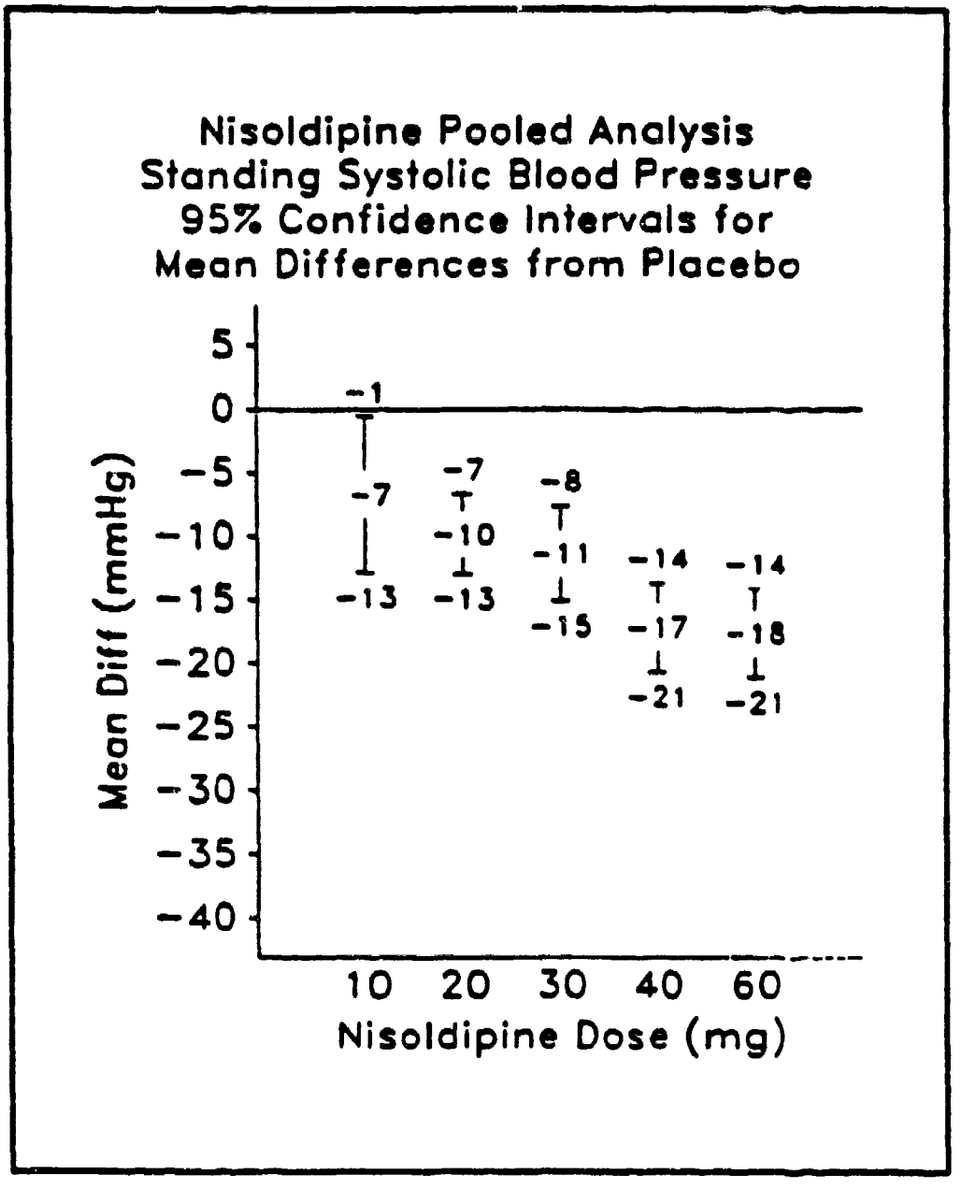
A linear relationship is not as evident as in previous graph but the maximum effect was achieved with 60 mg Nisoldipine dose

Similar results for STDBP are shown in the following figure :



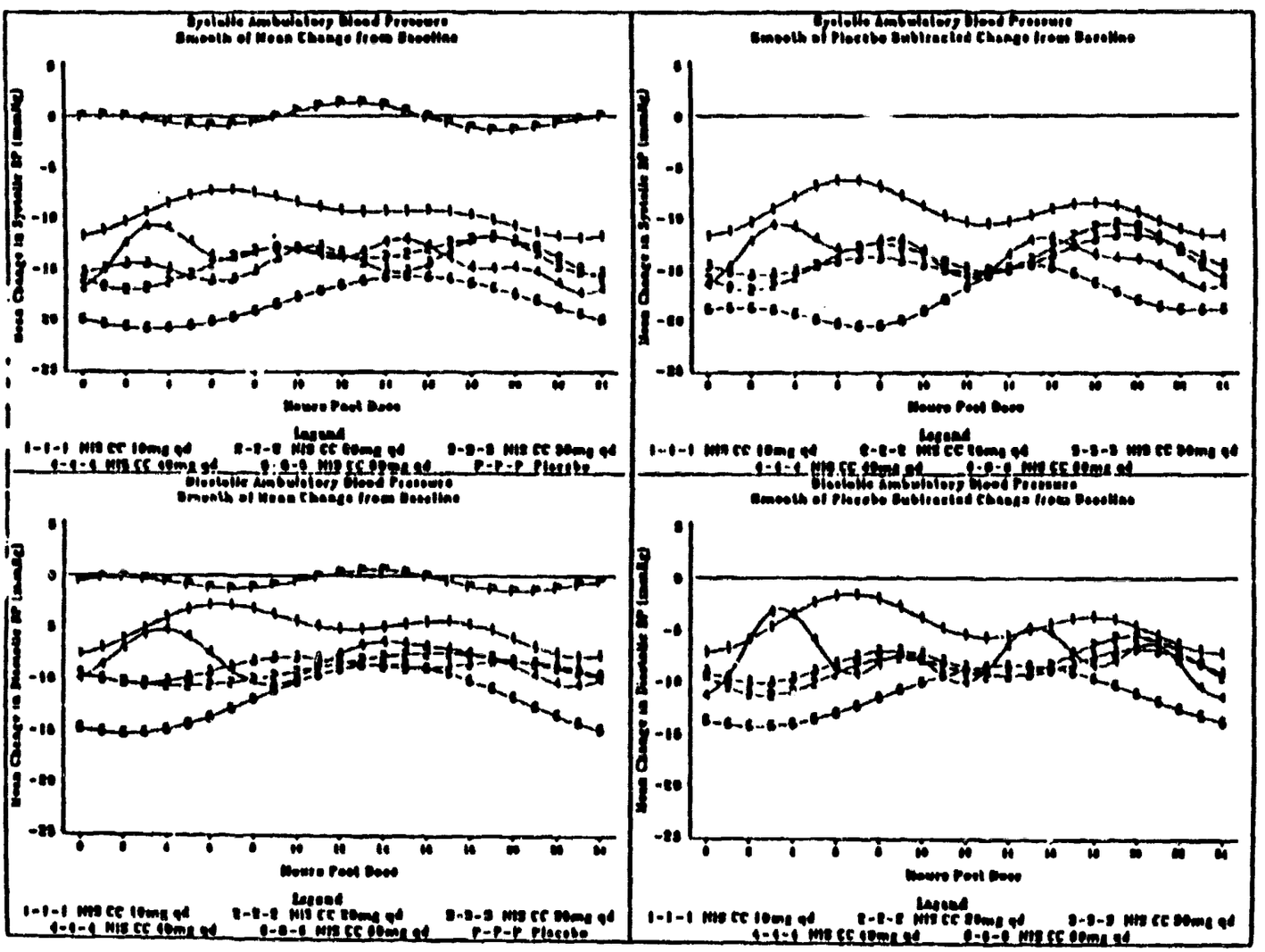
In this case the relationship of blood pressure reduction to dosage is roughly sigmoidal with an apparent plateau at 60 mg.

Similar results for STSBP are shown in the following figure :



The relationship of blood pressure reduction to dosage is sigmoidal with an apparent plateau at 40 mg

A pooled analysis of 24 hour ambulatory blood pressure monitoring is demonstrated in the following 4 graphs :



Through the 24-hour recording there seems to considerable overlapping especially among the higher doses but at trough there is evidence of blood pressure reduction that seems to be dose related.

The effects on diastolic blood pressure at peak and trough and the trough/peak ratios according to dose are given in the following table :

Dosage	Trough/Peak Ratio Diastolic Blood Pressure
10 mg	73 %
20 mg	75 %
30 mg	93 %
40 mg	100 %
60 mg	97 %

Time Course Effect of Nisoldipine. The therapeutic effect of Nisoldipine was achieved early in the course of treatment (approximately 2 weeks) and gradual incremental gain is evident for another 2-4 weeks.

The mean changes in sitting blood pressure from baseline after first dose is given in the following table :

Dose (mg)	N	8 Post-dose Systolic/ Diastolic	24 post-dose Systolic/ Diastolic
Placebo	10	-4.9/-1.9	3.8/-2.2
5	11	-10.4/-4.2	0.3/2.3
10	13	-6.7/-7.1	-0.7/-4.5
20	12	-11.3/-7.8	-5.8/-1.9
30	7	-15.4/-9.6	-13.3/-1.9

Pharmacokinetic and Blood Pressure Results. The mean sitting blood pressure change (mmHg) from baseline at peak and pharmacokinetic parameters (Mean ±SD) at steady state at each dose level is given below :

Dose (mg)	N	8h Post-Dose Sys/Dia	24h Post-Dose Sys/Dia	AUC (0-24h) (ng.h/ ml)	C_{max} (ng/ml)	T_{max} (h)
Placebo	10	-2.5/ -5.9	3.8/ 0.3			
5	11	-9.6/ -4.7	1.9/ -4.3	9.1±5.0	0.7±0.3	9.2±3.0
10	13	-8.9/ -7.1	-5.0/ -4.6	16.2± 3.0	1.1±0.3	6.3±4.8
20	12	-13.2/ -7.6	-5.4/ -4.3	29.4± 11.8	2.3±0.9	4.0±2.4
30	7	-21.7/ -10.5	-11.8/ -5.9	43.2± 23.1	2.9±1.1	5.4±5.0

The mean supine blood pressure change (mmHg) from baseline and pharmacokinetic parameters (mean±SD) at steady state for each dose level is given in the following table :

Dose (mg)	N	8h Post Dose	24 h Post Dose	AUC (0-24h) (ng.h/ ml)	C_{max} (ng/ml)	T_{max} (h)
30	18	-16.4/ -8.4	-14.0/ -10.2	74.28± 7.96	4.79± 0.68	7.22± 0.93
60	18	20.8/ 13.2	16.8/ 15.0	129.76± 12.74	8.48± 0.81	9.08± 1.97
90	9	-22.1± 12.1	-23.0± 13.4	199.31± 16.45	13.02± 1.20	6.78± 2.30
120	3	-30.7/ 25.0	-44.3/ -19.0	226.58± 12.41	14.92± 2.01	4.00± 1.00

To bring up more clearly the relationship between plasma Nisoldipine concentrations and blood pressure decrease, supine diastolic blood pressure changes from baseline at peak (8 h) and trough (24 h) were related to plasma Nisoldipine concentration at this time points using a simple linear regression. Placebo patients were used in this analysis with a plasma Nisoldipine level of Zero. The results for 30 and 60 mg are summarized in the table below :

Timepoint	Nisoldipine Mean Plasma Conc. (ng/ml)	Mean Change in SUDBP (mmHg)	Estimated Slope	Estimated Slope (P-Value)
Day 4, 30 mg (N=18)				
8 hours	3.5	-8.4	-2.55	0.0118
24 hours	2.6	-10.2	-1.42	(0.0689)
Day 8, 60 mg (N=17-18)				
8 hours	6.2	-13.2	-1.14	0.0507
24 hours	5.2	-15.0	-1.39	(0.0027)

Blood Pressure Rebound Upon Withdrawal. Blood pressure rebound was determined 24, 48 and 72 hours after cessation of Nisoldipine 60 mg qd in patients who had reached steady state. There was no evidence of for an exaggerated rebound effect on blood pressure after discontinuance of Nisoldipine at this high dose.

Maintenance of Blood Pressure Reduction in Long Term Studies. There was no evidence of tolerance to the antihypertensive effect of Nisoldipine over 6 months to 1 year of therapy.

Demographic Subgroups. Gender. Trough SUDBP changes from baseline to endpoint for male and female patients are given in the following table :

	Female	Male
Dosage	Nisoldipine-Placebo	Nisoldipine-Placebo
10 mg	-8.85	-2.27
20 mg	-3.21	-5.87
30 mg	-8.47	-6.1
40 mg	-7.82	-8.43
60 mg	-10.31	-10.79

Although dose-response profiles are somewhat erratic the overall effects are similar for men and women.

Race. A comparable analysis of efficacy for race related to dose is demonstrated in the following table :

	White	Black
Dosage	Nisoldipine-Placebo	Nisoldipine-Placebo
10 mg	-3.59	-4.37
20 mg	-4.54	-6.39
30 mg	-7.51	-8.82
40 mg	-6.69	-11.61
60 mg	-11.51	-11.1

Black patients responded with a greater decline in trough SUDBP than did white patients.

Age. In the following table the dose response for patients divided by age less than 65 years and equal or greater than 65 years is demonstrated.

	<65	≥65
Dosage	Nisoldipine - Placebo	Nisoldipine - Placebo
10 mg	-3.69	-6.2
20 mg	-4.98	-5.15
30 mg	-7.31	-5.48
40 mg	-8.21	-8.09
60 mg	-11.08	-8.14

The elderly demonstrated a greater low-dose response and a lesser high-dose response.

Quartile of Baseline Blood Pressure. For Nisoldipine as well as for many other antihypertensive drugs, a higher baseline blood pressure is associated with larger decline on medication. In the table below a dose response according to baseline SUDBP by quartile is demonstrated :

	Q1	Q2
Dosage	Nisoldipine-Placebo	Nisoldipine-Placebo
10 mg	-4.38	-5.97
20 mg	-4.23	-8.18
30 mg	-2.27	-11.49
40 mg	-6.36	-13.81
60 mg	-9.66	-14.16

The relationship of Nisoldipine dosage and decline in blood pressure is least evident in the first quartile and strongest in fourth quartile.

Combination Antihypertensive Therapy. Addition to a background of a beta blocker. One the pivotal studies (D89-029) evaluated the combination of Nisoldipine CC and a beta blocker.To patients who were already receiving Atenolol Nisoldipine was added. The sponsor claims the efficacy of Nisoldipine under these conditions. However there seems to be a drug interaction between these drugs that the sponsor has not recognized (see p. 35 this review).

Long Term Extension Trials. Based on open-label controlled trials and uncontrolled studies of one year duration the sponsor claims that meaningful responses were elicited by the combination of Nisoldipine with diuretics and or/ a beta blocker.

Recommendations. Nisoldipine should be approved as monotherapy for hypertension. The recommended dosage should be 10 mg to 40 mg.

Although the sponsor states that there is no drug interaction between Nisoldipine and beta blockers there are publications stating that such interaction exists (1, 2). This should be stated in the package insert.

Consideration should be given to advising that the dosage may need to be adjusted in patients with renal failure.

There were not well controlled studies of the combination of Nisoldipine with diuretics or other antihypertenive agents. Therefore the claim of efficacy with other drug combination is not well substantiated.

Cristobal G. Duarte, MD - HFD-110

CC.
ORIG. NDA
HFD-110
HFD-110/ CSO/Roeder
HFD-110/CGD/30Jul93

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Table of Contents

75 pages
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DiRocco

SEP 27 1993

NDA20356 P1

DIVISION OF CARDIO- RENAL DRUG PRODUCTS
MEDICAL OFFICER'S NDA REVIEW

NDA: 20 356

DRUG: Nisoldipine Core- coat

SPONSOR: Miles inc (Pharmaceutical Division)

DATE SUBMISSION: 3 March, 1993

DATE REVIEW: 20 August 1993

REVIEWER: Philip L. Dern M.D. P.L. Dern

RESUME:

This review deals entirely with safety aspects of the above submission; not efficacy. The primary approach is via examination of individual pools of data based on similar studies and provided by the Sponsor in this submission.

I. Hypertension

A. Exposure

Although the number of cases treated world-wide with nisoldipine exceeds 6000, according to the Sponsor, relatively few of these, N= 1292, were given nisoldipine core- coat (NIS cc) as shown below in completed studies:

PATIENTS EXPOSED TO NISOLDIPINE IN EACH CATEGORY

FORMULATION	NDA STATUS	INDICATION										TOTAL
		ANGINA		HYPERTENSION		HEART FAILURE		CLIN. PHARM.		OTHER		
		NON-US	US	NON-US	US	NON-US	US	NON-US	US	NON-US	US	
COAT-CORE	INCLUDED	624	474	516	776	142		210	183			2325
	EXCLUDED											0
	TOTAL	624	474	516	776	142	0	210	183	0	0	2325
IMMEDIATE RELEASE	INCLUDED	3472	521	2371	10	414	14	799	83	131		7721
	EXCLUDED	411		334		188		421		136		1487
	TOTAL	3883	521	2705	10	599	14	1176	83	267	0	9208
OTHER	INCLUDED	8				103		678				789
	EXCLUDED					31		126				157
	TOTAL	8	0	0	0	134	0	701	0	0	0	943
TOTAL	INCLUDED	4104	995	2887	786	659	14	1540	266	131	0	11382
	EXCLUDED	411	0	334	0	219	0	547	0	136	0	1644
	TOTAL	4515	995	3221	786	878	14	2087	266	267	0	13026

STUDIES IN EACH CATEGORY

FORMULATION	NDA STATUS	INDICATION										TOTAL
		ANGINA		HYPERTENSION		HEART FAILURE		CLIN. PHARM.		OTHER		
		NON-US	US	NON-US	US	NON-US	US	NON-US	US	NON-US	US	
COAT-CORE	INCLUDED	4	3	4	7	2		11	6			37
	EXCLUDED											0
	TOTAL	4	3	4	7	2	0	11	6	0	0	37
IMMEDIATE RELEASE	INCLUDED	111	16	87	1	33	2	67	3	8		328
	EXCLUDED	36		21		14		38		7		116
	TOTAL	147	16	108	1	47	2	105	3	15	0	444
OTHER	INCLUDED	1				9		55				65
	EXCLUDED					3		13				16
	TOTAL	1	0	0	0	12	0	68	0	0	0	81
TOTAL	INCLUDED	116	19	91	8	44	2	133	9	8	0	430
	EXCLUDED	36	0	21	0	17	0	51	0	7	0	132
	TOTAL	152	19	112	8	61	2	184	9	15	0	562

The following table provides the total duration of treatment by total daily dose of longest duration for the US NIS CC (total controlled and uncontrolled) cases. The second table provides duration of treatment in the non- US studies.

TABLE 2
TOTAL DURATION OF TREATMENT WITH NISOLDIPINE
BY TOTAL DAILY DOSE OF LONGEST DURATION

POOL OF US NIS CC HYPERTENSION
TOTAL CONTROLLED + TOTAL UNCONTROLLED

	ALL	DURATION									
		2-7 DAYS		8-30 DAYS		31 DAYS-60 DAYS		61 DAYS-180 DAYS		181 DAYS-360 DAYS	
	N	N	%	N	%	N	%	N	%	N	%
DRUG											
NIS CC [illegible] MG QD	37			33	89.2	2	5.4	2	5.4		
NIS CC [illegible] MG QD	263	9	3.4	37	14.1	87	33.0	64	24.3	36	13.8
NIS CC [illegible] MG QD	130	1	0.7	32	24.6	46	35.4	29	22.3	29	22.3
NIS CC [illegible] MG QD	164	3	1.8	13	7.9	56	34.1	81	49.4	15	9.1
NIS CC [illegible] MG QD	109	6	5.5	11	10.1	44	40.4	23	21.1	25	22.9
NIS CC [illegible] MG QD	11	4	36.4	6	54.5	1	9.1				
ALL	776	23	3.0	132	17.0	281	36.2	199	25.6	125	16.0

2. DURATION BY DOSE TABLE NISOLDIPINE (BAY K 5552) - DATA POOL / NON US-STUDIES 10:12 TUESDAY, SEPTEMBER 22, 1992
TOTAL DURATION OF TREATMENT WITH NISOLDIPINE BY TOTAL DAILY DOSE OF LONGEST DURATION

POOL 80: CC - HYPERTENSION - TOTAL STUDIES

INSTITUTE OF BIOMETRY

DRUG	ALL	DURATION																	
		NOT RECORDED		1 DAY		2-7 DAYS		8-30 DAYS		31-60 DAYS		61-120 DAYS		121-360 DAYS		> 360 DAYS	
	N	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
>5- <=10 MG	114	1	0.9	2	1.8	1	0.9	6	5.3	49	43.0	5	4.4	17	14.9	33	28.9
>10- <=20 MG	192	1	0.5					5	2.6	56	29.2	6	3.1	39	20.3	85	44.3
30 MG	106	1	0.9							47	44.3	1	0.9	22	20.8	35	33.0
40 MG	104									2	1.9	7	6.7	64	60.8	11	10.6
ALL IN POOL	516	3	0.6	2	0.4	1	0.2	11	2.1	154	29.8	19	3.7	162	31.4	164	31.8

In the non- US studies N= 326 received NIS CC for more than 6 months; N= 164 for more than 1 year. In the US studies the figure for greater than 6 months was N= 131 but, apparently, none were treated longer than 1 year.

Demographic characteristics in this safety evaluation will be related primarily to adverse effects and other safety- related features.

B.Safety

1. Deaths

No deaths occurred in the US NIS CC hypertension studies other than for a single subject receiving placebo. He was aged 68 years, collapsed, and failed to respond to resuscitation efforts. In the non-US NIS CC hypertension studies a placebo case died of cerebral hemorrhage; N= 2 NIS CC cases died, one due to an accident, another due to cerebral metastases from prostatic cancer.

2. Serious ADE

The next table shows the number and percentage of subject in the US NIS CC studies by dose for both cases with serious ADE, which display dose response, and for those withdrawing due to serious ADE, in whom dose response is probably present. The dose response for % of patients with ADE versus dose varys from 0.7% (10mg) to 9.1%(80mg).

Number (%) of Patients with Serious Adverse Events and Withdrawals from Study Participation because of Those Events by Dose of NIS CC							
DOSE OF NIS CC (n)	10mg (151)	20mg (395)	30mg (244)	40mg (292)	60mg (199)	80mg (11)	Total (1292)
NO. OF PATIENTS WITH SERIOUS AEs (% OF PTS ON DOSE)	1 (0.7)	8 (0.2)	2 (0.8)	5 (1.7)	9 (4.5)	1 (9.1)	26 (2.0)
NO. OF PATIENTS WHO WITHDREW BECAUSE OF SERIOUS AE (% OF PTS WITH SAE)	0	3 (38)	0	4 (80)	5 (55)	1 (100)	13 (50)

3. Discontinuations due to ADE

Of the N= 1292 patients (combined US+ non- US NIS CC), N= 25 (2.0%) ADE reports were received. Of these cases N= 13 were withdrawn because of these events. N= 17 of the 25 reports occurred during the double- blind phase of trials.

These N= 13 cases, among others, have narrative comments in Table 15a Pool 6 Vol 521. Each of the narratives on these cases was examined by the Reviewer. The final diagnoses were angina, MI, cellulitis of legs, possible MI, CVA, possible MI, infection, flu, pleural effusion, cholelithiasis, CVA, chest pain, pituitary tumor and berry aneurysm, elevated liver enzymes, edema and erythema plus petechiae, pain in legs with elevated CPK, chest pain.

Of the non- US NIS CC completed studies N= 35 cases on NIS withdrew due to ADEs. A listing, Vol 523 Table 15, provides reasons for discontinuation in N= 11 cases: These include tinnitus, non-response, pheochromocytoma, headache plus edema, atrial fibrillation, impotence, edema(2), vertigo, lack of efficacy, non- compliance. In the remaining cases a cause for discontinuation was not given although co-start terms for side effects were. There were a variable number of such terms for different patients. Most were manifestations of vasodilatation. No withdrawals for laboratory abnormalities were listed.

In the N= 6 placebo- controlled trials (US and non- US) with NIS CC 55/828 (6.6%) discontinued. Another N= 68 cases (11.5%) discontinued from among N= 590 patients on long- term uncontrolled studies. Since all but one of the 6 trial was a US study, the Sponsor focused on them. The following table shows that a dose response exists, except at 30 mg, for withdrawal due to ADE in the US placebo- controlled trials. The Sponsor does not believe dose response is evident, but this reviewer's logistic regression (below) shows a slope coeff t of 3.5 std errors.

Number and Percent of Patients in US Placebo-Controlled Studies Withdrawing Due to Adverse Experiences, by Dose of NIS CC							
	PLA (n=280)	10mg (n=37)	20mg (n=180)	30mg (n=125)	40mg (n=184)	60mg (n=137)	80mg (n=15)
N (%)	9 (3.21)	2 (5.4)	13 (7.2)	5 (4.0)	15 (8.2)	15 (10.9)	3 (20.0)

Table 15a Vol 521, not attached, provides a complete listing of reasons for withdrawal in this group. What is striking is that many subjects have multiple reasons for withdrawal. When more than one reason is listed, no single one is given most weight in this table. There was a suggestion, based on inspection of the table, that peripheral edema and rash might be associated but the number of cases is not more than a few.

The following table shows the most frequently reported ADE in subjects withdrawing due to ADE in the controlled US studies.

Incidence (%) of Most Frequently Reported Adverse Events in Patients Withdrawn Due to Adverse Events in U.S. Placebo-Controlled Studies		
ADVERSE EVENTS	PLA (n=280)	ALL NIS CC (n=678)
Any Body System	3.2	7.8
Headache	0.4	3.8
Peripheral Edema	0.4	2.9
Vasodilatation	0	1.5
Nausea	0	0.9
Palpitation	0	0.9
Dizziness	0.4	0.7

The Sponsor reports that the ratio of the number of ADE to the number of patients discontinuing was greater at lower doses than at higher ones.

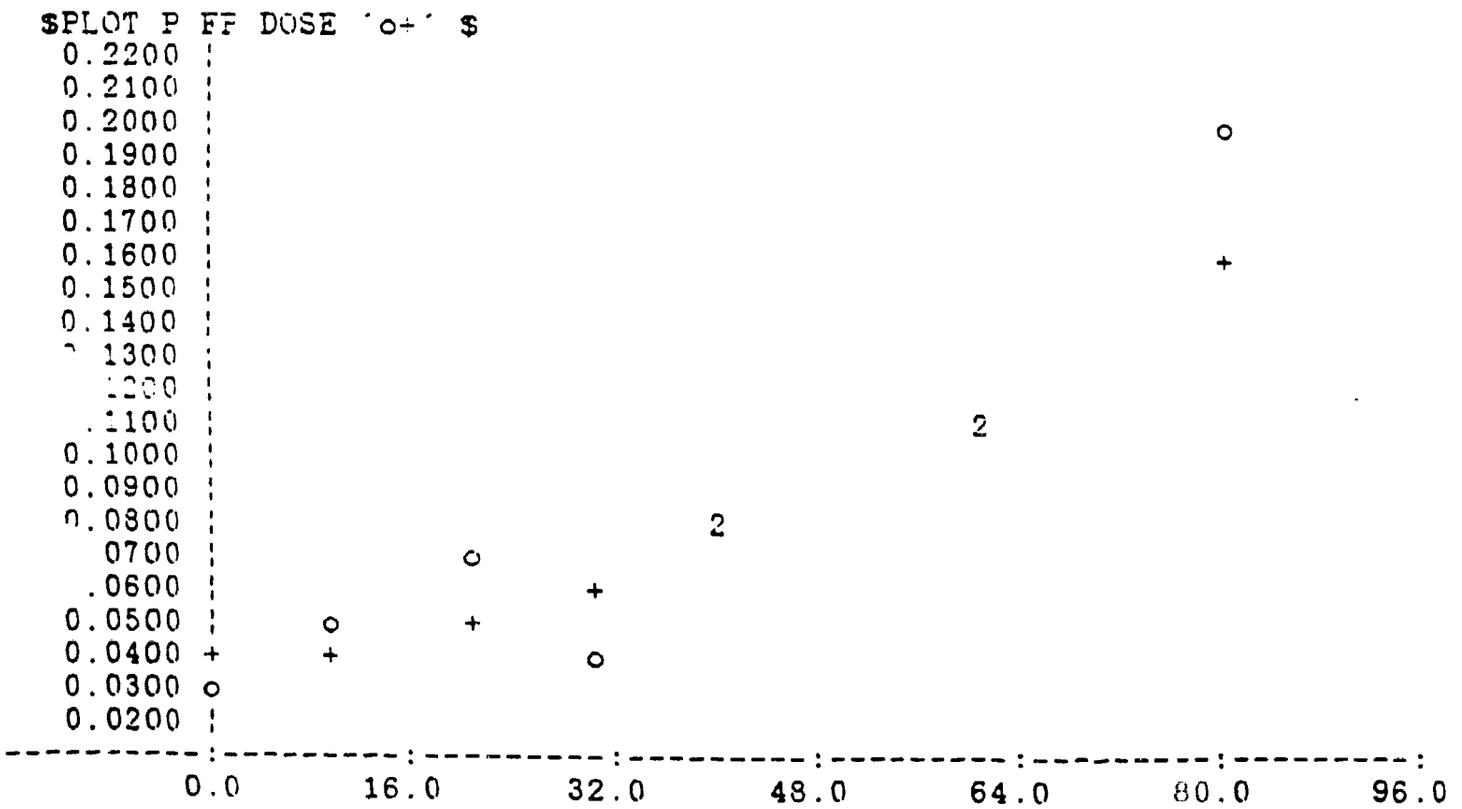
LOGISTIC REGRESSION OF WITHDRAWAL PROPORTION ON DOSE
Y AXIS: PROPORTION WITHDRAWN DUE TO ADE
X AXIS: DOSE NIS MG/DAY

Method: Iteratively- reweighted least squares (GLIM)
(analysis by reviewer)

o = OBSERVED

+ = PREDICTED

US PLACEBO- CONTROLLED HYPERTENSION TRIALS



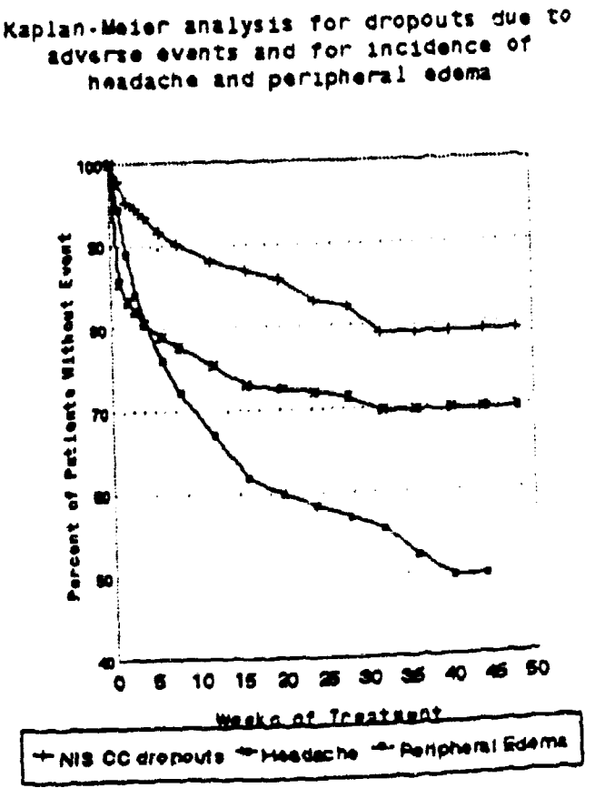
The Sponsor suggests that multiple, but mild, events could be occurring at low doses with more severe ones, though fewer, at high doses. No analysis of this hypothesis is provided.

The following table shows cases withdrawing due to ADE from US uncontrolled studies. Patients are assigned the dose they were on for the longest time. There is a counterintuitive inverse association of withdrawal and dose. One possibility is that subjects on higher doses had the opportunity to have withdrawn on lower ones during the process of upward dose adjustment in these long- term studies.

Number and Percent of Patients in U.S. Uncontrolled Studies Withdrawing Due to Adverse Experiences, by Dose of NIS CC				
	20mg (n=55)	30mg (n=46)	40mg (n=34)	60mg (n=89)
N (%)	12 (21.8)	9 (19.6)	6 (17.6)	8 (9.0)

The most frequent ADE in patients withdrawn during the US uncontrolled studies, shown below, are ordered somewhat differently than in the short- term studies. In particular, peripheral edema is more frequent in the long- term trials probably because of a time-dependence for withdrawal such that headache occurs earlier than edema. This is shown in the following table and in a Kaplan-Meier plot.

Incidence (%) of Most Frequently Reported Adverse Events in Patients Withdrawn Due to Adverse Events in U.S. Uncontrolled Studies	
ADVERSE EVENTS	ALL NIS CC (n=224)
Any Body System	15.6
Peripheral Edema	12.1
Headache	7.6
Rhinitis	4.0
Asthenia	3.6
Dizziness	3.1
Chest Pain	2.7
Vasodilatation	2.7



The Sponsor states events causing discontinuation are more severe or higher doses, say 60 mg, than on lower ones. In the absence of a specific analysis taking account of the correct denominators, this may be questioned.

4. Most frequent ADE

The following table lists the most frequent ADE regardless of whether they were associated with withdrawal. Data from US and non- US NIS CC cases are given. The prominance of symptoms related to vasodilation is again seen. The relatively greater incidence of edema in the pooled controlled + uncontrolled studies is also shown for both US and non- US data. In addition, the rates in the non- US studies are overall less than in the US ones.

Incidence Rate (%) of Adverse Experiences (≥3%) in Patients Treated in U.S. and Non-U.S. Studies						
Study Location	Studies conducted in the U.S.			Studies conducted outside the U.S.		
Type of Studies	Placebo-Controlled		Controlled + Uncontrolled	Placebo-Controlled		Controlled + Uncontrolled
Adverse Events	PLA (n=280)	NIS CC (n=678)	NIS CC (n=778)	PLA (n=58)	NIS CC (n=150)	NIS CC (n=516)
Headache	15	22	23	21	23	18
Peripheral Edema	10	22	29	7	12	15
Dizziness	4	5	7	9	4	5
Asthenia	4	4	6	0	1	3
Vasodilatation	2	4	5	0	3	5
Palpitation	1	3	3	3	4	3

5. ADE by Demographic features

ADE by demographic sub- groups is examined in the US placebo- controlled trials. The incidence is given below of ADE selected by the Sponsor for "common observance" with the type of compound used. These are mostly those with higher incidence. There is no aggregation by sex though one might have expected this, say, for edema in females.

Breakdown by Gender of the Incidence (%) of Selected* Adverse Events† in US Placebo-Controlled Studies				
ADVERSE EVENT	NIS CC		PLACEBO	
	Male (n=424)	Female (n=254)	Male (n=172)	Female (n=108)
Any Body System	67	69	50	58
Headache	20	24	14	16
Peripheral Edema	22	21	6	14
Dizziness	5	5	2	6
Asthenia	4	4	4	3
Vasodilatation	4	4	1	4
Palpitation	3	4	1	1

* The above events were selected because of their common observance with dihydropyridine therapy

† The US data includes both adverse events and intercurrent illnesses

In the non- US placebo- controlled trials there was an increase in edema in females treated with NIS CC but data for the placebo group is not provided. The incidence of edema in treated females is about the same as in the US placebo group, above. The more frequent ADEs are shown below by race in all placebo- controlled studies. Rates tend to be higher for headache and edema in Caucasians.

Breakdown by Gender of the Incidence (%) of Selected* Adverse Events in Non-US Placebo-Controlled Studies		
ADVERSE EVENT	NIS CC	
	Male (n=65)	Female (n=85)
Any Body System	33.8	38.8
Headache	22.1	22.4
Peripheral Edema	7.7	16.3
Dizziness	3.1	4.7
Vasodilatation	< 3	4.7
Palpitation	3.1	4.7

* The above events were selected because of their common observance with dihydropyridine therapy

ADE by age are shown below for placebo- controlled studies. Headache is more frequent in younger subjects, interestingly, though edema may be less frequent than in older cases. These trends are present in both US and non- US studies.

Breakdown by Age of the Incidence (%) of Selected Adverse Events† in US and Non-US Placebo-Controlled Studies				
ADVERSE EVENT	US Studies		Non-US Studies	
	≤ 65 Years (n=601)	> 65 Years (n=77)	≤ 65 Years (n=131)	> 65 Years (n=19)
Any Body System	69	87	37	37
Headache	24	5	24	11
Peripheral Edema	21	27	12	16
Dizziness	5	5	4	5
Asthenia	4	0	< 3	5
Vasodilatation	4	4	4	< 3
Palpitation	3	1	5	< 3

† The US data includes both adverse events and intercurrent illnesses

Peripheral edema was more frequent in heavier subjects than in the lighter ones shown below both in US and non- US studies.

The table below shows selected adverse events with incidence rate ≥ 3% broken down by median weight in the US and non-US placebo-controlled studies.

Breakdown by Weight of the Incidence (%) of Selected Adverse Events[1] in US and Non-US Placebo-Controlled Studies				
	US Studies		Non-US Studies	
ADVERSE EVENT	≤ 185 lbs (n=280)	> 185 lbs (n=396)	≤ median weight (n=61)	> median weight (n=69)
Any Body System	64	70	33	39
Headache	20	23	24	22
Peripheral Edema	16	28	10	15
Dizziness	5	5	4	4
Asthenia	4	4	< 3	< 3
Vasodilatation	5	4	< 3	6
Palpitation	3	3	< 3	6

[1] The US data includes both adverse events and intercurrent illnesses

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A table, not attached, shows that when ADE are stratified by baseline BP below and above 108 mmHg diastolic, that cases with lower BP tended to have more vasodilatation. The respective rates, 5/537 and 1/141. These are not very impressive.

6. Hemodynamic safety

A. Hypotension

ADE suggestive of hypotension, syncope and "hypotension" were sought. Asymptomatic hypotension was determined by "first dose effect", by trough/peak ratios from in- clinic and 24hr ambulatory BP readings, and by examining supine and standing BP plots. No cases (Sponsor) of syncope in the US NIS CC trials on NIS. N= 6 patients in the US placebo- controlled studies had either "hypotension" or "postural hypotension" on NIS CC. The next table shows data from US placebo- controlled trials.(INSERT tp15 v209)
Note that only a few of the cases show orthostatic hypotension in casual blood pressures. It seems worth while to point out that "dizziness" occurred in about 7% of all the US NIS CC studies and that it is possible that a number of cases had this symptom due to hypotension. Clinical experience shows that "dizziness" is not often distinguished from light- headedness without vertigo due to inadequate questioning of patients.

A first- dose effect was examined in two studies without showing adverse symptomatology but with BP reductions. The following table shows the results of in- clinic BP monitoring. Dose related peak effects are seen.

Mean Pre-dose and Peak Supine and Standing Blood Pressure Changes During In-Clinic Monitoring Periods in US Placebo-Controlled Studies								
		Mean Change in Supine Blood Pressures (mmHg)		Time to peak (hr)	Mean Change in Standing Blood Pressures (mmHg)		Time to peak (hr)
Drug Group	n	Pre-dose	Peak		Pre-dose	Peak	
STUDY CSS-054 (24-hour series, BPs every hour)							
Placebo	8	- 0.9/- 3.4	- 5.0/- 6.8	3	- 2.3/- 3.8	- 6.3/- 7.0	22
NIS CC 10mg	7	-11.8/-10.3	-12.8/-11.1	8	-17.4/- 8.7	- 9.7/-10.8	9
NIS CC 20mg	7	- 6.0/- 6.3	- 7.1/-12.0	14	- 9.8/- 4.8	- 7.1/-12.8	11
NIS CC 30mg	9	- 8.8/-12.0	-18.0/-15.8	12	- 5.8/- 7.4	-14.9/-16.2	11
STUDY 089-039 (12-hour series, BPs every two hours)							
Placebo	35	- 4.9/- 5.0	- 3.4/- 8.3	8	- 5.7/- 3.8	- 1.8/- 4.9	8
NIS CC 20mg	37	-11.7/-10.8	-16.7/-13.5	8	-14.3/- 8.3	-18.2/-12.5	8
NIS CC 40mg	38	-18.0/-12.9	-19.1/-15.5	4	-18.2/-12.1	-22.8/-16.9	4
VERAPAMIL	40	-14.4/-14.8	-17.4/-15.1	2	-16.2/-14.9	-21.0/-15.8	8
STUDY 090-019 (12-hour series, BPs every hour)							
Placebo	27	- 1.9/- 7.2	- 8.0/- 8.1	7	- 7.1/- 8.0	- 4.8/- 7.5	4
NIS CC 30mg	32	-13.3/-13.6	-19.0/-14.4	8	-14.6/-12.8	-16.9/-15.8	9
NIS CC 60mg	28	-19.2/-17.8	-16.8/-18.7	9	-18.1/-16.0	-21.1/-20.3	7

Symptomatic Hypotensive Adverse Events
Occurring in US Placebo-Controlled Studies

Study Number	Patient No. / Drug & Dose	Investigator Term	Days on Drug	Duration (days)	Intensity	Baseline BPs (supine & standing)	BPs (day and blood pressures)
		SYNCOPE					
D89-039	16020 / PLA	Vasovagal Attack	43	1	Moderate	165/107 168/105	day 43: 142/99 143/90
D90-019	1012 / PLA	Feeling Faint at Times	30	> 13	Mild	149/107 14[illegible]/10[illegible]	day 28: 155/113 171/113
		HYPOTENSION					
D89-039	13012 / NIS 20mg	Hypotension Post-Dose	62	1	Mild	136/95 135/97	day 62: 129/82 108/70
D89-039	16015 / VER SR 240mg bid	Hypotension	23	> 5	Severe	129/100 115/91	day 23: 114/93 98/74
		POSTURAL HYPOTENSION					
D89-039	1005 / NIS 20mg	Dizziness upon standing	30	12	Mild	129/95 130/89	day 41: 136/91 135/85
D90-019	16004 / NIS 30mg	Dizziness (Postural upon standing up)	10	9	Mild	165/105 162/106	day 14: 151/99 146/98
D89-039	1008 / NIS 40mg	Postural Hypotension	16	1	Mild	155/92 141/99	day 16: 139/89 129/85
D89-039	12007 / NIS 40mg	Postural Hypotension	25	1	Mild	157/97 150/97	day 25: 146/89 136/88
D90-019	16007 / NIS 60mg	Postural Dizziness	28	> 6	Mild	170/105 166/101	day 33: 145/89 147/90
D89-029	4001 / ATN + PLA	Dizziness upon standup	3	9	Mild	187/105 146/90	day 6: 183/98 150/93 day 14: 153/94 93/67

N= 5 ambulatory monitoring studies were done. The distribution of doses by study is provided. The second table shows the trough/peak ratios from these studies.

Number of Patients Undergoing 24-Hour ABPM by Dose of NIS CC						
Study & (location)	PLA	10mg	20mg	30mg	40mg	60mg
D88-064 (US)	18	12	11	12		
D88-029* (US)	31		32		32	30
D88-039 (US)	26		24		24	
D90-006 (non-US)	33	33	32	37		
D90-019 (US)	31			39		25

* This study was conducted on the background of atenolol 50mg od

Trough/peak ratios from the 24-hr ambulatory data are provided below. Note that for systolic BP two methods are used depending on whether peak systolic is a) determined at the time of peak diastolic BP or, b) whether the true peak is used. These ratios are consistant with peaks that are not substantially below the trough values. Note, however, that if in a given subject the trough readings are quite low that a small, further drop at peak might be hypotensive. For that reason trough/peak ratios may not be very good means of exploring for BP reductions for safety purposes.

TABULATION OF TROUGH-TO-PEAK RATIOS ANALYZED DURING 24-HOUR AMBULATORY BLOOD PRESSURE MONITORING (BASELINE- AND PLACEBO-SUBTRACTED RESULTS)						
	DOSE OF NIS CC					VER SR
PARAMETER	10mg	20mg	30mg	40mg	60mg	240mg bid
Diastolic BP Trough/Peak Ratio (%)	73	75	93	100	97	86
Systolic BP* Trough/Peak Ratio (%)	75	83	114	100	101	78
Systolic BP† Trough/Peak Ratio (%)	75	83	93	100	89	78

* Peak values correspond to the time of peak diastolic effect.
† Peak values are the actual maximum systolic effect.

The table below shows the percentage of patients having either a change of 20 mm from baseline or a BP below 100mm Hg. There appears to be a fairly consistant percentage of cases with a fall

In systolic BP regardless of dose except for low numbers in the small sample on 40 mgm NIS. Note that a substantial number of placebo cases also show this degree of fall. Subtraction of the placebo values gives about 4- 8% of cases with reduction below 100 mm systolic. Thus supine reductions of note occurred in some subjects.

TABULATION OF SAFETY PARAMETERS ANALYZED FROM 24-HOUR AMBULATORY BLOOD PRESSURE MONITORING (BASELINE-SUBTRACTED RESULTS)

SAFETY PARAMETER	DOSE OF NIS CC					PLA	VER SR
	10mg (n=46)	20mg (n=67)	30mg (n=98)	40mg (n=24)	60mg (n=29)	(n=106)	240mg qd (n=29)
Diastolic BP Change > 20mmHg from Baseline for at least 1 Hour (% of patients)	82	86	78	79	96	82	96
Systolic BP < 100mmHg for at least 1 Hour (% of patients)	24	26	22	4	20	16	24

The last method of assessing hypotension was to compare supine and standing blood pressures at trough. The correlation was near 1.0 and consistant with little orthostatic hypotension.

B. Reflex tachycardia

One of the effects of a vasodilator is reflex tachycardia. The Sponsor examined this by dose response of pulse rate; by frequency of tachycardia as an ADE; and by ECG HR.

In the US monotherapy studies the mean placebo- subtracted change in HR varied from -1.53/min to +0.18 over the dose range of 10 to 60mgm NIS CC. The change in HR for the combined doses was 0.52. Note that these are not specified as standing readings.

In the US placebo- controlled studies, tachycardia was an ADE in 1% of NIS CC patients. One patient in these studies withdrew for supraventricular tachycardia.Three patients in the US uncontrolled studies had tachycardia contributing to withdrawal. The Sponsor analyzed the transition from normal or low heart rate to high values in the US controlled trials and found this to have occurred in 1.4% of NIS CC patients and in 0.9% of placebo cases. Again, none of these readings are specified as taken standing, a position that might have exaggerated pulse change.

C. Rebound hypertension

A placebo- controlled study D90-022 in hypertensive patients treated for as long as 21 days sought evidence for rebound blood pressure elevations by a 72- hr follow-up after discontinuation of NIS CC. Examination of the Sponsor's table showed that the group means for diastolic BP show no evidence of rebound. However, the systolic BP values are higher at 72 hrs than at baseline except for the highest dose level, 120mg NIS. The systolic BP mean for the placebo group is also elevated at 72 hrs so that it is difficult to ascribe the increase in systolic BP to "rebound". It is more likely that loss of both the placebo and therapeutic effects are involved.

V. Clinical Laboratory Tests

A. US NIS CC placebo- controlled studies

1. Incidence rates of "high" lab abnormalities

The Sponsor provides Table 17a (not attached), in which the rates for "high" abnormalities are given by dose level from 0 mgm (PLAC) to 80mg. Sample sizes are very small for the lowest active dose, 10mgm and the highest, 80mgm. Examination of these rates show no evidence for dose response nor is it likely they would given the exceedingly small rates for the data pooled over doses. In particular, for items with overall higher rates including blood glucose, no dose response trend is seen. BUN has a 3% rate at 40mg NIS, 2% at 60mg, and 0% for placebo. No such trend is seen for creatinine. No trend is seen for increase with dose of serum calcium, alkaline phosphatase, or SGPT is seen.

Rates by dose/body weight are also provided but do not show trends of interest except, possibly, for alkaline phosphatase, which has a rate of 5% at the highest dose/weight level, >.55- <1.2 mgm/kg, versus a placebo rate of 2% and rates in lower active dose/weight levels of 1%.

For "low" values the hematologic values for all US NIS CC studies showed 2% of NIS patients with neutrophils below 1700/microl and 1% in the placebo group. The rate was also 2% in the pooled controlled and uncontrolled studies. No patients with platelets below 100,000/microl are shown. Thus addition of cases with long- term followup did not increase the rate of low values for these two tests.

Hematopoietic Parameter Abnormalities from Studies Conducted in the US

BLOOD CELL LINE	Placebo-Controlled		Controlled + Uncontrolled
RBC	PLA	NIS CC	NIS CC
	n=250	n=588	n=680
% PATIENTS LOW ABNORMAL	3	3	5
WBC	PLA	NIS CC	NIS CC
	n=232	n=553	n=636
% PATIENTS LOW ABNORMAL	5	4	5
% PT WITH NEUTROPHILS < 1700/μL	1	2	2
PLATELETS	PLA	NIS CC	NIS CC
	n=267	n=655	n=751
% PATIENTS LOW ABNORMAL	0		0
% PT WITH PLATELET < 100,000/μL	0	0	0

This submission contains a tabulation of mean difference from baseline for both NIS CC (N= 650) and placebo (N= 280) for US placebo- controlled subjects. A tabulation on the following page contains selected laboratory tests from the larger tables. The larger table, Table 21 Vol 522, also has standard deviations. The Reviewer calculated the mean difference +/- 2 SE limits for NIS cc and for placebo for hematocrit, platelets, %neutrophils, glucose, BUN, alkaline phosphatase, and SGPT. All of the 2SE limits for the NIS group overlapped those for the PLAC group for these particular tests so that the treatment groups are not likely to differ by this method.

Examination of 10 lowest or highest values of selected laboratory tests in all US studies(controlled and uncontrolled for individual subjects showed, among the lowest 10, N= 3 instances in which falls in hematocit occurred. None were associated with the lowest 10 values for total wbc or %neutrophils. Respective baseline and lowest values were 34,29: 36,30; and 36,33. The baseline values tended towards being low. The subject with the lowest hematocrit,29, was on many medicines at baseline including Naproxin, insulin, enalapril,labetalol, and glyburide. During the

Renal Function Parameter Abnormalities from Studies Conducted in the US			
RENAL FUNCTION TEST	Placebo-Controlled		Controlled + Uncontrolled
CREATININE	PLA	NIS CC	NIS CC
	n=271	n=646	n=739
% PATIENTS HIGH ABNORMAL	0	0	1
BUN	PLA	NIS CC	NIS CC
	n=269	n=645	n=738
% PATIENTS HIGH ABNORMAL	0	1	1

Hepatic Parameter Abnormalities from Studies Conducted in the US			
LIVER FUNCTION TEST	Placebo-Controlled		Controlled + Uncontrolled
SGOT	PLA	NIS CC	NIS CC
	n=248	n=603	n=692
% PATIENTS HIGH ABNORMAL	3	2	3
% PT >3X NORMAL	0	0	0
SGPT	PLA	NIS CC	NIS CC
	n=224	n=562	n=647
% PATIENTS HIGH ABNORMAL	3	2	4
% PT >3X NORMAL	0	0	0
ALKALINE PHOSPHATASE	PLA	NIS CC	NIS CC
	n=262	n=623	n=713
% PATIENTS HIGH ABNORMAL	2	2	4
% PT >1.25X NORMAL	0	0	0
LDH	PLA	NIS CC	NIS CC
	n=257	n=628	n=717
% PATIENTS HIGH ABNORMAL	3	2	4

All cases of high blood glucose on treatment were high at baseline, usually above 200mg%.

N=7 instances of mildly elevated BUN on treatment were found. Only one of these (value =27) was associated with an increased serum creatinine(1.4,2.0).
Serum calcium increased in association with treatment in N=3 cases baseline, on treatment:9.8,10.5; 10.0,10.5;9.1,10.4) but alkaline phosphatase was not in the highest N=10 for any of these. The last of the above N=3 cases had a low phosphate (2.3,1.9).

Although a number of cases had elevation of alkaline phosphatase during treatment, all were high at baseline. One instance of notable change (112,248), but with a subsequent fall to 150, despite some increase at baseline was more closely examined. The patient was a 64- year-old female with diabetes mellitus, hyperlipidemia, and edema. During a long-term extension trial she was on concomittant medications including atenelol, niacin, and glyburide. A slow rise in alkaline phosphatase over 1 and 1/2 years occurred with 3 high readings.

N=3 instances of elevation of SGPT with concomittant elevation of SGOT were noted (baseline, on treatment: 25,384; 39,209;35,88). The first of these also had elevated total bilirubin (0.6,3.3). This patient was a 63-yr-old male with a history of elevated transaminases on ACE inhibitors.During treatment he received Lovastatin. Despite continued treatment with NIS the final day SGPT and SGOT were well within normal limits though the previous two values, both obtained within a one-week period, were elevated. An additional N=2 instances of elevation of SGOT occurred in the absence of enough rises in the other enzymes to reach the level of the highest 10 values.

Increases in serum bilirubin occurred in N=4 cases. One of these had enzyme elevations described just above. Total CPK was elevated during treatment in N=2 patients. In one the MM and BB fractions were normal.

In the N=516 (depending on test) non- US controlled plus uncontrolled studies hematocrits were low in N= 3 cases but in N=2 they tended to increase subsequently. Total leucocytes were low in N=4 cases. In two of these the baseline value was also low. None of these cases were among the 10 lowest %granulocyte values. N=8 low platelet counts occurred in N= 8 cases. In N= 2 of these the baseline values were normal. Except for N=2 cases the on- Rx values were not very low, and though below the assigned normal range, were all above 100,000. In the one of N=2 cases with platelets below 100,000 total wbc was low both at baseline and on treatment. In the other the wbc count was not among the N=10 lowest.

In N= 10 cases elevation of SGOT occurred but baseline values were elevated in these. In two cases use of country- specific normal values might have reduced baseline values to normal. N=7 cases with elevated alkaline phosphatase values occurred. In N= 5 of these the baseline values were also elevated. These elevations are not associated with values for SGOT in the highest 10. N=6 cases of elevated serum bilirubin were found; in N=4 baselines were high. None were associated with SGOT values in the highest 10.

Serum creatinine was not elevated above normal in any of the values in the highest 10.

8. ECG

A. Background

Because of the finding of t-wave changes in study D90-022 (hypertension), the Sponsor examined that study and three phase III NIS CC trials for such alterations. The Sponsor has provided background information from the medical literature. T wave inversion or flattening occur during rapid reduction of BP with vasodilators. These reductions in BP do not appear to be associated with wall motion abnormalities on 2D echocardiography. Long- term treatment with minoxidil has been carried out with improvement in the initial t-wave changes.

B. The phase II trial D90-022

This was a trial in N= 26 patients with mild to moderate hypertension, mean age about 60 years. This was a forced titration trial 30- 120mg NIS CC with the first two doses given for 4 days each; the next two for 7days each. N=8 subjects were randomized to NIS CC and N=5 developed t- wave flattening on the ECG. The N=120mg dose level was discontinued due to poor tolerance (severe peripheral edema, ECG changes).A second group, N= 10, was randomized to NIS. N=6 of these cases developed t wave flattening and/orinversion with occurrence equal, N=2 cases, at each of the doses, 30,60, and 90 mg. No angina occurred. Thallium scans were reported as negative in N=5 of the first cohort.

The percent with T-wave changes and dose were, respectively, 0%(PLAC); 22%(30mg); 39%(60mg); 64%(90mg); 80%(120mg). The respective sample sizes were 5,18,18,11,5. n.b. The excess over the N=23 that were randomized must represent titration steps.

Stratifying results into cases with normal and abnormal ECG and into 6 and 24 hrs after dosing, showed significant differences between BP falls at 6hrs between the two ECG groups. Differences at 24 hrs (trough) were not significant.

Stratifying normal and abnormal ECGs by AUC and Cmax showed that the abnomal ECG group had larger pharmacokinetic parameters.

The Sponsor relates the ECG changes to the forced- titration design and, by analogy, to the literature reports of T- alterations in rapidly- induced hypotension.

C. Other Phase III trials

ECGs from trials D89-029,039,and D90-019 coded blindly and read by a cardiologist. These ECGs were usually taken at trough. Peak ECGs were obtained in one study. Dosage was up to 80mg NIS CC (N=494 in the pooled studies) or placebo. Mean age was about 55. One study had background atenolol.

In these titration studies the dose assigned to an ECG was, for one analysis, that to which a patient was randomized, not to, say, a lower dose they might have transited from. Another analysis assigned the dose as that on which the event occurred. Two analyses were done; one ignoring baseline ECG events and another eliminating cases with these. It seems likely that more than one event per person could occur since rates were calculated from the "total number of events" divided by the number of cases at risk for the first type of analysis. In the second analysis all subjects were at risk. One analysis was done for peak ECG responses.

The table below shows some evidence of dose response except for the small sample at 80mg.

STUDY D89-039						
ECG abnormality	PLA	ALL NIS CC	NIS CC 20 mg	NIS CC 40 mg	NIS CC 80 mg	VER SR
T flattening	5/70 (7%)	10/155 (6%)	3/71 (4%)	7/69 (10%)	0/15 (0%)	4/71 (6%)
T inversion	3/73 (4%)	11/157 (7%)	4/72 (6%)	6/71 (8%)	1/14 (7%)	3/72 (4%)
either	8/74 (11%)	20/166 (12%)	7/75 (9%)	12/76 (16%)	1/15 (7%)	7/76 (9%)

Similar results were obtained when 'all patients' (regardless of baseline findings) were studied and when the number of ECG tracings was used as the denominator. n.b. there tends to be a dose response in each of the above results except at 80mg.

Results for ECGs taken at peak were unrevealing. The sample sizes were far too small, about N=5 per active dose group.

Study DS029. This is the study with background atenolol in addition to NIS CC.The doses of NIS were 0,20,40, and 60mg.

The respective rates of T flattening or inversion at these doses were 18%,7%,20%13% so there was not much evidence of dose response.

For all cases, regardless of baseline status, there was a significant difference among doses for T-flattening (PLAC 21%; 20 21%; 40mg 41%; 60mg 27%).

For events per number of ECGs results were weaker.

Study D90-019. The rates for T-wave flattening or inversion by dose were PLAC 13%; 30mg 9%; 60mg 5%. Therefore no dose response is seen.

Rates using all patients or number of ECGs in the denominator were not more useful.

ST- segment elevation or depression: Rates for these were very low in each of the three studies (1-2%). Comparing the incidence in the PLAC and pooled NIS CC groups by ST depression and by elevation showed that placebo and active dose rates were each no more than 2%.

There is little evidence from these three studies, taken together, of a dose response for the primary ECG T-wave changes of interest. However, the findings in the Phase II trial with forced titration do show a trend for dose response as well as effects of BP reduction and plasma NIS concentrations on T abnormalities. Thus the findings in the Phase III trials may just be at the opposite end of a spectrum of effects.

Conclusions for Hypertension Safety (NIS CC):

In placebo- controlled trials there is a dose- related incidence of ADE over the range 0 to 80 mgm of NIS CC. The most frequent ADE are those related to the vasodilatory action of the drug - headache and edema. The occurrence of these two ADE is time- dependent with headache occurring relatively early versus edema.

Marked symptomatic hypotension was not prominant with NIS CC although "dizziness" occurred and may have been a manifestation of hypotension. Asymptomatic hypotension in trough readings was not frequent though routine systolic BP values in the region of 100mm Hg were not infrequent in 24- hr ambulatory BP readings. Rebound hypertension was not present overall during monitored withdrawal. ECG T- wave abnormalities, inversion and flattening, occurred during forced titration in a phase II study but in studies in which dosage was increased slowly was not prominant. There was an association of these ECG changes to the degree of BP reduction and to drug blood levels In the forced titration study ECG S-T alteration was infrequent.

Clinical laboratory abnormalities: In the US, placebo controlled (shorter- term) studies, evaluations by overall rates of abnormality, transition from normal, and overall rates by dose were not very revealing. present. Examination of the N= 10 highest or lowest values, as appropriate, in all US studies, controlled or uncontrolled (long-term), showed a few instances of falls in hematocrit, total wbc count, and %neutrophils. Several instances of increased transaminases, one with increased bilirubin were found. An instance of substantial elevation of alkaline phosphatase occurred. Serum calcium increased in three cases without increases in alkaline phosphatase.

In the non- US controlled and uncontrolled trials (approximately N= 516 NIS cases) several instances of decreases in hematocrit or wbc occurred. Platelet counts fell in some cases but not below 100,000. A number of cases had increases in SGOT but from elevated baseline levels. Serum creatinine was not elevated in the highest ten values.

38 pages

PURGED

Overall Conclusions for Safety of Nisoldipine:

Two major findings in this submission are 1) a substantial incidence of withdrawal associated with signs/symptoms of vasodilation and 2) an increased rate of withdrawal for angina/cad in trials for the angina indication. In the short- term trials the latter withdrawals tended to occur early and to be associated with signs/symptoms of vasodilation but, in the long- term trials they were primarily manifested by an increased rate of withdrawal.

In the US, placebo- controlled, hypertension trials there was a significant dose response for overall withdrawal of about 11% at 60mg NIS and 5.4% at 10 mg. The incidence of headache, not necessarily associated with withdrawal was about 20%. In the long- term, uncontrolled hypertension studies, about 20% of cases had withdrawn by 30 weeks and the cumulative incidence of peripheral edema was more than 40%. Thus whatever blood pressure reduction that is achieved is associated with substantial side effects, most of which are due to the pharmacologic action of the drug in causing vasodilation or to the compensatory mechanisms such as tachycardia.

One might expect some myocardial ischemic phenomena if the vasodilation and tachycardia increased cardiac work out of proportion due the benefits of reduction of afterload through lowering of blood pressure. There was little evidence that the balance was unfavorably affected since, in the hypertension studies the incidence of withdrawal due to angina/cad was about 1%. However, there were instances, especially in the phase 2 rapid, forced- titration trial, of the development of t wave abnormalities.

It is in the angina patients that evidence for ischemic effects of nisoldipine are of greater concern. Even in the short- term, US placebo- controlled trials the rates of withdrawal for angina/chd exceed those on placebo. In addition, it is of particular interest to note that a high proportion of such withdrawals occurred very early at times close to those for withdrawal due to vasodilation. Thus the close similarity of the distribution of withdrawal for vasodilation and that for angina/cad supports a similar mechanism for both. Symptoms of vasodilation were not prominant in the long- term angina trials but in those the number of events was higher, about 10%. Thus for the short- term trials one may use the rates, the timing of withdrawal and/or association of vasodilation; for the long- term trials only the rates are useful. Note that the use of timing of withdrawal and/or any associated vasodilation constitutes an "internal control.

From a purely safety standpoint, this reviewer is not in favor of approval for the angina pectoris indication in view of the increased rate of chest pain/cad in angina subjects. It is possible that a combination of a beta- blocker and nisoldipine would allow use of the latter drug in angina pectoris. n.b. a single study (0702) carried out in Canada, US, and Israel had very few withdrawals due

to ADE. N= 1 subject withdrew for increasing angina and N= 1 for myocardial infarction out of N= 200 NIS+ atenolol cases. The experience with this combination is as yet insufficient to recommend this combination but it may be a justifiable treatment to explore in future trials.

Note that if the NIS formulation dumps early, one would expect to see early withdrawal or the early occurrence of signs/symptoms of vasodilation.

The hypertension indication, at least for subjects without notable coronary heart disease, is supported by the safety data. . However, if the spectrum of patients selected for treatment includes patients whose hypertension is associated with CHD or as hypertensive subjects develop CHD, some of these may be unable to tolerate NIS therapy. Alternative therapy should be considered in such cases.

The ECG findings in rapid- dose escalation (intervals of less than a week) in hypertensive subjects, while not clearly established as adverse, are in an unfavorable direction and suggest that titration be carried out over the longest intervals consistant with the patient's need for blood pressure control.

Summary of safety recommendations:

1. Nisoldipine, as studied,

2.

3. Subjects with asymptomatic coronary disease constitute a somewhat difficult group with respect to suitability for NIS therapy since many hypertensives responding to this treatment undoubtedly have this condition. Perhaps some clinical judgement needs to be invoked here.

4. In view of the findings of ECG T abnormalities on rapid titration in hypertensive patients, it is suggested that dosage increments be made at the greatest intervals consistant with the need for BP control. The Sponsor's dosing recommendations do not specify the interval between dosage increments. Intervals of only a few days may be too frequent since they were associated with "adverse" ECG changes in hypertensiv. .

5. Although mono- therapy for hypertension with NIS appears justifiable due to the lack of serious drug- induced effects, there is still a substantial incidence of troublesome symptoms of vasodilation that might be diminished with combination of NIS and beta- blockade. This may also be a suitable and informative area for further trials.

6. Pharmacokinetic studies by the Sponsor show that the mean Cmax was 48% higher when NIS was administered with a meal. It is not clear whether this explains the very early occurrence of vasodilation after dosing and, in the angina patients, withdrawal associated with chest pain. It may not be correct to state that it is known that there are no clinical consequences from dose dumping.

7. Since elderly subjects have a 2- 3 fold higher plasma NIS concentration than younger ones, it may be best to follow these subjects more closely and increase dosage slower.

Philip L. Dern M.D.

cc:original
HFD-110
HFD-110/CSO
HFD-110/pld

Pharmacologist Review

DR

NDA 20-356

SEP - 2 1994

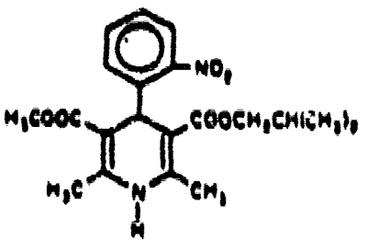
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Sidney Stolsenberg, Ph.D.
Xavier Joseph, D V.M.

ORIGINAL NDA DATED: March 31, 1993
CENTER RECEIPT DATE: April 1, 1993
REVIEWERS RECEIPT DATE: April 9, 1993

SPONSOR: Miles Inc. Pharmaceutical Division
400 Morgan Lane, West Haven, CT 06516

DRUG: Proprietary name - not established
Generic name - nisoldipine
Code name -



M.W. 388.4

FORMULATION: Coat core (extended release) tablets containing 10, 20, 30 or 40 mg of nisoldipine are formulated with following inactive ingredients: hydroxypropylcellulose, lactose, corn starch, crospovidone, microcrystalline cellulose, sodium lauryl sulfate, povidone 25, magnesium sulfate and magnesium stearate (core and outer coat); hydroxypropylmethylcellulose, polyethylene glycol 4000, ferric oxide and titanium dioxide (film coat).

PHARMACOLOGICAL CLASS: Calcium channel blocker

PROPOSED INDICATION: Treatment of hypertension

PROPOSED DOSAGE REGIMEN: 10-40 mg once daily

IND UNDER WHICH CLINICAL TRIALS WERE CONDUCTED: IND -

Table of Contents

SUMMARY OF PHARMACOLOGICAL STUDIES (X. Joseph)

A. Studies Related to Therapeutic Indications

1. Effects on Blood Pressure

a. Rats

The effects of nisoldipine on blood pressure and heart rate were studied and compared with appropriate reference drugs (nifedipine, nicardipine and hydralazine) in normotensive (NT) and spontaneously hypertensive (SH) rats. Single oral doses of nisoldipine (3-30 mg/kg) produced a dose-dependent decrease in blood pressure in normotensive rats. Although the hypotensive effect at 3 mg/kg was statistically not significant, doses of 9 and 30 mg/kg produced significant reductions in blood pressure lasting for 2 and 4 hr, respectively, after nisoldipine administration (Table 1). A significant dose-dependent increase in heart rate was observed in all nisoldipine treated groups for 2-6 hr postdose (Table 2). Reference drugs also produced similar dose dependent hypotensive effects and increased heart rates. However, in normotensive rats, hypotensive effects produced by reference drugs at 9 mg/kg were almost equivalent to the effect produced by the high dose level, 30 mg/kg, of nisoldipine (Table 1).

Nisoldipine produced a more pronounced antihypertensive effect in SH rats than in normotensive rats. Dose dependent significant reductions in blood pressure were seen at all levels of nisoldipine tested (3-30 mg/kg, po), beginning at 30 min and lasting until 4 hr (3 mg/kg) or 6 hr (9 mg/kg and above) postdose (Table 3). Heart rate was significantly increased up to 2 hr postdose in all nisoldipine treated groups and until 6 hr at the highest dose level (Table 4). At 24 hr, no significant differences in blood pressure or heart rate were seen between control and treated groups. Reference drugs also produced dose dependent decrease in blood pressure and increases in heart rates in SH rats.

Studies in other experimental animal models of induced chronic hypertension (renal hypertensive rats and deoxycorticosterone-NaCl hypertensive rats) also revealed a dose related hypotensive effect for nisoldipine.

The doses of nisoldipine and reference drugs required to decrease blood pressure by 20% or increase heart rate by 20% of the initial values (ED20 values) in normotensive and SH rats and also in other experimental animal models of hypertension are given in Table 5.

Table 1: Effects of nisoldipine and reference drugs on blood pressure in normotensive rats.

Drugs	Dose (mg/kg)	Mean blood pressure (mmHg) ± S.E.M.						
		Before	30 min	60 min	120 min	240 min	360 min	24 h
Control	-	108 ± 5	109 ± 5	110 ± 4	190 ± 5	180 ± 5	109 ± [illegible]	109 ± 5
Nisoldipine	3	109 ± 6	99 ± 5	100 ± 5	101 ± 5	103 ± 6	107 ± 6	109 ± 6
	9	111 ± 3	94 ± 6	93 ± 5*	92 ± 4*	99 ± 5	103 ± 5	112 ± 3
	30	112 ± 5	87 ± 4**	83 ± 3**	85 ± 3**	89 ± 3**	97 ± 3	114 ± 3
Nifedipine	1	112 ± 1	103 ± 1	105 ± 1	107 ± 1	107 ± 1	108 ± 1	113 ± 2
	3	109 ± 3	92 ± 3*	91 ± 3**	94 ± 3*	93 ± 2*	94 ± 2*	109 ± 3
	9	107 ± 5	79 ± 4**	76 ± 2**	77 ± 3**	77 ± 3**	80 ± 2**	108 ± 4
Nicardipine	3	113 ± 2	104 ± 2	106 ± 2	106 ± 1	109 ± 2	113 ± 2	115 ± 2
	6	112 ± 3	98 ± 3*	98 ± 3*	98 ± 2	100 ± 3	103 ± 3	113 ± 3
	9	109 ± 3	75 ± 2**	77 ± 2**	84 ± 3**	91 ± 1**	98 ± 1	111 ± 1
Hydralazine	3	113 ± 1	95 ± 3*	99 ± 2	101 ± 1	103 ± 1	107 ± 1	113 ± 1
	6	113 ± 2	70 ± 2**	89 ± 2**	90 ± 1**	91 ± 3**	91 ± 2**	112 ± 1
	9	108 ± 3	82 ± 4**	84 ± 4**	84 ± 3**	87 ± 3**	89 ± 2**	107 ± 3

Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$
In the control group, the vehicle alone (0.5 % CMC suspension) was administered

Table 2: Effects of nisoldipine and reference drugs on heart rate in normotensive rat.

Drugs	Dose (mg/kg)	Mean heart rate (beats/min) ± S.E.M						
		Before	30 min	60 min	120 min	240 min	360 min	24 h
Control	-	361 ± 17	358 ± 17	358 ± 15	355 ± 12	350 ± 10	350 ± 7	356 ± 15
Nisoldipine	3	359 ± 9	417 ± 20*	405 ± 7*	400 ± 9*	391 ± 17	376 ± 15	356 ± 7
	9	359 ± 7	451 ± 24**	469 ± 24**	469 ± 25**	413 ± 22*	385 ± 13*	356 ± 9
	30	353 ± 10	498 ± 15**	500 ± 14**	484 ± 20**	468 ± 14**	433 ± 11**	342 ± 20
Nifedipine	1	349 ± 7	395 ± 17	389 ± 17	374 ± 17	367 ± 12	362 ± 9	349 ± 7
	3	351 ± 7	489 ± 13**	491 ± 14**	477 ± 14**	462 ± 20**	429 ± 15**	350 ± 7
	9	352 ± 17	487 ± 11**	491 ± 11**	483 ± 13**	476 ± 11**	463 ± 12**	349 ± 12
Nicardipine	3	363 ± 13	443 ± 20**	400 ± 16	389 ± 11	38[illegible] ± 10	368 ± 9	349 ± 12
	6	354 ± 20	461 ± 12**	451 ± 14**	429 ± 17**	413 ± 12**	387 ± 17	357 ± 17
	9	366 ± 9	532 ± 15**	513 ± 15**	476 ± 10**	446 ± 16**	402 ± 10**	364 ± 9
Hydralazine	3	361 ± 17	446 ± 12**	439 ± 12**	424 ± 10**	411 ± 22**	387 ± 17	361 ± 9
	6	357 ± 17	479 ± 33**	460 ± 12**	446 ± 13**	429 ± 15**	399 ± 22	359 ± 9
	9	366 ± 7	514 ± 18**	473 ± 17**	449 ± 13**	433 ± 13**	423 ± 12**	368 ± 9

Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$.

Table 3: Effects of nisoldipine and reference drugs on blood pressure in spontaneously hypertensive rats.

Drugs	Dose (mg/kg)	Mean blood pressure (mmHg) ± S.E.M.						
		Before	30 min	60 min	120 min	240 min	360 min	24 h
Control	-	181 ± 5	179 ± 7	183 ± 6	182 ± 5	175 ± 4	176 ± 5	184 ± 4
Nisoldipine	3	180 ± 5	157 ± 4*	159 ± 4*	159 ± 4**	158 ± 4**	160 ± 3	181 ± 5
	9	180 ± 5	140 ± 5**	144 ± 4**	144 ± 5**	146 ± 5**	150 ± 4**	181 ± 5
	30	180 ± 7	115 ± 6**	117 ± 6**	127 ± 5**	131 ± 5**	136 ± 5**	179 ± 7
Nifedipine	1	188 ± 3	171 ± 6	167 ± 5	172 ± 5	170 ± 6	171 ± 2	199 ± 5
	3	177 ± 2	147 ± 5**	153 ± 2**	152 ± 5**	153 ± 2**	162 ± 4	175 ± 4
	9	176 ± 4	137 ± 8**	134 ± 7**	131 ± 7**	144 ± 11*	140 ± 10**	168 ± 9
Nicardipine	3	180 ± 5	157 ± 8	160 ± 6*	162 ± 6*	159 ± 7	164 ± 6	180 ± 4
	6	180 ± 5	145 ± 6**	152 ± 7**	154 ± 7**	160 ± 5*	169 ± 4	180 ± 5
	9	180 ± 4	122 ± 9**	120 ± 7**	134 ± 7**	142 ± 5**	153 ± 5**	173 ± 4
Hydralazine	3	184 ± 8	160 ± 10	151 ± 5**	149 ± 5**	141 ± 7**	147 ± 7**	169 ± 6
	6	179 ± 2	133 ± 10**	132 ± 6**	133 ± 8**	132 ± 6**	139 ± 6**	163 ± 4
	9	178 ± 5	82 ± 8**	80 ± 7**	86 ± 7**	92 ± 7**	102 ± 5**	146 ± 4

Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$.

Table 4: Effects of nisoldipine and reference drugs on heart rate in spontaneously hypertensive rats.

Drugs	Dose (mg/kg)	Mean heart rate (beats/min) ± S.E.M.						
		Before	30 min	60 min	120 min	240 min	360 min	24 h
Control	-	397 ± 12	357 ± 15	352 ± 9	356 ± 11	369 ± 15	363 ± 12	403 ± 15
Nisoldipine	3	400 ± 16	402 ± 12*	394 ± 12*	398 ± 10*	404 ± 10	387 ± 10	415 ± 6
	9	400 ± 11	445 ± 21**	446 ± 18**	436 ± 17**	400 ± 10	403 ± 16	403 ± 9
	30	400 ± 9	423 ± 25*	435 ± 17**	452 ± 19**	436 ± 9**	410 ± 14*	390 ± 19
Nifedipine	1	385 ± 18	341 ± 10	343 ± 14	344 ± 13	351 ± 15	363 ± 12	404 ± 15
	3	399 ± 10	403 ± 11*	400 ± 16*	360 ± 15	373 ± 16	369 ± 18	391 ± 12
	9	413 ± 33	424 ± 26*	403 ± 21*	423 ± 24*	400 ± 15	400 ± 18	393 ± 15
Nicardipine	3	400 ± 9	395 ± 22	397 ± 21	389 ± 22	369 ± 12	358 ± 15	381 ± 5
	6	400 ± 14	453 ± 16*	406 ± 21*	414 ± 10*	419 ± 5*	396 ± 5*	419 ± 11
	9	397 ± 13	541 ± 9**	515 ± 27**	504 ± 19**	491 ± 15**	433 ± 27**	385 ± 10
Hydralazine	3	409 ± 6	461 ± 11**	443 ± 13**	425 ± 8**	422 ± 6**	413 ± 7**	416 ± 7
	6	394 ± 13	472 ± 8**	446 ± 9**	453 ± 12**	445 ± 3**	430 ± 13**	423 ± 6
	9	429 ± 20	537 ± 9**	508 ± 12**	504 ± 18**	500 ± 15**	470 ± 1[illegible]**	457 ± 13

Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$.

Figure 1

Antihypertensive effect of nisoldipine after oral administration to spontaneously hypertensive rats.

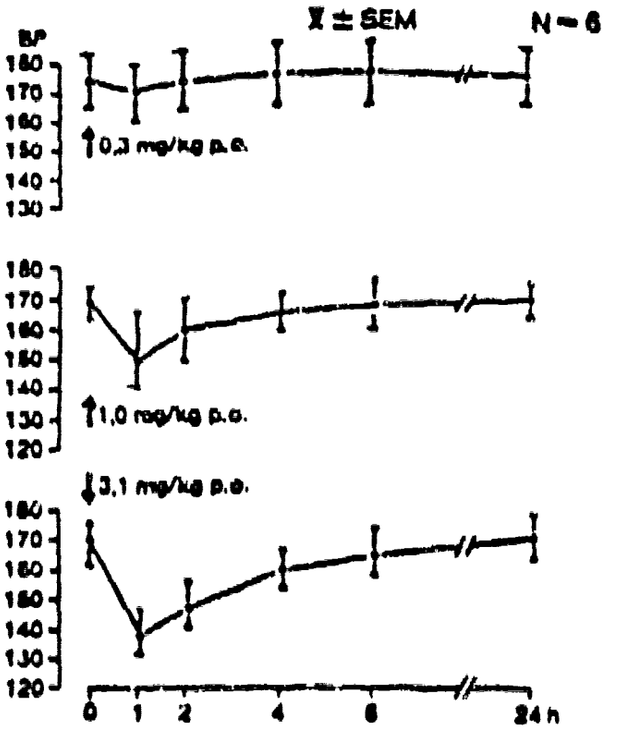


Figure 2

Antihypertensive effect of nisoldipine) by oral administration to one-kidney renal hypertensive rats.

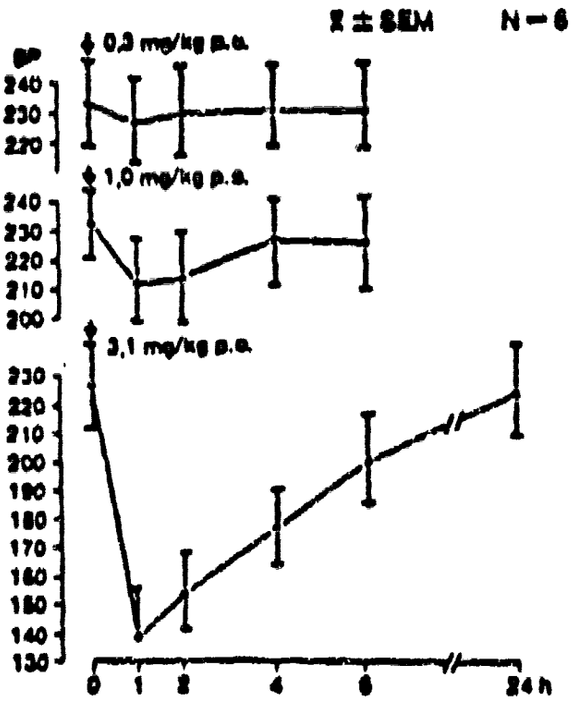


Table 5. Comparative effects (ED_{30}) of nisoldipine and reference drugs on blood pressure (BP) and heart rate (HR) in certain types of hypertensive rats and in normotensive rats

Drugs	ED_{30} (BP) (mg/kg p.o.)				ED_{30} (HR) (mg/kg p.o.)			
	NR	SHR	DNR	RHR	NR	SHR	DNR	RHR
Nisoldipine	12.0 (1)	4.0 (1)	7.21 (1)	4.04 (1)	3.80 (1)	1.40 (1)	>30.00 (1)	1.60 (1)
Nifedipine	4.10 (0.34)	3.60 (0.90)	1.48 (0.21)	1.50 (0.37)	1.40 (0.37)	9.20 (1.46)	11.5 (0.38)	5.61 (0.37)
Nicardipine	6.80 (0.57)	4.00 (1)	2.52 (0.35)	1.93 (0.48)	3.00 (0.79)	5.00 (0.79)	9.68 (0.32)	7.80 (0.51)
Hydralazine	4.30 (0.17)	4.10 (1.03)	2.90 (0.40)	1.91 (0.47)	1.60 (0.42)	1.80 (0.29)	5.23 (0.17)	4.41 (0.29)

Relative values of ED_{30} are dipicted in parenthuses (nisoldipine = 1).

NR=normotensive rat, SHR=spontaneously hypertensive rat, DNR= DOCA-NaCl hypertensive rat, RHR=renal hypertensive rat.

In terms of blood pressure lowering effect, nisoldipine was about equipotent to nifedipine, nicardipine and hydralazine in SH rats; however, it was less potent than the other drugs in other hypertensive models and in normotensive rats. The positive chronotropic effects of nisoldipine were less remarkable than those of reference drugs except in SH rats.

In another study, single oral doses of nisoldipine (0.315, 1.0 and 3.15 mg/kg) produced a dose dependent reduction of systolic blood pressure in conscious female SH rats (Fig.1). Although the lowest dose (0.315 mg/kg) produced only a slight reduction in blood pressure (3% reduction from the base value), doses of 1.0 and 3.15 mg/kg reduced blood pressure 12 and 18%, respectively. The maximum effect, at all dose levels, was seen at 1 hr after drug administration and the blood pressure returned completely or nearly to pretreatment level by 6 hr postdose. When the above doses of nisoldipine were given orally to one-kidney renal hypertensive rats, a significant decrease (39%) in blood pressure was seen at 3.15 mg/kg and moderate (9%) and slight (3%) reductions were observed at 1 and 0.315 mg/kg, respectively (Fig.2).

In conscious normotensive rats, nisoldipine (0.3 mg/kg po) significantly reduced systemic vascular resistance (0.58 to 0.38 mm Hg/kg/min/ml) and mean arterial pressure (122 to 108 mm Hg), and increased heart rate (395 to 447 beats/min), stroke volume (0.57 to 0.72 ml/beat/kg) and cardiac index (225 to 326 ml/min/kg). Left ventricular end-diastolic pressure (LVEDP) was slightly decreased (9.6 to 8.8 mm Hg, $p<0.05$) but no significant change in left ventricular systolic pressure was seen.

The effect of chronic dietary administration of nisoldipine on the development of hypertension was studied in SH rats.

Fig. 3

Effect of Long-term Treatment (60 weeks) with Nisoldipine on Systemic Blood Pressure in SH Rats

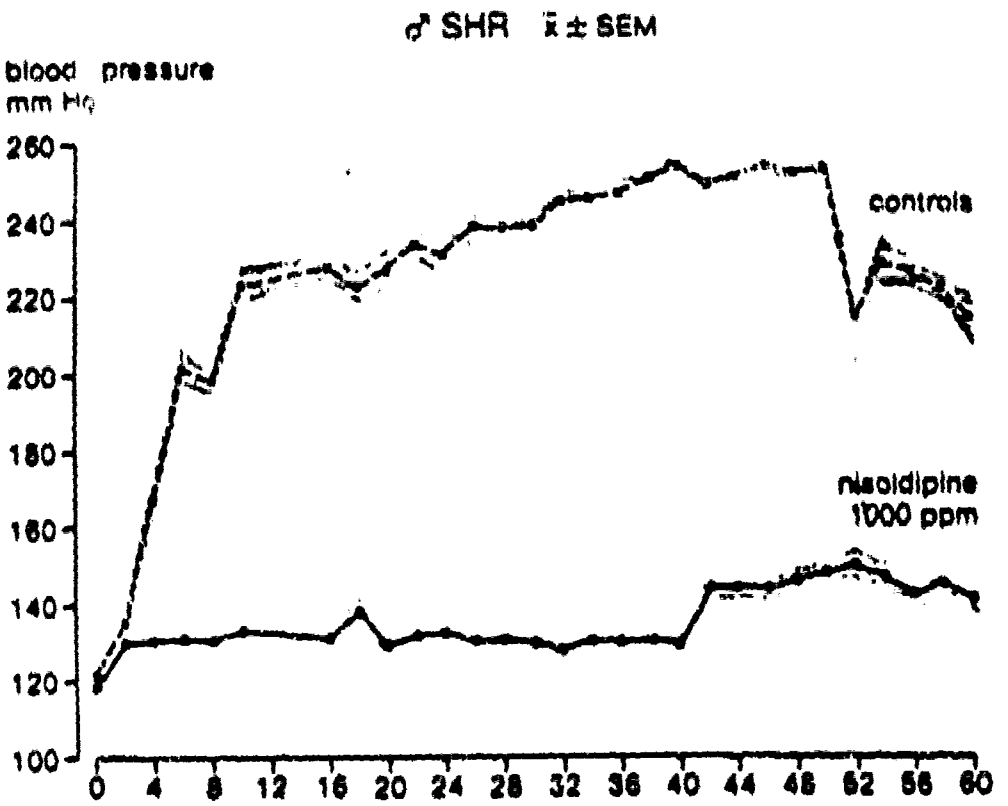


TABLE 6. *Preventive Experiment: The Effect of Long-term Treatment (60 weeks) with the Calcium Antagonist Nisoldipine on Systolic Blood Pressure, Plasma irANP, Relative Heart Weight, Body Weight, PRA, and Plasma Aldosterone Concentration in SHR and WKY*

Parameter measured after 60 weeks	SHR		WKY	
	Controls ($n = 7$)	Nisoldipine ($n = 10$)	Controls ($n = 8$)	Nisoldipine ($n = 8$)
SBP (mm Hg)	214 ± 7	141 ± 3‡	145 ± 3‡	137 ± 3
Plasma irANP (pg/ml)	470 ± 38	139 ± 35‡	88 ± 23‡	107 ± 39
Relative heart weight (mg/100 g body wt)	376 ± 29	313 ± 4*	277 ± 16†	284 ± 16
Body wt (g)	388 ± 8	377 ± 9	380 ± 16	381 ± 20
PRA (ng ANG I/ml/hr)	2.9 ± 0.3	1.9 ± 0.4	3.3 ± 0.4	2.4 ± 0.7
PAC (pg/ml)	332 ± 36	242 ± 16†	369 ± 30	454 ± 28

Values are means ± SEM. SBP = systolic blood pressure; irANP = immunoreactive ANP; ANG I = angiotensin I; PAC = plasma aldosterone concentration.
*$p < 0.025$; †$p < 0.01$, ‡$p < 0.001$, compared with values in untreated SHR.

Administration of nisoldipine to male SH rats (8 weeks old at the initiation of treatment) at 1000 ppm (50-100 mg/kg/day in diet) for 60 weeks prevented the development of hypertension during the treatment period [mean systolic blood pressure of 141 mm Hg in the treated group vs 214 mm Hg in the control group at the end of the study (Fig.3)]. The final blood pressure of treated SH rats was nearly the same as that of treated or untreated normotensive Wistar Kyoto (WKY) rats (Table 6). (However, it is noted that blood pressure in treated SH rats rapidly increased to the untreated control level when the treatment was stopped.) On the other hand, in untreated concurrent control SH rats, blood pressure increased progressively till week 48, to a maximum of 250 mm Hg, and declined thereafter to 214 mm Hg at the termination of the study. In WKY rats, no significant treatment related blood pressure changes were seen in the nisoldipine treated group compared to the control group. Furthermore, the results of the above study also showed that long term treatment with nisoldipine significantly decreased plasma atrial natriuretic peptide-like immunoreactivity (ANP-IR) and plasma aldosterone concentrations (PAC) and attenuated cardiac hypertrophy in SH rats (Table 6).

It was also shown that a 10 week dietary treatment with nisoldipine (50-100 mg/kg) in old SH rats (69 weeks old) with end-stage hypertensive disease caused significant reductions in systolic blood pressure [from 210 to 169 mm Hg (20%)], ANP-IR (20%) and relative heart weight (20%).

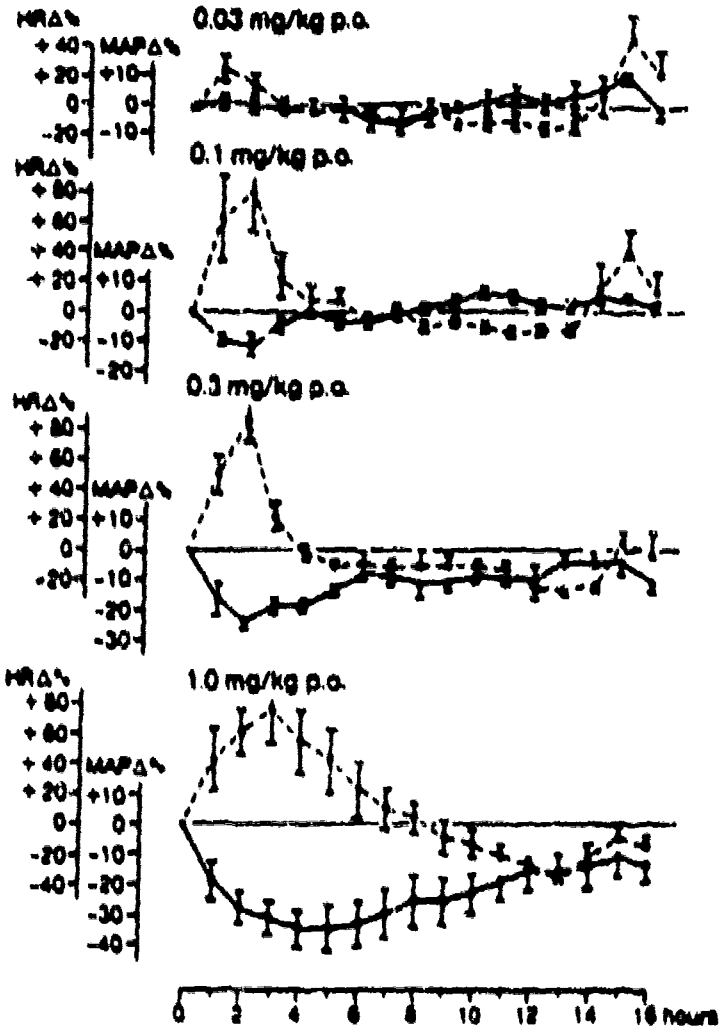
In inbred Dahl salt sensitive (DS) rats on a high salt diet (8% NaCl), dietary administration of nisoldipine at 1000 ppm (100 mg/kg/day) for 5 weeks produced significant reductions in systolic blood pressure (168 mm Hg in nisoldipine treated group vs 236 mm Hg in control DS rats) and plasma ANP-IR and renin activities. No nisoldipine treatment related effects were seen in Dahl salt resistant rats. Although treatment with the arteriolar vasodilator minoxidil (10 mg/kg, in drinking water) caused reduction of blood pressure in DS rats, the plasma ANP-IR levels and heart weights were significantly increased in treated rats compared to control DS rats.

In diabetic SH rats (streptozotocin induced), nisoldipine (9 mg/kg po for 10 weeks) significantly reduced blood pressure and inhibited the progress of renal lesions. No nisoldipine treatment related effects were seen on blood glucose, body weight gain, heart rate and heart and kidney weights.

Nisoldipine (0.3-0.6 mg/kg in food for 20 weeks) prevented the development of hypertension in rats subjected to 5/6 th nephrectomy (147 mm Hg in treated vs 237 mm Hg in untreated group).

Nisoldipine infusion (0.7 µg/min) decreased mean arterial pressure in anesthetized normotensive rats from 107 to 76 mm Hg; and in conscious rats, a bolus administration of nisoldipine (100 µg) caused a reduction of blood pressure from 1 2 to 76 mm Hg.

Figure 4



Effect of nisoldipine on mean arterial blood pressure (MAP; ●——●) and heart rate (HR; ○– – –○) of conscious, unrestrained renal hypertensive dogs.

Fig. 5

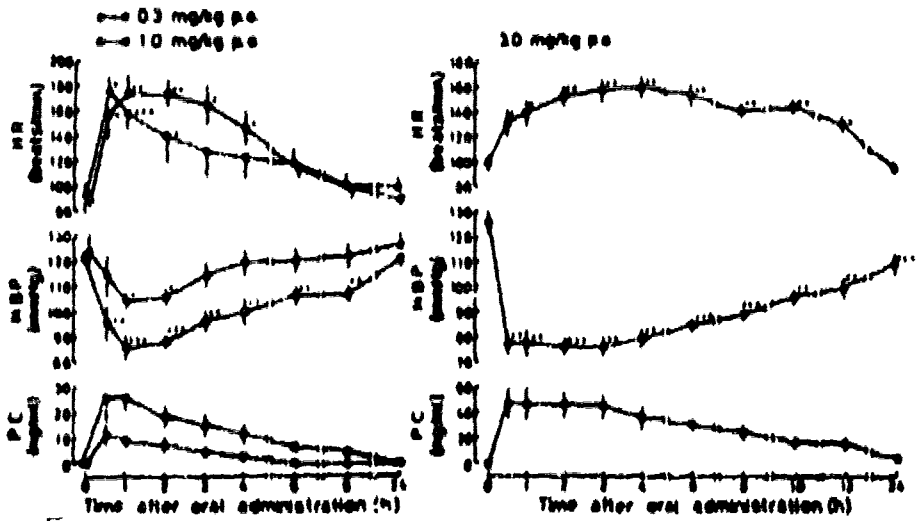


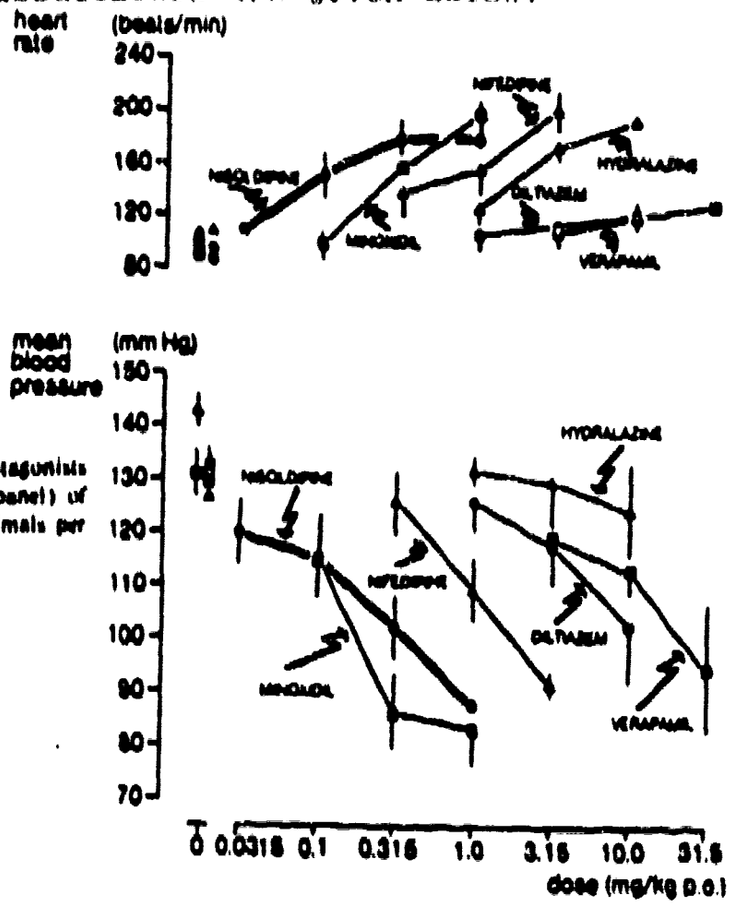
Fig. 5: Time course of the effects of single oral administration of nisoldipine on mean blood pressure (MBP), heart rate (HR) and plasma concentration (PC) in conscious renal hypertensive dogs. Values are expressed as the mean ± S.E.M. from 4 to 6 dogs. Asterisks indicate significant differences from the pre-drug values recorded at the zero time: *P < 0.05, **P < 0.01 and ***P < 0.001.

b. Dogs

The effects of oral nisoldipine on blood pressure and heart rate were studied in conscious, unrestrained, renal hypertensive (unilateral renal artery stenosis) beagle dogs using a radio-telemetric method, and compared with effects of other calcium antagonists (nifedipine, diltiazem and verapamil) and vasodilators (hydralazine and minoxidil). Single oral doses of nisoldipine (0.03 1.0 mg/kg) produced a dose-dependent decrease in mean arterial blood pressure in renal hypertensive dogs (Fig.4). At 0.3 mg/kg, a marked reduction in blood pressure (24%) was produced within 2 hr after drug administration and the hypotensive action lasted for 12 hours. A reflex tachycardia, lasting for about 3 hr, occurred at the above dose level. A more pronounced hypotensive effect was seen at 1 mg/kg. Nifedipine produced about the same degree of hypotension as that produced by 0.3 mg/kg po of nisoldipine at a 10 fold higher dose level (3.15 mg/kg po). The hypotensive effect and the reflex tachycardia lasted for 6 hr. Diltiazem and verapamil produced comparable antihypertensive effects at higher dose levels with slight to moderate increase in heart rates. [At the highest tested dose level of verapamil (31.5 mg/kg po), 3/5 dogs showed marked bradycardia.] The antihypertensive effect of minoxidil was more pronounced (34% blood pressure reduction at 0.3 mg/kg po) than that of hydralazine (about 15% reduction at 10 mg/kg po), and persistent reflex tachycardia was seen for the entire period of blood pressure reduction in both cases.

The ED20 (mg/kg) values (the dose that causes a 20% reduction in mean blood pressure) and the dose response curves for nisoldipine and other calcium antagonists and vasodilators are given below.

	ED20 (mg/kg)
nisoldipine	0.14
nifedipine	1.68
diltiazem	6.21
verapamil	8.39
minoxidil	0.14
hydralazine	>10.00



Dose response curves for the influence of nisoldipine and some other calcium antagonists and vasodilators on heart rate (upper panel) and mean blood pressure (lower panel) of conscious, unrestrained renal hypertensive dogs. Given are means ± S.E. of 4-6 animals per dose. Pre drug levels of heart rate and blood pressure are indicated by 0.

The above ED20 data indicate that nisoldipine and minoxidil are more potent antihypertensive agents in dogs than the other drugs studied. However, the dose response curves show that the antihypertensive effect of minoxidil is markedly more steep than that of nisoldipine. While diltiazem, verapamil and hydralazine are shown to be much less potent than nisoldipine and minoxidil in reducing blood pressure in renal hypertensive dogs, the antihypertensive action of nifedipine is rated as intermediate between nisoldipine or minoxidil and the other reference drugs studied.

Nisoldipine (31-315 µg/kg po) decreased MAP and TPR, and increased HR in anesthetized normotensive dogs. One hour after 100 µg/kg nisoldipine, MAP and TPR were decreased 20 and 45%, respectively, HR increased 76%, and LVEDP was unchanged. In another study in anesthetized dogs, nisoldipine (0.3 µg/kg iv) decreased TPR by 20% and increased stroke volume 28% without decreasing MAP, however, a dose of 30 µg/kg iv decreased MAP by 14% and TPR by 66%, increased HR 110% and stroke volume by 38% without changing EDP.

The antihypertensive effects and the pharmacokinetics of nisoldipine were compared with those of nifedipine, nimodipine, nicardipine and hydralazine in conscious, renal hypertensive (one-clip, two-kidney type hypertension of Goldblatt et al) male mongrel dogs. Single oral doses of nisoldipine (0.3, 1.0 and 3.0 mg/kg with Cmax values of 13, 33 and 60 ng/ml, respectively, or AUC values of 36, 132 and 523 ng/ml/hr, respectively) produced dose-dependent reductions in mean arterial blood pressure, which were significantly different from pre-drug values at 30 min (1 and 3 mg/kg), with maximum effect seen at about an hour after dosing (Fig.5). At 1.0 and 3.0 mg/kg dose levels of nisoldipine, mean blood pressure reductions of 36 and 50 mm Hg, respectively, were observed. Significant antihypertensive activity lasted up to 24 hr after the 3.0 mg/kg dose. Although not dose dependent, increased heart rate was seen at all dose levels and remained significantly higher than pre-drug levels for 4 (1 mg/kg) to 12 hr (3.0 mg/kg) after dosing. Peak plasma concentrations of nisoldipine were seen 0.5 hr after oral administration and the antihypertensive activity significantly correlated with plasma concentrations of the drug ($r=0.727$, $p<0.001$). Like nisoldipine, other calcium antagonists (nifedipine, nimodipine or nicardipine) also dose-dependently lowered mean blood pressure, attaining peak effects at 1-2 hr after dosing. Hydralazine had a slow onset and its effect peaked 3 hr postdose. In the above study, it was found that nisoldipine was 5-6 times more potent than nifedipine, nicardipine and nimodipine and its antihypertensive effect lasted 3-6 times longer.

In another study in conscious renal hypertensive beagle dogs, single doses of nisoldipine (0.03-1.0 mg/kg po) produced the following dose-dependent decreases in mean arterial blood pressure (MAP) and increases in heart rate (HR).

Dose (mg/kg po)	MAP (% decrease)	HR (% increase)
0.03	n.s.	30
0.1	20	45
0.3	30	47
1.0	45	82

In conscious normotensive coronary artery occluded mongrel dogs, infusion of nisoldipine (1 and 3 µg/kg/min) for 15 min produced the following changes in blood pressure and heart rate.

	Control	(1 µg/kg/min)	(3 µg/kg/min)
SBP, mmHg	**133±3**	**126±4**	**119±6***
DBP, mmHg	**92±2**	**74±3***	**59±5***
MAP, mmHg	**105±2**	**91±3***	**79±4***
HR, bpm	**103±10**	**146±9***	**163±12***

***Significantly (p<0.05) different from control.**

In conscious, nonsedated, chronically instrumented mongrel dogs, iv administration of nisoldipine (10 100 µg/kg) decreased MAP by 11 and 29 mm Hg at 30 and 100 µg/kg dose levels, respectively.

In anesthetized mongrel dogs with cardiac tamponade, nisoldipine (2 µg/kg/min, iv for 15 min) caused mean blood pressure to fall from 120 to 71 mm Hg and reduced heart rate from 212 to 167 bpm.

c. Cats, Pigs and Sheep

In conscious cats, oral nisoldipine at 0.1 and 0.5 mg/kg dose levels produced 21 and 18% reductions in mean blood pressure and 23 and 63% increase in heart rates, respectively.

Nisoldipine (10, 30 and 60 µg iv) dose-dependently reduced MAP and total peripheral resistance (TPR) and increased cardiac output in anesthetized cats.

In anesthetized Yorkshire pigs, infusions of nisoldipine (0.25, 0.5 and 1.0 µg/kg/min for 10 min) produced dose-dependent decreases in arterial blood pressure (30%), systemic vascular resistance (30%) and left ventricular filling pressure (15%), and increases in heart rate (25%) and LV dP/dt max (20%). Cardiac output was not significantly affected.

Nisoldipine (0.6 mg/kg/day iv for 4 days) prevented the development or reversed established hypertension induced by ACTH in sheep.

d. Antihypertensive Activity of Enantiomers

The antihypertensive activities of orally administered stereoisomers of nisoldipine were compared with the antihypertensive activity of the racemic compound in conscious SH rats and renal hypertensive dogs. In SH rats, (+)nisoldipine ED20=2.1 mg/kg) was only slightly more potent (1.4 times) than the racemic compound but was about 20 times more potent than the (-)nisoldipine . No significant difference in antihypertensive activity was seen between (+)nisoldipine and the racemic compound in dogs.

In a study in anesthetized normotensive dogs, 10 and 30 µg/kg po (+)nisoldipine decreased MAP by 10 and 38% respectively, while (-)nisoldipine had no significant effects at doses up to 300 µg/kg.

e. Antihypertensive Activity of Metabolites

(Unless otherwise noted, the following studies were done in anesthetized normotensive dogs.)

, a major metabolite of nisoldipine, showed weak peripheral vasodilator activity at iv doses of 1 mg/kg and above. No significant changes in blood pressure were seen at dose levels (0.3 to 3 mg/kg iv) tested in this study.

another metabolite, had relatively minor peripheral vasodilator activity at doses of 0.3 and 1.0 mg/kg iv in dogs. Slight blood pressure reduction was noted following the 1 mg/kg dose.

Metabolites had no hemodynamic effects (total peripheral resistance, cardiac output and LV dP/dt) at 1 mg/kg iv and had only minor peripheral vasodilator effect at 3.0 mg/kg.

, a dihydropyridine metabolite of nisoldipine, caused a dose-dependent drop in blood pressure at doses of 10 µg/kg iv and above, the effect lasting about 60 min at 30 µg/kg. Tachycardia and increased cardiac output were seen at the above dose level. (iv administration) was found to be 1/3rd to 1/10th as potent as nisoldipine in dogs.

In conscious renal hypertensive dogs, oral doses of (1 mg/kg) had a much weaker and shorter duration of action than the parent compound.

2. Effects on Coronary Blood Flow and Coronary Vessels

In-vitro Studies

a. Rat

Nisoldipine (3 nM) doubled coronary blood flow, compared to control, during reperfusion in isolated rat hearts subjected to 15 min ischemia followed by 15 min reperfusion. This increased blood flow was associated with decreased purine efflux from ischemic hearts. Nisoldipine (20 nM) partially prevented the decrease in ATP levels in hearts subjected to 30 min of ischemia. At 30 nM, nisoldipine caused negative inotropy.

In isolated rat heart, addition of nisoldipine (1 nM) 10 min before and during ischemia (33 min) and also during reperfusion (30 min) increased coronary blood flow (31% higher than control), recovery of contractile functions and tissue ATP levels. Another study in isolated rat heart showed that nisoldipine administration (1 nM) 10 min before zero-flow global ischemia did not depress preischemic contractile function and did not delay or reduce the development of ischemic contracture or the breakdown of high energy phosphate compounds during ischemia. However, on reperfusion, nisoldipine treated hearts showed enhanced reflow to the subendocardium, a dramatic improvement of contractile function and increased endocardial energy levels.

Nisoldipine (1 nM) prevented the increased sensitivity of ischemic and reperfused rat hearts to the vasoconstrictor effects of endothelin-1 (released by endothelial cells).

Low concentrations (0.5-5 nM) of nisoldipine prevented the reduction in endothelium dependent vasodilator responses that follow ischemia and reperfusion or after oxidative stress [induced by tert-butyl hydroperoxide (tBHP)] in isolated perfused rat hearts. At higher concentrations (50 nM), the drug reduced the incidence of arrhythmias caused by free radicals generated by xanthine-xanthine oxidase in isolated rat hearts, and improved the recovery of myocardial function to 90% of control hearts in tBHP-treated rat hearts.

b. Pig

Nisoldipine inhibited the anoxia-potentiated K^+-, histamine-, and acetylcholine-induced contractions of porcine coronary arteries with IC50 values of 9 to 20 nM.

In another study, nisoldipine was found to be twice as potent as nifedipine (IC50 of 5.9 and 12 nM, respectively) in inhibiting K^+-induced contraction of isolated pig coronary artery, but in contrast, it was only 1/3 as potent as nifedipine (IC50 of 13 and 3.6 nm) in inhibiting K^+-induced contraction of femoral artery.

Nisoldipine and nifedipine inhibited serotonin-induced contraction of isolated pig coronary artery with IC50 values of 22 and 83 nM, respectively. However, the IC20 values for nisoldipine and nifedipine for inhibiting serotonin-induced porcine femoral artery contraction were 1.6 and 0.53 µM, respectively, indicating that these drugs preferentially inhibited the serotonin-induced contractions of the coronary artery.

c. Rabbit

Nisoldipine (200 nM) reduced the constriction of endothelium-deprived large coronary arteries and the decrease in coronary flow induced by histamine in isolated working rabbit hearts. Nisoldipine also abolished or reduced the decrease in cardiac output, left ventricular end-systolic pressure, dP/dt and myocardial oxygen consumption.

In isolated rabbit heart, subjected to 40 min of global zero-flow ischemia and 2 hr of reperfusion, addition of 5 nM nisoldipine prior to ischemia prevented the increased vascular resistance during reflow and the increase in vascular volume, but had little or no effect on the increased vascular leakage of albumin or the rate of recovery of left ventricular function.

Nisoldipine inhibited potassium-induced contractions of rabbit coronary artery with an IC50 of <1 pM.

d. Human

Nisoldipine inhibited the spontaneous contraction of isolated human coronary arteries with an IC50 of 0.04 nM, and K^+- and serotonin-induced contractions were inhibited with IC50 values of 0.2 and 0.3 nM, respectively. For human ventricle and atria, the IC50 values for the inhibition of isoprenaline-induced contractions were 300 and 2.1 nM, respectively.

In-vivo Studies

a. Rat

In studies in conscious rats, using radioactive microspheres, oral nisoldipine (0.3 mg/kg) produced a pronounced increase in coronary blood flow as well as decreased coronary vascular resistance, and lesser increases in blood flow to gut and renal circulatory beds. Coronary and skeletal muscle blood flow was primarily increased after iv administration.

Intravenous administration of nisoldipine (1.6 µg/kg/min for 20 min) in conscious rats at rest significantly reduced vascular resistance in skeletal muscle and renal and coronary beds, which was not further reduced by nisoldipine during treadmill exercise.

b. Rabbit

In open-chest rabbits, injection of nisoldipine (1-100 nM through a left atrial cannula) significantly decreased left ventricular systolic pressure (1 nM), dilated large epicardial arteries and reduced large coronary artery vascular resistance (10 nM) and reduced maximal rate of left ventricular pressure rise (LV dP/dt max) and mean aortic pressure (100 nM). No significant change was seen in the left ventricular end-diastolic pressure.

c. Dogs, anesthetized

In anesthetized dogs, nisoldipine (10 to 315 µg/kg sublingual) increased coronary blood flow dose-dependently. At the highest dose, maximal values were more than 200% of the control values. A dose-dependent decrease in TPR and EDP and an increase in left-ventricular dP/dt max were seen.

Intragastric administration (100 µg/kg) of both nisoldipine and nifedipine produced increases in coronary sinus oxygen content by 151 and 119%, respectively; the duration of the effect of nisoldipine was considerably longer than that of the same dose of nifedipine (half times of 420 vs 260 min). Both drugs lowered diastolic blood pressure by 42%.

Intravenous administration of nisoldipine at 0.3, 1.0 and 3.0 µg/kg dose levels increased coronary oxygen saturation 19, 77 and 160%, respectively.

Both nisoldipine and nifedipine (10 µg/kg iv) decreased coronary vascular resistance more than renal vascular resistance or TPR. The duration of the increase in coronary, aortic and vertebral blood flow was about 3 times longer after nisoldipine than after nifedipine.

Nisoldipine (5 µg/kg iv) increased coronary sinus flow from 62 ml/min to a maximum of 131 ml/min (111% increase) and increased coronary sinus oxygen content by 50%.

Intracoronary injection of nisoldipine (0.2 to 0.8 µg/kg) decreased coronary vascular resistance by 46 to 62% and increased myocardial blood flow by 109 to 141% without changing myocardial contractility or peripheral circulatory parameters. Increasing the dose to 1.6 µg/kg did not cause a further increase in myocardial blood flow or a further decrease in coronary vascular resistance.

In anesthetized dogs, nisoldipine (0.1 µg/kg iv) was about 10 times more potent than nifedipine in inhibiting coronary vasoconstriction induced by neuropeptide-Y, a possible endogenous mediator of coronary spasm.

d. Dogs with Myocardial Ischemia

In conscious dogs with Ameroid constrictor chronically implanted on the proximal left anterior descending coronary artery (for gradual occlusion and subsequent stimulation of coronary collateral development), nisoldipine (1 and 3 µg/kg/min iv for 15 min) increased perfusion distal to coronary artery occlusion. The drug significantly increased mean coronary flow velocity and decreased mean coronary vascular resistance in these dogs. Despite a reduction in arterial pressure, nisoldipine preserved renal cortical, intestinal and skeletal muscle blood flow while increasing flow within liver and cerebral cortex.

The effects of equihypotensive doses of nisoldipine (1 and 3 µg/kg iv), verapamil (0.1 and 0.3 mg/kg iv) and diltiazem (15 and 30 µg/kg/min iv for 15 min) on cardiac hemodynamics and regional myocardial perfusion were studied in anesthetized dogs with acute coronary artery occlusion. Each drug, at the above dose levels, reduced arterial blood pressure and increased total coronary blood flow. Regional myocardial perfusion in non-ischemic areas was increased significantly in a dose-related manner by each agent. In ischemic areas, nisoldipine and verapamil increased total coronary collateral perfusion. Following nisoldipine, the increase in collateral flow was equally distributed to both subendocardium and subepicardium, but after verapamil, it was distributed primarily to subendocardium. With diltiazem there was no change in total perfusion within the ischemic zone. Verapamil (0.3 mg/ kg) and diltiazem (30 µg/kg/min) decreased heart rate and increased left ventricular EDP, but nisoldipine (1 and 3 µg/kg) had no effects on these parameters. Verapamil, but not nisoldipine and diltiazem, produced negative inotropy.

In dogs with acute myocardial infarction (produced by clamping the left anterior descending coronary artery for 6 hr), nisoldipine (5 µg/kg iv 15 min, 2 and 4 hr after occlusion) reduced myocardial infarct size by 31.4% ($p<0.05$) compared to controls. Although nisoldipine produced a moderate decrease in MAP, it had no effect on heart rate, mean left atrial pressure, left ventricular EDP, coronary blood flow, cardiac output, myocardial contractility or myocardial intracellular calcium concentrations. Nisoldipine showed no negative inotropic or negative chronotropic effects.

In anesthetized dogs subjected to 15 min of occlusion of left circumflex coronary artery and subsequent 4 hr reperfusion, nisoldipine (5 µg/kg iv) improved functional recovery of reperfused myocardium when given before, but not after, ischemia. This improved functional recovery of reperfused myocardium in dogs pretreated with nisoldipine was not attributable to increased regional myocardial blood flow during ischemia or reperfusion, but to the attenuation of myocardial calcium overload during the first few minutes of ischemia.

e. Baboons

The effects of nisoldipine on left ventricular function, regional myocardial blood flow, the incidence of arrhythmias and the extent of necrosis were studied in chronically instrumented, conscious baboons with coronary artery occlusion for 3 hours and reperfusion for 1 week. Nisoldipine administration (0.1 µg/kg/min iv) from 1 hr after coronary occlusion through the first 3 hr of coronary reperfusion increased regional blood flow significantly in the endocardium and epicardium of nonischemic and ischemic zones. During reperfusion, the incidence of arrhythmias in the nisoldipine-treated group were significantly lower than in the vehicle group. However, mean arterial pressure, left ventricular systolic pressure, heart rate, left ventricular dP/dt and the size of myocardial areas at risk and the infarct size were unaffected by the nisoldipine treatment.

f. Pigs

In anesthetized pigs, nisoldipine (0.25, 0.5 and 1.0 µg/kg/min, three 10 min infusions) dose dependently increased transmural myocardial blood flow, especially in epicardial layers. Endocardial blood flow was increased despite lowered perfusion pressure, but the endo/epi blood flow ratio decreased from 1.16 to 0.7.

The effects of nisoldipine on coronary blood flow and creatine phosphate levels were studied in pigs in which left anterior descending coronary artery blood flow was reduced to 20% of baseline for 60 min and reperfused for 2 hr. Nisoldipine (0.1 µg/kg/min), started after 30 min of ischemia and continued throughout reperfusion, increased coronary blood flow and creatine phosphate levels. Post-ischemic myocardial work was significantly increased in nisoldipine treated animals, indicating that nisoldipine attenuated the "no-reflow" phenomenon. Moreover, nisoldipine increased the sarcoplasmic reticular calcium uptake independent of ischemia, thus reducing the calcium overload.

Nisoldipine treatment (30 µg/kg po, every 6 hr for 1 month) in pigs undergoing gradual occlusion of the left circumflex artery (using Ameroid constrictors) significantly reduced infarct size ($p<0.01$) and increased endocardial and transmural blood flow ($p<0.01$) compared to the control group. Epicardial flow and the ratio of subendocardial to subepicardial blood flow were not significantly higher in treated groups. No differences were observed in heart rate and aortic pressure between control and treated pigs. Results of this study indicated that chronic nisoldipine treatment enhanced the endocardial collateral circulation in this animal model.

3. **Mechanism of Action**

Nisoldipine is a dihydropyridine calcium channel blocker which binds with very high affinity to L-type calcium channels. By blocking calcium entry into vascular smooth muscle cells, it inhibits muscular contractions, thereby causing vasodilation of peripheral and coronary vasculatures.

The receptor binding characteristics of nisoldipine and its optically pure enantiomers, BAY R 1224 (+ isomer) and (- isomer), were studied in rat cerebral cortical membranes using labelled nitrendipine. The concentration that causes 50% inhibition of ^{3}H nitrendipine binding (IC50), the inhibition constant (Ki) and the Hill Coefficient (nh) for the above compounds and the reference drugs are given below.

Substance	IC_{50} [nM]	K_i [nM]	nH	N
(Nisoldipine)	1.2 ± 0.1	0.769 ±0.064	1.0 ± 0.05	34
((+) enantiomer)	1.1 ± 0.08	0.705 ±0.051	1.0 ± 0.09	34
((-) enantiomer)	109 ± 0.15	70 ± 10	1.0 ± 0.01	34
Nifedipine (Reference)	2.7	1.7	1.15	
(Reference)	16	10	0.76	
Verapamil (Reference)	173	110	0.6	

Nisoldipine showed competitive displacement of ^{3}H nitrendipine with a Ki of 0.769 nM. The (+) enantiomer has a comparable Ki of 0.705 nM, while the affinity of the (-) enantiomer was reduced 100 fold to 70 nM. The Hill Coefficient for all three compounds was same, representing a linear Scatchard Plot and a homogenous population of binding sites. The above data indicate that the biologically active component of nisoldipine seems to be the (+) enantiomer.

Further studies in guinea pig ileal smooth muscle have confirmed that the receptor binding was of high affinity, saturable, reversible and stereoselective with high structural specificity, and correlated well with the pharmacologic activities. Binding studies in partially purified rat brain membranes using ^{3}H

nimodipine showed Ki values of 0.24 and 7 nM for nisoldipine and nifedipine, respectively. Studies in rat and rabbit ventricular membranes showed that nisoldipine differs from nifedipine in its high affinity binding (about 20 times greater), slow dissociation rate and large partition coefficient. The binding characteristics of both drugs are given below.

Comparison of Binding Characteristics of Nisoldipine and Nifedipine

	(+)Nisoldipine	Nifedipine
K_d, nM	0.04	0.81
Dissociation		
$t_{1/2}$, min	12	1.2
B_{max}, pmol/mg	0.69	0.17
Association Rate ($\times 10^6 M^{-1}min^{-1}$)	6.7	3.1
Entropy of binding	large positive	negative
Partition coefficient into biological membrane	6,000–27,000	2,900

The above biochemical and biophysical differences can be expected to result in a longer duration of action for nisoldipine.

Studies in isolated rat aorta revealed that there is good agreement among binding affinity and the IC50 values both for inhibiting ^{45}Ca influx and aortic contraction. The IC50 values for the inhibition of rabbit aortic ring contraction (potassium stimulated) were 1.8, 0.15 and 81 nm for racemic nisoldipine, (+) isomer and (-) isomer, respectively, indicating that these findings were in reasonable agreement with the results of the ligand binding studies.

Voltage-clamp studies in isolated calf Purkinje fibers have shown that nisoldipine binding is one thousand times stronger to inactivated channels (Kd=1 nm) than to resting channels (Kd=1.3 μM), indicating that nisoldipine blocks calcium channel current in a voltage dependent manner. Nisoldipine (10 μM) completely blocked the slow inward current and contractile activation in the above tissue. Moreover, nisoldipine reduced the transient outward, but not the delayed rectifier K^+ current, in calf cardiac Purkinje fibers.

Nisoldipine was a more potent inhibitor of BHT 920 (α2-adrenoceptor agonist)-induced contraction of isolated aortic rings when these rings were taken from stroke-prone SH rats than when they were taken from normotensive WKY rats (IC50=0.15 nM vs 7 nM).

Several studies have shown that the degree of nisoldipine inhibition of calcium channel currents (as well as contractions) increases with membrane depolarization. In patch-clamp studies in isolated smooth muscle cells from canine coronary artery, membrane depolarization from -65 mV to -30 mV increased the apparent affinity of nisoldipine binding about 9 fold (in the presence of 1 μM Bay K 8644, a calcium agonist). The calculated dissociation constant in this study was 0.07 nM, which was identical to the value obtained with radioligand binding

studies. In rabbit mesenteric artery, depolarization from -100 to -55 mV decreased the concentration of nisoldipine needed for 50% inhibition from 12 to 1.9 nM. Because of the very high affinity binding of nisoldipine to depolarized membranes, it is suggested that nisoldipine could preferentially bind to those depolarized arteries which increase total peripheral resistance of hypertension, and also to those producing coronary spasms.

B. Additional Cardiovascular Studies

1. Effects on Other Vascular Beds

Under conditions of controlled blood flow, nisoldipine infusion (1 µg/min) dilated the hindquarters vascular bed of anesthetized cats and inhibited vasoconstrictor responses to sympathetic nerve stimulation, norepinephrine, tyramine, methoxamine and BHT 933 (α2-adrenoceptor agonist).

The effects of nisoldipine on vascular resistance and vasoconstrictor responses were studied in the pulmonary vascular bed of anesthetized cats under conditions of controlled blood flow. Nisoldipine (1 µg/min) infused into the lobar artery caused only a small reduction in basal lobar resistance. It reduced pulmonary vasoconstrictor responses to methoxamine, BHT 933 and U46619 (thromboxane A2 mimetic).

2. Other Myocardial/Cardiovascular Effects

In isolated Langendorff-perfused rat hearts, nisoldipine (30 nm) prevented the ischemia-reperfusion-induced depletion of the cardiac stores of norepinephrine. In the above hearts, pretreatment with the drug (10 nm) 2 min before the onset of ischemia significantly improved cardiac output following global ischemia (20 min) and reperfusion (5 min). Pretreatment with nisoldipine (1 µM) for 5 min before ischemia prevented transcoronary macromolecular leakage after ischemia-reperfusion in isolated rat hearts.

In isovolumic coronary artery-perfused ferret hearts subjected to global ischemia for 3 min followed by 10 min reperfusion, nisoldipine (10 nm) significantly reduced the ischemia-induced rise in diastolic and systolic intracellular free ionized calcium (FIC, determined with the bioluminescent protein aequorin), and lessened the decrease in contractile function. Moreover, nisoldipine significantly accelerated the decline in FIC during reperfusion and improved recovery of contractility and relaxation.

Nisoldipine (5 µM) had no significant effect on dopamine-induced inhibition of nerve stimulated vasoconstriction of isolated perfused rabbit ear artery.

In anesthetized rats, nisoldipine (3 mg/kg p.o., 1-1.25 hr before acute coronary ligation) greatly reduced the duration of ventricular tachycardia and fibrillation occurring in the first 30 min postligation period. None of the treated animals died compared with a 40% mortality in controls.

Long-term dietary administration of nisoldipine (50-100 mg/kg for 22 weeks) prevented the rarefaction of myocardial capillarization in SH rats. Drug treated rats had lower arterial blood pressure and decreased left ventricular and septal weights compared to untreated rats.

In conscious chronically instrumented dogs, nisoldipine did not influence cardiac impulse formation or impulse conduction at 10 and 30 µg/kg iv, but at 100 µg/kg iv (strongly hypotensive dose), it produced reflex increase in the rate of atrioventricular conduction.

In dogs subjected to occlusion of the left anterior descending coronary artery for 1.5 hr followed by reperfusion, nisoldipine (3.5 µg/kg iv 10 min before the occlusion and again 10 min before reperfusion) suppressed the ischemia-induced increase in phospholipid breakdown as well as the increase in serum CPK activity. The drug also prevented ischemia-induced myocardial hemorrhage and premature ventricular contraction in a similar study.

Cumulative 10 min infusions of nisoldipine (0.05, 0.1, 0.25 and 0.5 µg/kg/min) in pigs with chronic left ventricular dysfunction (produced by the ligation of the left circumflex coronary artery 2-3 weeks before the study) improved ventricular function to the same extent as pimobendan, a phosphodiesterase inhibitor (2.5, 5, 12.5 and 25 µg/kg/min). Both drugs normalized cardiac output and exhibited similar cardiovascular effects (systemic vasodilation, reduction in left ventricular filling pressure, and increased heart rate) except for the significantly greater increase in left ventricular dP/dt max with pimobendan (85%) than with nisoldipine (45%). Thus, nisoldipine, despite of its lack of inotropic properties, improved ventricular function to about the same extent as pimobendan.

Infusion of nisoldipine (10 µg/kg over a 5 min period, 30 min before coronary artery occlusion) in open-chest pigs subjected to the occlusion of the left anterior coronary artery, completely prevented the increase in lipid peroxidation products associated with ischemia.

C. General Pharmacological Studies

1. Central Nervous System Effects

The analgesic activity of nisoldipine (25-500 mg/kg po) was assessed in female Wistar rats by the failure of the animal to withdraw its tail within 20 seconds after exposure to a focused heat ray. Nisoldipine had no analgesic activity at 25 mg/kg; however, at higher dose levels (100 mg/kg and above) 40-60% of animals showed evidence of analgesic activity.

To study the effects of nisoldipine on orientation motility, mice were placed in cages in the dark and their orientation motility was assessed at 5 min intervals for a total of 25 min after the light was turned on. Nisoldipine (25, 100 and 250 mg/kg po) had no significant effect on orientation motility in this test. However, in a separate study in which mice were kept initially in a dark chamber and then exposed to daylight after drug treatment, nisoldipine at 10 or 31.5 mg/kg po, but not at 3.15 mg/kg, reduced orientation motility by 20% in mice. The above dose levels had no effect on spontaneous motility.

The balancing ability of male mice to maintain their position on a round horizontal wood bar (diameter 8 mm) was tested at 60 min after oral treatment with nisoldipine (3.15, 10 or 31.5 mg/kg). The drug inhibited the balancing ability by 10% at 31.5 mg/kg. The ability of the mice to grasp a horizontal metal bar (diameter 3 mm) was not inhibited at the above dose levels.

There was no evidence of any muscle relaxant or sedative effects for nisoldipine in mice, as assessed by the measurement of the holding and climbing ability on a horizontal bar with at least one hind paw within 5 sec after they were suspended on the bar by their front paws, at 25, 100 and 250 mg/kg po dose levels.

The anticonvulsant activity of nisoldipine was determined by the ability of the drug to antagonize either electroshock- or pentylenetetrazole (PTZ)-induced tonic convulsions in mice. Nisoldipine showed no anticonvulsant activity when administered 30 min before electroshock at 25, 100 or 250 mg/kg po. In the PTZ test, the drug (25-250 mg/kg po, 30 min before PTZ administration) had a dose-dependent anticonvulsant effect with an estimated ED50 of 98 mg/kg.

In a subsequent study, nisoldipine (3.15, 10 or 31.5 mg/kg po), given 60 min before electroshock or PTZ administration, antagonized the electroshock-induced tonic convulsions in 20% of mice at 3.15 and 10 mg/kg and in 30% at 31.5 mg/kg. PTZ-induced convulsions were antagonized by nisoldipine at 10 (30%) and 31.5 (80%) mg/kg, but not at 3.15 mg/kg.

The depth of hexobarbital-induced anesthesia was not affected by nisoldipine at 3.15, 10 and 31.5 mg/kg po, but the duration of

anesthesia was prolonged at 31.5 mg/kg.

To evaluate the possible tranquilizing effect of nisoldipine on defensive behavior, fighting episodes were induced in mice by weak electric foot shocks of 0.2 msec duration at a frequency of 5 per min. Nisoldipine at 25 mg/kg po was ineffective, but a dose-dependent antagonism of fighting was observed at higher doses (50 to 400 mg/kg po) with an ED50 of 82 mg/kg.

The (-)enantiomer had no significant CNS effects in mice at oral doses of 3, 10 and 30 mg/kg. The (+)enantiomer at 10 and 30 mg/kg po impaired motor coordination of mice in the balance rod test and increased the threshold dose of PTZ required to produce convulsions. The no-effect dose was 3 mg/kg. No significant effects were seen in mice on traction ability, analgesic and anticonvulsive responses or depth of hexobarbital-induced anesthesia at 3-30 mg/kg po dose levels. In rats, administration of (10 and 30 mg/kg po) produced ptosis, salivation, sedation, hypothermia, prone position, reduced muscle tone and ataxia during walking.

2. Gastrointestinal Effects

Nisoldipine (3, 10 and 30 mg/kg po) significantly reduced the intestinal transit time in mice, as measured by the length of the intestine covered by charcoal which was given orally 40 min after nisoldipine administration, at all tested dose levels with an estimated ED50 of 17.19 mg/kg.

(-)Nisoldipine, at the above dose levels, had no effect on intestinal transit time in the rat, whereas (+)nisoldipine significantly reduced transit time dose-dependently.

Nisoldipine or its stereoisomers (10 or 30 mg/kg po) did not induce any gastric lesions in rats.

At 3, 10 and 30 mg/kg po, (-)nisoldipine had no effect on indomethacin-induced ulcers in rats, while (+)nisoldipine significantly reduced these lesions at all dose levels.

Nisoldipine (3 or 30 mg/kg intraduodenal) or (+) nisoldipine (3, 10 or 30 mg/kg id) had no significant effects on basal gastric acid secretion, whereas (-)nisoldipine significantly reduced basal gastric acid secretion at 30 mg/kg id.

Nisoldipine antagonized acetylcholine- and histamine-induced spasms of isolated guinea pig ileum at 1 mg/L and barium chloride-induced spasms at 3 mg/L.

3. Metabolic Effects

In fasted rats nisoldipine at 10 and 30 mg/kg po significantly and dose dependently elevated blood glucose at 1, 2 and 4 hr after drug administration, while the serum triglyceride

concentration was slightly reduced in a dose-dependent manner. At 3 mg/kg po, the drug had no significant effect on either blood glucose or triglycerides.

In fed rats nisoldipine elevated blood glucose and lowered serum triglyceride concentrations at 3, 10 and 30 mg/kg po.

4. Effects on Respiration

Nisoldipine (0.26 nm to 26 µM) had no effect on resting tone of the isolated guinea pig trachea. Histamine- and LTD4-induced contractions were significantly reduced by nisoldipine at 26 nM.

Nisoldipine (0.32, 1.0 and 3.2 µg/kg iv) had no significant effect on spontaneous respiration in anesthetized dogs.

The enantiomers of nisoldipine ((3, 10 and 30 mg/kg po) had no effect on airway resistance or lung compliance, and did not modify histamine-induced increases in lung resistance in anesthetized, spontaneously breathing guinea pigs.

5. Renal Effects

The diuretic activity of nisoldipine was tested in normotensive male Wistar rats loaded with 0.5% methylhydroxyethylcellulose (10 ml/kg). Nisoldipine at 1.0, 3.15, 10.0 and 31.5 mg/kg po produced no significant effects on urine volume or urinary excretion of Na^+ or K^+ over a 6 hr collection period. However, at 100 mg/kg, the drug reduced urinary volume and Na^+ and K^+ excretion. Urinary pH was not changed over the entire dose range.

In another study in liquid-loaded rats (with a solution containing 0.2% NaCl, 0.4% KCl and 0.1% tragacanth), nisoldipine (10, 30 and 100 mg/kg po) increased urinary volumes and excretion of Na^+ and K^+ at all dose levels, the effects being more pronounced at 10 mg/kg than at higher dose levels.

the (+)enantiomer of nisoldipine, had no significant effects on urine volume or electrolyte excretion in normotensive rats at 3 and 10 mg/kg po; however, at 30 mg/kg, it significantly reduced urine volume and the excretion of Na^+ and K^+.
the (-)enantiomer, had no significant effects on the above parameters.

In stroke-prone SH rats, administration of nisoldipine (0.315 to 10 mg/kg po) significantly increased urine volumes and excretion of Na^+ at 3.15 and 10 mg/kg. Although excretion of K^+ was increased after 10 mg/kg of nisoldipine, the increase failed to achieve statistical significance.

To elucidate the mechanism of diuretic effects of nisoldipine in SH rats, renal clearance and micropuncture studies were carried out in moderately saline-loaded animals. At dose levels of 0.1, 0.15 and 0.2 µg/kg/min iv for 15 min, the drug produced a

significant fall in blood pressure accompanied by increased sodium excretion and glomerular filtration rate (GFR). This natriuretic effect was attributed to the suppression of distal tubular sodium reabsorption. In another study in SH rats, nisoldipine (10 µg/kg/hr iv) produced diuretic and natriuretic effects without any changes in GFR. It was also shown that the diuretic and saluretic effects were considerably more pronounced in hypertensive rats than in normotensive Wistar-Kyota rats.

Nisoldipine (10 nm) completely reversed the norepinephrine-induced reduction in GFR in the isolated perfused rat kidney.

In the rat acute renal failure model (glycerol induced), nisoldipine treatment (10 mg/kg b.i.d for 2 days) increased urine volumes and significantly reduced glycerol-induced increases in serum creatinine and urea and renal tissue calcium levels.

In conscious dogs, nisoldipine at 0.1 mg/kg po had no significant effect on renal function (GFR and inulin and paraaminohippurate clearances). However, at 0.3 mg/kg the drug reduced all measured parameters of renal function, the maximum effects observed 20 min after drug administration.

The interaction of nisoldipine with angiotensin II (AII) or norepinephrine (NE) was studied in anesthetized mongrel dogs. Intrarenal infusion of nisoldipine (2, 10 or 50 ng/kg/min for 20 min) produced a dose-dependent increase in urine flow and urinary excretion of sodium, chloride and potassium, although no significant change in GFR was seen. Renal blood flow was significantly increased only at the highest dose level. AII and NE reduced renal blood flow and urine volumes. The decreased urine flow induced by AII, but not by NE, was completely blocked by nisoldipine, while the effect of AII on renal blood flow was only partially antagonized.

6. <u>Hematological Effects</u>

Collagen-induced platelet aggregation, coagulation time, thrombus elasticity and partial thromboplastin time were not affected by nisoldipine administration (10, 30 or 100 mg/kg po; blood sampling 90 min postdose) in rats. Nisoldipine (2.6 nm) had no effect on factor XIII activity in bovine plasma.

Neither (+) nor (-) enantiomer (3, 10 and 30 mg/kg po) had effects (60 min postdose) on hematological parameters in rats (hemoglobin, hematocrit, platelet count, fibrinogen levels, sedimentation rate, thrombin or thromboplastin time and collagen-induced platelet aggregation).

7. <u>Antiatherogenic Activity</u>

In rabbits fed a cholesterol (2.5%) supplemented diet, administration of nisoldipine (1 mg/kg/day for 7 weeks) significantly reduced the serum and aortic concentrations of cholesterol and

preserved endothelium-dependent relaxation of the isolated aortic rings.

8. Antiinflammatory Effects

Oral administration of nisoldipine (5 to 315 mg/kg, 1 hr before kaoline injection) showed antiinflammatory activity against edema (caused by intraplantar injection of kaolin into the hind paw) in rats at dose levels of 10 mg/kg and above (ED50=46 mg/kg po).

9. Effects on Histamine Release

Nisoldipine (0.26 to 260 µM) had no significant effect on histamine release from rat peritoneal mast cells in vitro and also did not modify antigen-induced histamine release from these cells.

D. Antidote Studies

In anesthetized rats, 100 µg/kg/min iv infusions of nisoldipine produced pronounced decreases in arterial blood pressure, heart rate, cardiac output and peripheral resistance, followed by death within about 55 min after the initiation of the infusion. EKG changes included sinus bradycardia, partial or complete AV block and shifting of the pacemaker to the AV node. Infusion of calcium gluconate (15 mg/kg/min), isoproterenol (20 µg/kg/min) or dopamine (100 µg/kg/min) simultaneously with nisoldipine prolonged survival time by more than 100%. Norepinephrine (20 µg/kg/min) had no significant effect.

In anesthetized dogs, calcium gluconate (100 mg/kg iv) reversed the hypotension and tachycardia produced by 10 µg/kg iv nisoldipine, but exacerbated the hypotension produced by 30 and 100 µg/kg.

E. General Pharmacological Studies of Metabolites

Nisoldipine was shown to be about 21 times and the (a dihydropyridine metabolite of nisoldipine) was twice as potent as diphenhydramine (reference compound) in inhibiting histamine-induced spasms of isolated guinea pig ileum. The other metabolites
had no significant effect on the above parameter.

had no significant CNS (rat and mouse), pulmonary (guinea pig), hematologic (rat), gastrointestinal (rat) and urinary (rat) effects. This metabolite at 0.26 to 26 µM did not induce histamine release from rat peritoneal mast cells in vitro; however, at the same concentration range, it significantly inhibited the antigen-induced histamine release from the cells.

BAY R 9425 inhibited LTD4-induced contraction of isolated guinea pig trachea at 260 nM, but not at 26 nM. This compound was about 10 times less potent than nisoldipine in inhibiting K^+-induced contractions of isolated pregnant (IC50=86 nM) or nonpregnant (IC50=138 nM) rat uterus. Other metabolites were effective only at very high concentrations (above 50 µM).

F. Interaction of Nisoldipine with Propranolol

Administration of nisoldipine (0.315 mg/kg po) in conscious unrestrained renal hypertensive dogs after ß-adrenoceptor blockade by propranolol (3.15 mg/kg po) increased the maximal reduction in systolic blood pressure from 15 to 23% and reduced the maximal reflex increase in heart rate from 109 to 50%.

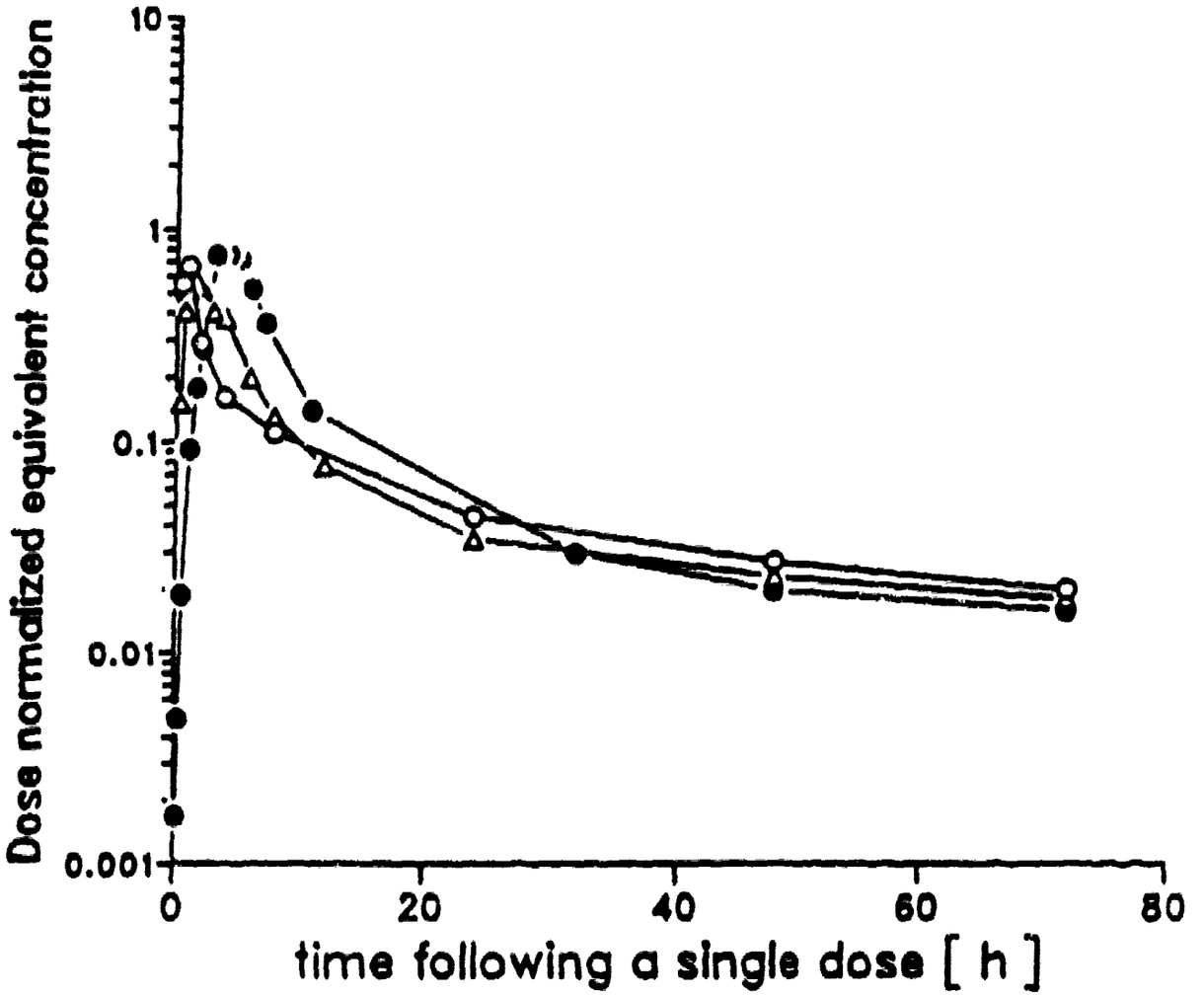
Propranolol (2 mg/kg iv) attenuated the reflex increase in heart rate produced by nisoldipine (2.5 to 25 µg/kg/min iv) in conscious instrumented dogs and prevented an increase in the heart rate-systolic pressure product (an index of myocardial oxygen consumption). Propranolol potentiated the hypotensive effect of nisoldipine (5-25 µg/kg/min), but did not further increase mean coronary blood flow.

Propranolol (4.4 mg/kg po) attenuated both the positive chronotropic and inotropic effects and the changes in systolic wall thickening caused by exercise in pigs with coronary artery stenosis. Nisoldipine (0.5 mg/kg po) alone did not modify cardiovascular effects of exercise, but it further improved wall function in the presence of propranolol.

SUMMARY OF PHARMACOKINETICS STUDIES (S. Stolzenberg)

1. Absorption and Excretion: Nisoldipine was measured by gas chromatography and electron capture. A summary of urinary, fecal and CO_2 recoveries following administration of a single dose of ^{14}C-labelled nisoldipine in four species, including man, is given on the page 30 of this review. In the rat, dog and pig, the primary route of excretion was the biliary-fecal route, whereas in the monkey and man, the urinary route predominated. Based on the ratio of the % of Dose Excreted in urine for i.v./p.o. routes multiplied by 100, nisoldipine was considered to be rapidly and almost completely absorbed in male rats (107%), dogs (10/%) and man (89.7%). For the pig, monkey (rhesus) and rat, where i.v. doses were not given, the figure under this column represents the amount excreted in the urine, which is regarded as "the lower limit of absorption".

2. Plasma Pharmacokinetics: The figure which follows illustrates the plasma concentrations vs time, following a single oral dose in rats, dogs and monkeys. The table on selected plasma pharmacokinetics on page 31 reflects these observations and includes results from i.v. administration in the same 3 species and in man. After oral administration, no distinct species differences were noted in the rat, dog or monkey; $CEQ_{max,norm}$ (normalized to 1 mg/kg dose, based on radioactivity equivalence of parent compound) ranged between 0.49 - 0.79 $kg*l^{-1}$, but was 4.7 to 7.5 times higher in man (3.7 $kg*l^{-1}$). AUC_{norm} for total radioactivity was also similar in the 3 species, but was higher in man by a factor of 6 to 10. $C_{max,norm}$ and AUC_{norm} for unchanged nisoldipine following oral administration were very low in all four species, including man, which was attributed to an extensive first pass effect, known for this compound. In humans given immediate release tablets of 2.5, 5, 10 and 20 mg, or core coated tablets of 10, 20, 40 and 60 mg, dose proportionality was observed for plasma nisoldipine C_{max} and AUC_{0-24}. At steady state (8th day of dosing) in humans, both AUC_{0-24} and C_{max} showed a "dose dependent and broadly dose proportional increase" at 30, 60, 90 and 120 mg. Correspondingly, both systolic and diastolic blood pressures "showed a general dose related decrease from baseline at steady state". Despite the high rates of absorption, bioavailabilities (F) of parent compound after oral doses were correspondingly low; 2.7, 11.7 and 8.4% in the rat, dog and man, respectively. The core coated preparation had an F value of 5.5% in man. In the dog, it was shown that both the gut wall and the liver contributed to the first pass effect and resulting low F values for the parent compound. The plasma half-life of unchanged drug was essentially similar in dog, monkey and man (2.3 to 4 h). The shorter $t_{1/2}$ of unchanged compound listed in the table for rats following oral or i.v. dosing does not represent a true terminal half-life because plasma levels were measured for only 2 hours post-dosing. In rats, the pharmacologically more potent (+)- enantiomer had a five fold higher bioavailability than the (-)- enantiomer.



Dose-normalized equivalent concentrations of total radioactivity after single oral administration of [^{14}C]nisoldipine to male rats (n = 5), female dogs (n = 3) and female monkey (n = 1) (mean of each).

o = 5 mg·kg^{-1}, rat

Δ = 5 mg·kg^{-1}, dog

● = 10 mg·kg^{-1}, monkey

ABSORPTION/EXCRETION OF NISOLDIPINE IN ANIMAL SPECIES AND MAN FOLLOWING ADMINISTRATION OF [^{14}C]NISOLDIPINE.

Species	Route	Dose (mg/kg)	% of Dose[1] Excreted in:			% absorbed (minimum)
			urine	feces	CO_2	
Rat (M)	p.o.	5.0	30.8 (2.4)[a]	75.0 (0.7)[a]	0.5[b]	107.0
	i.v.	1.0	28.7 (3.5)[a]	71.8 (6.5)[a]	0.6[b]	-----
Rat (F)	p.o.	5.0	41.9 (2.9)[a]	62.0 (2.8)[a]	-----	41.9
Dog (F)	p.o.	5.0	38.9 (5.1)[d]	55.2 (2.3)[d]	-----	107.0
	i.v.	0.5	36.3 (5.2)[d]	59.1 (8.2)[d]	-----	-----
Pig (M)	p.o.	1.0	38.9[d]	54.9[d]	0.3[b]	38.9
Monkey (F)	p.o.	10.0	76.5[e] 77.6[d]	14.5[e] 16.2[d]	-----	77.6
Man (M)	p.o.	12.0 mg	73.7 (5.4)[f]	12.3 (2.4)[f]	-----	89.7
	i.v.	0.8 mg	82.2 (7.4)[f]	14.4 (9.5)[f]	-----	-----

[1]Values are arithmetic means (sd)

n = 5 (rat), 3 (dog) and 10 (man). For monkey and pig, n=1

Collection periods:

a) 48 h. d) 144 h

b) 24 h e) 240 h

c) 72 h f) 96 h

SELECTED PLASMA PHARMACOKINETIC PARAMETERS IN SEVERAL SPECIES FOLLOWING ADMINISTRATION OF [^{14}C]NISOLDIPINE							
Species	Rat (M)		Dog (F)		Monkey (F)	Man (M)	
Route Dose (mg/kg)	i.v. 1	p.o. 5	i.v. 0.5	p.o. 5	p.o. 1	p.o. 12*	i.v. 0.8*
Radioactivity $CEQ_{max,norm}$ ($kg*l^{-1}$)	---	0.49	---	0.59	0.79	3.7	---
t_{max}(h)	---	0.87	---	1.45	3.64	0.77	---
AUC_{norm}($kg*h*l^{-1}$)	13.6	4.0	4.2	5.9	7.1	40.1	50.2
$t_{½norm}$(h)	23.9	14.9	37.9	54.4	41.8	80.3	85.8
Parent $C_{max,norm}$ ($kg*l^{-1}$)	---	0.009	---	0.017	0.003	0.054	---
t_{max}(h)	---	0.5	---	1.0	4.0	0.42	---
AUC_{norm}($kg*h*l^{-1}$)	0.36	0.0097	0.46	0.054	0.02	0.077	0.954
$t_{½}$(h)	0.36	0.70	4.0	2.3	4.0	3.8	3.8
F(%)	---	2.7	---	11.7	---	8.4	---

n = 5 (rat), 3 (dog), 1 (monkey), 12 (man)
Rat data for parent (p.o.) from 1 mg/kg dose.

*Total mg dose per volunteer

Immediate Release

Dose (mg)	C_{max} (ng/mL)	AUC_{0-24} (ng.h/mL)
2.5	0.43 (58)	1.26 (64)
5	0.85 (46)	2.89 (48)
10	1.44 (59)	6.52 (46)
20	3.42 (57)	14.5 (42)

Coat Core

Dose (mg)	C_{max} (ng/mL)	AUC_{0-48} (ng.h/mL)
10	0.90 (43)	15.2 (33)
20	1.45 (39)	27.1 (37)
40	3.07 (49)	54.3 (47)
60	4.28 (52)	83.3 (43)

Study D90-022

Pharmacokinetic Parameters at Steady-State (Mean ± SD)

Dose (mg)	N	AUC(0-24h) (ng·h/ml)	C_{max} (ng/ml)	T_{max} (h)
30	18	74.28 ± 7.96	4.79 ± 0.68	7.22 ± 0.93
60	18	129.76 ± 12.74	8.48 ± 0.81	9.08 ± 1.97
90	9	199.31 ± 16.45	13.02 ± 1.20	6.78 ± 2.30
120	3	226.58 ± 12.41	14.92 ± 2.01	4.00 ± 1.00

Study D90-022

L.S. Mean Supine BP Change from Baseline at Steady-State (mmHg)

		Systolic		Diastolic	
Dose (mg)	N	8h post dose	24h post dose	8h post dose	24h post dose
30	18	-16.4	-14.0	-8.4	-10.2
60	18	-20.8	-16.8	-13.2	-15.0
90	9	-22.1	-23.0	-12.1	-13.4
120	3	-30.7	-44.3	-25.0	-19.0

3. Tissue Distribution: Quantitative tissue distribution in Sprague-Dawley rats was determined after 0.5, 1, 4, 8, 24, 48 and 72 hours (although the tables which follow provide 4, 24 and 72 hour values only). After oral administration, maximum concentrations in virtually all organs were reached within one hour, with liver, fat, kidney and adrenal gland generally containing the highest levels, brain and skeletal muscle the lowest. After a single dose, terminal elimination half-lives ranged from 42.2 h for plasma to 123 h for brain. With repeated daily dosing (5 mg/kg for 3 weeks), steady state was reached within 8 days. The CEQ at 24 h after the last (21st) dose and the $AUC_{1-3\,d}$ (based on radioactivity) were increased by a factor of 5 to 9, compared to a single dose. After 21 days of dosing, a slower elimination phase, characterized by half-lives of 2.66 days for plasma, 6.13 days for lung and up to 28 days for brain (generally 6-11 days in most other organs), was found.

After a single dose, there was no indication for changes in organ distribution pattern at later time intervals (up to 72 hours). By 72 hours, 1.1% of the administered radioactivity remained in the body of the rat (excluding the intestinal tract). In beagle dogs, tissue levels were measured only after 72 hours and the pattern of distribution was found to be similar to that in rats. Corresponding residue values in the body of dogs after 3 days were around 1% (i.v.) or 2% (oral) of the administered dose.

In pregnant rats following single oral or intravenous dosing, placental transfer was observed, with total radioactivity in the fetus reaching 17% of maternal plasma and 39% of the average maternal tissue concentration within one hour.

In lactating rats, secretion of nisoldipine and its metabolites into milk was noted after an oral dose of 5 mg/kg, with concentrations in milk being lower than in plasma at all time periods up to 48 hours post-dosing.

Whole-body autoradiography indicated rapid tissue distribution and penetration of the blood-brain barrier within 5 minutes after an intravenous dose. In addition, autoradiography essentially confirmed the widespread tissue distribution that was observed with the quantitative tissue measurements, including placental transfer and secretion into milk.

Placental transfer and milk secretion studies in rats are summarized in the 11/7/89 review of IND by X. Joseph, D.V.M., Ph.D.

QUANTITATIVE[1] TISSUE DISTRIBUTION OF TOTAL RADIOACTIVITY IN THE RAT AFTER ORAL (MALE AND FEMALE) AND INTRAVENOUS (MALE ONLY) ADMINISTRATION OF [^{14}C]NISOLDIPINE.						
Time post-dose	4 h			24 h		
Route	i.v. (male)	p.o. (male)	p.o. (female)	i.v. (male)	p.o. (male)	p.o. (female)
Dose (mg*kg-1)	1	5	5	1	5	5
Organ/Tissue:						
body excl. g.i.t.	0.11	0.11	0.29	0.026	0.021	0.032
plasma	0.35	0.16	0.36	0.13	0.044	0.056
erythrocytes	0.09	0.05	0.14	0.038	0.015	0.018
liver	0.54	0.56	1.8	0.13	0.11	0.36
kidneys	0.26	0.19	0.44	0.057	0.034	0.031
lungs	0.17	0.11	0.26	0.068	0.027	0.034
heart	0.09	0.07	0.20	0.029	0.016	0.016
brain	0.04	0.03	0.046	0.012	0.011	0.0062
adrenal gland	0.13	0.15	0.32	0.11	0.034	0.043
testes	0.06	0.04	----	0.019	0.014	-----
ovaries	----	----	0.38	----	----	0.029
renal fat	0.33	0.32	0.70	0.031	0.025	0.025
skin	0.06	0.06	0.16	0.022	0.018	0.021
skel. muscle	0.05	0.03	0.11	0.011	0.011	0.0081
resid. carcass	0.10	0.09	0.22	0.018	0.015	0.015

[1]Mean dose-normalized equivalent concentrations ($kg^{*}l^{-1}$). n = 5 per group.

QUANTITATIVE[1] TISSUE DISTRIBUTION IN THE RAT AND DOG AT 72 H FOLLOWING ADMINISTRATION OF [^{14}C]NISOLDIPINE.			
Species	Dog (F)		Rat (M)
Time after application	72 h		72 h
Route of administration	i.v.	p.o.	p.o.
Dose [mg*kg^{-1}]	0.5	5	5
body excl. g.i.t.	0.0095	0.017	0.012
plasma	0.011	0.017	0.020
erythrocytes	0.0083	0.011	0.0095
liver	0.078	0.090	0.057
kidney	0.020	0.034	0.017
lungs	0.016	0.024	0.009
heart	0.0057	0.014	0.009
brain	0.0016	0.0034	0.008
adrenal gland	0.032	0.072	0.025
renal fat	0.016	0.026	0.015
skin	0.0071	0.012	0.012
skel. muscle	0.0034	0.010	0.007
uterus	0.0080	0.018	-----
ovary	0.0090	0.016	-----

[1]Mean dose-normalized equivalent concentrations (kg*l^{-1}). n = 3 (dog) and 5 (rat) per group.

4. Protein Binding: As determined by equilibrium dialysis, ^{14}C-nisoldipine was highly bound to plasma proteins of the rat (97.8-99.1%), dog (97.6-97.1%) and man (>99.4%) and was not influenced by sex in any of the three species. In humans, ^{14}C-labelled drug was bound predominently to the serum albumin, and the extent of binding was not influenced by plasma concentration over a broad range; i.e., between 0.1 and 10 ug/ml. Similar levels of protein binding were found in human plasma whether it was measured by equilibrium dialysis or ultrcentrifugation, and degree of binding of the (+) and (-) ^{14}C-enantiomers was similar (around 99.4%), with no indication of preferential stereospecific binding. In the "expert opinion" on pre-clinical pharmacokinetic studies, it is claimed that when protein binding was measured *ex vivo* (dialysis method) in rats and dogs after i.v. or oral administration, nisoldipine was highly bound initially, but the protein bound fraction dropped to 50 to 80% between 30 and 180 minutes after dosing, indicating lower binding affinities for the metabolites.

5. Metabolism

a. Biotransformation: Nisoldipine is rapidly and extensively metabolized in the rat, dog, monkey and man. Only a small percentage of unchanged ^{14}C-labelled substance could be found in the circulation of the rat or dog at 30 and 60 minutes after oral administration, when plasma radioactivity was at the maximum level. No unchanged drug was eliminated in the urine or feces of all 4 species, or in bile of bile duct cannulated rats that received the drug either by i.d. or i.v. route. Partial enterohepatic recirculation of metabolites was demonstrated in rats. A schematic for the biotransformation of the drug is shown on the page which follows. The investigators describe the biotransformation steps as follows:

-hydroxylation of the isobutyl ester
-dehydrogenation to the pyridine derivative
-cleavage of the ester to form the carboxylic acid
-reduction of the nitro group to the amino group
-glucuronidation (phase II enzymatic reaction)

In urine of all 4 species, at least 12 biotransformation products were detected, with 6 of them, M-1 to M-5 and M-12, accounting for 80% of radioactivity in urine; all other metabolites were minor products. M-5 was the major urinary metabolite, accounting for 24 to 46% of the renal eliminated radioactivity in all 4 species. Only 1 metabolite, M-12, was hydrolyzable with B-glucuronide, to give M-5 as the aglycone. Metabolic profile in urine was essentially similar in all 4 species.

In bile, metabolic profiles of rats "were quantitatively identical *in vitro* (isolated perfused rat liver model) and *in vivo* following intraduodenal administration". At least 24 metabolites have been detected, but only 8 of them, M-3 to M-5, M-10, M-12, M-14 to M-16, were quantitatively important. The

major metabolic products in bile of rats were M-5 and its conjugate, M-12.

In serum, at least 12 metabolites were observed in rats within 30 minutes of dosing, and the main ones were M-2 and M-5, together with their gamma-lactones, R-3 and R-5. In dogs, at least 11 biotransformation products were isolated from the serum, and a similar pattern was seen as in rats, with M-2 and M-5 being the main products.

A total of 18 biotransformation products have been identified in urine and serum. The main biotransformation products in all 3 animal species and humans, based on findings in the urine and serum (also in the bile of rats), are M-5 plus M-12 which is the glucuronide of M-5, and R-5 which is the gamma-lactone of M-5.

main metabolites M-5, M-12 and R-5 in urine and serum

	rat serum	rat urine	dog serum	dog urine	monkey urine		
					0-7 h	7-11 h	11-24 h
	[%]	[%]	[%]	[%]	[%]		
M-5	13.3	34.9	46.5	44.9	27.2	23.7	26.7
M-12		3.1		11.7	30.8	31.1	15.5
R-5	11.7						
total:	25	38	46.5	56.6	58	54.8	42.2

In studies with dogs, the liver and gut wall were identified as the primary sites of biotransformation (Arzneim. Forsch./Drug Res. 38: 1093, 1988) after oral administration. It was estimated that around 60% is metabolized pre-hepatically in the gut and 30% in the liver.

b. Effects on Hepatic Enzymes: In two experiments with male rats, nisoldipine was administered orally at doses of 0, 10, 50 and 200 mg/kg for 2 weeks, followed by a 1 week recovery period in the second experiment. The positive control was phenobarbital at 25 mg/kg. The hepatic levels of cytochrome P-450, aminopyrine N-demethylase and aniline hydroxylase activities were decreased at mid and high dose, whereas phenobarbital caused significant increases in levels of all 3 enzymes. The decreases in all 3 enzyme levels were found to be reversible after a 1-week recovery period.

SUMMARY OF TOXICOLOGICAL STUDIES

A. **Acute Toxicity Studies** (X. Joseph)

Acute oral and iv toxicity studies were done in mice, rats, rabbits and dogs at Bayer AG, Institute of Toxicology, Wuppertal, FRG. For oral toxicity studies, the drug (suspended in a solution of glycerol, lutrol and demineralized water) was given via stomach tube (20 ml/kg) to mice, rats and rabbits; and in dogs the drug was given in gelatin capsules. For iv studies, the drug suspended in the above solution was given at a volume of 5 ml/kg for rodents and at 1 to 4 ml/kg for dogs. After drug treatment animals were observed for a period of 14 days. No clinical signs or mortality were seen after oral administration. However, tonic/clonic convulsions, gasping, cyanosis, exophthalmos and respiratory disturbances (all species) were seen after iv administration. All deaths occurred either during or within 10-20 min of drug administration. The surviving animals were free of symptoms within 1 (mice, rabbit and dog) to 48 hr (rat) postdose. The autopsies (dead or sacrificed at the end of the study) showed no pathological findings. The LD50 values for different species are below.

ACUTE TOXICITY OF NISOLDIPINE IN MICE, RATS, RABBITS, AND DOGS

Species	Sex	Route of Administration	LD_{50} - mg/kg (95% Conf. Limits)
Mouse (CFW1/W)	M	p.o.	> 10,000
Mouse (CFW1/W)	M	i.v.	2.20 (2.0-2.5)
Rat (Wistar)	M	p.o.	> 10,000
Rat (Wistar)	F	p.o.	> 10,000
Rat (Wistar)	M	i.v.	2.32 (2.06-2.65)
Rat (Wistar)	F	i.v.	1.86 (1.77-1.97)
Rabbit (Lge Chinchilla)	M,F	p.o.	> 5000
Rabbit (Lge Chinchilla)	M,F	i.v.	ca. 2.5
Dog (Beagle)	M,F	p.o.	> 5000
Dog (Beagle)	M,F	i.v.	ca. 2.0

A separate study showed that pretreatment with propranolol (1 mg/kg ip for 4 or 5 days) had no effect on the acute iv toxicity of nisoldipine in male Wistar rats (iv LD50 = 1.6 mg/kg with or without propranolol pretreatment).

B. **Subchronic, Chronic and Carcinogenicity Studies**

RAT STUDIES (X.Joseph)

a. Four Week Dietary Dose Rangefinding Study

Testing Facility:

Study Number:

Study Dates: August - September, 1980

GLP Compliance: Study was not conducted according to GLP regulations. The deviations were as follows: 1. no phase 1-3 GLP audits. 2. no checking of physico-chemical properties of test substance.

Animals: Wistar strain TNO-74 rats, individually housed in Macrolon cages, Type II, were 5-6 weeks old (average weights: males - 131 g; females - 112 g) at the initiation of dosing.

Dose Levels: was mixed with powdered rat diet to obtain drug concentrations of 0, 300, 1000, and 3000 ppm.

Achieved Dose Levels:

Dose (ppm)	Average Drug Intake (mg/kg/day)	
	Male	Female
0	0	0
300	26	26
1000	86	89
3000	258	265

(Note: The drug intake was calculated from the average daily food intake for the whole duration of the study. No significant difference in drug intake was seen with time.)

Number of Animals: 10/sex/group

Parameters Evaluated: Appearance and behavior (at least once daily), body weight and food consumption (weekly), organ weights (heart, liver, kidneys and adrenal glands) and gross pathology. (No histopathological examinations were conducted.)

Results: No treatment-related clinical signs or mortalities were observed in this study. A significant reduction in body weight, compared to concurrent controls, was observed throughout the treatment period in high dose males (10-18%) and females (6-10%) except in females at week 4. No significant body weight differences were seen between control and mid or low dose groups (both sexes). Food consumption was reduced in the high dose group. Though not measured quantitatively, water consumption appeared to be increased in all treated groups. Organ weight findings (both absolute and relative) are given below.

Mean Organ Weights of Male and Female Rats

		Sex	Dose Group (ppm in diet)			
			0	300	1000	3000
Body Weight (g)		M	225	219	224	196*
		F	142	140	144	131
Adrenals	(Absolute, mg)	M	36	38	38	39
	(Relative, mg/100g)	M	16	18	17	21*
	(Absolute, mg)	F	50	49	53	49
	(Relative, mg/100g)	F	36	35	37	38
Heart	(Absolute, mg)	M	663	669	675	677
	(Relative, mg/100g)	M	295	304	301	349**
	(Absolute, mg)	F	490	517	555**	542
	(Relative, mg/100g)	F	346	371	387*	417**
Kidney	(Absolute, mg)	M	1489	1392	1409	1295**
	(Relative, mg/100g)	M	663	634	630	669
	(Absolute, mg)	F	986	978	1036	975
	(Relative, mg/100g)	F	697	702	719	749
Liver	(Absolute, mg)	M	8219	8074	8599	8699
	(Relative, mg/100g)	M	3650	3655	3825	4436**
	(Absolute, mg)	F	5300	5452	5857	6105*
	(Relative, mg/100g)	F	3761	3895*	4064*	4652**

** Significantly different from control at 0.05 level.*
*** Significantly different from control at 0.01 level.*

Relative mean heart and liver weights of the high dose group (both sexes) were significantly increased with no significant changes in absolute weights except for the mean liver weight of high dose females which was significantly higher (15%) than the control value. Both absolute and relative heart weights were increased in mid dose females.

It is stated that dietary dose levels, 0, 50, 300 and 1800 ppm, for the 2 year carcinogenicity study in rats were selected on the basis of the above study, and also based on the previous experience from long-term studies with other dihydropyridines [amendment (no serial number) dated May 31, 1994].

b. Three Month Oral (Gavage) Toxicity Study in Rats

Testing Facility:

Study Number:

Study Dates: September - December, 1980

GLP Compliance: Not addressed.

Animals: Wistar (TNO/W 74, SPF) rats, individually housed in type 11 Makrolon cages, were 7-8 weeks old (males 115-155 g; females 120-145 g) at the initiation of dosing.

Mode of Administration: dissolved in a solvent mixture, containing placebo solution (polyethylene glycol, glycerol and distilled water) and distilled water, was given by oral intubation. It is stated that the test formulation was stable at room temperature for over a week.

Dose Levels: 0 (vehicle control), 10, 30 and 100 mg/kg/day (5 ml/kg)

Number of Animals: 15/sex/group

Parameters Evaluated: Appearance and behavior (daily), body weight and food and water consumption (weekly), hematology, blood chemistry and urinalysis (5 rats/sex/group; weeks 4/5 and 13), major organ weights and gross and microscopic pathology (more than 20 different tissues/rat; control and high dose groups).

Major Findings: Two low dose females (on days 8 and 68) died during the study. Gross pathology findings in the above animals included enlarged kidneys, bladder and adrenals in one rat and discolored lung and pulmonary emphysema in the other. Labored breathing was noticed in high dose rats during the first 5 weeks of treatment. No significant differences in body weights were seen between treated and control groups except for the lower body weights observed in the high dose group, compared to concurrent controls, during the first one or two weeks of treatment. Food consumption was unaffected. Water intake of high dose females was about 20% higher than that of concurrent control. Although hemoglobin and hematocrit values in mid and high dose males were lower than concurrent control values at 13 weeks, it is stated that all values were within the historical control range for Wistar rats. High dose females had significantly higher plasma urea levels, compared to control, after either 4 or 13 weeks of treatment. In mid and high dose males, both absolute and relative thymus weights were significantly higher than control. Heart and liver weights (both absolute and relative) in high dose females were significantly higher than control; absolute and/or relative

weights of these organs in mid dose females and mid- and high-dose males were also higher than control. There were no significant histopathological findings in this study.

Mean Organ Weights of Male and Female Rats

		Sex	Dose Group (mg/kg)			
			0	10	30	100
Body Weight (g)		M	338	347	330	326
		F	206	207	200	204
Adrenals	(Absolute, mg)	M	36	39*	37	37
	(Relative, mg/100g)	M	11	11	11	11
	(Absolute, mg)	F	52	55	53	56
	(Relative, mg/100g)	F	25	27	26	27*
Brain	(Absolute, mg)	M	1788	1860*	1849	1843
	(Relative, mg/100g)	M	530	538	569	568**
	(Absolute, mg)	F	1652	1676	1649	1685
	(Relative, mg/100g)	F	802	815	829	829
Heart	(Absolute, mg)	M	955	1022*	1000*	1004
	(Relative, mg/100g)	M	283	295	304**	308**
	(Absolute, mg)	F	688	704	706	758**
	(Relative, mg/100g)	F	334	342	354**	372**
Liver	(Absolute, mg)	M	10966	11179	11777	11523
	(Relative, mg/100g)	M	3243	3220	3602*	3537*
	(Absolute, mg)	F	6394	6822	6907	7588**
	(Relative, mg/100g)	F	3100	3304	3460*	3716**
Lung	(Absolute, mg)	M	1172	1202	1000*	1004
	(Relative, mg/100g)	M	347	347	366	366*
	(Absolute, mg)	F	900	937	913	911
	(Relative, mg/100g)	F	436	455	457*	447
Thymus	(Absolute, mg)	M	196	222	235*	233*
	(Relative, mg/100g)	M	58	64	72*	72*
	(Absolute, mg)	F	206	213	180	185
	(Relative, mg/100g)	F	100	104	90	91

** Significantly different from control at 0.05 level.*
*** Significantly different from control at 0.01 level.*

c. Two Year Carcinogenicity Study in Rats

Testing Facility:

Study Number: T 1000876

Study Dates: November 1980 - November 1982

GLP Compliance: Study was conducted in accordance with GLP regulations

Animals: Wistar strain TNO/W 74 rats, individually housed in type II Makrolon cages, were 5-6 weeks old (mean body weights: males - 79 g; females - 75 g) at the initiation of the study.

Dose Levels and Mode of Administration: . purity - about 99.2%) was mixed, weekly, with powdered rat diet at concentrations of 0, 50, 300 and 1800 ppm. The stability and the concentration of the test substance in diet were determined pretest and then every three months. The concentrations of the drug in diet were found to be in good agreement with theoretical values. However, there is no indication that concentrations of drug in diet were adjusted periodically to maintain a constant mg/kg body weight exposure.

Number of Animals: 50/sex/group (An additional 10 rats/sex included in each group were sacrificed after 12 months of treatment - interim sacrifice.)

Observations/Measurements: Rats were observed at least once daily for general appearance, behavior and clinical signs. Body weights were recorded weekly until week 27 and biweekly thereafter. Food and water consumption were determined weekly and once every 3 months, respectively. Hematological [erythrocyte, leucocyte (total and differential), platelet and reticulocyte counts, hemoglobin, hematocrit, MCV, MCH and thromboplastin time (only at the termination of the study)] and blood chemistry (alkaline phosphatase, transaminases, creatine kinase, urea, creatinine, blood sugar, cholesterol, total bilirubin, total protein, corticosterone, aldosterone and serum electrolytes) evaluations and urinalyses were conducted on 10 rats/sex/group (selected at random) at 6, 12, 18 and 24 months. Complete autopsies were performed on animals that were sacrificed at 12 months and at study termination. Animals were examined grossly and heart, lung, liver, spleen, kidneys, adrenals and testes were weighed. All protocol specified tissues (more than 30 different tissues/rat) and gross lesions were fixed in buffered formalin. In addition, left liver lobe from all rats was fixed in formol-calcium, and lower jaw from 5 rats/sex/group was fixed in buffered formalin. Autopsies were also performed on rats that died or were sacrificed in extremis and all evaluable tissues were preserved. All protocol specified tissues from control and

high dose groups, all tissues from animals that died or were sacrificed moribund, as well as adrenals, genital organs, areas of skin change and kidneys (females) of low and mid dose animals and all grossly abnormal tissues were examined histologically.

Differences between treated and control groups were analyzed using the significance test (U-test) of Mann and Whitney and of Wilcoxon. Mortality and tumor data were analyzed by Fischer's exact test.

Achieved Dose Levels:

Dose (ppm)	Average Drug Intake (mg/kg/day)	
	Male	Female
0	0	0
50	2.15	2.78
300	13.13	18.04
1800	82.40	110.68

(Note: The drug intake was calculated, at the termination of the study, from the average daily food intake/animal/group for the whole duration of the study. Periodical drug intake determinations were not made in this study.

Results: No treatment-related clinical signs were seen in this study. The mortality data (cumulative) at different intervals are given below and it is presented graphically in Figures 6 and 7.

Mortality of Rats Receiving Nisoldipine in Diet for 24 Months

Daily Dose (ppm in diet)	Number of Rats (M/F)	Number of Dead (M/F)	% Mortality (M/F)
12 Months			
0	50/50	1/1	2/2
50	50/50	0/1	0/2
300	50/50	1/0	2/0
1800	50/50	0/2	0/4
18 Months			
0	50/50	2/3	4/6
50	50/50	0/2	0/4
300	50/50	2/5	4/10
1800	50/50	5/4	10/8
24 Months			
0	50/50	4/8	8/16
50	50/50	8/8	16/16
300	50/50	11/15	22/30
1800	50/50	11/13	22/26

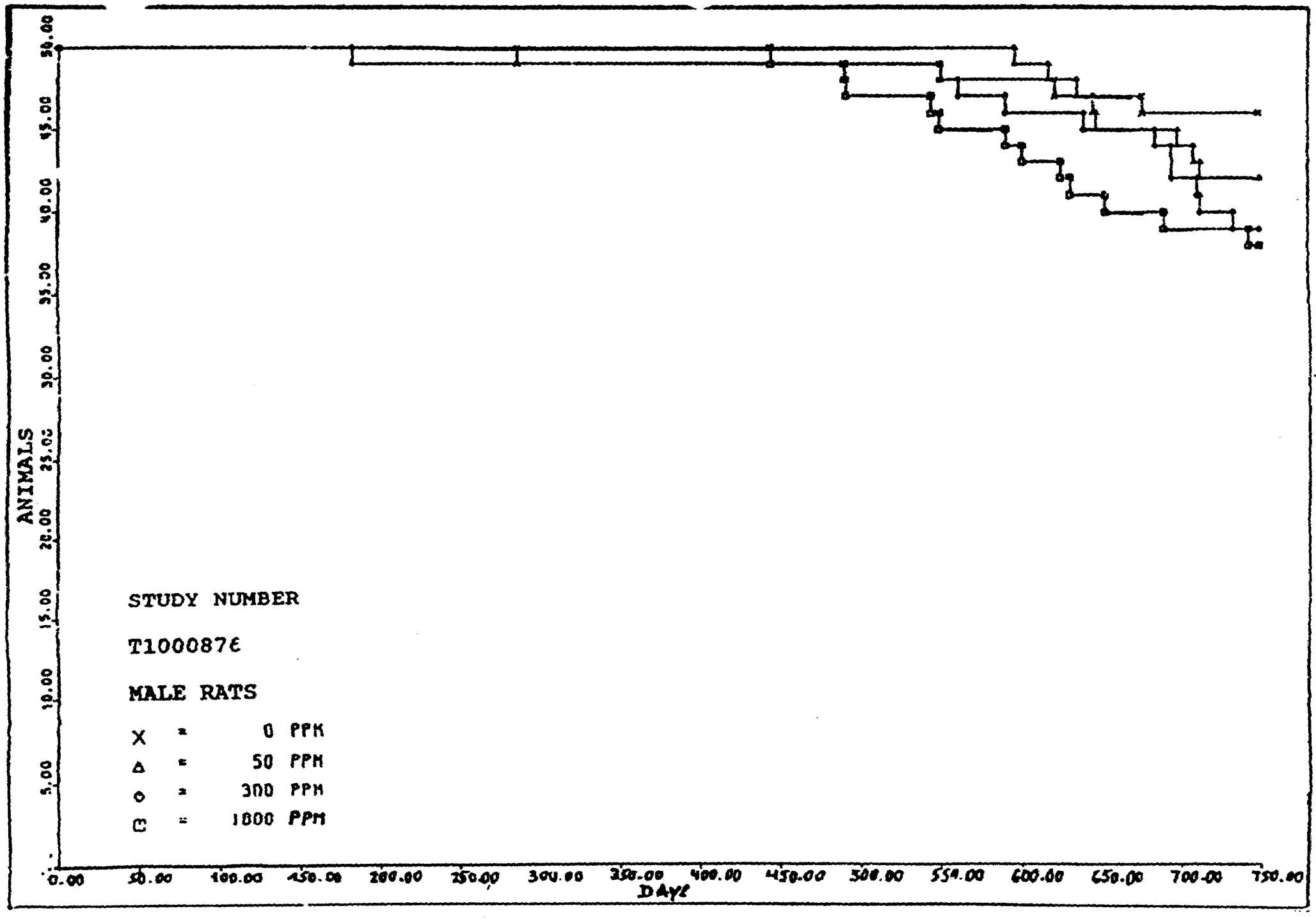


Fig. 6: Mortality curves of male rats which received for 24 months in their feed

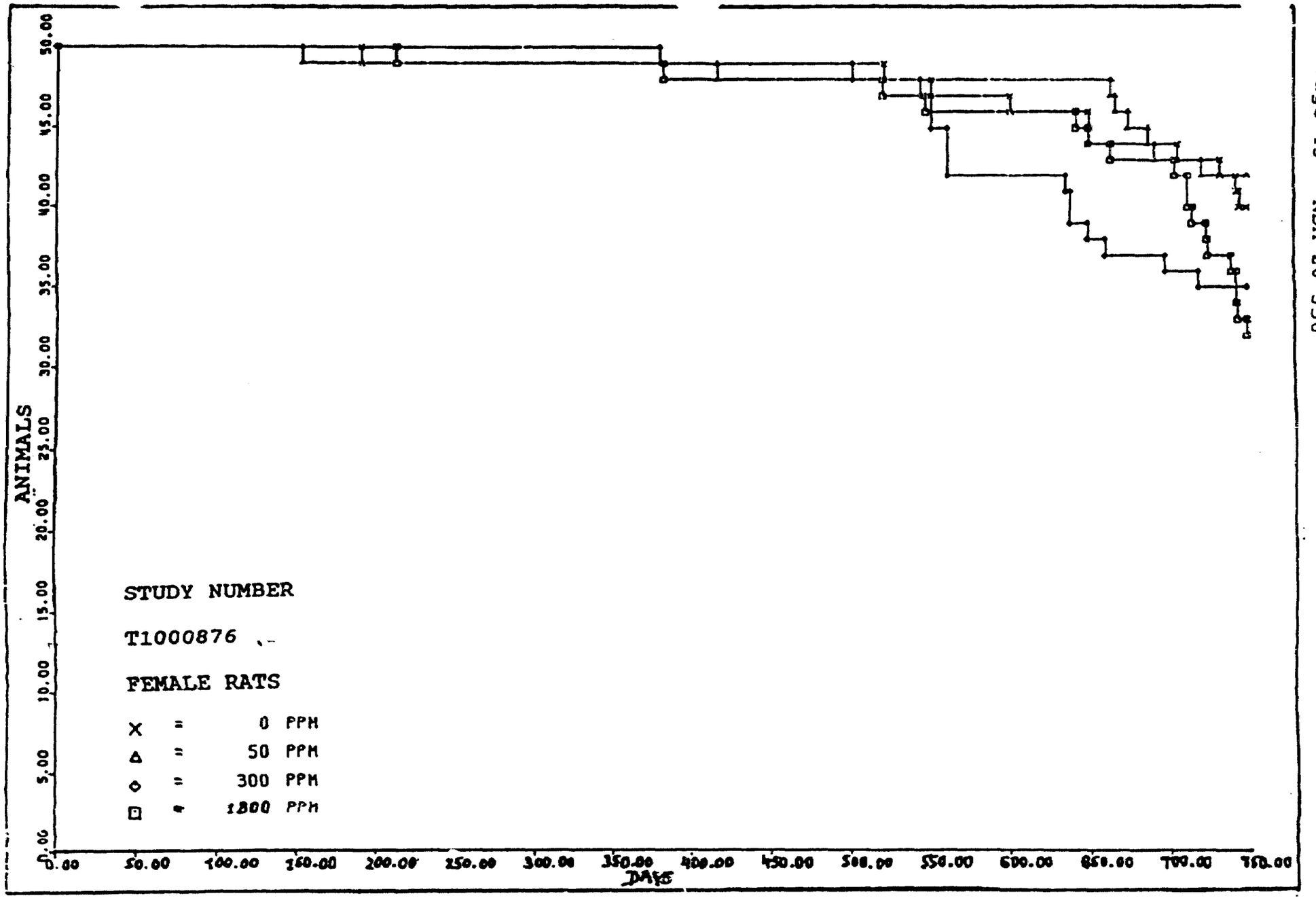


Fig. 7 : Mortality curves of female rats which received for 24 months in their feed

Although more animals (both sexes) died during the study in mid and high dose groups than in the concurrent control group, the differences were statistically not significant (sponsor's analysis - Fischer's exact test).

When tested for heterogeneity in survival distribution, FDA statisticians observed that there was no significant difference (at 0.05 level) in the survival distribution for either sex (both Cox test and generalized Wilcoxon test). Additionally, no significant linear trend (at 0.05 level) was seen in the intercurrent mortality rate for either males or females.

Mean body weight values (presented graphically in Fig.8) for the high dose group (both sexes) were significantly lower than control values for the whole duration of the treatment period, except on four occasions (weeks 25, 29, 51 and 63) in males. At the termination of the study, body weights of high dose males and females were 5.6% and 22%, respectively, lower than concurrent control. The body weight gain values of high dose males and females were 7% and 31%, respectively, lower than concurrent control. No significant treatment-related reductions in body weight were seen in low and mid dose groups except on a few occasions (weeks 13, 14 and 39 for males and weeks 77, 79, 85, 87 and 89 for females) at mid dose level. While food consumption was unaffected, water consumption in high dose animals, especially in females, was increased.

Although statistically significant hematological findings were occasionally observed in drug treated groups, no dose dependence or consistency at different intervals was observed. Statistically significant clinical chemistry findings and the time points of their occurrence are given on pages 48 & 49. In the high dose group, significant reductions in alkaline phosphatase levels (both sexes) and increases in GOT (males), GPT and CPK levels (females) were seen. Although a dose-dependent increase in bilirubin levels was seen in both sexes during the early part of the study, these levels in treated females were lower than that of control (no significant difference in males) at the termination of the study. Blood urea levels in mid and high dose females were significantly higher than control at the end of the study; however, these levels were lower than control during week 28. Decreased calcium levels were seen in treated animals, especially at the high dose level (both sexes). Plasma aldosterone levels in high dose males and plasma corticosterone levels in high dose females were significantly lower than respective control values at week 55.

A significant increase in urinary protein excretion was seen in high dose females. While urinary calcium excretion was decreased in treated female groups, especially in mid and high dose groups, urinary potassium levels were increased in high dose males. Urinary aldosterone excretion was significantly higher in high dose males than in control males.

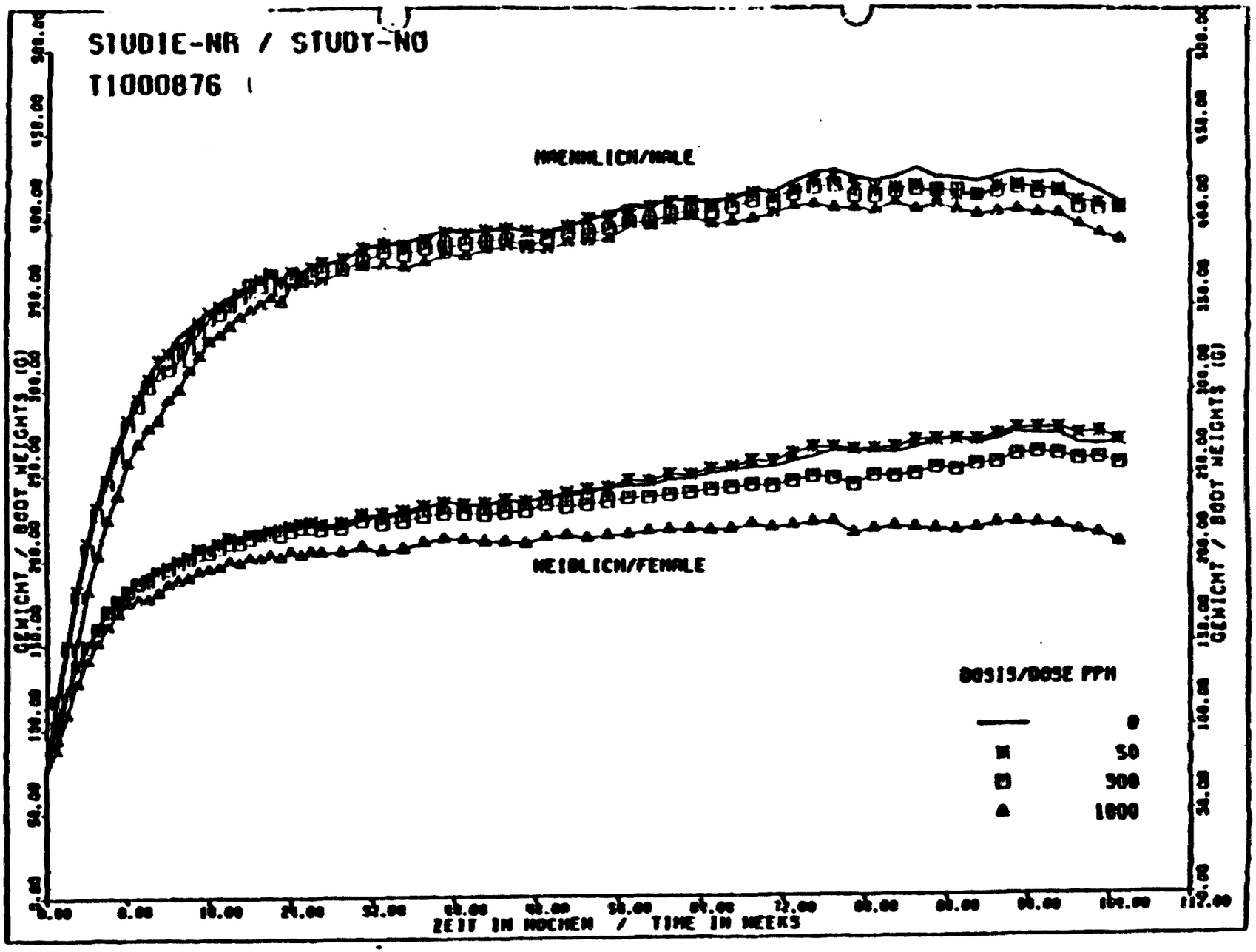


Fig.8 : Body weight curves for male and female rats which received with the feed for 24 months.

Mean Clinical Chemistry Parameters (Male Rats)					
		Dose Group (ppm in diet)			
Parameter	Week	0	50	300	1800
ALP	28	211	201	201	175*
U/L	54	182	174	186	145*
	79	180	156	176	136**
GOT		38.8	39.0	38.9	52.7*
U/L					
Bilirubin	28	3.6	3.1	4.0	4.8*
mcmol/L					
Creatinine	79	53	50	47*	51
mcmol/L	105	57	63	46**	50**
Urea	105	5.80	7.01*	5.69	5.27
mmol/L					
Cholesterol	28	1.98	2.16	2.21*	2.21
mmol/L					
Protein	54	66.5	64.2**	61.0**	61.4**
g/L	105	68.4	67.1*	66.3*	67.8
Sodium	28	142	143	140*	140*
mmol/L	54	141	142	142*	142
	79	140	139	138*	141
Potassium	79	4.8	5.0	5.1*	5.1
mmol/L					
Calcium	28	2.64	2.54*	2.49*	2.55*
mmol/L	79	2.76	2.66*	2.62**	2.63**
	105	2.69	2.66	2.63	2.58*
Aldosterone	55	349.7	360.2	334.9	245.1**
pg/mL					

** Significantly different from control at the 0.05 level*
*** Significantly different from control at the 0.01 level*

Mean Clinical Chemistry Parameters (Female Rats)					
		Dose Group (ppm in diet)			
Parameter	Week	0	50	300	1800
ALP	28	174	140	153	133*
U/L					
CPK	28	98	54*	72	85
U/L	79	43	75	64	77*
GPT	28	54.1	50.5	52.9	66.3*
U/L					
Bilirubin	54	3.0	3.1	3.2	4.3**
mcmol/L	105	4.7	2.9**	3.2*	2.9*
Creatinine	79	56	50	59	57*
mcmol/L	105	71	59	55**	61
Urea	28	7.54	7.27	6.56*	6.30**
mmol/L	79	6.22	5.96	6.51	7.56**
	105	6.09	6.43	6.67*	7.28*
Cholesterol	28	7.54	7.27	6.56*	6.30**
mmol/L	105	2.46	3.01*	2.76	2.96
Glucose	105	4.71	5.25	5.52*	5.22
mmol/L					
Sodium	54	140	138	135**	138
mmol/L					
Potassium	54	4.8	4.8	5.0	5.2*
mmol/L	105	4.5	4.6	4.8*	4.8*
Calcium	28	2.58	2.65	2.59	2.47*
mmol/L	54	2.71	2.65	2.58*	2.52**
Corticosterone	55	36.6	41.7	20.4	19.2*
mcg/DL					

** Significantly different from control at the 0.05 level*
*** Significantly different from control at the 0.01 level*

At the termination of the study, the relative mean weights of adrenals, heart, kidneys and liver of the high dose group (both sexes) were significantly higher than respective control values (page 50A). However, no significant differences were seen in the absolute weights of the above organs except for the increased mean kidney weight of the high dose males. At the interim sacrifice, relative heart and liver weights (high dose males and females) and relative adrenal and kidney weights (high dose females) were significantly increased without any significant changes in absolute weights.

No significant treatment related gross lesions were seen in this study.

At the interim sacrifice, no treatment-related histological findings were observed except for the moderate widening of the zona glomerulosa region of the adrenal cortex of high dose animals. The cells of this zone were large and contained a foamy cytoplasm. Four benign tumors [2 in control females (cystadenoma of thyroid in one and pituitary adenoma in the other) and 2 in high dose males (Leydig cell tumor of testis in one and meningioma of the cerebellum in the other)] were seen at the interim sacrifice.

[Note: The terms "blastoma" and "tumor" are used interchangeably in this NDA.]

The number of rats with benign and/or malignant tumors and the percent of these tumor carriers are given in Table 7. According to sponsor, no treatment-related increased incidence of tumor bearing animals was observed in this study. The incidence of various types of tumors observed at different locations are presented in Tables 8 and 9. Although the incidence of Leydig cell tumor of testes appears to be higher in treated male groups than in control, the differences were statistically not significant.

Analysis of the tumor data by FDA statisticians showed that there was a statistically significant (at 0.05 level) linear trend in brain granular cell tumor (listed also as meningioma in this NDA) in male rats (p=0.0411). The incidence of this tumor is as follows: control - 0/50, low dose - 0/50, mid dose - 0/50 and high dose - 3/50 (2 animals at the final necropsy and one at the interim sacrifice). However, pairwise comparison did not reveal any significant difference between control and high dose groups (p=0.1594). According to the sponsor, "the incidence rate for granular cell tumors among male rats at terminal kill in the study performed with lay within the spontaneous range for male rats at terminal kill" (Table-page 50e). Spontaneous tumors of meningeal origin (meningioma, meningeal sarcoma or granular cell tumor) were seen in 7 out of 30 studies in male Wistar rats (39-50 rats/study). In 2 studies, 2 rats each were diagnosed with such tumors at terminal kill, whereas in 4 other studies, only one rat each had above tumors.

[Note: Granular cell tumors are believed to be of meningeal origin and are considered to be a subclassification of meningiomas.(Boorman et. al. 1990. eds. Pathology of the Fischer Rat. Academic Press, Inc.)]

Relevant nonneoplastic findings observed in this study included hypertrophy of the cells of the zona glomerulosa of the adrenal cortex of high dose males and females and increased incidence of progressive nephropathy in high dose females.

d. Two-Week Intravenous Toxicity Study

Testing Facility:

Study Number: Not provided (Pharma Report No.7721)

Study Date: October, 1977

GLP Compliance: Not addressed

Animals: SPF Wistar albino rats, individually housed in Type II Macrolon cages, weighed 125 to 130 g at the initiation of dosing.

Dose Levels: 0, 0.1, 0.3 and 1.0 mg/kg. . dissolved in a 10%:90% mixture of Cremophor EL and physiological saline, was administered as a single iv bolus injection (caudal vein; 1 ml/kg) daily for 14 consecutive days.

Number of Animals: 10/sex/group

Parameters Evaluated: Appearance and behavior (daily), body weight and food and water consumption (weekly), hematology and clinical chemistry (at the termination of the study; 5 rats/sex/group), urinalyses (after 10th treatment), major organ weights and gross and microscopic pathology (more than 30 different tissues/rat; 5 rats/sex from control and high dose groups).

Results: High dose animals showed inertia and dyspnea for about 5 to 15 min following drug administration. Two high dose females died during the study, one after the third dose and the other after the ninth dose. No treatment-related clinical signs or mortalities were seen in low and mid dose group animals.

Intravenous administration of nisoldipine had no significant effect on body weight, food or water consumption and hematologic, blood chemistry and urinalyses parameters. There were no treatment-related gross or histopathological findings, or any evidence of local intolerance at dose levels tested in this study.

MOUSE STUDIES (X. Joseph)

a. 28-day Dietary Dose Rangefinding Study

Testing Facility:

Study Number: T 1003 576

Study Dates: June-July, 1981

GLP Compliance: Study was not conducted according to GLP regulations. The deviations were as follows: a. no phase 1-3 GLP audits, and b. no checking of physico-chemical properties of test substance.

Animals: SPF-bred NMRI mice, individually housed in Type I Macrolon cages, were 4-5 weeks old (average body weights: males-20.0 g, females-19.8 g) at the initiation of the study.

Dose Levels: was mixed with powdered diet by the addition of peanut oil DAB 7 (1%) to obtain dietary drug concentrations of 0, 400, 800, 1200 and 1600 ppm.

Achieved Dose Levels:

Dose (ppm)	Average Drug Intake (mg/kg/day)	
	Male	Female
0	0	0
400	110	124
800	226	271
1200	348	383
1600	429	523

(Note: The drug intake was calculated from the average daily food intake/animal/group for the whole duration of the study.)

Number of Animals: 10/sex/group

Parameters Evaluated: Appearance and behavior (daily), body weight, food and water consumption (weekly), organ weights (heart, lungs, liver, spleen and kidneys) and gross pathology.

Results: No treatment-related clinical signs or mortalities were observed. Significant reductions in body weights (3-7%), compared to concurrent control, were seen in females throughout the study

at dose levels of 800 ppm and above except at 1200 ppm at the end of the study. In males, although body weights were lower than concurrent control (4-9%) at 1200 and 1600 ppm levels, the differences were statistically significant only at 1200 ppm. No significant differences were seen in food and water consumption between treated and control groups. Organ weight findings are given below.

Mean Organ Weights of Male and Female Mice

		Sex	Dose Group (ppm in diet)				
			0	400	800	1200	1600
Body Weight (g)		M	31.8	30.1	31.0	29.5*	30.3
		F	25.5	25.4	24.0*	24.7	24.5*
Heart	(Absolute, mg)	M	0.14	0.15	0.17**	0.15*	0.15
	(Relative, mg/100g)	M	0.44	0.52**	0.53**	0.53**	0.49*
	(Absolute, mg)	F	0.13	0.14	0.14	0.15*	0.14*
	(Relative, mg/100g)	F	0.51	0.54*	0.58**	0.61**	0.57**
Kidneys	(Absolute, mg)	M	0.46	0.46	0.49	0.49	0.48
	(Relative, mg/100g)	M	1.44	1.53	1.59	1.66*	1.58
	(Absolute, mg)	F	0.35	0.34	0.33	0.33	0.34
	(Relative, mg/100g)	F	1.35	1.32	1.38	1.33	1.37
Liver	(Absolute, mg)	M	1.93	1.84	1.82	1.74*	1.72*
	(Relative, mg/100g)	M	6.08	6.09	5.66	5.90	5.66*
	(Absolute, mg)	F	1.47	1.40	1.34	1.39	1.36
	(Relative, mg/100g)	F	5.72	5.53	5.55	5.64	5.56
Lung	(Absolute, mg)	M	0.23	0.26*	0.25	0.23	0.22
	(Relative, mg/100g)	M	0.72	0.88**	0.80**	0.78*	0.74
	(Absolute, mg)	F	0.22	0.21	0.20	0.23	0.22
	(Relative, mg/100g)	F	0.87	0.82	0.82	0.92*	0.89
Spleen	(Absolute, mg)	M	0.09	0.09	0.10	0.09	0.09
	(Relative, mg/100g)	M	0.28	0.31	0.32	0.30	0.29
	(Absolute, mg)	F	0.10	0.09	0.10	0.10	0.09
	(Relative, mg/100g)	F	0.38	0.37	0.41	0.38	0.38

** Significantly different from control at 0.05 level.*
*** Significantly different from control at 0.01 level.*

Relative heart weights in all treatment groups (both sexes) were significantly higher than control; however, absolute heart weights were significantly higher in males only at 800 and 1200

ppm and in females at 1200 and 1600 ppm levels. Both absolute and relative liver weights were lower than control in 1600 ppm males.

No significant gross findings were observed. Histopathological evaluations were not performed in this study.

Based on the results of this study, dietary dose levels of 100, 300 and 900 ppm were selected for the mouse carcinogenicity study.

b. 21 Month Carcinogenicity Study in Mice

Testing Facility:

Study Number: T7010709 (Sponsor's number)

Study Dates: Initiation of dosing - 10/7/81
Autopsy of last animal - 7/7/83

GLP Compliance: Studies were done in accordance with GLP regulations.

Animals:

Strain: Bor:NMRI (SPF HAN)
Sex: Both sexes
Age and Wt: 4 to 6 weeks old/20-22 g
Housing: Individually housed in Makrolon Type I cages.

Mode of Administration of Test Agent: Powdered diet.

Dose Levels: 0, 100, 300 and 900 ppm dosage levels of (Batch No. 662845, purity-98.1%) were used on the basis of results of the 28-day dietary dose rangefinding study. The stability and the concentration of drug in the diet were determined periodically. The concentrations of the drug in diet, at all intervals, were more than 89% of the theoretical values, and the compound was found to be stable in the diet for at least 10 days. However, there is no indication that concentrations of drug in diet were adjusted periodically to maintain a constant mg/kg body weight exposure.

No. of Animals: Equal numbers of males and females (50+20*/sex/dosage level) were used. *Additional 20 mice included in each group were sacrificed 12 months after the initiation of dosing for interim investigations

Observations/Measurements:

Appearance/Behavior monitored twice daily and a detailed assessment of each individual animal was made on a weekly basis, with particular attention given to posture, general behavior, body surfaces, orifices and breathing and elimination products.

Body weight determinations were done at the beginning of the study, once a week until 27th week and every 2 weeks thereafter and also before the termination of the study.

Food intake was calculated on a weekly basis up to 23rd week and every two weeks thereafter.

Hematological (RBC, WBC, platelet and reticulocyte counts, differential white cell counts, hemoglobin, hematocrit, MCV, MCH and MCHC) and clinical chemistry [alkaline phosphatase, transaminases (ASAT and ALAT), plasma creatinine, urea, blood glucose, cholesterol, bilirubin and total plasma proteins] investigations were done at 12 months (interim sacrifice group) and also at the end of the study (10 animals/sex/treatment group).

Autopsies were done on all mice which died during the course of the study or that were killed in extremis, and also on those that were sacrificed at 12 months and at the termination of the study. Nine major organs were weighed and sections of various organs and tissues (about 38 different tissues/mouse) and gross lesions were preserved for histopathologic evaluation. At 12 months, these evaluations were done only on tissues from 0 and 900 ppm dosage groups and also on any tissue from 100 and 300 ppm groups which looked tumorous macroscopically. At the termination of the study, tissues from 0, 300 and 900 ppm dosage groups were examined histologically (only stomach, pituitary, uterus and liver were examined from 100 ppm group).

Statistical analysis on body weights, clinical laboratory values and organ weights were done using two-tailed U test according to Mann and Whitney, and Wilcoxon. The survival data were analyzed by the statistical software package using the generalized Wilcoxon test. Statistical analysis of tumor findings was done using the death rate method for malignant tumors and the prevalence method for benign tumors (Peto et al.). Because of the high mortality rate in high dose males, the death rate and the prevalence methods were used combined for the analysis of hepatocellular tumor data.

Interim Sacrifice:

Surviving animals from interim sacrifice groups (originally 20 mice/sex/dosage group) were killed at 12 months.

Achieved Dose Levels:

Average daily drug intake* (mg/kg body wt)

Sex	Dose (ppm) 0	100	300	900
Male		19.37	58.06	162.93
Female		24.99	74.36	217.28

(*The drug intake was calculated from the average daily food intake/animal/group for the whole duration of the study.)

Mortality:

Mortality data is summarized below and it is presented graphically in Figures 9 &10.

Mortality of Mice Receiving Nisoldipine in Diet for 21 Months			
Daily Dose (ppm in diet)	**Number of Mice (M/F)**	**Number of Dead (M/F)**	**% Mortality (M/F)**
	6 Months		
0	50/50	0/0	0/0
100	50/50	2/0	4/0
300	50/50	0/0	0/0
900	50/50	3/1	6/2
	12 Months		
0	50/50	0/2	0/4
100	50/50	3/5	6/10
300	50/50	0/3	0/6
900	50/50	8/5	16/10
	18 Months		
0	50/50	5/21	10/42
100	50/50	11/19	22/38
300	50/50	7/20	14/40
900	50/50	29/21	58/42
	21 Months		
0	50/50	14/28	28/56
100	50/50	15/34	30/68
300	50/50	19/34	38/68
900	50/50	40/32	80/64

The mortality rates in treated females (all groups) were not significantly different from controls at any given interval. However, the incidence of deaths in females was high in all groups including controls from 12 months onward. The mortality rates in males from 900 ppm group, especially at 21 months, were significantly higher ($p<0.001$) compared to controls or other

Fig.9 : Mortality curves of male mice which received in the diet 21 months

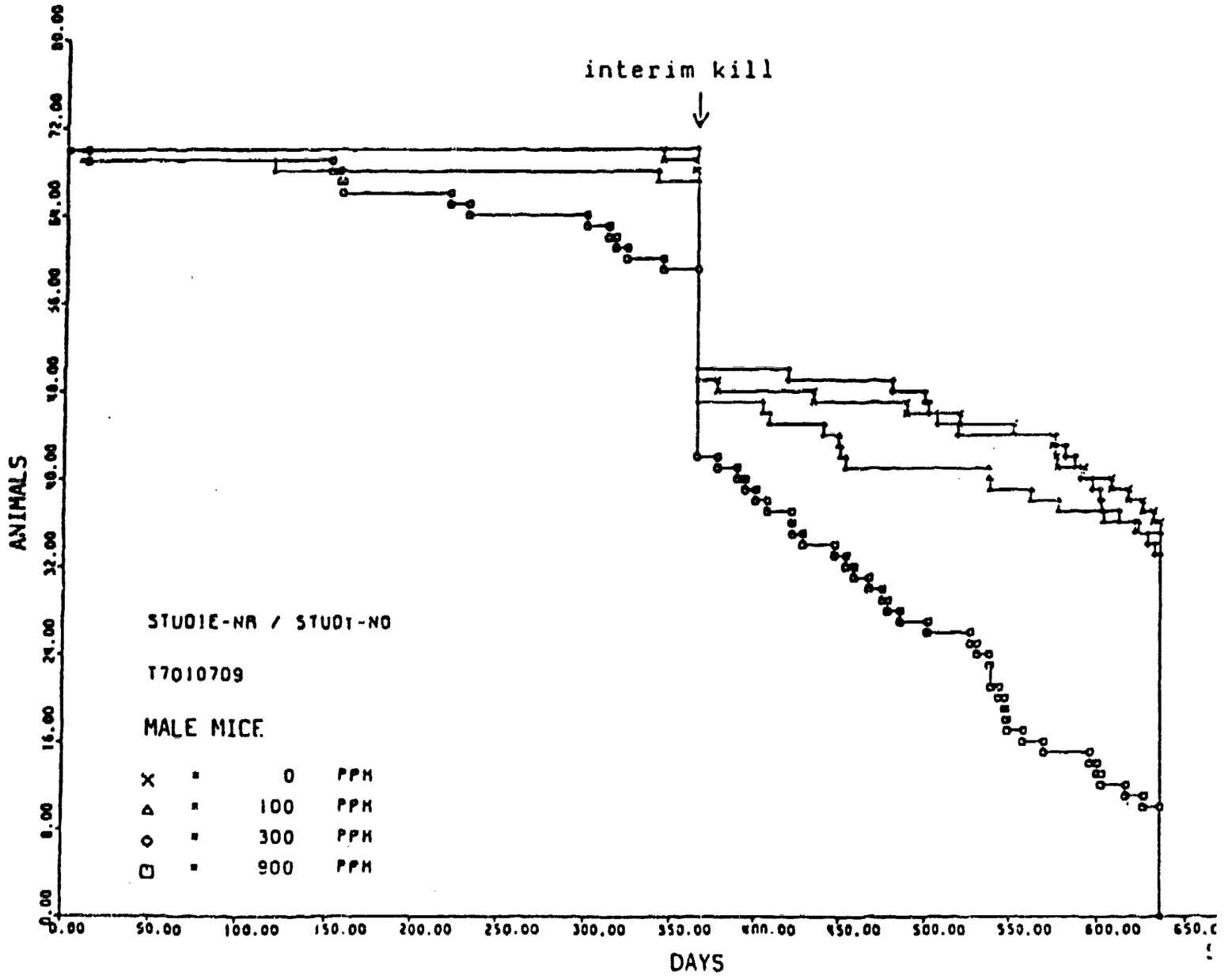
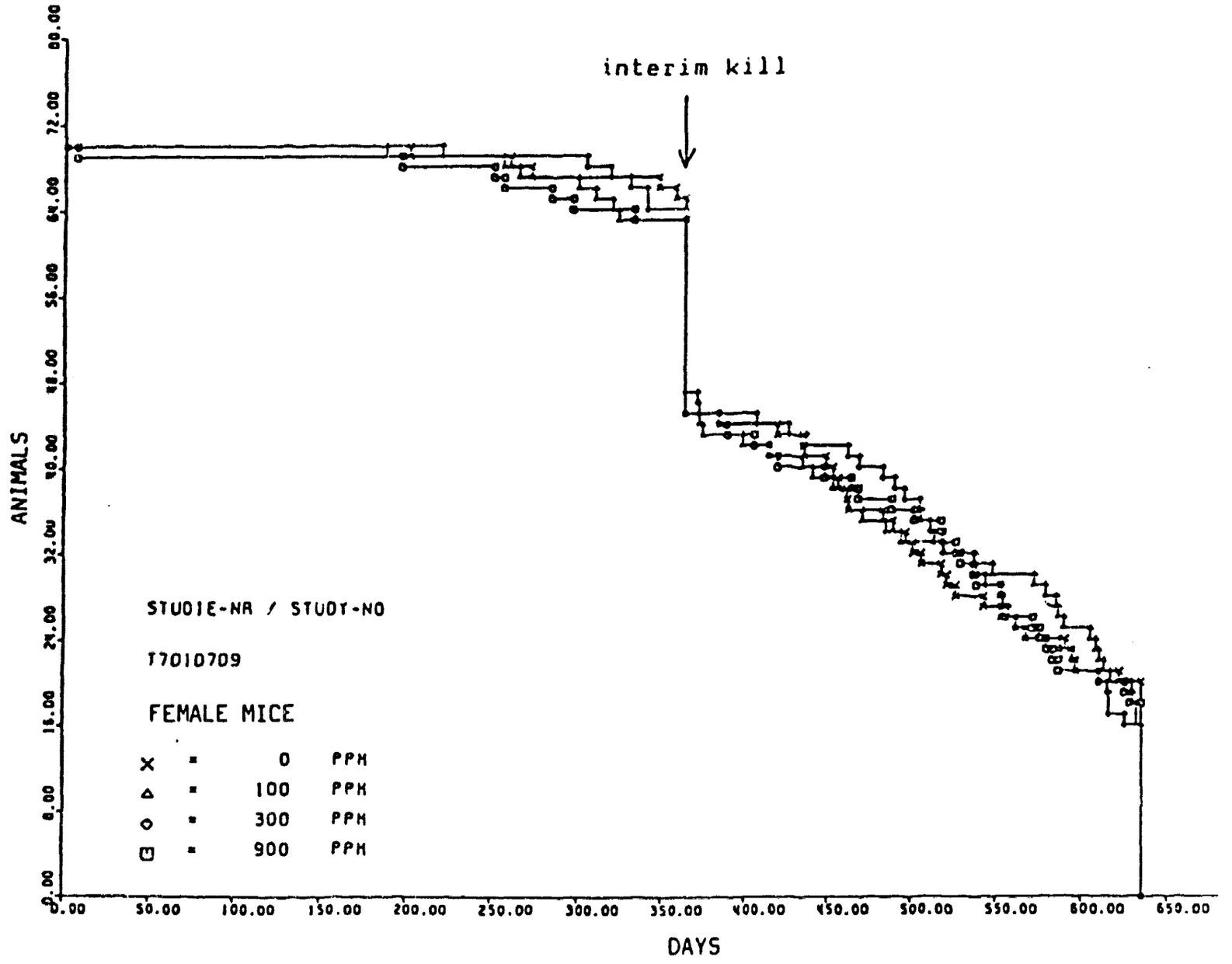


Fig.10: Mortality curves of female mice which received in the diet
21 months



lower dosage groups. No significant difference noticed in this parameter between males of low or middle dosage groups and controls. The increased mortality of males in the high dose group is partly attributed to pharmacodynamically induced colonic atonies. Autopsy of animals that died or were killed in extremis frequently showed that the large intestine was tightly filled with solid faeces resulting from colonic atony.

Using the Cox and the generalized Wilcoxon methods for testing the heterogeneity in survival distribution, FDA statisticians observed a statistically significant difference (at 0.05 level) in the survival distribution in males, but not in females (for both of the above tests, the p values for males were <0.00001).

Drug Associated Findings:

No treatment related clinical signs were seen in this study. The food intake in males from the 900 ppm group was about 9% less than in control males. Average body weights are presented graphically in Figures 11 & 12. Statistically significant reductions in body weights at certain weeks were seen especially in males of 900 ppm group and to a lesser extent in 100 ppm group. However, at the termination of the study, no significant body weight differences were seen between treatment and control groups (both sexes). Leukocyte counts at 21 months were significantly lower ($p<0.01$) in males (900 ppm) and females (300 and 900 ppm groups) compared to respective controls. However, differential counts did not show any significant variations in the proportion of different cell types between treated and control mice. The hemoglobin and hematocrit values in mid and high dose males were significantly lower at 12 months, but not at 21 months. The blood glucose concentration was significantly higher ($p<0.01$) in males (300 and 900 ppm) at 12 months and also at 21 months (all treatment groups). Females showed a similar increase only at 12 months. All these values were reported to be within the range of historical control values. Significant elevations in blood urea levels were seen only at 12 months in all treated male groups and in high dose females.

Macroscopically, swollen gastric mucous membranes were observed more frequently in treated males than in controls (0 ppm - 5; 100 ppm - 12; 300 ppm - 14; 900 ppm - 14). The incidence of enlarged hearts was more in males of 900 ppm group (0 ppm - 7; 100 ppm - 2; 300 ppm - 11; and 900 ppm - 23). In mice that died or were killed in extremis, the incidence of large intestines impacted with solid feces was higher in both sexes at the highest dosage level (0 ppm - males 5 and female 0; 900 ppm - male 13 and female 9).

The heart and liver weights (absolute and relative) in males (21 months) were significantly increased at 300 and 900 ppm dose levels, but the weights of adrenals (absolute and relative) were significantly decreased in all treated male groups compared to controls. In females, the heart and liver weights were

Figure II : Body weight curves for male mice receiving in their food for 21 months

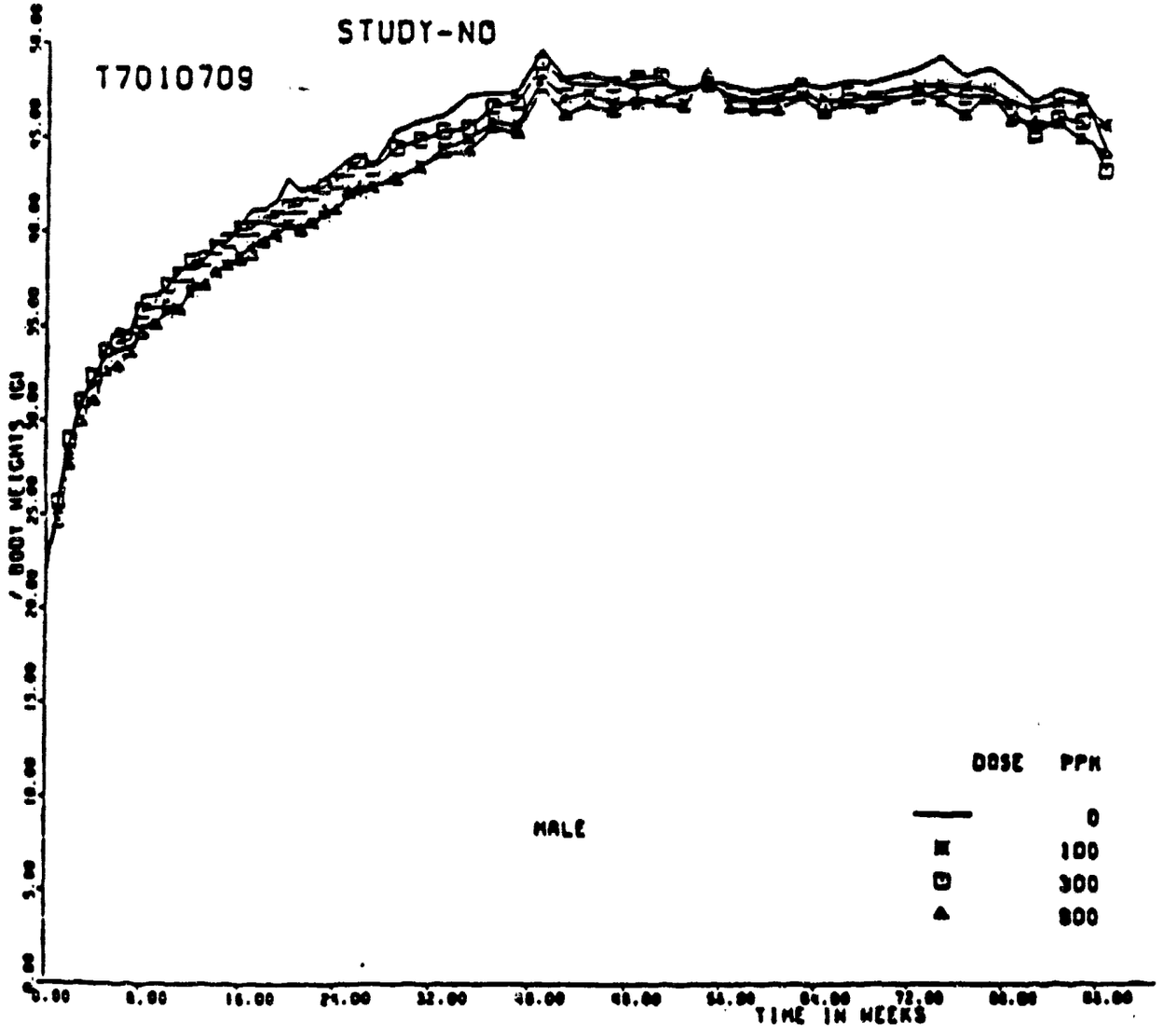
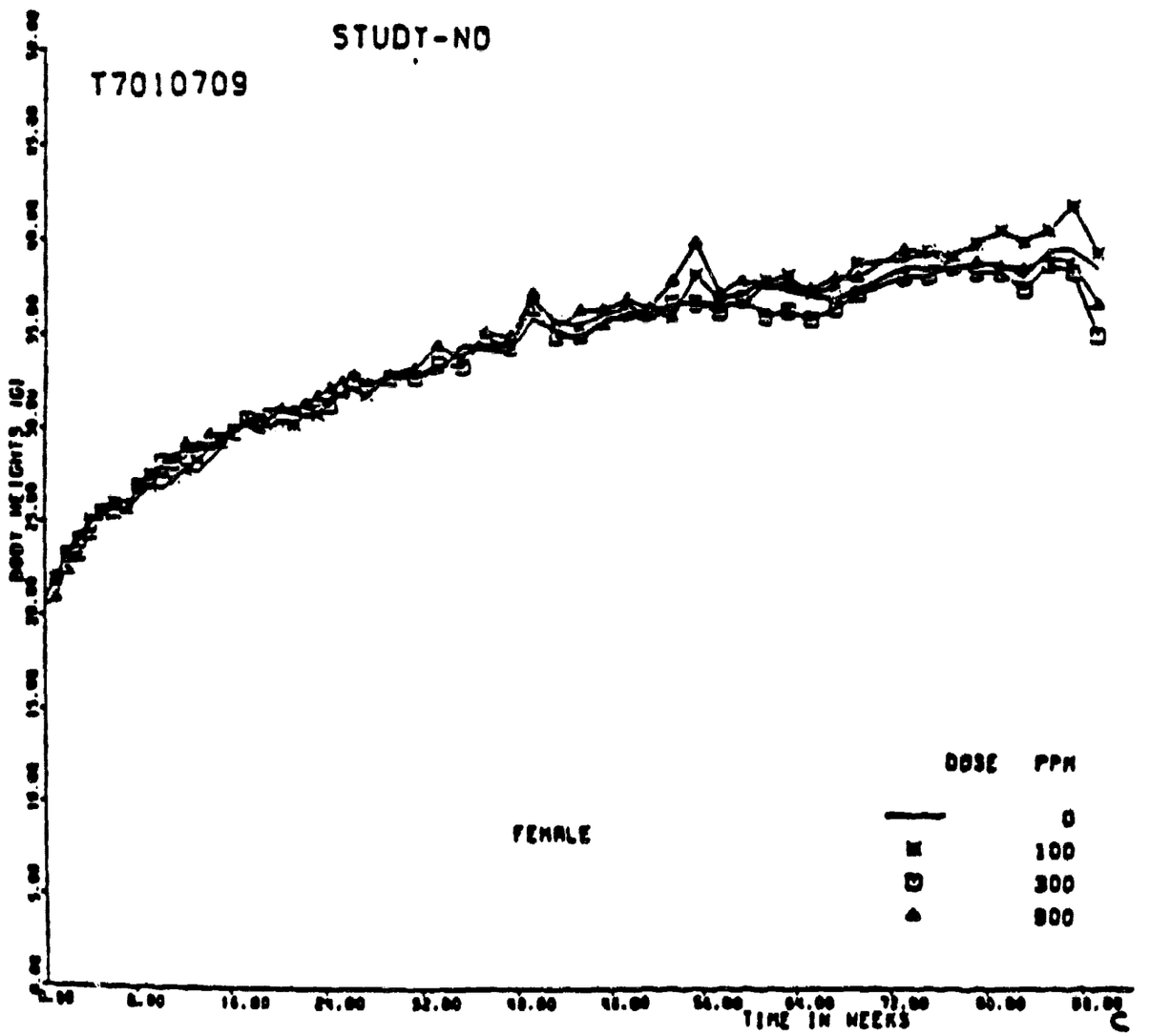


Figure 12: Body weight curves for female mice receiving . in their food for 21 months



significantly higher only at the highest dose level. Females that received either 300 or 900 ppm doses had significantly lower kidney weights at 21 months. Significantly increased liver weights (absolute and relative) were seen in mid and high dose females at the interim sacrifice.

Histologically, hyperplastic mucosa of the glandular stomach was found more frequent in treated males than in females. The incidence of this condition is given below.

Incidence of Hyperplastic Mucosa of the Glandular Stomach

Dose (ppm)	Percent affected	
	Male	Female
0	18	6
100	31	16
300	36	10
900	24	16

The above values are reported to be within the range of historical control values (Table 10).

A dose dependent increase in the occurrence of intracytoplasmic vacuoles near the nucleus was seen in the hepatic cells, more in females than in males. Round cell infiltrates in the kidney and senile nephropathy were predominantly found in females. The endometrium was often found to be hyperplastic (0 ppm - 13%; 100 ppm - 34%; 300 ppm - 33%; and 900 ppm - 28%). The above incidences are reported to be within the historical control ranges for this strain of mouse (Table). An increased incidence of pituitary hyperplasia was seen in females (0 ppm - 17%; 100 ppm - 13%; 300 ppm - 22%; 900 ppm - 42%).

The incidences of tumors observed at the interim sacrifice are given below.

Comparative Summary of Tumors at 12 Months According to Location, Type and Malignancy

Sex:	Males		Females	
Dose (ppm in diet):	0	900	0	900
Reticulocytary system: malignant lymphoma	1	1	2	4
Lung: alveologenic carcinoma (malignant)	0	2	1	2
Liver: hepatocellular carcinoma (malignant)	0	1	0	0
Stomach: kerato-acanthoma	0	0	1	0
Number of blastoma carriers	1	4	4	5
Number of malignant tumors	1	4	3	6
Number of benign tumors	0	0	1	0
Number of mice investigated	20	20	20	20

Table 10

Historic control values: NMRI mouse 1980 to 1984

	Number					
Test No.	1	2	3	4	5	6
Adenomatous gastric mucosal hyperplasias in males	9*	20	10	32	5	27
n	50	50	50	44	47	48
%	18	40	20	73	11	56
in females	1*	8	11	14	10	13
n	50	48	49	46	47	48
%	2	17	22	30	21	27
*classified as adenoma						
Liver tumour in males	7	3	9	5	1	6
n	50	50	50	45	46	48
%	14	6	18	11	2	12
Uterine hyperplasias	0	19	21	23	0	33
n	50	46	49	45	45	46
%	0	41	43	51	0	71

Glucose concentration in the plasma: 4.32 - 9.36 mmol/l (male)
4.51 - 7.75 mmol/l (female)
Urea concentration in the plasma: 5.96 - 15.12 mmol/l (male)
4.05 - 14.99 mmol/l (female)

n = Number of organs evaluated

The increased incidence of lymphoma of reticulohistiocytary system (RHS) observed in high dose females is considered to be incidental since the incidence of this tumor at 21 months was higher in the control group than in treated groups.

The overall incidences of benign and/or malignant tumors and the total number of tumor bearing animals (21 months) for both sexes at 0, 300 and 900 ppm dose levels are given in Table 11. There is no significant increase in the neoplasm incidence among treated animals of either sex compared to respective controls (sponsor's analysis). Moreover, there is also no difference in tumor occurrence between 300 and 900 ppm dosage groups. Incidence of tumors according to the location and the type is presented in Table 12. Because of the increased incidence of hepatocellular tumors in males especially in the 900 ppm group (only few females, 900 ppm group, had this type of tumor) at 21 months and also because of the occurrence of hepatocellular carcinoma in a male mouse from interim sacrifice, an additional investigation was carried out by examining more hepatic tissue sections (5 per animal) from male mice of each group for hepatocellular tumor occurrence. This second study showed additional cases of hepatocellular tumors as follows: 1 each from 100 and 900 ppm groups, 4 from 300 ppm and 1 from control groups. Combined incidences of these tumors (males) from the original and additional investigations (49-50 mice/group) and the p values from the trend test (death rate method) are given below. (The results of sponsor's statistical analyses of liver tumor data are summarized in Table 13.)

	Dose(ppm)				p value
	0	100	300	900	
hepatocellular adenoma	2	2	2	3	0.0735
hepatocellular carcinoma	3	4	5	8	0.0015
hepatocellular tumors (all)	5	6	7	11	0.0004

Thus, the sponsor's analysis showed significant positive linear trends (at 0.05 level) for the incidences of hepatocellular carcinoma and hepatocellular tumors (all) in male mice. Analysis of the tumor data by FDA statisticians showed that there were no significant positive linear trends for the incidences of hepatocellular carcinoma (p=0.0762) and hepatocellular tumors (p=0.0514) in male mice. According to FDA statisticians, the above discrepancies in p values observed in sponsor's and FDA analyses are attributed to "1. the sponsor did not apply the survival-adjusted method and 2. the ordinal dose levels 0, 1, 2 and 3 were used in sponsor's analysis."

FDA analysis of tumor data showed a significant positive linear trend for inverted papilloma of pars cutanea of the stomach in male mice (p=0.0072). The incidence of the above tumor is as follows: 0 ppm - 0/50; 100 ppm - 0/49; 300 ppm - 0/50; and 900 ppm - 2/50. Pairwise comparison also showed significant difference between high dose and control groups (p=0.0435). Historical control data from 21 month studies in NMRI mice, conducted during a 7 year period from July 1981 to August 1988, showed that papillomas of the stomach occurred in 2 of the 18 studies evaluated (page 65e), in 1/49 males and 1/47 females examined (amendment to original application dated May 31, 1994). Moreover, incidence rates upto 4% were seen for the above tumor in NMRI control male mice in carcinogenicity studies conducted between 1974 and 1979 (page 65f). Although statistically significant, the incidence rate (4%) observed in the present study for the stomach papilloma is considered to be within the historical control range for NMRI mice.

Significant positive linear trends were also reported by FDA statisticians for the urinary bladder benign stromal tumor in male mice and RHS malignant lymphoma* in females. However, when the incidences of urinary bladder stromal tumors are combined (benign + malignant, benign + polypous, or benign + malignant + polypous tumors), no statistically significant trend was seen. In the case of RHS malignant lymphoma also, if all malignant lymphomas of different locations are combined, then, no significant linear trend was observed.

*Note: The sponsor has listed <u>all</u> malignant lymphomas, irrespective of locations, under <u>RHS system</u>; however, for some lymphomas, the anatomic site (organ) is specified (e.g. lymphoma of adrenal or heart etc.) but for others no site is given (listed only as lymphomas). By using the combined incidences of all lymphomas, no treatment-related increased incidence of this tumor was seen in sponsor's statistical analysis. [According to NTP guidelines (McConnel et al, 1986. Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. JNCI 76: 283-289), lymphomas of all types can be combined for statistical evaluation.]

DOG STUDIES (S.Stolzenberg)

a. 4-Week Oral Administration Study

Testing Facility

Pharma Report No: 7075
Study No: Not given

Study Dates: 11/8/76 to 12/9/76

GLP compliance: This study predates GLP compliance requirements.

Animals: Purebred beagles, 2 males and 2 females per group were used. At the start of dosing, the animals were 25 to 30 weeks old, with body weights of 7.4 to 11.8 kg.

Dose Levels/Mode of Administration: The test substance (Batch 2/76) was administered at doses of 0, 1, 3 and 10 mg/kg, once daily, 4 to 6 hours before feeding, in a vehicle of polyethylene glycol 400, glycerol and water, in gelatin capsules.

Observations/Measurements: Body weights were obtained before the start of treatment and weekly thereafter. Appearance, behavior, body posture, appetite and feces were checked daily. Neurological investigations (pupillary reflex, patellar reflex and extensor postural reflex) and body temperature measurements (rectal) were conducted pretreatment and after 2 and 4 weeks. Ophthalmoscopy (direct) was performed at pre-treatment and after 4 weeks. ECG measurements (Leads I, II and III) were recorded on the 1st, 11th and 23rd day, immediately before administration and 1 and 24 hours after administration. Femoral artery blood pressure was measured on the 1st, 11th and 23rd day, before administration and 1 and 24 hours after, via a Stratham element, Hellige measuring bridge and Hellige recorder. Blood and 6 hour urine samples were obtained before treatment, then after 1 and 4 weeks, for hematology, blood chemistry and urinalysis. Post-mortem examination included weights of 12 or 13 major organs (including gonads and prostate), gross pathology and complete histopathology (31 or 32 organs).

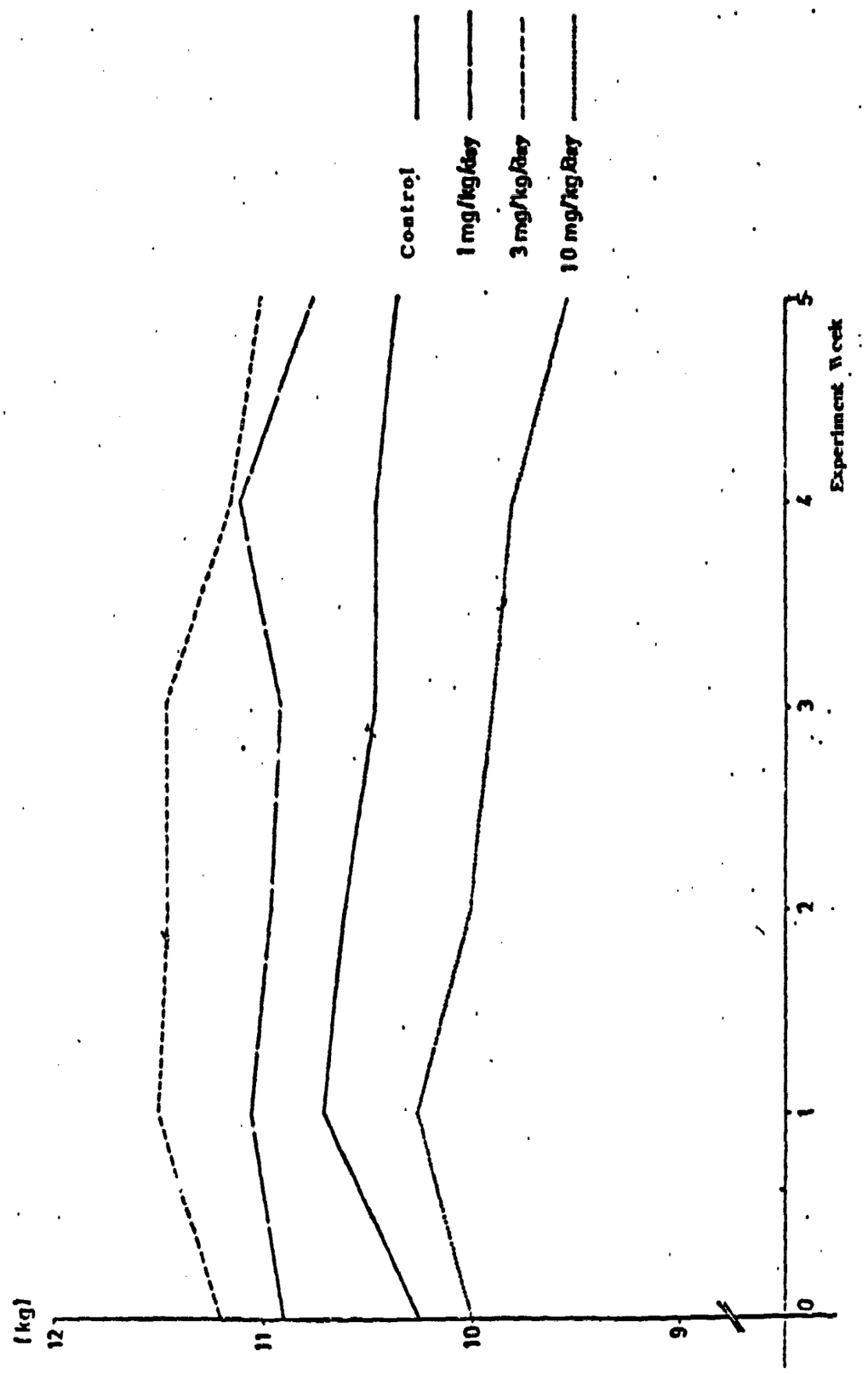
Mortality: There were no deaths.

Drug Associated Findings: Slightly reduced weight gain was observed in the high dose males, with a reduced food consumption in both high dose females and in one high dose male, from the middle of the third week to the end of the study. In the 10 mg/kg treated animals, a distinct ST drop (manifestation of a possible myocardial ischemia) was observed in one male 1 hour after the 1st and 23rd dose, and in one female 1 hour after the 1st dose. No treatment related effects on P or Q waves or QRS complex were observed at any time. Heart rates determined from

ECGs, showed dose dependent increases one hour after dosing on days 1, 11 and 23, and with the high dose, bradycardia was still evident 24 hours after dosing on days 11 and 23. Systolic and diastolic blood pressures at 1 hour post dosing were decreased by a mean of 30 to 50% in all treated groups (dose dependent) on days 1, 11 and 23. As a rule, blood pressures returned to pre-treatment levels by 24 hours after treatment, except after day 1, when they remained lower for the 1 and 10 mg/kg groups.

Although no gross pathology or organ weight changes due to treatment were noted, histopathology revealed that the hearts of both females and 1 of the 2 males on the high dose had myocardial scars in one or both left ventricular papillary muscles. The effect was attributed to hypoxic damage related to vasodilator-induced heart rate increase, "a known damage mechanism in the dog". The ST drops noted above were observed in two of the dogs with myocardial scars. The ST drops and the bradycardia (which was most pronounced in a male with the most severe lesions) were attributed to the heart muscle damage.

Weight Gains of the Male Dogs. The weights were measured in each case at the end of the experimental week.



BAY k 5552

Heart Rate (Beats per Minute)

(Average Values)

Dose mg/kg	Time of Investigation	Before Administration	1 Hour After Administration	% Deviation from 1-Hour Value	24 Hours After Administration
0	Preliminary Investigation	136			
	1st Administration	148	115	- 22	140
	11th Administration	118	108	- 8	125
	23rd Administration	123	123	0	118
1	Preliminary Investigation	158			
	1st Administration	143	253	+ 77	160
	11th Administration	120	235	+ 96	113
	23rd Administration	113	223	+ 97	110
3	Preliminary Investigation	128			
	1st Administration	133	195	+ 47	143
	11th Administration	113	213	+ 88	110
	23rd Administration	98	213	+ 117	90
10	Preliminary Investigation	133			
	1st Administration	133	210	+ 58	148
	11th Administration	68	223	+ 228	75
	23rd Administration	80	200	+ 150	81

Blood Pressure (mmHg)

(Average Values)

Dose mg/kg	Time of Investigation	Before Administration		1 Hour After Administration		% Deviation from 1-Hour Value		24 Hours After Administration	
		s	d	s	d	s	d	s	d
0	Preliminary Investigation								
	1st Administration	172	107	179	99	+ 4	- 7	181	101
	11th Administration	176	95	171	102	- 3	+ 7	174	110
	23rd Administration	181	78	191	96	+ 6	+ 24	187	97
1	Preliminary Investigation								
	1st Administration	171	95	115	63	- 33	- 34	116	83
	11th Administration	176	101	96	54	- 45	- 47	181	110
	23rd Administration	173	94	114	68	- 34	- 28	193	118
3	Preliminary Investigation								
	1st Administration	177	101	99	52	- 44	- 49	179	96
	11th Administration	178	106	78	49	- 56	- 54	176	109
	23rd Administration	178	99	107	53	- 40	- 46	188	96
10	Preliminary Investigation								
	1st Administration	177	94	114	51	- 36	- 47	149	74
	11th Administration	194	111	98	51	- 49	- 54	194	111
	23rd Administration	203	111	116	53	- 43	- 52	231	133

s = systolic pressure d = diastolic pressure

Histological Data

Oral, Dogs (2 Week Experiment)

Animal No.	Sex	Dose and Frequency of Administration	Heart	Lung	Liver	Spleen	Kidney	Adrenals
F 813	♂	Control (0 mg/kg)	0	Ici +	0	0	0	0
F 823	♂	"	0	Ici +	Ici +	0	0	0
F 800	♀	"	0	Ici +	Ici +	0	0	0
F 802	♀	"	0	Ici +	Ici +	0	0	0
F 807	♂	10 mg/kg	Fi2	Ici +	0	0	0	0
F 817	♂	"	0	Ici +	0	0	0	0
F 814	♀	"	Fi1-2	Ici1	Ici +	0	0	0
F 818	♀	"	Fi +	Ici2	Ici +	0	0	V+

List of Abbreviations

Histological Data

At	=	Atrophy
Cy	=	Cyst
Fi	=	Focal fibrosis with isolated mononuclear cells (Figures 4 and 5)
Icl	=	Cellular or inflammatory-cellular infiltration
P	=	Parasitic lesion (bore hole, granuloma, eosinophilic infiltration)
0	=	Finding within the normal variability, which, in particular, corresponds to the species and to the age of the experimental animals and to their conventional housing conditions
ø	=	Not investigated (section missing)
Pi	=	Yellow-green (hematogenous) pigment
Th	=	Thrombus
V	=	Cytoplasmic vacuoles

Intensity of the Changes

+	=	very slight, indicated
1	=	slight
2	=	moderate
3	=	severe

b. 13-Week Oral Administration Study in Dogs

Pharma-Report No: 10,380
Study No: B/K 5552/023

Performing Laboratory:

Dates Performed: 8/21/80 to 11/25/80

Quality Assurance: No statement of GLP compliance is included.

Test Animals: Purebred beagles, 3 males and 3 females per group, were used. At the start of dosing, the animals were 38 to 51 weeks old, with body weights of 6.8 to 10.8 kg.

Dose Levels/Mode of Administration: The test substance (Batch 576,923) was administered at doses of 0, 1, 2.5 and 6.25 mg/kg, once daily, 4 to 6 hours before feeding, in a vehicle of polyethylene glycol 400, glycerol and water, in gelatin capsules.

Observations/Measurements: Body weights were obtained before the start of treatment and weekly thereafter. Appearance, behavior, body posture, appetite and feces were checked daily. Neurological examinations (pupillary reflex, patellar reflex and extensor postural reflex), ophthalmoscopic examinations (direct) and body temperature measurements (rectal) were performed pretreatment and after 2, 5 and 12 weeks. Femoral artery blood pressure w[illegible]s measured at the time of the first dose, and in weeks 3, 6 and [illegible] before administration, and 1 and 24 hours after, via a Strath[illegible]ment, Hellige measuring bridge and Hellige recorder; [illegible]easurements (Leads I, II and III) were recorded at the same time [illegible]eriods. Blood and 6 hour urine samples were obtained before treatment, then after 3, 6 and 13 weeks, for hematology, blood chemistry and urinalysis. Postmortem examination included weights of 12 (female) or 13 (male) organs, gross pathology and complete histopathology (31 or 32 organs for control and high dose, but all 3 doses for heart).

Mortality: There were no deaths.

Drug Associated Findings: Circumoral reddening of the skin and reddening of the conjunctiva in the mid and high dose groups, and ataxia in the high dose group, occurred regularly throughout the treatment period, around 1 hour after dosing. Blood pressure decreased (systolic decreased to a greater extent than the diastolic), and heart rate increased (data on heart rate not provided by sponsor) at 1 hour post dosing in all 3 treated groups. Neither of these two effects were considered to be dose related, and values returned to pretreatment levels by 24 hours post-treatment. No changes in ECG occurred at low and mid doses. One high dose male developed a ventricular tachycardia with a "bundle-branch-block-like deformation of the QRS complex",

diagnosed 1 hour after the first dose. For this animal, another ECG was taken on the following day 2 hours after dosing; the P wave was still elevated and the ST segment again showed sagging depression. "On the 19th day in this dog, no pathological finding in the ECG was observed" but this animal showed extra systoles and an elevated P wave. Serum chemistry effects included a small increase in GOT during week 6. The only compound related post-mortem finding noted was scaring of the left ventricular papillary muscles of 1 male and 1 female at the high dose, and 1 female at the mid dose. Histopathology revealed focal fibrosis with isolated mononucler cells and a cellular and inflammatory-cellular infiltration.

Study No. 5552/023 Blood Pressure (mm Hg) - Average Values

Dose mg/kg	Time of Examination	Before administration		1 hour after administration		% Deviation of 1 hour value		24 Hours after administration	
		s	d	s	d	s	d	s	d
0	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration	210	130	180	110	-14	-15	180	110
	in the 3rd week	200	120	190	120*	- 5	0	205	125**
	in the 6th week	225	135	170	90	-24	-33	165	105**
	in the 13th week	200	120	205	130	+ 2	+ 8	195	120*
1.0	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration	190	115	140	75*	-26	-35	175	115
	in the 3rd week	195	115	135	75	-31	-35	190	120
	in the 6th week	185	115	85	50	-54	-57	175	100**
	in the 13th week	190	120	130	80	-32	-33	195	115**
2.5	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration	190	105	145	70	-24	-33	180	105
	in the 3rd week	195	115	115	60	-41	-48	200	120
	in the 6th week	195	115	100	50	-49	-56	175	105
	in the 13th week	195	120	130	70	-33	-42	175	110
6.25	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration	185	115	110	60	-41	-48	185	125
	in the 3rd week	190	115	110	55	-42	-52	170	110**
	in the 6th week	185	115	80	45	-57	-61	170	105
	in the 13th week	195	120	100	60**	-49	-50	190	125

s = systolic blood pressure; d = diastolic blood pressure *n = 5; **n = 4

5552/Study 023

Animal No.	Sex	Dose	Esophagus	Stomach	Intestine	Mesenteric Lymph Nodes	Thymus	Gall-bladder	Urinary Bladder
K 103	♂	Control	0	0	0	0	0	0	0
K 109	♂	Control	0	0	0	ø	At3	0	0
K 121	♂	Control	0	0	0	0	0	0	0
K 108	♀	Control	0	0	0	0	0	0	0
K 112	♀	Control	0	0	0	0	At2	0	0
K 120	♀	Control	0	0	0	0	ø	0	0
K 93	♂	6.25 mg/kg	0	0	0	0	ø	0	0
K 115	♂	6.25 mg/kg	0	0	0	0	0	0	0
K 117	♂	6.25 mg/kg	0	0	0	0	ø	0	0
K 102	♀	6.25 mg/kg	0	0	0	P/Ic11	At1	0	0
K 104	♀	6.25 mg/kg	0	0	0	P/Ic12	0	0	0
K 118	♀	6.25 mg/kg	0	0	0	0	0	0	0

5552/Study 023

Animal No.	Sex	Dose	Heart
K 103	♂	Control	0
K 109	♂	Control	0
K 121	♂	Control	0
K 108	♀	Control	0
K 112	♀	Control	0
K 120	♀	Control	0
K 93	♂	6.25 mg/kg	0
K 115	♂	6.25 mg/kg	Fi2-3 Ici1
K 117	♂	6.25 mg/kg	0
K 102	♀	6.25 mg/kg	0
K 104	♀	6.25 mg/kg	Fi1
K 118	♀	6.25 mg/kg	0

Animal No.	Sex	Dose	Heart
K 113	♂	2.5 mg/kg	0
K 119	♂	2.5 mg/kg	0
K 123	♂	2.5 mg/kg	0
K 122	♀	2.5 mg/kg	Fi1 Pi+
K 124	♀	2.5 mg/kg	0
K 126	♀	2.5 mg/kg	0
K 79	♂	1.0 mg/kg	0
K 105	♂	1.0 mg/kg	0
K 107	♂	1.0 mg/kg	0
K 110	♀	1.0 mg/kg	0
K 114	♀	1.0 mg/kg	0
K 116	♀	1.0 mg/kg	0

c. 52-Week Oral Administration Study in Dogs

Study No: T 20 10 506

Performing Laboratory:

Dates Performed: July 13, 1981 to July 11, 1982

Quality Assurance: No statement of GLP compliance is included.

Test Animals: Purebred beagles, 4 males and 4 females per group. At the start of dosing, the animals were 38 to 51 weeks old, with body weights of 6.9 to 11.2 kg.

Dose Levels/Mode of Administration: The test substance (batch 57 69 23) was administered at doses of 0, 0.3, 1.0 and 3.0 mg/kg, once daily, 7 days per week, 4 to 6 hours before feeding, in a vehicle consisting of 85.3% polyethylene glycol 400, 4.8% anhydrous glycerol and 9.9% water, contained in gelatin capsules.

Observations/Measurements: Body weights were obtained before the start of treatment and weekly thereafter. General appearance was checked "several times a day". Neurological exams were conducted and body temperatures were checked pretreatment and after 3, 6, 17, 29, 39 and 50 weeks. Ophthalmoscopy was performed pre-treatment and after 12, 31, 38 and 51 weeks. Blood pressure and ECG were measured pre-treatment and after 3, 6 and 17, 29, 39 and 50 weeks, before dosing, then 1 and 24 hours after dosing. The methods and instruments used were the same as in the preceding dog studies. Blood and 6 hour urine samples were obtained before treatment, then after 3, 6, 13, 26, 39 and 52 weeks, for hematology, blood chemistry and urinalysis. Postmortem examination included weights of 11 or 12 organs, gross pathology and complete histopathology (31 or 32 organs for all animals on test). Liver enzyme induction of O-demethylase, N-demethylase and cytochrome P_{450} content of liver homogenates were measured.

Mortality: No deaths occurred.

Drug Associated Findings: Slight reddening of the mucosa and skin, observed in all nisoldipine treated groups, was considered to be due to the vasodilator effect of the drug. Dose related decreases in blood pressure and resultant increases in heart rate were observed. Twenty-four hours after dosing, all values had returned to normal. Slight ST segment depression, T wave inversion and QT segment shortening were observed, which were all reversible (data on ECG could not be found) and considered to be due to increased heart rate.

Study No: T 20 10 506

BLOOD PRESSURE (mm Hg)

(Means n = 8)

DOSE	TIME	before admin.	1 h after admin.	% difference **	24 h after admin.
0.0 mg/kg	1st admin.	195/112	198/113	+1.5/+0.9	199/114
	17th admin.	194/112	193/119	-0.5/+6.3	
	38th admin.	184/102	187/106	+1.6/+3.9	
	114th admin.	210/116	205/116	-2.4/0.0	
	200th admin.	199/108	204/113	+2.5/+4.6	
	269th admin.	211/108	195/110	-7.6/+1.9	
	347th admin.	201/118	204/119	+1.5/+0.8	
0.3 mg/kg	1st admin.	208/119	*167/ 97	-19.7/-18.5	193/115
	17th admin.	203/111	164/ 98	-19.2/-11.4	
	38th admin.	180/ 98	163/ 88	- 9.4/-10.2	
	114th admin.	219/118	171/ 98	-21.9/-16.9	
	200th admin.	191/113	153/ 86	-19.9/-23.9	
	269th admin.	205/114	168/ 90	-18.0/-21.1	
	347th admin.	215/124	184/105	-14.4/-15.3	

* n = 7

** Calculation using unrounded figures

Study No: T 20 10 506

BLOOD PRESSURE (mm Hg)

(Means n = 8)

DOSE	TIME	before admin.	1 h after admin.	% difference**	24 h after admin.
1.0 mg/kg	1st admin.	179/104	141/ 76	-21.2/-26.9	173/104
	17th admin.	177/101	136/ 76	-23.2/-24.8	
	38th admin.	181/ 98	116/ 68	-35.9/-30.6	
	114th admin.	184/106	133/ 75	-27.7/-29.0	
	200th admin.	178/107	135/ 79	-23.6/-26.3	
	269th admin.	196/109	135/ 73	-31.1/-33.0	
	347th admin.	195/115	161/ 93	-17.6/-19.1	
3.0 mg/kg	1st admin.	195/114	116/ 64	-40.7/-43.9	194/115
	17th admin.	200/127	133/ 71	-33.5/-44.1	
	38th admin.	186/112	*106/ 59	-43.0/-47.3	
	114th admin.	212/126	124/ 66	-41.6/-47.6	
	200th admin.	197/119	133/ 76	-32.5/-36.1	
	269th admin.	218/123	115/ 62	-47.2/-49.6	
	347th admin.	206/124	134/ 69	-35.0/-44.4	

* n = 7

** Calculation using unrounded figures

Study No: T 20 10 506

HEART RATES (beats/min)

(means, n = 8)

(calculation using unrounded figures)

DOSE (mg/kg)	Initial Figure	TIME OF INVESTIGATION															
		1st admin.			17th admin.		38th admin.		114th admin.		200th admin.		269th admin.		347th admin.	
		before	1 h	24 h	before	1 h	before	1 h	before	1 h	before	1 h	before	1 h	before	1 h
0.0	134*	120	113*	134	128	132*	136	133	133	121	129	138	119	119	101**	112
Diff. %			-5.9		+3.1		-2.2		-9.0		+7.0		0.0		+10.9	
0.3	138	136	172	141	134	190	137	194	123	177	128	205	118*	178	113*	161
Diff. %			+26.5		+41.8		+41.6		+43.9		+60.2		+50.8		+42.5	
1.0	147	134	191	135	130	209	131	218	106	221	113	220	96	207	111	153
Diff. %			+42.5		+60.8		+66.4		+108.5		+94.7		+115.6		+37.8	
3.0	146	134	211	128	120	234	113	201	101	224	99	215	90	209	93	186*
Diff. %			+57.5		+95.0		+77.9		+121.8		117.2		+132.2		+100.0	

*n = 7
**n = 6

CHRONIC TOXICITY STUDY ON DOGS — B A Y K 5 5 5 2 — STUD.T2 810 506

SYNOPSIS OF GROUP MEANS — CHRONISCHER TOXIZITAETSVERSUCH AN HUNDEN

ANLISTUNG ALLER MITTELWERTE

		HEART / HERZ	LUNG / LUNGE	LIVER / LEBER	KIDNEYS / NIEREN	SPLEEN / MILZ	TESTES / HODEN	OVARIES / OVARIEN	THYROID / SCHILD-DRUESE	ADREN. / NEBEN-NIEREN	THYMUS / THYMUS	PROSTA. / PRO-STATA	BRAIN / GEHIRN	PANC. / PAN-KREAS
ABSOLUTE ORGANWEIGHTS (G) / ABSOLUTE ORGANGEWICHTE (G)														
MALES / MN.TIERE														
CNTR./KONTR.		98.8	96.8	436.2	57.0	30.3	17.30	-	0.825	1.277	5.15	8.887	78.8	33.8
GROUP/GRUPPE	I	108.0	91.8	423.0	56.5	29.8	18.38	-	0.727	1.162	5.32	6.910	75.5	35.8
GROUP/GRUPPE	II	103.3	109.5	484.3	66.3	37.3	26.92	-	0.860	1.335	5.20	5.787	82.3	36.0
GROUP/GRUPPE	III	111.3	93.8	472.0	61.3	68.3	28.42	-	0.805	1.318	5.65	7.340	78.5	33.5
FEMALES / WB.TIERE														
CNTR./KONTR.		95.8	86.8	407.8	53.0	24.3	-	0.937	0.867	1.385	6.96	-	76.5	25.5
GROUP/GRUPPE	I	102.8	96.5	385.5	57.5	27.5	-	0.832	0.797	1.558	7.27	-	73.3	29.5
GROUP/GRUPPE	II	96.8	82.5	393.5	53.3	38.8	-	1.123	0.807	1.672	7.67	-	77.8	32.3
GROUP/GRUPPE	III	103.3	98.3	395.8	59.3	41.8	-	1.787	0.860	1.537	7.17	-	77.8	31.8
BOTH SEXES / ALLE TIERE														
CNTR./KONTR.		97.3	91.4	424.3	55.8	28.3	17.30	0.937	0.846	1.331	6.05	8.887	77.6	29.3
GROUP/GRUPPE	I	105.4	94.1	404.3	57.0	43.6	18.38	0.832	0.762	1.356	6.30	6.910	74.4	32.3
GROUP/GRUPPE	II	100.0	91.5	438.9	59.8	37.6	26.92	1.123	0.834	1.504	6.44	5.787	80.0	33.9
GROUP/GRUPPE	III	107.1	92.0	432.9	60.3	54.5	28.42	1.787	0.832	1.449	6.41	7.340	77.8	32.3
RELATIVE ORGANWEIGHTS (G/KG) / RELATIVE ORGANGEWICHTE (G/KG)														
MALES / MN.TIERE														
CNTR./KONTR.		9.12	9.00	40.25	5.25	2.85	1.667	-	0.0780	0.1200	0.480	0.7577	7.42	3.85
GROUP/GRUPPE	I	10.17	8.62	39.77	5.32	2.85	1.760	-	0.0687	0.1100	0.516	0.6585	7.35	3.30
GROUP/GRUPPE	II	9.15	8.87	42.67	5.85	3.30	1.842	-	0.0757	0.1175	0.462	0.5085	7.30	3.20
GROUP/GRUPPE	III	10.02	8.45	42.73	5.52	6.25	1.842	-	0.0730	0.1109	0.510	0.6627	7.10	3.05
FEMALES / WB.TIERE														
CNTR./KONTR.		9.50	8.55	40.77	5.25	2.50	-	0.0912	0.0865	0.1372	0.667	-	7.65	2.55
GROUP/GRUPPE	I	9.37	8.85	34.88	5.25	5.20	-	0.0755	0.0722	0.1415	0.652	-	6.70	2.65
GROUP/GRUPPE	II	9.72	8.25	44.17	5.32	3.77	-	0.0869	0.0812	0.1698	0.767	-	7.82	3.22
GROUP/GRUPPE	III	9.87	8.62	37.32	5.65	3.85	-	0.1642	0.0805	0.1535	0.670	-	7.45	3.05
BOTH SEXES / ALLE TIERE														
CNTR./KONTR.		9.31	8.77	40.51	5.25	2.67	1.667	0.0912	0.0822	0.1286	0.556	0.7577	7.54	2.80
GROUP/GRUPPE	I	9.77	8.74	41.18	5.29	4.82	1.760	0.0755	0.0705	0.1257	0.575	0.6585	6.92	2.97
GROUP/GRUPPE	II	9.44	8.56	43.42	5.59	3.54	1.842	0.0869	0.0785	0.1432	0.615	0.5085	7.56	3.21
GROUP/GRUPPE	III	9.94	8.54	40.34	5.59	5.05	1.842	0.1642	0.0767	0.1358	0.590	0.6627	7.27	3.05

REPRODUCTIVE TOXICITY STUDIES (S. Stolzenberg)

1. Fertility and Reproduction Ability in Wistar Rats

Bayer Study No: T0002152

This report is accompanied by a "first amendment to report no. 12691", dated 8/11/93. Tables in the original English translation of the report were of very poor quality, not legible, contained errors in translation and typing, and a few tables were not logically organized. Some of these faults are listed in the amendment under the "rationale for the first amendment".

Performing Laboratory:

Dates Performed: 2/81 to 9/81

Quality Assurance: No statement on GLP compliance was found in the original report. In the amendment, it is claimed, "...there were no legally binding GLP regulations in force" during the time that this study was performed.

Test Animals: Mura:WIST (SPF 67 HAN), 24 males and 60 females per group. At the start of dosing, males were 5-7 weeks old, and weighed 74-110 g, females were 8-10 weeks old and weighed 158-190 g.

Procedure: The test substance (batch 576 923) was administered at doses of 0, 3, 10 and 30 mg/kg, once daily, by oral gavage in a vehicle consisting of polyethylene glycol 400:glycerol:water in a ratio of 969:60:100. Males were dosed starting 10 weeks before mating and during the 3 week mating period, females were dosed for 3 weeks prior to mating until the 7th day of pregnancy. Except during mating and lactation, both the males and females were kept in individual Makrolon cages. Each male was paired with 2 or 3 females, which were placed together in a Makrolon cage each night and the females were examined for vaginal sperm in the morning. Half the pregnant females in each group, selected by "statistical methods", were C-sectioned on day 20 of gestation, the remaining half were allowed to litter and raise their young to postpatum day (PPD) 21. All C-sectioned fetuses were examined for external anomalies, 1/3 from each dam were examined for soft tissue anomalies (modified Wilson method) and 2/3 for skeletal malformations (alizarin red S). In addition to examining the F_0 parents for reproductive performance, the F_0 females for lactational performance and the F_1 offspring for survival and weight gain during lactation, one male and one female from each litter of the control group and of the highest dose group were reared to sexual maturity to determine F_1 reproductive capacity. The mated F_1 dams were allowed to litter, and testicular weights for F_1 males were obtained after mating.

Test substance administered was Batch 576 923. It is claimed that the preparations for oral gavage were tested for stability and concentration but the data and details for these tests were not included in the report.

There is no statement on why these doses were selected for this study.

Effects on *F_0 Males*

All treated and control males survived to scheduled necropsy and no compound related clinical signs were evident in males of any treated group. There were no effects on weight gain, mating behavior or fertility in males of any treated group compared to controls. There were no effects on gross pathology observed at necropsy (presumably sacrificed after mating and while still on drug treatment). The drug had no effect on testicular weights (See page which follows).

STUDY ON FERTILITY T0002152

BODYWEIGHTS (G) OF THE MALES BEFORE MATING
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEEK 10	90.5	87.3	91.0	89.9
	9.6	7.7	7.9	7.4
WEEK 9	136.5	132.5	135.4	131.7
	12.7	11.0	10.0	9.2
WEEK 8	175.0	172.0	174.3	173.3
	16.8	14.8	14.3	13.6
WEEK 7	215.5	212.3	215.7	213.5
	22.0	17.0	16.7	17.3
WEEK 6	252.5	249.6	249.6	252.1
	26.4	19.5	18.6	20.2
WEEK 5	279.0	276.3	276.1	280.6
	29.9	21.6	21.9	23.4
WEEK 4	291.5	291.5	287.6	294.5
	31.9	23.4	26.1	23.6
WEEK 3	311.4	309.9	308.8	314.0
	33.5	25.2	25.1	26.0
WEEK 2	331.5	329.7	327.1	331.6
	34.8	27.1	28.5	28.5
WEEK 1	344.5	345.9	341.6	346.9
	35.0	28.8	29.8	30.0
WEEK 0	360.7	358.9	354.7	360.4
	35.6	29.5	31.4	30.8

TESTICLE WEIGHTS (G)

GROUP MEAN VALUES AND STANDARD DEVIATIONS

0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
3.23	3.26	3.17	3.26
0.30	0.20	0.31	0.25

Effects on *F_0 Females*

Mortality: Deaths are listed only in the narrative portion of this report. In both the original report and the amendment, there is no indication of the time of death; not even if the deaths occurred before mating, during pregnancy or lactation. Deaths occurred in two rats at 3 mg/kg, in one at 10 mg/kg and in two at 30 mg/kg, but none of the deaths were attributed to treatment. Based on scrutinization of tables in the original report, the 2 animals in the 30 mg/kg group which died had both been assigned to "rearing animals". Deaths were attributed to misintubation for a low and mid dose rat, "gastrointestinal disorders" for the second low dose rat, to a lung tumor and to pneumonia for the two high dose rats. In addition, one dam in the control group, which had littered 12 pups and died shortly after birth, was not included in the results because at necropsy only 4 nidation sites were found.

Even in the amended tables for individual animal data, there is no indication of which animals died and the time of the deaths. Numerous animals were dropped from the study for a variety of reasons, which included, "not inseminated", "not pregnant", and for a few, there is a statement "animal dropped from the study" but no reason is given. Most summary tables do not specify the number of animals per group upon which the data are based. Therefore, the following table lists the total number of females in each group that were included in the results, based on a count taken from the individual animal body weight data.

Dosage Group	# C-Sectioned*	# Littered*
Control	25	22
3 mg/kg/day	2[illegible]	27
10 mg/kg/day	24	20
30 mg/kg/day	27	22

* There were 60 mated females per group at initiation of the study, 30 of which were designated for C-section or littering.

Body Weight and Body Weight Gain: Mean body weight gains and body weights of pregnant females, those that were C-sectioned and those that were allowed to litter, are given on the two pages which follow. Body weights 3 weeks before mating (prior to initiation of treatment) and during pregnancy, were significantly lower for the high dose group of the C-sectioned animals, but there was no effect on body weight gain. Although a small increase in body weight gain was noted for the low dose C-sectioned group between days 7 and 20 of gestation, there was obviously no effect that could be attributed to treatment. No effects on mean body weight or body weight gain were observed in the females selected for delivery of litters during the 3 weeks prior to gestation, during gestation or during lactation.

STUDY ON FERTILITY T0002152

WEIGHT DEVELOPMENT [G] OF THE FEMALES UNDERGOING CESAREAN SECTION
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEIGHT GAIN				
DAY 0 - 7 P.C.	22.2	23.9	21.7	23.0
	4.7	5.1	5.1	5.6
DAY 7 - 20 P.C.	73.3	82.1*	76.2	71.4
	10.1	12.6	10.8	17.1
DAY 0 - 20 P.C.	95.5	106.0*	97.9	94.4
	12.4	15.5	12.6	18.1
BODYWEIGHTS BEFORE MATING				
WEEK 3	173.6	172.3	170.3	168.6**
	7.0	6.8	6.8	6.3
WEEK 2	187.1	187.1	186.0	184.9
	8.7	9.5	8.7	9.1
WEEK 1	198.9	197.3	198.3	194.9
	10.0	9.3	9.5	9.7
WEEK 0	209.7	210.2	210.8	204.8
	10.9	11.3	11.6	9.7
BODYWEIGHTS DURING GESTATION				
DAY 0 P.C.	223.8	223.2	223.9	213.5**
	11.5	13.9	13.6	12.4
DAY 7 P.C.	245.9	247.0	245.6	236.5*
	13.2	14.3	13.4	13.5
DAY 20 P.C.	319.2	329.1	321.8	308.0
	17.2	23.2	17.4	23.3

* SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.01$
** SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.005$

T0002152

WEIGHT DEVELOPMENT (G) OF THE DAMS
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEIGHT GAIN				
DAY 0 - 7 P.C.	23.7	19.9	23.7	21.4
	6.2	5.1	5.2	4.4
DAY 7 - 20 P.C.	73.5	70.3	77.0	71.7
	13.3	13.8	13.0	14.2
DAY 0 - 20 P.C.	97.2	90.2	100.7	93.1
	14.6	14.5	11.7	15.0
BODYWEIGHTS BEFORE MATING				
WEEK 3	173.7	171.6	172.5	172.4
	8.2	7.9	8.2	8.9
WEEK 2	186.1	186.1	188.8	187.6
	9.2	10.2	10.1	10.1
WEEK 1	194.7	196.8	200.2	198.6
	11.1	11.1	10.3	12.4
WEEK 0	206.3	208.7	211.0	208.7
	13.1	12.8	10.8	14.2
BODYWEIGHTS DURING GESTATION				
DAY 0 P.C.	216.4	225.0	224.1	217.5
	16.4	16.8	13.6	18.7
DAY 7 P.C.	240.1	244.9	247.8	238.9
	19.4	16.5	11.0	20.1
DAY 20 P.C.	313.5	315.3	324.8	310.6
	25.3	26.1	19.6	31.2
BODYWEIGHTS DURING LACTATION				
DAY 1 P.P.	244.6	250.0	253.7	240.3
	18.9	18.1	15.1	21.3
WEEK 1 P.P.	272.3	277.8	281.6	267.1
	20.5	19.4	14.3	24.4
WEEK 2 P.P.	272.5	278.7	283.3	271.7
	18.8	16.8	14.0	20.1
WEEK 3 P.P.	258.6	265.5	266.7	259.4
	19.0	17.8	15.3	18.2

C-Section *F_0 Females*

As seen in the tables which follow, there were no effects of compound treatment on number or percentages of animals inseminated, with implantations and with live fetuses, mean corpora lutea count, nidations, average number of male or female live fetuses, sex ratio or fetal loss. From these data, it is evident that there were no effects on pre- or post-implantation losses.

The mean fetal weights were significantly increased (apparently dose related) in the 10 and 30 mg/kg groups, and the mean placental weight was slightly but significantly increased in the 3 mg/kg group. The investigators claimed that the mean placental weight increase was incidental, and that the mean fetal weights for the mid and high dose groups were within the norm for this strain (given as 3.5 ± 0.27, based on 268 litters).

There were no compound related effects on mean numbers of gross, visceral or skeletal malformations, nor were there any effects on "underdeveloped forms" (fetuses weighing <3 g). There were also no effects on minor skeletal variations.

STUDY ON FERTILITY

NUMBER OF ANIMALS - RESULTS OF THE STUDY

ANIMALS UNDERGOING CESAREAN SECTION

DOSE (MG/KG)	USED	INSEMINATED N	INSEMINATED % OF THOSE USED	NUMBER OF FEMALES WITH IMPLANTATIONS N	NUMBER OF FEMALES WITH IMPLANTATIONS % OF THOSE INSEMINATED	FEMALES WITH FOETUSES N	FEMALES WITH FOETUSES % OF THOSE WITH IMPLANTATIONS
0	30	27	90.0	25	92.6	25	100.0
3	29	27	93.1	23	85.2	23	100.0
10	30	26	86.7	25	96.2	24	96.0
30	30	28	93.3	27	96.4	27	100.0

STUDY ON FERTILITY

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RESULTS OF THE CESAREAN SECTION (MEAN VALUES)

DOSE (MG/KG)	WEIGHT GAIN (G) 0-20 P.C.	WEIGHT GAIN (G) 7-20 P.C.	NUMBER (PER DAM) OF CORP. LUTEA	IMPL.	MALE	FEM.	SUM	LOSS	MEAN-WEIGHT IN GRAMMS FETUSES	PLACENT.	NO. OF FOETUSES EXAMINED BY WILSON	DAWSON	FOETUSES WITH MINOR SKELETAL DEVIAT.	MALFORMATIONS	NO. OF RUNTS (<3G)
0	95.5	73.3	12.4	11.9	6.3	4.9	11.2	0.7	3.49	0.50	3.3	7.9	3.52	0.04	0.52
3	106.0**	82.1**	12.4	12.0	6.3	5.0	11.4	0.6	3.58	0.53*	3.5	7.[illegible]	3.48	0.09	0.30
10	97.9	76.2	11.4	11.0	5.6	5.0	10.6	0.4	3.60*	0.50	3.4	7.6	2.67	0.00	0.25
30	94.4	71.4	11.6	11.0	5.4	4.9	10.3	0.7	3.63**	0.52	3.0	7.3	3.22	0.00	0.19

* SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.025$
** SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.01$

F_0 Females Allowed to Litter

Pregnancy duration was slightly increased in all 3 treated groups (statistically significant for the 3 and 30 mg/kg groups; see table below). This effect was considered to be "incidental" because the mean durations for these groups were within the norm for this strain. No effects during lactation were noted.

Postpartum Examination of Pups: There were no significant effects on total number of live pups at birth per group, nor on number of viable pups after 1, 2 or 3 weeks postpartum (See table below). It was claimed there were no treatment related effects on number of stillborn pups, and on sex ratio at birth or at the 3 weekly intervals. Mean birth weight was slightly higher for all 3 treated groups (statistically significant for low and high dose), but mean weight and weight increase during the 3 weekly intervals were not influenced by treatment (See table below).

Maturational Development: There were no effects on age of pinna unfolding of the ears, hair coat, eye opening or normal gait.

Function Tests: There were no effects on sight or pupillary reflexes to light, hearing ("pinna twitch reflex", tested by means of a Galton whistle with a set frequency and duration). In a proprioreceptive reflex test (running roller brought from stationary position to 10 revolutions per minute) there was a decrease in performance at 30 mg/kg during the first test but no effect in the second or third test. The age of the animals when these tests were done was not indicated.

Fertility Test of F_1 Generation: There was no effect of treatment with 30 mg/kg on mating, fertility, duration of pregnancy, litter size, live and dead pups, sex ratio, mean weights of the pups or external anomalies at birth.

T0002152

DAMS

DOSE (MG/KG)	USED	NUMBER OF FEMALES WITH IMPLANTATIONS N	% OF THOSE INSEMINATED	N	% OF THOSE USED	THAT LITTERED N	% OF THOSE WITH IMPLANTATIONS	THAT REARED THEIR PUPS N	% OF THOSE THAT LITTERED
0	29	24	82.8	22	91.7	22	100.0	22	100.0
3	29	28	96.6	27	96.4	27	100.0	27	100.0
10	29	22	75.9	20	90.9	20	100.0	20	100.0
30	28	26	92.9	22	84.6	22	100.0	22	100.0

DURATION OF PREGNANCY IN DAYS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
21.9	22.2*	22.1	22.2**
0.5	0.6	0.6	0.4

* SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.05
** SIGNIFICANT DIFFERENCE TO CONTROL P < 0.025

NUMBER OF IMPLANTATIONS OF THE DAMS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
10.8	10.4	11.0	11.2
3.0	2.9	2.4	2.8

PRENATAL LOSS OF DAMS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
0.5	0.6	0.4	0.9
0.7	0.9	1.0	1

STUDY ON FERTILITY

T0002152

NUMBER AND WEIGHT DEVELOPMENT OF THE VIABLE PUPS
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION		0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
NUMBER OF PUPS					
AT BIRTH	TOTAL	10.4 3.0	9.8 2.8	10.4 2.5	9.7 2.7
	MALES	5.2 1.8	5.2 2.3	5.2 1.6	4.9 1.8
	FEMALES	5.2 2.3	4.6 1.7	5.3 2.0	4.8 2.1
AFTER 1 WEEK	TOTAL	10.2 3.0	9.7 2.7	10.4 2.5	9.4 2.9
	MALES	5.3 1.9	5.2 2.3	5.2 1.6	4.8 1.8
	FEMALES	5.1 2.3	4.5 1.7	5.3 2.0	4.5 2.3
AFTER 2 WEEKS	TOTAL	10.0 3.0	9.6 2.7	10.3 2.5	9.3 3.0
	MALES	5.0 1.9	5.1 2.2	5.1 1.7	4.7 1.9
	FEMALES	5.0 2.3	4.5 1.7	5.1 2.0	4.5 2.3
AFTER 3 WEEKS	TOTAL	10.0 3.0	9.6 2.7	10.2 2.5	9.3 3.0
	MALES	5.0 1.9	5.1 2.2	5.0 1.7	4.7 1.9
	FEMALES	5.0 2.3	4.5 1.7	5.1 2.0	4.5 2.3
WEIGHT (G) OF THE VIABLE PUPS					
AT BIRTH		5.9 0.5	6.2** 0.5	6.1 0.6	6.2* 0.6
AFTER 1 WEEK		14.0 2.2	14.8 1.6	14.9 1.8	15.0 2.2
AFTER 2 WEEKS		24.7 4.4	26.1 3.5	26.1 3.3	26.4 4.4
AFTER 3 WEEKS		38.1 6.1	40.4 5.8	39.7 5.8	41.5 7.4

* SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.05$
** SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.025$

2. Embryotoxic and Teratogenic Action in Long-Evans Rats

Pharma Report No: 7596 Study No: T2012540

Performing Laboratory:

This study was originally presented as a translation from German with only a few brief summary tables; no individual animal data. Amendments received at CDER on 9/29/93 and 10/8/93 contain tables with individual animal findings and summaries. It is claimed that the study was carried out between January and May, 1977, "in accordance with FDA recommendations", but there is no statement of GLP compliance.

Procedure: Naturally inseminated Long-Evans female (strain FB 30) rats, 20 or 21 per group, 2.5 to 3.5 months of age and weighing 195 to 262 g prior to mating, received 0, 10, 30 or 100 mg nisoldipine/kg/day by oral gavage (batch 3/76, micronized), from days 6 to 15 of gestation. The drug was dissolved in polyethylene glycol 400/glycerol/water. A C-section was performed for each dam on day 20 of gestation and the fetuses were examined for external, visceral (Wilson technique) and skeletal (alizarin red stain) anomalies.

Effects on Survival and Body Weights of Dams: One control rat died on gestation day 13 or 14, due to improper intubation into lungs, and was excluded from results. There was no compound related effect on mortality, nor on "general appearance or behavior" of the dams, but there was a dose related decrease in mean weight gain (see table which follows).

Dose (mg/kg)	Weight Gain in Grams	
	Treatment Period	Total Pregnancy
0	62.3	182.4
10	56.1	140.3
30	53.0*	132.6*
100	49.6*	131.8*

*) Significant difference from the control, $P < 0.01$

C-Section of Dams: Of the 21 inseminated rats in each of the 3 compound treated groups, 20 were pregnant, and all 20 surviving rats in the control group were pregnant. All pregnant treated and control rats had live fetuses at necropsy on day 20 of gestation. Corpora lutea count for each rat was not determined in this experiment, but no statistically significant differences between the treated groups and control were found for mean number of implantations, mean number of fetuses, mean number of dead fetuses and resorbed embryos, mean fetal weight, underdeveloped forms (fetuses <3 g in weight), mean placental weight, frequency of fetuses with minor skeletal deviations, sex distribution (see page 94), nor on external, soft tissue or bone deformations (see table below on this page).

Group	Dam No.	Number of Malformed Fetuses	Malformation
Control	604	2	Rib dysplasia (hump formation)
10 mg/kg	581	1	Edematous head
30 mg/kg	—	—	None
100 mg/kg	605	1	Rib dysplasia (hump formation)
	639	1	Cryptorchidism
	647	1	Kinking of the tail
	683	1	Hydrops universalis, micrognathia, kinking of the tail

T2012540

Results of the Caesarean Section

Mean values of the groups and standard deviations

Note: The mean fetal and placental weights given in the report no. 7596 were calculated by adding all litter weights of the group and by dividing these sums by the number of fetuses or placentas per group. In the following table these mean values are marked with "a".
For the calculation of the standard deviation the mean fetal and placental weights per litter were calculated first and were used for further calculation. Mean fetal and placental weights obtained by this procedure are marked with "b".

Dose mg/kg	Weight gain [g] during pregnancy	Weight gain [g] during treatment period	Number (per dam) of fetuses impl.	fetuses male	fetuses female	fetuses total	Res.**	Mean weight [g] of fetuses	Mean weight [g] of placentas	Number of fetuses with minor skeletal deviations	Number of fetuses with malformations	Number of fetuses runts < 3 g
0	152.4	62.3	11.6	5.8	5.4	11.1	0.4	4.26[a] 4.27[b]	0.57[a] 0.58[b]	2.95	0.10	0.00
	18.5	11.7	1.4	2.3	2.3	1.7	0.7	0.29	0.06	2.11	0.45	0.00
10	148.3	56.1	11.8	5.5	5.0	10.5	0.5	4.07[a] 4.07[b]	0.59[a] 0.59[b]	2.60	0.05	0.05
	23.9	9.7	2.9	1.8	1.6	2.8	0.8	0.29	0.11	2.79	0.22	0.00
30	132.6*	53.0*	11.3	5.3	5.1	10.3	0.9	4.08[a] 4.09[b]	0.57[a] 0.57[b]	3.50	0.00	0.05
	19.9	8.1	2.5	2.4	2.5	2.6	1.3	0.33	0.05	2.61	0.50	0.22
100	131.8*	49.6*	11.9	5.5	5.2	10.7	1.1	4.12[a] 4.14[b]	0.57[a] 0.57[b]	4.10	0.20	0.00
	21.2	11.2	2.7	2.6	2.1	2.7	1.4	0.23	0.05	2.45	0.41	0.00

* significant difference to control, $p < 0.01$ (WILCOXON-MANN-WHITNEY-U-TEST)

** Res. is the abbreviation for resorptions, which the sponsor defined as the total of resorbed embryos and dead fetuses

3. Teratology Study in Sprague Dawley (CD) Rats

Study No: Not provided. LSR Report No. 87/0938

Performing Laboratory:

Sponsor:

Dates Performed: 8/5/87 to 12/2/87

Quality Assurance: A signed statement of GLP compliance is included.

Test Animals: Charles River CD (Sprague Dawley derived) females, 9-10 weeks of age and weighing 200-248 g on the day of insemination, were mated on a 1:1 basis with stock males of the same strain.

Procedure: The test substance (batch number 500139) was administered to 32 inseminated females per group, once daily by oral gavage as a suspension in 0.5% aqueous Tylose, prepared fresh each day, from days 7 to 17 of gestation, at doses of 0, 10, 30 and 100 mg/kg. On day 20 of gestation, 21 dams per group were C sectioned and the fetuses were examined for external anomalies; 1/2 from each dam were examined by free hand serial sectioning (Wilson technique) for soft tissue anomalies. The remaining half were first dissected (neck, thoracic and abdominal cavities) to evaluate for soft tissue anomalies, then were prepared by a modification of Dawson's alizarin staining technique for evaluation of skeletal malformations.

The remaining 11 dams/group were allowed to litter and raise their young to postpatum day 25. On PPD 4, litters with more than 8 were reduced to 8 by random culling, leaving, if possible, 4 of each sex per litter. After weaning, the offspring were housed on a litter basis, but the sexes were separated and there was a maximum of 5 of the same sex per cage. At approximately 5 weeks of age, following completion of behavioral and neuromuscular function tests, 20/sex/group were randomly selected for further assessment of physical, sexual maturation and reproductive performance; unselected ones were killed and grossly examined. At 9 or 10 weeks of age, F_1 males and females were paired 1:1 within treatment groups, avoiding sibling matings. All F_1 mated females were laparotomized on day 20 of gestation; the fetuses were examined only for external malformations and discarded. After gross examination of the F_1 females, F_1 males were killed, then examined externally and internally for macroscopic abnormalities.

Stability of Test Substance: Test formulations for all 3 concentrations, taken from the first and last weeks of treatment,

were generally found to be within approximately 82 to 90% of the target concentrations, and were stable for at least 4 hours after preparation.

Results

F_0 Females

Mortality and Clinical Signs: No data on mortality, and no statement in the text pertaining to mortality, were found; all rats apparently survived. It is claimed that one dam receiving 100 mg/kg showed flaccid muscle tone and piloerection during the early stages of treatment (possibly compound related), but other females in that group were not affected.

Body Weights/Food Consumption: Small reductions in body weight gain (not statistically significant) were evident in all 3 treated groups during the initial day or 2 of treatment (See table on the page which follows), and this was accompanied by significant reductions in food intake by the mid and high dose groups, limited to the first 3 days of treatment. Subsequent body weight gains in all 3 treated groups were not affected by treatment, but the body weights remained below control to the day of necropsy. The lower mean body weights of the mid and high dose groups compared to controls were statistically significant only on day 18 of gestation.

Laparotomy Observations: There were no effects on mean corpora lutea counts, total implantations, viable males or females. There was a slight increase in total resorptions ($P < 0.05$) predominently due to number of late resorptions, and in percent post implantation loss ($P < 0.05$), in the high dose group. Fetal weights were depressed in all 3 treated groups; statistically significant and dose related at mid and high dose (See table two pages ahead).

Fetal Evaluation: There was an increased incidence of small fetuses (<2.7 g) and litters with one or more small fetuses in the 100 mg/kg group. An increased number of fetuses with slightly increased dilatation of lateral ventricles and/or space between the body wall and organs, occurred mainly in two litters, and this was associated with fetuses of low body weight in these two litters. The investigators considered this to be indicative of fetal immaturity. Also associated with the fetuses weighing <2.7 g in 2 litters of the high dose group and considered to be due to fetal immaturity, was an increased incidence of incomplete ossification of basisphenoid, first thoracic vertebral centrum, sacral vetebral arches, ischia, metacarpals and metatarsals.

Group mean bodyweights (g) of females during gestation

Group	:	1	2	3	4
Compound	:	Control	--		--
Dosage (mg/kg/day)	:	0	10	30	100

Group		Day of gestation														
		0	3	7	8	9	10	11	12	13	14	15	16	17	18	20
1	Mean	219	238	255	261	266	272	278	284	291	298	307	317	330	346	378
	SD	8	9	12	11	12	12	12	13	13	13	14	15	16	17	18
	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
2	Mean	217	237	251	256	261	267	273	279	285	292	300	312	323	338	368
	SD	10	12	14	12	13	12	14	13	14	14	15	16	16	17	18
	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
3	Mean	217	237	253	255	260	266	272	278	284	291	299	311	323	338*	367
	SD	11	13	14	13	14	14	14	15	15	15	17	17	18	19	21
	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
4	Mean	215	235	253	250	252	261	267	272	278	285	293	303	316	327***	361
	SD	8	9	12	12	12	13	12	12	13	13	13	16	17	18	20
	n	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31

SD Standard deviation.
n Number of pregnant animals.
* Bodyweight gain from Day 7 significantly different from Controls, P<0.05 (one way analysis of variance and Student's t-test).
*** Bodyweight gain from Day 7 significantly different from Controls, P<0.001 (one way analysis of variance and Student's t-test).

Group mean litter data - females killed on Day 20 of gestation

Group	: 1	2	3	4
Compound	: Control	---		-
Dosage (mg/kg/day)	: 0	10	30	100

Group	Number of pregnant animals		Corpora lutea count	Implantations	Viable young			Resorptions			Implantation loss (%)		Foetal weight (g)	Placental weight (g)
					M	F	Total	Early	Late	Total	Pre-	Post-		
1	21	Mean	17.1	15.6	8.0	6.9	14.9	0.5	0.1	0.7	9.4	4.3	3.50	0.53
		SD	1.7	1.3	2.3	1.9	1.1	0.7	0.4	0.8			0.06	0.01
2	21	Mean	16.9	14.9	7.0	7.0	14.0	0.8^{NS}	0.1	0.9^{NS}	11.6	6.1^{NS}	3.45	0.53
		SD	1.5	1.4	1.8	2.0	1.5	0.9	0.4	1.0			0.06	0.02
3	21	Mean	16.4	14.9	6.4	7.1	13.5	1.2^{NS}	0.1	1.4^{NS}	9.8	9.3^{NS}	3.34*	0.54
		SD	1.7	1.4	2.3	2.3	2.4	1.1	0.4	1.2			0.06	0.02
4	20	Mean	16.9	15.1	6.7	6.8	13.5	0.9^{NS}	0.8^{NS}	1.6^{+}	11.2	10.6^{+}	3.19***	0.52
		SD	1.9	3.3	2.5	2.6	3.6	0.9	0.9	1.3			0.09	0.03
Background control (159 studies)														
Mean			15.9	14.5	6.7	6.9	13.7	0.69	0.18	0.87	8.7	6.0	3.32	0.58
Low			13.9	12.0	5.2	5.6	11.1	0.05	0.00	0.25	1.6	1.7	3.00	0.43
High			19.0	16.7	8.2	8.7	15.3	1.68	0.58	1.79	16.5	12.7	3.55	0.57

SD Standard deviation.
* Significantly different from Control, P<0.05 (Nested analysis of variance and weighted t-test).
*** Significantly different from Control, P<0.001 (Nested analysis of variance and weighted t-test).
NS Not significant (Mann Whitney 'U'-test).
+ Significantly different from Control P<0.05 (Mann Whitney 'U'-test)

Summary of foetal observations at necropsy

Group	: 1	2	3	4
Compound	: Control	-		--
Dosage (mg/kg/day)	: 0	10	30	100

Group :	1	2	3	4	Control data	
External examination						
Number of foetuses (litters) examined:	313(21)	294(21)	284(21)	269(20)	39809 foetuses	159 studies
Number of male : female foetuses:	168:145	147:147	135:149	134:135		
Observations: % incidence[4] (number of litters)					Mean	Study ranges
Small foetus (less than 2.70 g)	1.0(3)	0.7(2)	1.4(4)	11.9(6)	3.5	0 - 16.9
Large foetus (more than 4.00 g)	2.9(3)	1.4(3)	1.4(2)	-	1.3	0 - 8.7
Shiny pup	-	0.3(1)	-	1.1(1)	0.3	0 - 4.1
Pale pup	-	-	-	0.4(1)	0.02	0 - 1.1
Domed head	-	0.3(1)	-	-	0.01	0 - 0.4
Subcutaneous haemorrhage on chin	0.3(1)	-	-	-	0.1	0 - 0.7
Small placenta (less than 0.30 g)	-	-	-	0.4(1)	0.2	0 - 2.3
Large placenta (more than 0.70 g)	2.2(4)	2.0(6)	3.2(4)	3.0(3)	1.3	0 - 6.2
Conjoined placentae	-	0.3(1)	-	-	0.03	0 - 0.8
Dark green material surrounding placenta	-	0.3(1)	-	-	0.1	0 - 6.9
Short tail	-	-	-	0.4(1)	0.01	0 - 0.5
Threadlike tail	0.3(1)	-	-	-	0.02	0 - 0.8
Imperforate anus	0.3(1)	-	-	-	0.04	0 - 0.8

[4] One foetus may have more than one observation.

continued

Summary of foetal observations after free-hand serial sectioning

Group	1	2	3	4
Compound	Control			--
Dosage (mg/kg/day)	0	10	30	100

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined.	156(21)	148(21)	143(21)	134(20)	12835	126
Number of males : females	82:74	78:70	78:73	68:66	foetuses	studies
Observations: % foetal incidence (number of litters affected)					Mean	Study ranges
Abdomen:						
Diaphragmatic hernia	0.6(1)	-	-	0.7(1)	0.1	0 - 1.9
Small additional liver lobe(s)	25.6(19)	32.4(18)	23.1(18)	28.4(17)	0.8	0 - 10.9
Hepatic haemorrhage(s)	9.0(8)	12.2(15)	7.7(7)	9.0(11)	10.3	0 - 27.7
Localised internal abdominal haemorrhage	1.9(3)	-	0.7(1)	2.2(3)	1.3	0 - 6.7
Haemorrhagic peritoneal fluid	0.6(1)	-	0.7(1)	-	2.4	0 - 18.0
Haemorrhagic abdomen	0.6(1)	0.7(1)	0.7(1)	-	1.7	0 - 8.9
Left kidney displaced slightly towards midline	-	-	-	0.7(1)	0.05	0 - 1.7
Small haemorrhage within capsule of right kidney	-	0.7(1)	-	-	0.02	0 - 1.8
Unilateral hydronephrosis	1.3(1)	3.4(3)	1.4(2)	1.5(2)	2.6	0 - 11.7
Bilateral hydronephrosis	-	1.4(1)	-	-	0.9	0 - 9.8
Unilateral hydroureter	13.5(12)	7.4(6)	7.7(6)	14.2(13)	6.7	0 - 24.2
Bilateral hydroureter	2.6(2)	7.4(4)	2.8(4)	4.5(4)	4.4	0 - 21.1
Testis(es) displaced slightly†	11.8(7)	12.8(7)	8.6(6)	10.3(7)	3.7	0 - 23.5
Fluid-filled vesicle at anal edge of genital tubercle	-	0.7(1)	-	-	*	*
Genital tubercle slightly elongated	-	-	-	3.0(2)	0.4	0 - 6.3
Blood in anus	-	1.4(2)	0.7(1)	1.5(2)	0.2	0 - 7.2
Threadlike tail; imperforate anus; displacement of adrenal glands and kidneys	0.6(1)	-	-	-	0.08	0 - 1.8
Tip of tail threadlike and hooked	0.6(1)	-	-	-	*	*

* One foetus may have more than one observation.
† Percentage calculated on number of male foetuses.
* No record in background control data.

continued

Summary of foetal observations after free-hand serial sectioning

Group	:	1	2	3	4
Compound	:	Control	---		-
Dosage (mg/kg/day)	:	0	10	30	100

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined.*	156(21)	148(21)	143(21)	134(20)	12835	126
Number of males : females	82:74	78:70	70:73	68:66	foetuses	studies
Observations: % foetal incidence (number of litters affected)					Mean	Study ranges
Thorax:						
Oesophagus displaced to right of trachea	-	-	-	0.7(1)	0.01	0 - 1.2
left lobe of thyroid gland very reduced in size/absent	-	-	-	0.7(1)	0.1	0 - 2.2
Space between bodywall and organs	-	2.0(2)	-	11.2(5)	10.9	0 - 47.4
Retro-oesophageal right subclavian artery and misshapen thymus gland	-	0.7(1)	-	-	0.02	0 - 1.2
Blood-filled thoracic lymph duct	0.6(1)	1.4(2)	0.7(1)	-	0.3	0 - 2.9
Innominate artery reduced in length/absent	-	1.4(2)	1.4(1)	0.7(1)	0.1	0 - 1.4
Gross cardiovascular abnormality with valve defects	-	-	-	0.7(1)	*	*
Gross cardiovascular abnormality with double outlet right ventricle	-	-	-	0.7(1)	*	*
Slightly increased amount of pericardial fluid	1.3(2)	0.7(1)	1.4(2)	0.7(1)	0.3	0 - 5.3
Slightly haemorrhagic pericardial fluid	-	0.7(1)	1.4(1)	-	0.2	0 - 9.1
Haemorrhages on edge of lung lobes	-	-	-	1.5(2)	0.1	0 - 2.2

* One foetus may have more than one observation.
* No record in background control data.

continued

Summary of foetal observations after free-hand serial sectioning

Group	:	1	2	3	4
Compound	:	Control	---		-
Dosage (mg/kg/day)	:	0	10	30	100

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined.*	156(21)	148(21)	143(21)	134(20)	12835 foetuses	126 studies
Number of males : females	82:74	78:70	70:73	68:66		
Observations: % foetal incidence (number of litters affected)					Mean	Study ranges
Others:						
Subcutaneous haemorrhage(s):						
Lower/side of jaw	3.2(3)	7.4(4)	4.2(4)	5.2(3)	0.8	0 - 8.9
Submandibular	2.6(2)	3.4(5)	6.3(7)	6.0(6)	1.0	0 - 8.8
Nasal	1.3(2)	3.4(5)	0.7(1)	2.2(2)	1.1	0 - 5.9
Cranial	1.9(3)	4.1(6)	1.4(2)	2.2(3)	2.5	0 - 8.2
Ventral/dorsal cervical	0.6(1)	2.7(3)	1.4(2)	6.7(5)	1.1	0 - 5.8
Scapular	11.5(12)	17.6(11)	16.1(12)	15.7(11)	28.6	6.4 - 90.5
Lateral/ventral/dorsal thoracic	2.6(3)	5.4(7)	7.7(8)	5.2(5)	1.5	0 - 5.8
Fore-/hind-limb(s)	17.3(10)	13.5(7)	10.5(7)	16.4(12)	*	*
Lateral/dorsal abdominal	0.6(1)	2.0(3)	0.7(1)	0.7(1)	0.6	0 - 3.8
Anal region	-	0.7(1)	-	0.7(1)	0.5	0 - 10.0
Tail	-	-	0.7(1)	0.7(1)	0.7	0 - 10.6
Subcutaneous oedema - trunk	2.6(2)	6.1(4)	4.2(5)	9.0(8)	3.6	0 - 17.5

* One foetus may have more than one observation.
* No record in background control data.

continued

Summary of foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	---		-----
Dosage (mg/kg/day)	:	0	10	30	100

Group :	1	2	3	4	Control data	
Number of foetuses (litters) examined:	157 (21)	146 (21)	141 (21)	135 (20)	19316 foetuses	129 studies
Observations :Grand Mean % foetal incidence @ (number of litters)					Mean	Study ranges
Vertebrae,limbs and girdles						
Ossification of ventral arch of 1st cervical vertebra.	7.0 (7)	4.8 (6)	7.1 (5)	2.2 (3)	6.94	0.0 - 22.2
Incomplete ossification, one or more cervical vertebral arches.	0.6 (1)	1.4 (2)	0.7 (1)	0.0 (0)	0.50	0.0 - 5.2
1st thoracic vertebral centrum unossified.	0.6 (1)	1.4 (1)	0.7 (1)	7.4 (2)	1.10	0.0 - 5.5
Incomplete ossification, one or more thoracic vertebral centra.	27.4 (15)	19.9 (16)	25.5 (17)	27.4 (14)	26.66	8.6 - 58.3
Incomplete ossification of one or more lumbar vertebral centra.	0.0 (0)	0.7 (1)	0.0 (0)	0.7 (1)	0.41	0.0 - 2.5
Incomplete ossification of one or more lumbar vertebral arches.	0.0 (0)	1.4 (2)	0.0 (0)	0.0 (0)	0.12	0.0 - 1.8
Incomplete ossification of sacral vertebral centra.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	0.01	0.0 - 0.5
Incomplete ossification of one or more sacral vertebral arches.	1.9 (3)	2.1 (3)	3.5 (5)	9.6 (4)	1.17	0.0 - 6.2
Short tail, tip thickened.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	*	
25 pre-sacral vertebrae.	1.3 (2)	0.0 (0)	2.8 (4)	0.7 (1)	0.81	0.0 - 6.7
Incomplete ossification of caudal vertebrae (less than 5).	1.3 (2)	1.4 (2)	1.4 (2)	14.1 (5)	2.96	0.0 - 14.5
Metacarpals/metatarsals 3/4.	69.4 (20)	56.2 (20)	80.1 (21)	74.1 (19)	67.30	28.6 - 86.9
Metacarpals/metatarsals 4/4.	29.3 (14)	43.2 (16)	19.9 (10)	16.3 (12)	30.65	6.2 - 71.4
Metacarpals/metatarsals incompletely ossified or unossified.	6.4 (5)	4.8 (5)	5.0 (6)	14.1 (6)	3.05	0.0 - 10.8
One or more phalangeal bones ossified.	1.3 (1)	6.2 (5)	2.1 (2)	0.7 (1)	1.89	0.0 - 8.1
Inner corners of one or both scapulae unossified.	5.7 (6)	2.7 (2)	9.2 (9)	5.2 (4)	3.59	0.0 - 14.4
Pubic bones incompletely ossified or unossified.	7.6 (6)	4.1 (6)	7.1 (5)	14.8 (8)	7.43	0.0 - 18.6
Incomplete ossification of one or both ischial bones.	1.9 (3)	2.1 (3)	5.0 (3)	5.2 (5)	0.90	0.0 - 4.7
Asymmetric pelvis, ilial bones associated with different sacral vertebrae.	0.0 (0)	0.0 (0)	1.4 (2)	0.7 (1)	0.49	0.0 - 3.7

@ One foetus may have more than one observation
* New parameter, no control data available

- continued

Summary of foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	---		---
Dosage (mg/kg/day)	:	0	10	30	100

Group :	1	2	3	4	Control data	
Number of foetuses (litters) examined:	157 (21)	146 (21)	141 (21)	135 (20)	19316 foetuses	129 studies
Observations :Grand Mean % foetal incidence @ (number of litters)					Mean	Study ranges
Sternebrae and ribs						
Incomplete ossification of 1 sternebra.	18.5 (13)	19.2 (12)	5.2 (8)	11.9 (9)	13.53	0.0 - 40.0
Incomplete ossification of 2 sternebrae.	66.9 (21)	65.1 (21)	75.9 (21)	48.9 (18)	66.88	43.3 - 84.8
Incomplete ossification of 3 sternebrae.	7.6 (8)	11.6 (7)	11.3 (11)	23.0 (14)	11.80	1.1 - 23.3
Incomplete ossification of 4 sternebrae.	4.5 (4)	3.4 (4)	1.4 (2)	7.4 (9)	3.68	0.0 - 17.5
Incomplete ossification of 5 sternebrae.	0.0 (0)	0.0 (0)	2.1 (1)	3.0 (2)	0.00	0.0 - 3.8
Incomplete ossification of 6 sternebrae.	1.3 (2)	0.0 (0)	0.0 (0)	5.2 (2)	0.53	0.0 - 6.7
1st sternebra cleft.	0.6 (1)	0.7 (1)	0.7 (1)	5.2 (4)	0.86	0.0 - 7.6
One or more sternebrae offset.	2.5 (4)	1.4 (1)	2.1 (3)	0.8 (0)	1.32	0.0 - 5.2
Ribs 13/13.	100.0 (21)	97.3 (21)	98.6 (21)	97.0 (20)	98.1*	92.5 -100.0
Ribs 13/14.	0.0 (0)	1.4 (2)	1.4 (2)	1.5 (2)	1.20	0.0 - 4.2
Ribs 14/14.	0.0 (0)	1.4 (2)	0.0 (0)	1.5 (2)	0.51	0.0 - 3.5
14th rib enlarged.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	*	
13th rib or ribs reduced in length.	1.3 (2)	2.7 (3)	2.8 (3)	2.2 (2)	2.31	0.0 - 13.4
Slight medial thickening of one or more ribs.	0.0 (0)	0.7 (1)	0.0 (0)	0.0 (0)	0.02	0.0 - 1.4

@ One foetus may have more than one observation
* New parameter, no control data available

Summary of foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	-----	---	---
Dosage (mg/kg/day)	:	0	10	30	100

Group :	1	2	3	4	Control data	
Number of foetuses (litters) examined:	157 (21)	146 (21)	141 (21)	135 (20)	19316 foetuses	129 studies
Observations :Grand Mean % foetal incidence @ (number of litters)					Mean	Study ranges
Head						
Small anterior fontanelle.	1.9 (2)	0.0 (0)	0.7 (1)	0.0 (0)	1.33	0.0 - 11.2
Medium anterior fontanelle.	96.2 (21)	98.6 (21)	99.3 (21)	91.9 (19)	96.20	76.4 -100.0
Large anterior fontanelle.	1.9 (3)	1.4 (2)	0.0 (0)	8.1 (2)	2.46	0.0 - 23.6
Incomplete ossification of supra-occipital bone.	24.2 (12)	14.4 (10)	12.8 (11)	19.3 (11)	13.48	0.0 - 29.2
Incomplete ossification of interparietal bone.	49.0 (20)	40.4 (18)	40.4 (18)	41.5 (18)	28.40	4.9 - 91.0
Incomplete ossification of parietal bone.	0.6 (1)	1.4 (2)	0.0 (0)	2.2 (1)	1.30	0.0 - 7.1
Incomplete ossification of squamosal bone.	0.0 (0)	0.7 (1)	0.0 (0)	3.0 (2)	0.84	0.0 - 4.7
Incomplete ossification of frontal bone.	1.3 (2)	0.0 (0)	0.0 (0)	3.0 (2)	0.09	0.0 - 1.7
Discrete unossified area in frontal bone.	0.6 (1)	2.7 (3)	2.1 (3)	2.2 (2)	0.43	0.0 - 4.6
Incomplete ossification of nasal bone.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	0.02	0.0 - 0.6
Discrete unossified area in basioccipital bone.	0.0 (0)	0.0 (0)	0.7 (1)	0.0 (0)	0.12	0.0 - 2.4
Incomplete ossification of basioccipital bone.	0.0 (0)	0.7 (1)	0.0 (0)	0.6 (8)	0.01	0.0 - 0.6
Incomplete ossification of basisphenoid, craniopharyngeal canal enlarged.	0.0 (0)	0.0 (0)	1.4 (2)	2.2 (2)	0.19	0.0 - 14.3
Incomplete ossification of basisphenoid bone.	4.5 (7)	7.5 (8)	11.3 (9)	13.3 (8)	0.94	0.0 - 12.6
Presphenoid bone incompletely ossified or unossified.	0.6 (1)	0.0 (0)	0.0 (0)	4.4 (2)	0.12	0.0 - 5.4
Fronto-nasal suture enlarged.	1.9 (3)	0.7 (1)	0.7 (1)	4.4 (2)	1.13	0.0 - 5.3
Incomplete ossification of hyoid bone.	7.0 (6)	5.5 (6)	9.9 (3)	5.9 (4)	7.28	0.0 - 25.0
Hyoid bone unossified.	12.7 (8)	11.6 (9)	7.1 (8)	5.2 (6)	8.52	0.0 - 18.5

@ One foetus may have more than one observation

F_0 Dams: Postnatal Phase

There was a slight increase in gestation length in the 30 mg/kg (n.s.) and 100 mg/kg ($P< 0.05$) groups, from a mean of 22.5 days in control to a mean of 23.0 days in the 100 mg/kg group). Although body weights of the drug treated animals tended to be higher than control, no significant intergroup differences in body weight were observed during lactation.

F_1 Offspring to 5 Weeks of Age

There were no compound related effects at any dose level on number of stillbirths, litter size or sex ratio at birth, number of implantation sites, survival indices throughout lactation, body weight or body weight gain during lactation.

There were no compound related effects on physical development (time of pinna unfolding, hair growth, testis descent, tooth eruption, eye opening and vaginal opening).

There were no effects of compound treatment on auditory and visual function (tested on PPD 25), within-cage activity (measured by means of electronic detectors and infra-red light apparently on PPD 26 to 27), learning ability (water filled Y-maze on PPD 27) and neuromuscular function (traversing flat and round rods, rotorod treadmill, mid-air righting reflex, fore- and hind-limb wire hanging and grid-griping ability on PPD 28-30). There were no effects on body weight or body weight gain to 5 weeks of age.

F_1 Offspring Between 5 and 10 Weeks of Age

The 20 males and 20 females per group, selected at 5 weeks of age, were assessed primarily for physical and sexual maturation and reproductive performance.

There were no compound related effects in males or females on appearance, behavior, body weights, mating performance at 9 or 10 weeks of age or on fertility.

In F_1 females killed on day 20 of gestation, there was no evidence of effects on implantation, embryo/fetal survival, fetal weights or placental weights.

No gross pathology abnormalities were observed in F_1 males or females that were considered to be related to treatment of the F_0 females.

4. Embryotoxic and Teratogenic Effects in Rabbits

Report No: 7595
Study No.: Not given

Performing Laboratory:

Dates Performed: 10/77 to 1/78 (first test)
1/78 to 4/78 (second test)

Quality Assurance: These studies were performed prior to the time that GLP compliance was required. The investigators in Germany claim that to the best of their knowledge, the study was performed "according to the state of the art".

Test Animals: Sexually mature female Himalayan rabbits, around 2 to 3.5 kg body weight, inseminated twice by means of copulation with males of the same strain and similar age.

Procedure: There were two separate but related studies in which batch 1/77, micronized were used. In the first study, the test substance was administered once daily by stomach tube, as a suspension in a vehicle consisting of "60 g anhydrous glycerol, 100 g demineralized water and polyethylene glycol 400 to make up 1129 g". Twelve or 13 does/group were treated from days 6 to 18 of gestation with doses of 0, 3, 10 or 30 mg/kg. On day 29, a C-section was carried out and each doe was examined for number of implantations (no data on corpora lutea count), number of live and dead fetuses and embryos, weight of litter and placentae. Each fetus was sexed, then examined for external, visceral and skeletal anomalies. Because of diarrhea in 4 animals of the 30 mg/kg group and spontaneous abortion in 2 of these 4 does, a second study limited to 0 and 30 mg/kg (n = 12 or 13/group), with a vehicle consisting of 0.5% aqueous Tylose, was performed. It was suspected that the vehicle contributed to the diarrhea in the first study. In all other respects, the procedure was the same as in the first study.

Results of the First Study

Maternal Survival, Clinical Signs and Body Weight Gain

One death at high dose (day 27 p.c.) was attributed to diarrhea on multiple days; 1 death at mid dose (day 9 p.c.) was attributed to an intubation accident.

The 30 mg/kg dose "caused" diarrhea in 4 animals (1, with multiple days of diarrhea, died; the remaining 3 had diarrhea on only 1 day, either on day 16 or 17 p.c.). Spontaneous abortions occurred in 1 doe at 3 mg/kg (day 25 p.c.), 1 at 10 mg/kg (day 28 p.c.), and 2 at 30 mg/kg (days 18 and 27; both had diarrhea). None of the control does aborted. Although there was only one

more spontaneous abortion in the high dose group than in the low or mid dose groups, the investigators nevertheless suggested that the incidence in the high dose group was increased, and attributed this effect to diarrhea associated with the vehicle and treatment. The abortions at low and mid doses were considered to be neither treatment related nor significant because the observed rates were considered normal for the strain of rabbit used.

Only females found to be pregnant were included in calculation of mean body weight values (N = 13, 12, 12 and 10 for control, 3, 10 and 30 mg/kg groups). Decreases in body weight gain between days 6 and 18 and over the entire period of gestation in all 3 treated groups were not statistically significant (See table on page 105A).

Laparoscopic Observations

There were no significant effects on mean numbers of implantations or resorptions per doe. The mean number of live male fetuses per doe was reduced in the 30 mg/kg group vs control ($P< 0.05$) resulting in a reduction in ratio of males:females but it was considered to be a random occurrence. There was no effect on fetal or placental weights, and no increase in number of "underdeveloped forms" (i.e. fetuses lower than 2.5 g body weight). There was an increase in malformation rate in the high dose group; the high dose malformations occurred in the offspring of the three animals that had diarrhea and were suggested to be the result of maternal stress (see pages 104 to 105A).

Results of the Second Study:

Maternal Survival, Clinical Signs and Body Weight Gain

Diarrhea or other clinical signs did not occur in does of the 30 mg/kg group, but one doe on 30 mg/kg died of an intubation accident (sometime between days 18 and 29, p.c., based on individual animal weight gain tables). Final results were based on 11 surviving does in each group. There was a decrease in mean body weight in the treated group, compared to an increase in control, between days 6 and 18 (treatment period), resulting in a compound related decrease in body weight gain over the entire gestation period.

Laparoscopic Observations

There were no compound related effects on pregnancy rate, spontaneous abortion rate, mean number of implantations (corpora lutea were not counted) or live fetal count, but mean fetal weight and placental weight were lower ($P<0.05$) and the number of "underdeveloped forms" was higher in the treated group. One of the dams on 30 mg/kg had 5 fetuses with "reduced motility". In this second study, there was no effect on sex ratio at birth, as had been observed in the first study. There was no increase in

external, visceral or skeletal malformations in any treated group vs control (See tables on pages 105B, C & D).

Comment: There were indications of fetal toxicity at 30 mg/kg, a dose that was maternally toxic. For example, there was a reduction in live male fetuses per dam and suggestions of an increase in total number of fetuses with malformations and the total number of litters with fetuses that had malformations, compared to control, in the first study. The total number of runts was higher and mean fetal weights were lower than control in the second study. However, there was no increase in incidence of any specific form or class of terata and no clear indication that this substance was teratogenic in rabbits.

Report No. 7595

Incidence table of the findings of the fetuses#

Findings	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
MENINGOCELE	1(1)[a]			
TELENCEPHALON dysplasia	1(1)[a]			
CRAWN-HAND slight	1(1)[a]			
TONGUE small/sharp/thin		1(1)		
FORE-LIMB abnormal position				1(1)[a]
arthrogryposis	1(1)[b]			1(1)[a]/1(1)[b]
MULTIPLE MALFORMATION			1(1)	1(1)[a]/[illegible][b]
CLEFT PALATE				1(1)[a]
MOTILITY reduced				5(1)[b]

# on individual basis; values in () on litter basis
a = first study / b = second study

Report No. 7595

Individual clinical findings of the dams

Note: animals without findings are not listed

Dose (mg/kg)	Dam-No.	Findings
0 (1st study)	914	on day 26 p.c. no stool, from day 27 p.c. very reduced stool
3	899	abortion on day 25 p.c.: 3 placentas
10	876	abortion on day 28 p.c.: 4 fetuses with placentas
	896	found dead on day 9 p.c. (lung application)
30 (1st study)	877	abortion on day 27 p.c.: 3 fetuses and 3 placentas
	881	on day 13 p.c. red bordered eyes on day 16 p.c. scratch wounds on day 17 p.c. diarrhea
	885	on day 17 p.c. diarrhea
	905	on day 18 p.c. diarrhea
	921	on days 17 and 18 p.c. diarrhea from day 24 p.c sick, reduced stool on day 25 p.c. bloody urination found dead on day 27 p.c
	928	abortion on day 18 p.c.: 3 fetuses
0 (2nd study)	-	-
30 (2nd study)	950	from day 9 p.c. sick, decreased feed consumption, no stool on one day between day 18 and 25 p.c. diarrhea found dead on day 25 p.c

5. Teratogenicity Study in Cynomolgus Monkey

Bayer Study No: T 3 022 847

Performing Laboratory:

Sponsor:

Dates Performed: 2/23/87 to 6/16/87

Quality Assurance: A signed statement of GLP compliance was included. The statement notes two deviations from 21CFR 58: 1) "the stability of the test article/carrier mixture had not been determined at the time of the study", and 2) "the final report of the study does not contain all the information specified in subsection 58.185. In particular, no analytical data relating to the test article or test article/carrier mixture are included."

Justification for Species Selection: "...because of its similar hormonal profile during pregnancy to that of man and this particular non-human primate submits itself as a favourable species for reproductive toxicology studies."

Doses Tested: 0, 30 and 100 mg/kg

Procedure: Feral cynomolgus monkeys (Macaca fascicularis) were obtained from and from . The females were "sexually mature", quarantined for "at least 2 weeks" and acclimatized to laboratory conditions for 3 weeks, before initiation of the study. There were 10 (30 mg/kg) or 12 females (0 and 100 mg/kg) per group that had tested positive for pregnancy (by a mouse uterotropic test); these animals weighed 2.6 to 3.8 kg on day 20 post-coitum. Pregnancy was subsequently monotored by rectal palpation on specified days between GD 30 and 86. Test substance was administered by intragastric intubation between gestation days (GD) 20 and 50, and necropsies for C-section were done on GD 100 ±1 day. Examination for fetal malformations included a comprehensive external examination, including head and body size measurements and appearance of externally observable organs. Soft tissue examination consisted of "a full necropsy of each fetus with visual macroscopic inspection" of the organs and weight measurements of 12 organs. After weighing, the organs were fixed in formalin, but not further evaluated. The skeleton was cleared and stained and examined for skeletal anomalies.

Test Substance: Batch No. 828 305. The vehicle consisted of 9.9% demineralized water, 4.8% glycerine and 85.3% Lutrin (polyethylene glycol 400). Test mixtures, consisting of separate solutions for each dose level, were prepared daily. The report notes that "Analysis of formulations and proof of absorption were not required by the study sponsor."

Results:

Spontaneous Abortions, Clinical Observations and Mortality: One control and 1 high dose monkey were found to be not pregnant, thus leaving 11, 10 and 11 monkeys in control, low and high dose groups, respectively. Incidence of abortions and deaths are summarized below.

Dose mg/kg	#/Gp*	# Abortions	# Deaths
Control	11	6 (GD 26-56)	3 (GD 21-99)
30	10	7 (GD 28-53)	1 (GD 48)
100	11	8 (GD 33-59)	5 (GD 27-88)

*Includes only pregnant animals.

Symptoms included heavy bleeding (followed by abortion), reduced food intake, diarrhea and vomiting. Symptoms were observed only during intervals of treatment, and included animals in the control group. Although all of the symptoms were generally more frequent or of longer duration in drug treated monkeys, they are considered to be due to treatment with the vehicle.

Two of the 3 control animals that aborted, subsequently died. The increased incidence of spontaneous abortions and deaths in the 100 mg/kg dose treated group were considered treatment related. (See comments on next page.)

The death of an additional animal in the high dose group which was found to be not pregnant and is not included in these results, was also considered to be treatment related. Causes of death in the 3 pregnant control monkeys included gastro-enteritis, catarrhal enteritis and "signs of asphyxia". Of the drug treated animals which died, the one in the 30 mg/kg group, and 4 of the 5 in the 100 mg/kg group, each had a volvulus (an intestinal obstruction due to twisting of the bowel); 3 of these animals (all high dose) exhibited abdominal distention shortly before death, and one of these 3 had acute catarrhal enteritis. A volvulus was considered to be a compound related cause of death; no volvulus was seen in any control animals.

Blood Analyses: Blood samples were taken from each monkey on GD 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90 and 97. However, data on blood analyses of any kind could not be found.

Fetal Examinations: External, visceral and skeletal findings for each surviving fetus are shown on the page which follows. In spite of the very few surviving fetuses (3 control, 2 mid dose and 1 high dose), the investigators concluded that the external and skeletal malformations seen in the sole surviving high dose fetus were drug related and were due to severe maternal toxicity. It was claimed that these malformations had never

before been observed in controls.

Fetal Organ Weights: Although weights of 10 or 11 different fetal organs with means (and standard errors only for controls) are presented in Table 4 and Appendix III of the report, statistical comparisons were obviously not possible (only one or two surviving fetuses in the treated groups).

Comments: It should be noted that the control incidence of symptoms, mortality and spontaneous abortions were "unusually high". Although the investigators attributed the higher incidence of abortions and deaths in the high dose group to treatment, there are no indications that the differences were statistically significant. The animals used in the present study were feral monkeys. It seems reasonable to suggest that there may have been an interaction between disease or parasitic infestations (often inherent in feral monkeys), stress of handling or control vehicle administration, and treatment with the high dose, which would confound the outcome of this study. The monkey is not a commonly used model for reproductive toxicity tests and there is limited background information. The limited number of offspring (usually one per monkey) is considered to be a disadvantage for using this species for this type of study. The high mortality and abortion rates, even in control animals, further limits the usefulness of this study. However, the only surviving fetus in the in the high dose group showed malformations "which were never before observed in control fetuses".

Group 1 - 0 mg/kg

Female number	Fetus alive	Type of defect		
		External findings	Visceral findings	Skeletal findings
33797	+	bent tail end	left adrenal severely enlarged	6th to 10th and 12th rib on the right side and 7th to 9th rib on the left side of uneven thickness; 4th to 6th sternebra not ossified, 7th sternebra incompletely ossified
33383	+	no abnormalities detected	no abnormalities detected	5th to 11th rib on the right side and 4th to 11th rib on the left side of uneven thickness; 1st to 7th sternebra not ossified
28980	+	no abnormalities detected	no abnormalities detected	5th to 11th rib on the right side and 6th to 10th rib on the left side of uneven thickness; 6th sternebra not ossified and 7th sternebra incompletely ossified
149	+	no abnormalities detected	no visceral investigation due to caesarian section on day 76 p.c.	no skeletal investigation due to caesarian section on day 76 p.c.

Group 2 - 30 mg/kg

Female number	Fetus alive	Type of defect		
		External findings	Visceral findings	Skeletal findings
34738	+	prepuce not patent	no abnormalities detected	displaced zygostyle; 5th to 11th rib on the right side and 8th to 11th rib on the left side of uneven thickness; 1st to 2nd and 6th to 7th sternebra not ossified
34733	+	bent tail end	no abnormalities detected	parietals incompletely ossified; 6th to 13th rib on the right side and 2nd to 3rd, 7th to 11th and 13th rib on the left side of uneven thickness; 2nd and 6th to 7th sternebra not ossified

Group 3 - 100 mg/kg

Female number	Fetus alive	Type of defect		
		External findings	Visceral findings	Skeletal findings
34040	+	left forelimb appears thinner than normal; tail shortened and inwards curved tail end; only three fingers on the left side and 3rd finger with two nails	no abnormalities detected	additional ossification site between the metacarpals of the 2nd and 3rd finger, additional fingernail on the 3rd finger, proximal phalanx of the 2nd and 3rd finger and medial phalanx of the 3rd finger on the left side abnormally developed; the last three coccygeal vertebrae asymmetrically and incompletely ossified; 5th to 11th rib on the right side and 7th to 12th rib on the left side of uneven thickness; 1st to 3rd and 6th to 7th sternebra not ossified

6. Perinatal and Postnatal Effects Following Oral Administration to Rats

Study No: T1002153

This report is accompanied by a "first amendment to report no. 12801 A", dated 8/14/93. Tables in the original English translation of the report were of very poor quality, not well organized, not legible, and contained errors in translation and typing. Some of these faults are listed in the amendment under the "rationale for the first amendment".

Performing Laboratory:

Dates Performed: May 1981 to October 1981

Quality Assurance: No statement on GLP compliance was found in the original report. In the amendment, it is claimed, "...there were no legally binding GLP regulations in force" during the time that this study was performed.

Doses Tested: 0, 3, 10 and 30 mg/kg.

Test Animals: Mura:WIST (SPF 67 Han) female rats, 11-14 weeks of age and weighing 177-240 g, were mated with stock males of the same strain.

Procedure: Presumed pregnant females were dosed orally by gastric intubation from gestation day (GD) 16 to postpartum day (PPD) 21. Half the females in each group were C-sectioned on GD 20, the remainder were allowed to litter and rear their young to PPD 21. Of the C-sectioned animals, approximately 1/3 of all the fetuses in each group were examined for visceral anomalies by the Wilson technique, the remainder were evaluated for bone anomalies by staining with Alizarin red. The abdominal and thoracic organs of animals selected for bone anomaly examination were removed and "evaluated".

Test Substance: Batch No. 576 923. The vehicle consisted of Lutrol 400, anhydrous glycerol and demineralized water in a ratio of 969:60:100. A preparation of 0.6% nisoldipine was tested for stability and was found to be stable after 7 days.

Effects on All Pregnant Females: There were two deaths, one at 3 mg/kg (cause of death not determined), and one at 10 mg/kg (died of pneumonia), but no treatment related effects on mortality or clinical signs were evident. A small but significant decrease in body weight gain was found for the dams at 30 mg/kg (both the C-sectioned and rearing groups) between the first day of administration (GD 16) and GD 20 (See tables on pages 113 & 113A). Body weights of the low dose group of the C-sectioned rats were significantly higher than control throughout gestation, even on GD 0, but obviously this was not a compound related effect.

There were no compound related effects on reproduction parameters (percent inseminated, percent with implantations, etc.; see table on a page 114), nor were there any effects on gross pathology.

Examination of C-Sectioned Females: There were no effects found on mean number of implantation sites, live or dead fetuses or resorptions per dam. Furthermore, there were no effects of treatment on sex distribution, mean placental weight, number of runts (fetuses <3 g), or frequencies of external, visceral or bone deformations. A decrease ($P< 0.01$) in mean fetal weight at the 30 mg/kg dose was evident (See table on page 115). The table which follows immediately below is a summary of malformations found in all 4 groups, copied from the original report.

Group	Dam No.	Number of changed fetuses	Changes
Control	-	-	-
3 mg/kg	2995	1	no tail
10 mg/kg	2849	1	Otocephaly
	2852	2	slight dilation of the lateral ventricle of the brain
30 mg/kg	2991	1	Cryptorchism

Effects on F_0 Dams Allowed to Litter: There were no effects on duration of pregnancy at any dose level. The report indicates a significantly higher number of implantations per dam in the high dose group (probably incidental and not treatment related), and no effect on prenatal loss (implantations - surviving and dead pups). Complete litter losses were reported for 2 dams in the 30 mg/kg group (both did not suckle their young), and for 1 in the 10 mg/kg group (devoured its young during the 3rd week), but normal lactational behavior was observed in all other dams (not shown in tables but indicated narratively in the original report).

Effects on F_1 Offspring: There was an increase in number of stillborn pups, and a dose related increase in mortality of the newborn pups during the first week postpartum in the 10 and 30 mg/kg groups (See table on page 115B; no statistical analysis). The birth weight and the weight increase during the 3 weeks postpartum were both significantly reduced in the 30 mg/kg group vs control (See table on page 115C). The report claims no compound related effects on appearance or clinical signs of the F_1 offspring. In the maturational development tests, there were no effects of treatment on time to pinna unfolding, appearance of fur or eye opening, but there was a slight delay in time for normal walking in the 30 mg/kg group. For the functional tests, there was no effect on pupillary reflex ("following a light in a darkened room"; age when given is not stated), and no effect on hearing (stimuli from a Galton whistle "at the end of the lactation period"). Running performance on a running roller

(sensomotor behavior or proprioreceptor reflexes) was considerably reduced in the 3 mg/kg group, but this was considered an incidental finding because there was no effect at the 10 and 30 mg/kg dose levels. For the F_1 generation fertility test, 1 male and 1 female in each litter of the control and high dose groups were reared to 10 weeks of age, then mated. There were no differences between the 2 groups in rate of insemination or fertilization, duration of pregnancy, total number of live male or female pups at birth, body weight of pups or pups with external deformities.

PERI- AND POSTNATAL-STUDY T1002153

WEIGHT DEVELOPMENT (G) OF THE FEMALES UNDERGOING CESAREAN SECTION
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEIGHT GAIN				
DAY 0 - 16 P.C.	59.8	64.4	60.1	57.8
	9.6	9.9	11.1	10.3
DAY 16 - 20 P.C.	38.4	40.2	39.2	33.0*
	10.2	8.0	6.6	5.4
DAY 0 - 20 P.C.	98.3	104.5	99.3	90.8
	16.4	16.3	14.6	12.5
BODYWEIGHTS DURING GESTATION				
DAY 0 P.C.	201.0	210.0*	201.8	203.1
	11.8	12.2	10.1	11.3
DAY 16 P.C.	260.8	274.4*	261.9	260.9
	12.9	16.3	16.9	16.5
DAY 20 P.C.	299.3	314.5*	301.1	293.9
	20.8	20.0	20.6	16.8

* SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.025$
** SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.01$

PERI- AND POSTNATAL-STUDY T1002153

NUMBER OF ANIMALS RESULTS OF THE STUDY

DAMS

DOSE (MG/KG)	USED	NUMBER OF FEMALES							
		INSEMINATED		WITH IMPLANTATIONS		THAT LITTERED		THAT REARED THEIR PUPS	
		N	% OF THOSE USED	N	% OF THOSE INSEMINATED	N	% OF THOSE WITH IMPLANTATIONS	N	% OF THOSE THAT LITTERED
0	25	25	100.0	21	84.0	20	95.2	20	100.0
3	25	25	100.0	20	80.0	20	100.0	19	95.0
10	25	25	100.0	23	92.0	23	100.0	22	95.7
30	25	25	100.0	20	80.0	20	100.0	17	85.0

ANIMALS UNDERGOING CESAREAN SECTION

DOSE (MG/KG)	USED	NUMBER OF FEMALES					
		INSEMINATED		WITH IMPLANTATIONS		WITH FOETUSES	
		N	% OF THOSE USED	N	% OF THOSE INSEMINATED	N	% OF THOSE WITH IMPLANTATIONS
0	25	25	100.0	20	80.0	20	100.0
3	25	25	100.0	20	80.0	19*	95.0
10	25	25	100.0	21	84.0	19*	90.5
30	25	25	100.0	23	92.0	22	95.7

* ONE FEMALE DIED BEFORE CESAREAN SECTION

PERI- AND POSTNATAL-STUDY T1002153

RESULTS OF THE CESAREAN SECTION (MEAN VALUES)

DOSE [MG/KG]	WEIGHT GAIN [G] 0-20 P.C.	16-20 P.C.	NUMBER (PER DAM) OF IMPL.	MALE	FEM.	SUM	LOSS	MEAN-WEIGHT IN GRAMMS FETUSES	PLACENT.	NO. OF FOETUSES EXAMINED BY WILSON	DAWSON	FOETUSES WITH MINOR SKELETAL DEVIAT	MALFORMATIONS	NO. OF RUNTS [<3G]
0	98.3	38.5	11.0	5.1	5.2	10.3	0.7	3.50	0.50	3.1	7.1	4.80	0.00	0.60
3	104.5	40.2	11.4	5.1	5.5	10.6	0.8	3.52	0.52	3.3	7.2	6.11***	0.05	0.58
10	99.3	39.2	10.2	4.5	4.9	9..	0.8	3.54	0.53	2.8	6.9	4.68	0.05	0.32
30	90.8*	33.0*	10.7	5.3	4.6	9.9	0.8	3.38**	0.49	3.1	7.2	4.73	0.05	0.91

* SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.05
** SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.01
*** SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.002

7. Supplemental Perinatal and Postnatal Study in Rats

Study No: T3008898

Sponsor:

Dates Performed: February 1984 to March 1984

Quality Assurance: A signed statement of GLP compliance was included.

Doses Tested: 0 and 30 mg nisoldipine/kg/day. On the last 2 days of treatment, "several animals" received only 20 mg/kg due to an error, but the study results were not considered to have been affected by this error.

Procedure: Bor:WISW (SPF Cpb) naturally inseminated female rats (25 per group) were 12-14 weeks of age and weighed 178-216 g at the start of treatment. They were dosed orally by gastric intubation from gestation day (GD) 16 to postpartum day (PPD) 14. All the pregnant dams were allowed to litter and rear their young to PPD 14. The dams and offspring were evaluated for tolerance of the compound, effects of nisoldipine on birth and lactation and influence on post-natal development (similar to the observations in the main study). However, this study differed from the main study because the treatment and observation period extended to PPD 21 in the main study and only to PPD 14 in the present study.

Test Substance: Batch No. 907437. The vehicle consisted of Lutrol, glycerol and water.

Effects on the Dams: At 30 mg/kg, 1 died during parturition (with 6 dead fetuses). Treatment related effects noted were a highly significant decrease in weight gain between GD 16 and GD 20 (initial part of the treatment period), which resulted in a reduced mean body weight up to PP day 7, no longer evident by PPD 14, lightly colored feces, no longer evident after littering, and prolonged gestation (from 21.6 days in control to 22.1 days in the treated group; $P < 0.001$).

Effects on the Offspring: There were no significant intergroup differences in number of pups per litter at birth or after 1 and 2 weeks. However, there was a higher incidence of stillbirths and a higher rate of neonatal mortality throughout the 2 week postpartum study period for offspring of nisoldipine treated dams. The body weights of pups in the nisoldipine treated group were lower than control at the time of birth, and after 1 and 2 weeks. There was no effect on sex ratio at birth or at PPD 14 (See table which follows).

DOSE (MG/KG)	TOTAL NO. OF YOUNG (ALIVE + DEAD)	TOT. NO. OF DEAD YOUNG AT BIRTH	UP TO TIME OF 1ST WEIGHING	AFTER 1 WEEK	2 WEEKS
CONTROL	208	2	2	7	11
30	227	22*	30**	42**	50**

* significant difference from the controls, $p < 0.01$
**significant difference from the controls, $p < 0.001$

Comment: The increase in mean duration of gestation, followed by a higher incidence of stillbirths and a higher rate of neonatal mortality throughout the 2 week postpartum study period, and the lower body weight at birth for offspring of nisoldipine treated dams, were observed in both the main study and in the present study. These observations are suggestive of dystocia. Dystocia is defined as abnormal labor, which is usually accompanied by increased duration of labor and an increaed incidence of stillbirths and neonatal mortality.

MUTAGENICITY STUDIES (S. Stolzenberg)

1. Salmonella/Microsome Test

Study No.: Not provided. Pharma Report No. 9634

Performing Laboratory:

Date Performed: August, 1980 to September, 1980

Quality Assurance: A signed statement of compliance with GLP is not included.

Background: This widely used mutagenicity assay detects point mutations (base pair by TA 1535 and TA 100 and frame shift by TA1537 and TA 98), caused by chemical agents, *in vitro*. The reversion rates to prototrophy of histidine requiring (his-) mutants to the wild type histidine independent strain (his+), are evaluated in a medium with a low content of histidine. In the presence of test compound, an increase in reversion rate, measured by an increase in colony growth on the agar plate, is an indication of mutagenicity.

Procedure: The evaluation was performed with and without metabolic activation (provided by S-9 fraction of livers of Aroclor pretreated rats). *S. typhimurium* strains TA1535, TA100, TA1537 and TA98 were used. Two studies with TA 1535 but only one with the remaining 3 strains were carried out with 4 plates per concentration of test substance, DMSO control or postive control substances. Concentrations tested were 0, 20, 100, 500, 2500 and 12500 ug/plate.

Positive Controls:

a). without S-9 activation
Endoxan (cyclphosphamide) for TA1535 and TA100
Trypaflavin for TA1537 and TA98

b). with S-9 activation
2- aminoanthracene for all 4 tester strains

Results: 2500 ug/plate was toxic to bacterial growth for all 4 strains, whereas 12500 was toxic and caused precipitation, making it not possible to evaluate colony growth. 500 ug/plate was toxic for TA1535. In the first test, the positive control for TA1535 without S-9 showed no response, and the negative controls, both and without S-9, were unusually low. Therefore, the test with TA1535 was repeated. In the second test with TA1535, no indication of mutagenicity was seen with or without S-9. Similarly, Bay k 5552 showed no mutagenic effects on TA100, TA1537 and TA98. Positive controls gave adequate responses; i.e. well over double those of the negative controls.

Salmonella/Microsome Test with BAY k 5552 on Salmonella typhimurium TA 1535.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment / M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
12500			Could not be evaluated		
2500	0	0.8	64.4**	-	0.80
500	8.5	6.5	126.9**	8.50[a]	6.50[a]
100	9.0	6.3	158.4	9.00[a]	6.30[a]
20	14.5	5.0	146.0	14.50[a]	5.00[a]
Negative Control 0	1.0	1.0	152.4	1.00	1.00
Positive Control Endoxan 435	0.5	1.0	163.7	0.50[a]	1.00[a]
Positive Control 2-Aminoanthracene 10	32.8	19.0	5.2**	32.80[a]	19.00[a]

** Bacteriotoxic effect
a) See the "Results" section

In this test system, was considered not mutagenic at non-toxic, soluble concentrations.

Salmonella/Microsome Test with . on Salmonella typhimurium TA 100.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment / M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
12500			Could not be evaluated		
2500	4.3	19.5	2.0**	0.06	0.04
500	80.5	34.5	4.3	1.18	0.71
100	72.3	62.0	4.7	1.06	1.27
20	53.5	49.3	6.2	0.78	1.01
Negative Control 0	68.5	48.8	4.7	1.00	1.00
Positive Control Endoxan 435	273.8	82.0	3.9	4.00*	1.68
Positive Control 2-Aminoanthracene 10	1136.0	81.8	0.6**	16.58*	1.68

* Mutagenic effect
** Bacteriotoxic effect

Salmonella/Microsome Test with [redacted] on Salmonella typhimurium TA 1537.

Dose in µg per Plate	Mutants / Plate (M/P) + S-9 Mix	Mutants / Plate (M/P) - S-9 Mix	Total Organism Count per ml x 10^8	M/P Treatment M/P Negative Control + S-9 Mix	M/P Treatment M/P Negative Control - S-9 Mix
12500			Could not be evaluated		
2500	0	0.5	45.9**	-	0.13
500	3.3	1.5	49.1	0.66	0.38
100	7.3	2.0	51.3	1.46	0.50
20	5.0	1.5	51.5	1.00	0.38
Negative Control 0	5.0	4.0	50.9	1.00	1.00
Positive Control Trypaflavine 200	162.8	114.8	44.7**	32.56*	28.70*
Positive Control 2-Aminoanthracene 10	37.3	15.3	0.3**	7.46*	3.83*

* Mutagenic effect

** Bacteriotoxic effect

Salmonella/Microsome Test with on Salmonella typhimurium TA 98.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^6	+ S-9 Mix	- S-9 Mix
12500			Could not be evaluated		
2500	3.0	1.3	100.0**	0,18	0,27
500	22.0	3,0	127.3	1.31	0.63
100	19.3	4,8	151.70	1.15	1,00
20	14.0	5,5	-	0.83	1.14
Negative Control 0	16.8	4,8	121.5	1.00	1.00
Positive Control Trypaflavine 200	460.0	4.3	144.3	27.38*	0.90
Positive Control 2-Aminoanthracene 10	845.3	8.0	70,7**	50.32*	1.67

* Mutagenic effect
** Bacteriotoxic effect

Salmonella/Microsome Test with on Salmonella typhimurium TA 1535. Repeat.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
2500	0	0	toxic	-	-
500	0	0	toxic	-	-
100	6.5	6.0	non-toxic	1.08	1.33
20	6.8	7.0	non-toxic	1.13	1.56
Negative Control 0	6.0	6.5	non-toxic	1.00	1.00
Positive Control Endoxan 435	427.3	24.3	non-toxic	71.22*	5.40*
Positive Control 2-Aminoanthracene 10	266.0	8.5	non-toxic	44.33*	1.89

* Mutagenic effect

2. Salmonella/Microsome Test

Study No.: T 5027709

Performing Laboratory:

Date Performed: 1/29/88 to 3/11/88

Quality Assurance: A signed statement of compliance with GLP is included.

Procedure: Tester strains used were *S. typhimurium* TA1535, TA100, TA1537 and TA98. Two different forms of S-9 were used; from livers of Aroclor 1254 pretreated male NMRI mice, and from livers of NMRI male mice that had been pre-treated for 28 days with 2000 ppm Bay k 5552 in the diet. All the studies were carried out with 4 plates per concentration of test substance, control or postive control substances. Initially, concentrations tested both without and with metabolic activation were 0, 20, 100, 500, 2500 and 12500 ug/plate. Subsequently, concentrations tested were 0, 75, 150, 300, 600, 1200 and 2400 ug per plate. There is no explanation as to why two methods of enzyme activation were employed.

Positive Controls:

a). without S-9 activation
Sodium azide for TA1535
Nitrofurantoin for TA100
4-nitro-1,2-phenylene diamine for TA 1537 and TA98

b). with S-9 activation
2- aminoanthracene for all 4 tester strains

Results: No indication of mutagenicity was observed at any concentration tested. However, 20 ug/plate, the lowest concentration tested, was the only concentration at which bacteriotoxic problems were not encountered. Starting at 150 ug/plate, precipitation problems were also encoutered. Positive controls gave adequate responses; i.e. well over double the colony count of the negative controls.

Bay k 5552 was considered not mutagenic in this test system, but this study is valid only at 20 ug/plate because it was the only concentration at which toxicity and/or precipitation problems were not encountered with all 4 bacterial strains.

3. CHO HGPRT Forward Mutation Assay

Study No.: T 1023114 (sponsor's number)

Performing Laboratory:

Sponsor:

Date Performed: 7/11/86 to 10/29/86

Quality Assurance: A signed statement of compliance with GLP is included.

Background: "The objective of this study was to evaluate the test article for its ability to induce forward mutation at the HGPRT locus in the CHO-K1-BH Chinese hamster cell line, as assessed by colony growth in the presence of 6-thioguanine (TG). Hypoxanthine guanine phosphoribosyl transferase (HGPRT) is a cellular enzyme that allows cells to salvage hypoxanthine and guanine from the surrounding medium for use in DNA synthesis. If a purine analog such as TG is included in the growth medium, the analog will be phosphorylated via the HGPRT pathway and incorporated into nucleic acids, eventually resulting in cellular death. The HGPRT locus is located on the X chromosome. Since only one of the two X chromosomes is functional in the female CHO cells, a single-step forward mutation from HGPRT+ to HGPRT- in the functional X chromosome will render the cell unable to utilize hypoxanthine, guanine, or TG supplied in the culture medium. Such mutants are viable as wild-type cells in normal medium because DNA synthesis may still proceed by de novo synthetic pathways that do not involve hypoxanthine or guanine as intermediates. The basis for the selection of HGPRT- mutants is the loss of their ability to utilize toxic purine analogs (e.g., TG), which enables only the HGPRT- mutants to grow in the presence of TG. Cells which grow to form colonies in the presence of TG are therefore assumed to have mutated, either spontaneously or by the action of the test article, to the HGPRT- genotype."

Procedure: In preliminary, range finding tests, concentrations of 50 and 100 ug/ml (batch #828305) without metabolic activation were found to be 100% cytotoxic to the cell culture, but in the presence of metabolic activation (provided by S-9 fraction of livers of Aroclor 1254 pretreated rats), 100 ug/ml caused only a 39% decrease in survival index.

Three tests without S-9 included duplicate cultures with concentrations between 10 to 40 ug/ml, and two tests with S-9 included duplicate cultures with concentrations of 5 to 85 ug/ml. Positive controls were 5-bromodeoxyuridine without S-9 and 3-methylcholanthrene in the presence of S-9.

Results: Decreases in relative cell survival were seen at 30 ug/ml or higher concentrations without S-9, and at 50 ug/ml and higher with S-9. There were sporadic, small but statistically significant increases in mutant frequencies in each of the 3 tests without S-9 and in one of the two tests with S-9. In every case the increase occurred in only one of the two duplicate cultures. In spite of a suggestion of a concentration relationship at 40 and 60 ug/ml without S-9 (see first 2 tables which follow), it was claimed that a concentration relationship did not exist. It should, however, be noted that even among the duplicate controls, there were wide variations in mutant frequencies in most of the tests.

was considered not mutagenic in this test system.

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: _____ ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: August 20, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants
Expression Time: 7 days

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES/DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
NONACTIVATION:																		
Vehicle Control	258.0 ± 13.5	100.0	100.0	0	1	0	0	0	0	0	0	0	0	0	0	1	118.5 ± 8.7	0.4
	275.0 ± 1.0			2	2	0	1	0	1	2	1	2	3	0	4	18	108.8 ± 13.9	6.9*
Positive Control	145.7 ± 1.5	54.7	67.4	26	20	22	17	26	17	26	24	18	20	22	23	261	88.5 ± 1.3	122.9**
(50 µg/ml BrdU)[b]	149.3 ± 9.1	56.0	68.4	23	22	25	18	35	27	18	23	31	31	18	25	296	86.8 ± 7.3	142.1**
TEST ARTICLE																		
10.0 µg/ml	242.7 ± 6.5	91.1	169.6					NOT CLONED										
10.0 µg/ml	251.7 ± 12.7	94.4	159.6					NOT CLONED										
12.5 µg/ml	237.3 ± 7.6	89.1	131.4					NOT CLONED										
12.5 µg/ml	263.7 ± 32.0	98.9	139.6					NOT CLONED										
15.0 µg/ml	243.3 ± 7.5	91.3	127.3	0	2	0	0	0	0	0	0	1	0	0	0	3	83.5 ± 3.6	1.6
15.0 µg/ml	245.0 ± 22.6	91.9	164.6	0	0	1	0	0	0	0	0	0	0	0	0	1	80.5 ± 6.1	0.5
20.0 µg/ml	243.0 ± 21.7	91.2	143.7					NOT CLONED										
20.0 µg/ml	232.7 ± 11.6	87.3	136.2					NOT CLONED										
25.0 µg/ml	215.7 ± 10.2	80.9	124.1	1	1	1	1	3	1	2	0	2	0	1	0	13	84.0 ± 4.0	5.4
25.0 µg/ml	206.7 ± 28.6	77.5	115.2	2	0	1	1	0	0	1	1	1	1	2	0	10	100.3 ± 2.3	4.2
30.0 µg/ml	249.0 ± 6.6	93.4	99.7	C	C	C	C	C	C	C	C	C	C	C	C	-	100.0 ± 0.0[f]	-
30.0 µg/ml	227.3 ± 10.0	85.3	66.1	0	0	0	0	4	0	1	1	2	0	1	1	10	113.3 ± 4.6[g]	3.7
35.0 µg/ml	237.7 ± 21.5	89.2	59.8	C	C	C	C	C	C	C	C	C	C	C	C	-	122.5 ± 6.4[g]	-
35.0 µg/ml	227.7 ± 11.7	85.4	55.1	0	0	0	0	2	0	0	0	0	1	0	1	4	133.7 ± 4.4	1.2
40.0 µg/ml	193.3 ± 9.3	72.5	35.6	4	2	1	1	4	3	3	0	0	3	0	0	21	105.2 ± 5.5	8.3**
40.0 µg/ml	193.0 ± 13.0	72.4	42.3	0	0	1	0	0	0	0	0	0	0	0	1	2	109.8 ± 3.3	0.8

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: _____ ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: September 19, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants
Expression Time: 7 days

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
NONACTIVATION:																		
Vehicle Control	193.7 ± 6.0	100.0	100.0	0	3	1	1	0	2	2	0	1	0	0	0	10	102.3 ± 4.9	4.1
	187.0 ± 15.4			0	2	1	1	2	2	0	1	0	1	0	0	10	111.3 ± 13.7	3.7
Positive Control (50 µg/ml BrdU)[b]	148.3 ± 15.8	77.9	37.6	12	14	16	10	13	13	15	14	13	16	17	15	168	103.3 ± 4.8	67.8**
	139.7 ± 7.8	73.4	32.9	20	12	18	16	20	15	22	14	16	13	14	10	190	105.0 ± 6.6	75.4**
TEST ARTICLE																		
10.0 µg/ml	147.3 ± 12.1	77.4	43.6	0	4	0	1	0	1	0	0	0	1	0	0	7	129.0 ± 5.3	2.3
10.0 µg/ml	156.3 ± 11.8	82.1	59.5	3	0	2	2	1	3	3	0	3	3	1	0	21	125.0 ± 6.4	7.0
15.0 µg/ml	152.0 ± 17.3	79.9	44.0	1	1	1	1	1	1	1	0	2	0	1	2	12	127.0 ± 14.1	3.9
15.0 µg/ml	151.3 ± 11.7	79.5	39.8	1	0	1	0	0	4	1	0	3	1	3	0	14	142.7 ± 14.7	4.1
20.0 µg/ml	137.0 ± 23.1	72.0	44.8	0	0	2	0	1	0	0	0	1	0	2	0	6	112.3 ± 7.4	2.2
20.0 µg/ml	151.3 ± 11.2	79.5	31.8	1	2	4	5	2	2	1	2	1	0	1	1	22	141.3 ± 2.8	6.5
30.0 µg/ml	125.3 ± 3.2	65.8	42.9	0	2	1	1	0	0	0	1	2	0	2	0	9	110.2 ± 1.2	3.4
30.0 µg/ml	135.7 ± 8.6	71.3	49.9	3	1	0	1	1	2	2	2	3	5	2	0	22	115.5 ± 4.4	7.9*
40.0 µg/ml	42.7 ± 9.0	22.4	7.0	0	0	1	0	0	0	1	0	0	1	2	1	6	129.3 ± 15.7	1.9
40.0 µg/ml	42.3 ± 8.1	22.2	7.4	0	3	2	4	3	8	4	3	4	3	7	3	44	96.3 ± 1.5	19.0**
60.0 µg/ml	0.0 ± 0.0	0.0	< 0.1	NOT CLONED														
60.0 µg/ml	0.0 ± 0.0	0.0	< 0.1	NOT CLONED														

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: _____ ASSAY NO: C-9510

VEHICLE: DMSO TEST DATE: October 10, 1996

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants
Expression Time: 7 days

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
NONACTIVATION:																		
Vehicle Control	152.3 ± 11.2	100.0	100.0	1	2	0	0	1	2	0	6	0	0	0	3	9	112.8 ± 6.8	3.3
	172.0 ± 8.5			1	2	0	0	2	3	3	3	2	1	3	1	21	126.5 ± 11.0	6.9
Positive Control (50 µg/ml BrdU)[b]	84.7 ± 1.2	52.2	42.9	24	25	27	15	40	25	20	21	27	24	21	20	297	101.3 ± 11.5	122.2**
	84.7 ± 14.5	52.2	41.1	23	22	21	21	24	26	17	13	20	20	30	20	265	90.7 ± 5.5	121.7**
TEST ARTICLE																		
10.0 µg/ml	140.0 ± 6.1	86.3	76.0	2	2	1	3	5	2	2	3	1	2	2	0	25	108.7 ± 1.3	9.6*
10.0 µg/ml	145.7 ± 4.0	89.8	95.6	0	3	0	1	3	1	2	0	2	1	2	5	20	101.0 ± 11.3	8.2
15.0 µg/ml	146.7 ± 13.1	90.4	85.1	0	C	1	0	0	1	0	2	0	2	3	1	10	120.5 ± 1.7	3.5
15.0 µg/ml	136.7 ± 12.0	84.3	72.4	2	2	3	3	2	1	3	0	3	0	1	1	21	115.0 ± 13.0	7.6
20.0 µg/ml	130.7 ± 11.5	85.5	67.7	0	2	1	0	1	0	0	2	0	1	0	1	8	124.2 ± 6.6	2.7
20.0 µg/ml	120.0 ± 20.9	70.9	73.9	0	2	3	3	3	4	2	3	1	2	1	3	27	100.3 ± 9.4	11.2**
30.0 µg/ml	102.7 ± 7.4	63.3	65.0	1	2	1	1	1	3	2	1	1	1	3	3	20	104.2 ± 3.2	8.0
30.0 µg/ml	100.3 ± 9.0	66.0	57.7	T	T	T	2	2	1	1	5	2	1	4	0	10	122.3 ± 11.0	8.2
40.0 µg/ml	45.7 ± 12.1	20.2	0.2	6	0	0	1	2	4	4	0	1	0	3	1	22	110.0 ± 3.3	7.0
40.0 µg/ml	47.0 ± 5.3	29.0	9.1	1	0	1	0	1	1	3	0	1	1	2	0	11	113.5 ± 3.9	4.0
60.0 µg/ml	0.3 ± 0.6	0.2	< 0.1					NOT CLONED										
60.0 µg/ml	0.3 ± 0.6	0.2	< 0.1					NOT CLONED										
80.0 µg/ml	0.0 ± 0.0							NOT CLONED										
80.0 µg/ml	0.0 ± 0.0							NOT CLONED										
100.0 µg/ml	0.0 ± 0.0							NOT CLONED										
100.0 µg/ml	0.0 ± 0.0							NOT CLONED										

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: _____ ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: September 10, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants

Expression Time: 7 days S9 batch: 1-186

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES: DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
S9 ACTIVATION:																		
Vehicle Control	294.3 ± 12.9	100.0	100.0	0	2	2	2	1	0	4	1	0	2	0	0	14	110.3 ± 5.1	5.3
	396.3 ± 122.1			0	0	0	2	1	1	0	0	0	0	0	0	4	110.2 ± 5.8	1.5
Positive Control (5 µg/ml 3-MCA)[a]	276.0 ± 20.2	79.9	55.4	69	55	53	88	53	70	69	60	61	63	61	52	754	97.2 ± 9.0	321.2**
	256.7 ± 12.0	74.3	37.8	86	81	65	64	80	64	54	62	63	77	68	83	841	117.3 ± 5.1	298.7**
TEST ARTICLE																		
10.0 µg/ml	272.7 ± 16.3	79.0	194.8	NOT CLONED														
10.0 µg/ml	267.3 ± 4.5	77.4	167.8	NOT CLONED														
20.0 µg/ml	295.3 ± 11.7	85.5	111.4	NOT CLONED														
20.0 µg/ml	324.7 ± 8.4	94.0	145.5	NOT CLONED														
35.0 µg/ml	305.0 ± 26.6	88.3	140.3	1	0	1	0	1	0	0	0	0	1	0	0	4	96.2 ± 10.2	1.7
35.0 µg/ml	293.0 ± 9.5	84.9	153.9	0	1	0	0	0	0	0	0	1	0	0	0	2	94.5 ± 5.9	0.9
50.0 µg/ml	680.3 ± 43.7	197.0	108.1	0	0	3	0	1	2	2	3	0	3	1	1	16	94.7 ± 3.8	7.0*
50.0 µg/ml	250.0 ± 11.5	72.4	94.4	1	0	2	0	0	1	0	0	0	0	0	0	4	87.8 ± 5.0	1.9
60.0 µg/ml	215.3 ± 13.6	62.4	77.2	0	0	0	0	0	0	0	0	0	0	0	0	0	91.7 ± 6.0	0.0
60.0 µg/ml	250.0 ± 13.0	72.4	37.5	0	0	0	0	1	0	1	0	3	2	0	0	7	95.7 ± 4.5	3.0
70.0 µg/ml	124.7 ± 2.1	36.1	17.3	0	2	0	0	0	0	0	0	0	0	1	0	3	81.3 ± 5.9	1.5
70.0 µg/ml	119.7 ± 12.7	34.7	17.8	0	0	0	0	1	0	1	2	0	1	0	0	5	79.8 ± 1.3	2.6
85.0 µg/ml	22.0 ± 1.7	6.4	2.9	0	0	0	0	0	0	0	0	0	0	0	0	0	102.2 ± 5.5	0.0
85.0 µg/ml	24.0 ± 3.6	7.0	3.0	0	0	0	0	0	0	0	0	0	0	0	0	0	98.1 ± 4.9	0.0
100.0 µg/ml	6.3 ± 1.5	1.8	0.3	NOT CLONED														
100.0 µg/ml	5.0 ± 2.6	1.4	0.3	NOT CLONED														

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIEN _____ TEST ARTICLE: __ _____ ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: October 1, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants

Expression Time: 7 days S9 batch: 1-186

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES/DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
S9 ACTIVATION:																		
Vehicle Control	287.3 ± 12.7	100.0	100.0	0	C	0	0	0	C	C	1	2	1	0	1	5	101.8 ± 3.5	2.7
	347.3 ± 21.5			1	0	0	1	1	1	2	1	3	2	2	1	15	125.8 ± 9.5	5.0
Positive Control	259.7 ± 1.5	81.8	49.4	C	71	C	94	C	80	69	93	71	72	73	73	696	80.2 ± 7.2	482.1**
(5 µg/ml 3-MCA)[a]	266.3 ± 24.0	83.9	46.4	80	84	87	89	79	82	70	70	73	62	65	74	915	85.8 ± 7.7	444.3**
TEST ARTICLE																		
20.0 µg/ml	332.0 ± 26.5	104.6	90.3	0	0	1	0	1	0	2	2	1	0	1	0	8	119.7 ± 4.0	2.8
20.0 µg/ml	283.0 ± 43.8	89.2	80.6	2	2	0	1	1	1	1	0	1	0	1	3	13	127.3 ± 2.1	4.3
35.0 µg/ml	341.7 ± 13.1	107.7	99.2	0	1	0	0	2	0	1	0	0	1	0	0	5	122.3 ± 6.8	1.7
35.0 µg/ml	266.0 ± 16.6	83.8	132.5	0	0	0	0	0	0	0	1	0	0	1	0	2	129.2 ± 10.2	0.6
50.0 µg/ml	268.7 ± 7.6	84.7	C	NOT CLONED														
50.0 µg/ml	272.0 ± 7.8	85.7	C	NOT CLONED														
60.0 µg/ml	278.7 ± 39.4	87.8	75.4	2	0	0	1	3	2	3	0	1	1	1	C	14	117.3 ± 3.8	5.4
60.0 µg/ml	280.7 ± 4.6	88.5	82.3	1	1	>0	0	1	0	0	0	1	0	1	1	6	141.5 ± 4.8	1.8
70.0 µg/ml	169.3 ± 14.5	53.4	22.9	1	1	0	1	0	1	0	0	0	1	0	0	5	116.8 ± 9.4	1.8
70.0 µg/ml	142.7 ± 10.1	45.0	31.6	0	0	1	0	2	0	0	1	0	0	0	0	4	99.5 ± 4.8	1.7
85.0 µg/ml	46.0 ± 5.6	14.5	3.3	3	1	0	2	1	2	0	2	1	0	1	0	13	109.5 ± 2.8	4.9
85.0 µg/ml	34.0 ± 3.6	10.7	2.3	2	0	1	0	0	0	0	0	0	0	0	0	3	125.7 ± 0.6	1.0
100.0 µg/ml	5.0 ± 0.0	1.6	0.2	NOT CLONED														
100.0 µg/ml	3.3 ± 0.6	1.0	0.2	NOT CLONED														

4. Mouse Hepatocyte Primary Culture DNA Repair Assay

Study No.: T2 023 386 (sponsor's number)

Performing Laboratory:

Sponsor:

Date Performed: 8/14/86 to 2/25/87

Quality Assurance: A signed statement of compliance with GLP is included.

Background: Freshly obtained rodent, metabolically active hepatocytes are capable of limited biotransformation activity. Chemically induced damage to nucleic acid of the mammalian cells results in an effort by the enzyme systems to repair the defect(s), resulting in unscheduled DNA synthesis.

Procedure: Freshly prepared hepatocyte primary cell cultures from adult $B6C3F_1$ males were used, according to the method of Williams et al, Cancer Letters 6: 119-306, 1982. Eight concentrations between 1 mg/ml and 5×10^{-4} mg/ml (listed in the table which follows) were tested in triplicate against $5X10^5$ hepatic cells. DNA repair was determined by ^3H-thymidine uptake, to determine the net increase in nuclear grains induced by the test compound. Benzo(a)pyrene served as positive control and DMSO and pyrene served as negative controls. A test compound was considered positive when the mean net nuclear grain count exceeded that of the DMSO control by more than 2 standard deviations.

Results: Cytotoxicity was observed at concentrations of 1 and $5X10^{-1}$ mg/ml. No net increase in nuclear grain count was observed at concentrations between $5X10^{-4}$ and 10^{-1} mg/ml or with pyrene and DMSO (negative controls); the positive control, benzo(a)pyrene, was highly genotoxic, indicating that the hepatocytes were capable of metabolic transformation and DNA repair.

Under the conditions of this test system, Nisoldipine was considered to be not genotoxic at concentrations up to 10^{-1}M

NDI/IN VITRO Systems Facility
Report on: UDS/DNA Repair Assay

Date:	November 21, 1986
Expt #	M112186
Chemical	Nisoldipine
Molecular wt	
Source/Purity	
Lot #	
Solvent/vehicle	DMSO
Volatility	
Precautions	
Positive control	Benzo(a)pyrene (BaP)
Negative control	Pyrene

Assay method	Autoradiography
Species/strain/sex/age/wt	House/B6C3F$_1$/Male/Adult/28-32g
Organ or tissue/condition	Liver
Cells/primary or line	Primary
Medium used:	Williams Medium E
Duration of chemical exposure	18 hrs.
Chemical dose range	1 to 5 x 10^{-6} mg/ml
Exposure method	In Situ
Label/uCi/ml or Ci/mM. final	^{3}H-thymidine, 10uCi/ml
Interval between exp. and label	Simultaneous
Duration of label	18 hrs.

CONTROLS			TEST RESULTS				COMMENTS:
Positive	Conc.	Autoradiog. grains/nucleus (NET)*	Conc. mg/ml	Autoradiog. grains/nucleus (NET)*	Cytoxicity	Evaluation	* Mean ± standard deviation of triplicate coverslips.
BaP	10^{-5}M	43.7 ± 5.0	1		Toxic		**Mean of duplicate coverslips.
			5 x 10^{-1}		Toxic		
			10^{-1}	-11.6 ± 1.7	Subtoxic	Negative	
			5 x 10^{-2}	-15.2**	Subtoxic	Negative	
			10^{-2}	-16.5 ± 1.8	Nontoxic	Negative	
			5 x 10^{-3}	-15.3 ± 2.4	Nontoxic	Negative	
Negative	conc.	grains/nucleus (NET)	10^{-3}	-16.5 ± 2.1	Nontoxic	Negative	
Pyrene	10^{-5}M	-11.1 ± 4.2	5 x 10^{-4}	-12.8 ± 0.9	Nontoxic	Negative	
DMSO	1%	-13.6 ± 1.3					
Cell Control		-13.9 ± 3.7					

5. Micronucleus Test in Mice

Study No.: T 1010 875

Performing Laboratory:

Date Performed: 11/6/81 to 12/1/81

Quality Assurance: A signed statement of compliance with GLP is not included.

Background: The micronucleus test permits the recognition of a mutagenic action on a somatic tissue, the femoral bone marrow, of an intact animal. Erythrocytes are normally anuclear. The increased occurrence of micronuclei (chromosome fragments) in polychromatic erythrocytes, compared to negative controls, indicates that the test substance brings about chromosome breaks, or has effects on the spindles in the erythroblasts.

Procedure: Bor: NMRI (SPF Han) mice, 8 to 12 weeks of age, weighing 23 to 32 g, 5 of each sex per group, received 0, 100 and 200 mg Bay k 5552/kg/day, p.o., on two consecutive days; positive controls received cyclophosphamide (Endoxan^{R}) orally. All animals were sacrificed 6 hours after the second dose and femoral bone marrow samples were removed for the purpose of determining the frequency of micronuclei observed, by examination of 1000 polychromatic erythrocytes per animal.

Results: Since there was no difference between males and females, the results obtained for both sexes were pooled. No deaths or clinical signs were observed. The significant decrease in normochromatic erythrocytes in the 100 mg/kg group was considered "not biologically significant" (see table on page which follows). It is therefore claimed that produced no inhibition of erythropoiesis.

There was no increase in polychromatic erythrocytes with micronuclei. The positive control caused a high level of response.

It was concluded that there was no indication of a mutagenic effect by this test.

Survey of the experimental evaluation he micronucleus test

with

Experimental group	Number of investigated polychromatic erythrocytes	Number of normochromatic erythrocytes per 1,000 polychromatic erythrocytes	Number of cells with micronuclei	
			per 1,000 normochromatic erythrocytes	per 1,000 polychromatic erythrocytes
I Negative control	10000	682.6	1.0	1.6
II BAY k 5552 2 x 100 mg/kg	10000	465.6*	1.9	1.8
III BAY k 5552 2 x 200 mg/kg	10000	543.1	1.2	1.8
IV Positive control Endoxan 2 x 87 mg/kg	10000	724.0	1.2	31.4

* $P \leq 0.05$ in the distribution-free test of ranks according to NEMENIY related to the negative control (I)

6. Dominant-Lethal Test in Mice

Study No.: T 50 10 239

Performing Laboratory:

Date Performed: 5/11/81 to 11/10/81

Quality Assurance: A signed statement of compliance with GLP is not included.

Background: This test permits detection of mutations in germ cells, particularly stage-specific effects, during meiotic maturation of sperm cells, and is usually performed in mice or rats. A mutagenic test substance causes severe chromosomal damage or lethal germ cell mutations, resulting in embryo or fetal mortality. This is determined by increased occurrence of pre- or post-implantation loss in females (untreated) that were mated to test substance treated males.

Procedure: Bor: NMRI (SPF Han) mice, 8 to 12 weeks of age, body weights at initiation of study of 30-44 g for males and 25-30 g for females, were used. Males in the treated group (50/group) received 200 mg/kg /kg in aqueous hydroxyethylmethyl-cellulose, single oral dose (Batch No. 576923); control males received vehicle. Starting on the day of drug administation, a new, untreated female was placed in the cage of each treated or control male at the start of each of 12 mating periods, lasting 4 days each (48 days total). About 14 days after the middle of the relevant mating period, the uterus of each female was examined for live and dead embryos, resorption sites and corpora lutea.

Criteria for Dose Selection: Claimed to be based on a preliminary study with female mice, 5/group, which received 200, 500 or 1000 mg/kg, p.o., and in which "200 mg/kg was tolerated with only slight symptoms".

Results: There were no effects of treatment on impregnation rate, fertility, pre- or post-implantation loss. Because there was no indication of early death of embryos at any stage of mating due to compound treatment, there was no indication of a dominant lethal effect (see tables on next 2 pages).

In conclusion, was considered to be not mutagenic in this test system.

DOMINANT-LETHAL-TEST

SINGLE TREATMENT OF MALE MICE WITH BAY K 5552

STUDY .: 15010239

DOSE GROUP 200 MG/KG (P.O.)

PREIMPLANTATION LOSS

MATING PERIOD	CORPORA LUTEA TOTAL CONTROL	CORPORA LUTEA TOTAL DOSE GROUP	CORPORA LUTEA PER IMPREGNATED FEMALE CONTROL	CORPORA LUTEA PER IMPREGNATED FEMALE DOSE GROUP	IMPLANTATIONS* TOTAL CONTROL	IMPLANTATIONS* TOTAL DOSE GROUP	IMPLANTATIONS* PER IMPREGNATED FEMALE CONTROL	IMPLANTATIONS* PER IMPREGNATED FEMALE DOSE GROUP	PREIMPLANTATIONS LOSS TOTAL CONTROL	PREIMPLANTATIONS LOSS TOTAL DOSE GROUP	PREIMPLANTATIONS LOSS PER IMPREGNATED FEMALE CONTROL	PREIMPLANTATIONS LOSS PER IMPREGNATED FEMALE DOSE GROUP
1	621	584	12.9	13.3	577	453	12.0	11.9	44	51	0.92	1.34
2	472	535	12.8	12.4	442	502	11.9	11.7	30	33	0.81	0.77
3	543	596	12.3	13.8	518	566	11.8	12.3	25	30	0.57	0.65
4	572	425	13.6	12.1	532	388	12.7	10.9	40	45	0.95	1.29
5	419	433	13.1	12.4	408	400	12.8	11.4	11	33	0.34	0.94
6	518	558	12.6	13.8	474	503	11.6	11.7	44	55	1.07	1.28
7	531	544	14.8	13.6	504	503	13.3	12.6	27	41	0.71	1.02
8	591	503	13.7	13.2	536	469	12.5	12.3	55	36	1.28	0.89
9	541	512	12.9	13.1	507	463	12.1	11.9	34	49	0.81	1.26
10	624	514	13.8	13.5	581	462	12.1	12.2	43	52	0.98	1.37
11	568	561	13.5	13.7	543	527	12.9	12.9	25	34	0.60	0.83
12	604	525	14.4	12.5	568	491	13.5	11.7	36	34	0.86	0.81
1 -12	6604	6210	13.2	13.0	6190	5719	12.4	12.0	414	491	0.63	1.03

* Since it can occur that a placenta is found with two embryos on one implantation site, the number of implantations can be smaller than the total of living and dead implants.

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DOMINANT - LETHAL - TEST
SINGLE TREATMENT OF MALE MICE WITH BAY R 5552
STUDY : T5910239

DOSE GROUP 200 MG/KG (P.O.)

POSTIMPLANTATION LOSS

MATING PERIOD	LIVING IMPLANTS		PER IMPREGNATED FEMALE		DEAD IMPLANTS		PER IMPREGNATED FEMALE	
	CONTROL GROUP	DOSE GROUP	CONTROL GROUP	DOSE GROUP	CONTROL GROUP	DOSE GROUP	CONTROL GROUP	DOSE GROUP
1	547	427	11.4	11.2	31	24	0.65	0.68
2	414	481	11.2	11.2	31	24	0.84	0.56
3	492	531	11.2	11.5	27	36	0.61	0.78
4	488	346	11.4	9.9	52	38	1.24	1.09
5	338	361	12.1	10.3	22	40	0.69	1.14
6	446	461	10.9	16.7	29	46	0.71	1.87
7	472	479	12.4	12.8	33	25	0.87	0.63
8	497	468	11.6	11.6	42	29	0.98	0.76
9	485	429	11.5	11.8	24	34	0.57	0.87
10	546	434	11.4	11.4	38	31	0.79	0.82
11	501	481	11.9	11.7	43	46	1.02	1.12
12	544	463	13.6	11.6	27	30	0.66	0.71
1 -12	5812	5333	11.6	11.2	399	485	0.88	0.85

7. In Vitro Chinese Hamster Ovary Cell (CHO) Test for Clastogenic Potency

Study No.: 2528 MIC

Performing Laboratory:

Sponsor:

Date Performed: Not indicated. The report is dated 1/19/88

Quality Assurance: A signed statement of compliance with GLP is included.

Procedure: "CHO described by Puck" (Genetics 55:513-518, 1967), were used. After preliminary cytotoxicity tests, concentrations of Bay K 5552 tested without metabolic activation were 10, 20 and 30 uM, and with metabolic activation (provided by S-9 fraction of livers from Aroclor induced rats) they were 500, 600 and 800 uM. The highest dose levels were selected to give about a 50% inhibition of mitotic index, but allowed sufficient numbers of cells at the metaphase stage for analysis of chromosome and chromotid aberrations (breaks, gaps and exchanges); generally, a requirement for dose selection with this test. Positive control compounds were methyl-methane-sulfonate (MMS) without S-9 and cyclophosphamide (CP) with S-9. An 18 hour incubation period, which corresponds to one cell cycle, was selected for the main study. Aberrations were analyzed in 100 cells arrested at the metaphase stage of cell division for each concentration level. The assays with and without S-9 were performed twice.

Results: There were no increases in chromosome or chromatid aberrations at any concentration (see tables which follow). The positive controls (MMS or CP exposed cells) showed statistically significant, large increases in rates of aberration.

In conclusion, was considered to be not clastogenic in this test system.

ASSAYS WITHOUT S-9 MIX
Mean of both assays

A. Number of aberrations per 100 analysed metaphases

Chemical	Dose (µM)	Gap	Break	Chromatid Exchanges	Chromosome Exchanges	Multiple aberrations	Total number of aberrations	
							Including gaps	Excluding gaps
-	-	8.5	2.0		1.0		11.5	3.0
DMSO	-	5.5	0.5		1.0		7.0	1.5
	10	4.5	2.0			0.5	6.5	2.0
	20	2.5	5.5	1.0	2.5		11.5	9.0
	30	5.5	2.0				7.5	2.0
MMS	605	15.5	29.5	36.0	7.0	28.5	88.0	72.5

B. Percentage of cells containing aberrations

Chemical	Dose (µM)	Gap	Break	Chromatid Exchanges	Chromosome Exchanges	Multiple aberrations	Percentage of cells containing aberrations	
							Including gaps	Excluding gaps
-	-	7.0	2.0		1.0		9.5	3.0
DMSO	-	5.5	0.5		1.0		6.5	1.5
	10	4.5	2.0			0.5	7.0	2.5
	20	2.5	4.0	1.0	1.5		7.0	5.0
	30	4.5	1.5				6.0	1.5
MMS	605	13.0	18.0	26.5	6.5	28.5	73.0	65.0***

Break : chromatid and chromosome
Chromatid exchanges : inter and intrachange
Chromosome exchanges : inter and intrachange
Multiple aberrations : complex rearrangement

Statistical test used : X^2 *** $p \leq 0.001$

ASSAYS WITH S-9 MIX
Mean of both assays

A. Number of aberrations per 100 analysed metaphases

Chemical	Dose (µM)	Gap	Break	Chromatid Exchanges	Chromo-some Exchanges	Multiple aberra-tions	Total number of aberrations	
							Including gaps	Excluding gaps
-	-	8.0	1.0	1.0	1.5	2.0	11.5	3.5
DMSO	-	6.0	1.0	1.0	1.5		9.5	3.5
	500	3.5	2.0		0.5		6.0	2.5
	600	2.0	0.5		1.0		3.5	1.5
	800	3.5	2.5	1.5	1.0	0.5	8.5	5.0
CPA	130	17.0	29.5	24.5	6.5	3.0	77.5	60.5

B. Percentage of cells containing aberrations

Chemical	Dose (µM)	Gap	Break	Chromatid Exchanges	Chromo-some Exchanges	Multiple aberra-tions	Percentage of cells containing aberrations	
							Including gaps	Excluding gaps
-	-	8.0	1.0	1.0	1.5	2.0	13.0	5.0
DMSO	-	6.0	0.5	1.0	1.5		8.0	2.5
	500	3.5	2.0		0.5		6.0	2.5
	600	1.5	0.5		1.0		3.0	1.5
	800	3.5	2.0	1.0	1.0	0.5	7.5	4.0
CPA	130	15.0	25.5	20.0	5.5	3.0	53.5	44.0***

Break : chromatid and chromosome
Chromatid exchanges : inter and intrachange
Chromosome exchanges : inter and intrachange
Multiple aberrations : complex rearrangement
Statistical test used : X^2 *** $p \leq 0.001$

8. Test for Inhibition of Liver Cell Culture Intercellular Communication

Study No.: T2 023 386 (sponsor's number)

Performing Laboratory:

Sponsor:

Date Performed: 5/18/87 to 5/27/88

Quality Assurance: A signed statement of compliance with GLP is included.

Background Information: In this assay, the transfer of the toxic metabolite, 6-thioguanine (TG), from metabolically competant freshly isolated rat hepatocytes ($HGPRT^+$) to a mutant rat liver cell culture, ARL14-TG^R, which is purine analog resistant ($HGPRT^-$), is measured. When exposed to TG, the presence of the primary hepatocytes results in a reduction of TG^R colonies. The TG kills primary hepatocytes and ARL-TG^R cells to which the metabolite is transferred. If a test chemical inhibits membrane contact or intercellular communication (also referred to as metabolic cooperation), the reduction of TG^R colonies in the flasks containing primary hepatocytes will be diminished, leading to an increased survival and formation of colonies. It is claimed that "many but not all tumor promotors inhibit metabolic cooperation".

Procedure: Nisoldipine was tested at concentrations which ranged between $5X10^{-5}$ mg/ml and $5X10^{-4}$ mg/ml, to determine if it inhibited metabolic cooperation between rat primary hepatocytes (wild type cells) and ARL14-TG^R cells. It is claimed that toxicity had been previously observed at $5X10^{-3}$ mg/ml, but the data were not shown. Positive control used was DDT.

Results: A concentration dependent inhibition of metabolic cooperation was not observed with nisoldipine. DDT caused an inhibition of metabolic cooperation. (See table which follows).

It was concluded that did not inhibit metabolic cooperation in this test system.

Chemical	Concentration	Number of Colonies[a] No Hepatocytes	Number of Colonies[a] 1.25×10^6 Hepatocytes
None	-	233 ± 38 (226,276,185,245)	97 ± 19 (98,122,93,75)
DMSO	0.1%	221 ± 22 (252,222,206,205)	118 ± 11 (103,117,123,129)
DDT	5×10^{-5}M	157 ± 10 (145,163,153,167)	151 ± 14 (132,156,151,164)
Nisoldipine	5×10^{-4} mg/ml	187 ± 54 (235,230,160,124)	118 ± 7 (111,118,125)
	10^{-4} mg/ml	181 ± 9 (177,171,192,183)	118 ± 9 (114,112,129)
	5×10^{-5} mg/ml	202 ± 17 (227,197,198,187)	89 ± 28 (114,69,112,60)

[a] Mean ± standard deviation of three to four flasks.

LABELING

Under PRECAUTIONS, the first sentence of the subsection "Carcinogenesis, Mutagenesis and Impairment of Fertility" presently reads:

Nisoldipine was administered orally to mice and rats for 21 and 24 months respectively, and was not shown to be carcinogenic.

We recommend the following revision :

Dietary administration of nisoldipine at doses up to 111 mg/kg/day for 24 months to rats or at doses up to 217 mg/kg/day for 21 months to mice (125 to 250 times the maximum recommended human dose of 40 mg/kg, based on a mg/kg comparison assuming a patient weight of 60 kg) revealed no evidence of a tumorigenic effect.

Under the same subsection "Carcinogenesis, Mutagenesis and Impairment of Fertility", the statement, "_____nisoldipine did not interfere with fertility at a dose more than 30 times the maximum recommended human dose" is meaningless. This should be changed to indicate the dosage in terms of mg/kg, then converted to dosage based on surface area, i.e. in terms of mg/m^2, which may then be compared to human dosage.

Based on the outcome of all the *in vitro* and *in vivo* tests, the statement in labeling, "The results of *in vitro* and *in vivo* mutagenic studies were negative" is reasonable.

Under *Pregnancy Category C*, we agree with the Category C classification, even though animal studies are only suggestive of potential fetal risk. The labeling should be modified to indicate that the fetal toxicity observed in the studies with animals are suggestive, not conclusive. It should specify that the monkey study was confounded by 1) the use of feral monkeys which are particularly susceptible to the stress of handling, and 2) the high rate of abortion and mortality, in both treated and control groups, resulting in only one surviving fetus in the 100 mg/kg group (which presented with anomalies) and only 3 surviving control fetuses. It should be stated that although it cannot be concluded that nisoldipine was teratogenic in the monkey study, such a possibility cannot be ruled out because the teratogenic effects observed (multiple left forelimb, finger and tail abnormalities observed externally, and related forelimb and vertebral abnormalities observed with skeletal examination) had not been previously observed in untreated animals of this species.

The proposed labeling does not specify the form of maternal toxicity that was observed in either the rat or rabbit. The phrase which states that nisoldipine caused a slightly increased malformation rate in rabbits should be omitted from the labeling. Of the two rabbit studies, slightly increased malformation rate was attributed to the stress of diarrhea, which occurred in one

of these two studies. There was no increase in any specific form of malformation.

The statement on fetotoxicity in rats and rabbits should be revised to read as follows:

Fetotoxicity in rats and rabbits was usually observed only at doses which caused a decrease in body weight gain of dams compared to control. In rats, an increase in post-implantation loss was observed at 100 mg/kg, and a decrease in fetal weight was observed at 30 and 100 mg/kg. In rabbits, decreases in fetal and placental weights were observed at 30 mg/kg.

For the rat and rabbit Segment II studies, the dosages cited as "*30 (or 100) times the maximum human dose*", should be expressed in terms of both mg/kg and mg/m^2.

Under a new section, *Labor and Delivery*, the labeling should state that the drug caused a slight prolongation of pregnancy in rats. The prolongation of pregnancy is presently noted under the *Pregnancy Category* section but should be moved here.

Under *Nursing Mothers*, it should be pointed out that "nisoldipine was found in the milk of lactating rats at concentrations which were lower than levels found in the plasma".

OVERALL SUMMARY AND EVALUATION

Nisoldipine coat core (Nis CC) tablet, proposed for the treatment of hypertension (alone or in combination with other antihypertensive agents) and chronic stable angina on a once-a-day dosage regimen, is an extended release formulation of the dihydropyridine calcium channel blocker nisoldipine. Nis CC tablet has an external coat of slow release formulation and an internal core of fast release formulation of nisoldipine.

Calcium channel blockers have recently emerged as a promising new class of antihypertensive agents. By blocking the channels which mediate calcium entry into smooth muscle cells, these agents decrease intracellular calcium levels, thereby inhibiting vascular smooth muscle contractions, resulting in a decrease in peripheral vascular resistance and reduction in blood pressure. Because calcium channel blockers inhibit coronary vasoconstriction and reduce vascular resistance and increase coronary blood flow, thereby protecting the heart against ischemia and reperfusion damage, they are also effective in the treatment of angina pectoris.

Nisoldipine was developed with the aim of improving the pharmacologic properties of nifedipine. Despite its chemical similarity to nifedipine, nisoldipine is a more potent dilator of coronary as well as peripheral blood vessels. Nisoldipine is 3 to 10 times more potent than nifedipine in increasing coronary blood flow and coronary venous oxygen saturation in dogs. In isolated vascular preparations, nisoldipine inhibits calcium- and potassium-induced contractions at concentrations 4-10 times lower than that of nifedipine. A negative inotropic effect, an adverse effect usually observed with other calcium channel blockers, has not been shown with nisoldipine in its therapeutic dose range.

An oral dose of 10-40 mg once daily is proposed for the treatment of hypertension and a dose of 10-30 mg once daily is proposed for the treatment of angina pectoris.

This new drug application is supported by fairly extensive preclinical studies.

Nisoldipine was shown to produce dose-dependent reductions in blood pressure and total peripheral resistance in rats, dogs, cats and pigs. In SH rats, single oral doses of 0.315, 1.0 and 3.15 mg nisoldipine/kg produced 3, 12 and 18% reductions in blood pressure, respectively. The maximum effect was seen at 1 hr after drug administration and the blood pressure returned to the pretreatment level at 6 hr postdose. The antihypertensive effect of nisoldipine was much more pronounced in renal hypertensive rats than in SH rats; a dose of 3.15 mg/kg po produced a 39% reduction in blood pressure in renal hypertensive rats compared to an 18% reduction in SH rats. Long term treatment with nisoldipine (50-100 mg/kg/day in the diet for 60 weeks)

prevented the development of hypertension in SH rats (mean systolic blood pressure of 141 mm Hg in the drug treated group vs 214 mm Hg in the control group at the termination of the study) and other rat models. Chronic treatment with the test compound also significantly reduced the atrial natriuretic peptide and aldosterone concentrations in plasma and attenuated cardiac hypertrophy in SH rats.

The (+) enantiomer was found to be only slightly more potent than the racemic compound in its antihypertensive activity in SH rats, but it was about 20 times more potent than the (-) enantiomer.

In terms of antihypertensive effect, nisoldipine was about equipotent to nifedipine, nicardipine and hydralazine (ED20 = 4 mg/kg po) in SH rats; however, it was less potent than the other drugs in other hypertensive rat models.

Single oral doses of nisoldipine (0.03-1.0 mg/kg) produced dose-dependent decreases in mean arterial blood pressure in conscious renal hypertensive dogs. At 0.3 mg/kg, a 24% reduction in blood pressure was produced within 2 hr after drug administration and the hypotensive action lasted for about 12 hours. A tachycardia, lasting for about 3 hr, also occurred at the above dose level. Nifedipine produced about the same degree of hypotension (lasting about 6 hr) as that produced by an oral dose of 0.3 mg nisoldipine/kg at about a 10 fold higher dose level (ED20 - 0.14 mg/kg for nisoldipine vs 1.68 mg/kg for nifedipine). The antihypertensive activity of orally administered nisoldipine in renal hypertensive dogs significantly correlated with plasma concentrations of the drug.

Low doses of nisoldipine increased coronary blood flow and protected the heart against ischemia and reperfusion damage in various experimental models. In isolated rat hearts subjected to ischemia and reperfusion, nisoldipine (3 nM) doubled coronary blood flow. At 1 nM, nisoldipine increased coronary blood flow 31% and improved the recovery of contractile function and tissue ATP levels. Radioactive microsphere studies in conscious rats showed that oral administration of nisoldipine (0.3 mg/kg) produced a marked increase in coronary blood flow as well as a decrease in coronary vascular resistance. In anesthetized dogs, nisoldipine (5 µg/kg iv) increased coronary sinus blood flow by 111% and coronary sinus oxygen content by 50%. In dogs with acute myocardial infarction, nisoldipine (5 µg/kg iv 15 min, 2 and 4 hr after coronary artery occlusion) reduced myocardial infarct size by 31%. In pigs with gradual coronary occlusion, the test drug (30 µg/kg po, every 6 hr for 1 month) significantly reduced infarct size and increased endocardial and transmural blood flow by enhancing endocardial collateral circulation.

Nisoldipine did not depress cardiac function at doses needed to increase coronary blood flow or lower blood pressure in hypertensive animals. The drug reduced or abolished ventricular fibrillation and reperfusion arrhythmias, improved cardiac

output, reduced mortality, and improved ventricular function in several animal models. The beneficial effects are attributed to increases in perfusion of the ischemic zone, and a reduction in afterload through a decrease in peripheral resistance.

In vitro studies have shown nisoldipine to be twice as potent as nifedipine in inhibiting contractions of isolated pig coronary artery; however, it was only 1/3 as potent as nifedipine in inhibiting femoral artery contractions, indicating its selectivity for coronary vasculature.

Nisoldipine produced diuretic and natriuretic effects in rats, the effects being more pronounced in hypertensive than in normotensive rats. The natriuretic effect was attributed to the suppression of distal tubular sodium reabsorption.

Nisoldipine binds with very high affinity (Kd=<0.1 nM) to L-type calcium channels of various cell types. Compared to nifedipine, nisoldipine is found to have 2 to 30 times higher binding affinity depending on the cell type and experimental conditions. Several studies have shown that there is good agreement among binding affinity and the IC50 values both for inhibiting ^{45}Ca influx and aortic contraction. Moreover, the degree of nisoldipine inhibition of calcium channel current was found to increase with membrane depolarization.

Isolated membrane studies showed that the (+) isomer had a binding affinity 100 times higher than that of the (-) isomer.

General pharmacological studies in rodents showed analgesic and anticonvulsant effects, prolongation of anesthesia, attenuation of aggressive behavior, elevation of blood glucose and reduction of intestinal motility at dose levels 15 to 150 times the maximum recommended human dose on a body weight basis. (It is noted that the above effects were seen at dose levels 33 to 333 times the dose that produced the desired pharmacological effects in rats.)

Bay R 9425, an active metabolite with a dihydropyridine structure, exhibited pharmacological effects similar to the parent compound but was 1/3 to 1/10 less potent. The other metabolites had no significant effects.

Combined administration with propranolol prolonged the anti-hypertensive action of nisoldipine and reduced the reflex tachycardia in dogs.

Based on the ratio of percent of administered radioactivity excreted in urine for the i.v./oral doses, nisoldipine has been shown to be virtually completely absorbed in rats, dogs and humans, following oral doses of 5 mg/kg in the animals or 10 to 40 mg Nisoldipine CC tablets, in man. Despite the high rates of oral absorption, bio-availabilities (F) of parent compound were low, averaging 11.7% or less in the 3 species, which was attributed to an extensive first pass effect (shown in the dog to

be due to metabolism in both the gut and liver). In man, the F value for immediate release compound was 8.4%, but for the core-coated tablet, it was 5.5%. When administered by the oral route, $CEQ_{max,norm}$'s (C_{max} normalized to 1 mg/kg dose, based on radioactivity equivalence) for parent compound were similar in rat, dog, monkey and man, whereas $CEQ_{max,norm}$'s for total radioactivity (which includes parent compound plus all metabolites) were 4.7 to 7.5-times higher in man than in the 3 animal species. AUC_{norm}'s for parent compound were also similar in all 4 species whereas AUC_{norm}'s for total radioactivity (parent compound plus metabolites) were 6 to 10 times higher in man than in the three animal species. The plasma half-lives for parent compound following oral administration were only 0.7, 2.3, 3.8 and 4.0 hours, respectively, for the 4 species, whereas the plasma half lives for total radioactivity (parent compound plus metabolites) were 14.9, 54.4, 41.8 and 80.3 hours for the rat, dog, monkey, and man, respectively.

At steady state in humans, dose proportionality was seen for both immediate release and coat core tablets, based either on AUC or C_{max}. Correspondingly, decreases in systolic and diastolic blood pressures showed a general dose related decline from baseline.

Tissue distribution of total radioactivity following a single oral dose of 5 mg/kg in rats, determined between 0.5 and 72 hours post-dosing, reached maximum values at 1 hour, with liver, fat, kidney and adrenal glands generally containing the highest levels, brain and skeletal muscle the lowest. There was no indication of a change in organ pattern distribution with time. In dogs, tissue distribution (measured only 72 hours) after oral dosing was similar to that observed in rats. Placental transfer in pregnant rats, and secretion into milk of lactating rats, were observed. Whole-body autoradiography in rats indicated rapid tissue distribution and penetration of the blood-brain barrier within 5 minutes after i.v. dosing.

Protein binding, determined by equilibrium dialysis, was greater than 97.5% in rats and dogs and around 99.4% in humans. Nisoldipine was rapidly and extensively metabolized in the rat, dog, monkey and man; only a small percentage of unchanged labelled test substance could be found in the circulation within 30 or 60 minutes after an oral dose. Partial enterohepatic recirculation of metabolites was detected in rats. Hepatic enzyme levels of cytochrome P_{450}, aminopyrine N-demethylase and aniline hydroxylase were decreased following oral administration for 2 weeks, but these decreases were found to be reversible within one week following treatment termination.

At least 18 biotransformation products have been identified in urine and serum of rat, dog, monkey or man, 6 of which account for 80% of radioactivity in urine of all 4 species. After oral administration, there were no important differences in plasma or urinary metabolic profile between the 4 species. The investigators describe the biotransformation steps in all 4

species as follows:

-hydroxylation of the isobutyl ester
-dehydrogenation to the pyridine derivative
-cleavage of the ester to form the carboxylic acid
-reduction of the nitro group to the amino group
-glucuronidation (phase II enzymatic reaction)

In acute toxicity studies, oral LD50 values of nisoldipine were greater than 10,000 mg/kg for mice and rats and greater than 5000 mg/kg for rabbits and dogs. Acute iv LD50 values for the above four species ranged from 1.9 to 2.5 mg/kg. Propranolol pretreatment had no effect on the iv LD50 in the rat.

In the rat three month oral (gavage) toxicity study (0, 10, 30 and 100 mg nisoldipine/kg/day), elevation of plasma urea levels was seen in the high dose female group. Although absolute and/or relative weights of heart and liver (mid and high dose rats of both sexes) and thymus (mid and high dose males) were significantly higher than control, no significant histopathological findings were observed. The "no effect level" for liver and heart weight effects in the above study was found to be 10 mg/kg/day.

In reply to a request for justification of dose selection for the three dog studies, we were informed by the sponsor that doses were selected for the initial 4 week study on the basis of previous experience with the pharmacologically similar dihydropyridines, nifedipine and nitrendipine. The rationale for selection of the highest dose in the 52-week study was the avoidance of papillary muscle scars that were observed with the highest doses employed in the 4 and 13 week experiments. Such lesions were considered life threatening by the sponsor. Myocardial scars in one or both left ventricular papillary muscles, observed at 10 mg/kg in the 4 week study, and 6.25 mg/kg in the 13-week study, are generally attributed to hypoxic damage associated with vasodilator induced heart rate increase, a known damage mechanism in dogs. In the 4-week study, the associated ST drops and tachycardia were most pronounced in the dog with the most severe lesions. The pharmacologic effects (decreases in blood pressure and increases in heart rate) were usually reversible within 24 hours after treatment. No other treatment related effects were noted in the 4 and 13 week studies. The doses selected for the 52-week study were 0.3, 1.0 and 3 mg/kg,which caused dose-related decreases in blood pressure and increases in heart rate. Also noted were slight ST segment depressions, T-wave inversions and QT segment depressions, but no gross or histologically observable heart muscle damage or other indications of toxicity. In humans, nisoldipine is known to cause ST segment depression along with a decrease in peripheral vascular resistance, and side effects include palpitation and tachycardia.

The two year oral (dietary) carcinogen bioassay in the rat at doses of 0, 50, 300 and 1800 ppm nisoldipine (2.15, 13.13 and 82.40 mg/kg/day, respectively, in males and 2.78, 18.04 and 110.68 mg/kg/day, respectively, in females) did not show any evidence of a treatment-related increased incidence of tumors according to sponsor's analysis. However, a statistically significant (at 0.05 level) linear trend was reported by FDA statisticians for brain granular cell tumor ($p=0.0411$) in male rats; occurrence of the above tumor was limited to 3 (of 50) high dose males. Pairwise comparison showed no significant difference between high dose and control groups ($p=0.1594$). According to the sponsor, the incidence rate for the brain granular cell tumor is within the spontaneous range for male rats. It is noted that the above tumor incidence was observed at a dose level which is about 125 times the maximum recommended human dose of 40 mg/day, based on a mg/kg comparison assuming a patient weight of 60 kg. Hypertrophy of the zona glomerulosa cells of the adrenal cortex (high dose males and females) and increased incidence of progressive nephropathy (high dose females) were the major nonneoplastic findings of the above study. Although mean body weights for the high dose group (both sexes), for the duration of the study, were significantly lower than control values except on few occasions, the body weight decrement in high dose males was less than 10% throughout the study. In high dose females, beginning week 45 of treatment, the weight decrement was 10% or more, compared to control, till the end of the study. (The terminal weight decrements for high dose males and females were 5.6% and 22%, respectively.) There were no statistically significant differences in the survival of drug treated and control rats (male or female) in the above study.

In the mouse, dietary administration of nisoldipine at 0, 100, 300 and 900 ppm (19.37, 58.06 and 162.93 mg/kg/day, respectively, in males and 24.99, 74.36 and 217.28 mg/kg/day, respectively, in females) for 21 months showed no evidence of a drug related carcinogenic effect except for significant positive linear trends (at 0.05 level) for hepatocellular carcinoma and hepatocellular tumors (all) in male mice (sponsor's analysis). Analysis of the tumor data by FDA statisticians failed to confirm these trends. However, FDA analysis showed a significant positive linear trend ($p=0.0072$) for stomach papilloma in male mice [occurrence limited to 2 (of 50) high dose males]. Pairwise comparison showed the difference between high dose and control groups to also be significant ($p=0.0435$). The incidence rate for the stomach papilloma is reported to be within the historical control range for this tumor in NMRI mice. It is noted that the above tumor incidence occurred at dose level that is about 250 times the maximum recommended human dose on a body weight basis. Relevant non-neoplastic findings observed in this study included increased incidences of gastric mucosal hyperplasia (treated males and females - all groups) and pituitary hyperplasia (treated females - all groups). Chronic drug treatment had no significant effect on body weight in this study. The mortality rate of high dose males was significantly higher ($p<0.001$) than control (80%

in the high dose male group vs 28% in control). Though the mortality rate in high dose females (64% vs 56% in control) was also higher than control, the difference was not statistically significant.

Since the incidences of brain granular cell tumor in male rats and stomach papilloma in male mice are within the historical control range, and because nisoldipine has been shown not to be genotoxic, the drug-tumor association is considered to be not biologically relevant.

Only two of the 7 reproductive toxicology studies submitted were performed in accordance with GLPs; the Sprague-Dawley rat and the cynomolgus monkey teratology studies. All of the studies appear to be scientifically valid, but in the non-GLP studies, even with the amended tables, it was frequently not possible to determine the times of death.

In spite of the fact that the test substance had very low acute toxicity (LD_{50}> 10 g/kg) in Wistar rats, and could be administered chronically to Wistar rats in the carcinogenicity study at a dose as high as 220 mg/kg, doses given in the modified Segment I and III studies were only 3, 10 and 30 mg/kg. The only justification given for selection of these low doses was the undocumented statement, "The doses were chosen on the basis of toxicological results of other studies". Both studies included C-section of a proportion of the dams on day 20 of gestation.

In the Segment I study, nisoldipine produced no observable effects in the males (treatment started 70 days prior to mating) or females (treatment started 21 days prior to mating) in terms of clinical signs, body weight gain, mating, fertility and pregnancy rate. There were small but significant and dose related increases in mean fetal weight at 10 and 30 mg/kg but weights were said to have remained within normal limits for this strain. There were no differences from control in fetuses with external, soft tissue or skeletal malformations. In dams allowed to give birth, pregnancy duration was slightly, but significantly increased at all 3 dose levels.

In the Segment III study, nisoldipine administration was associated with a slight but statistically significant decrease in body weights (compared to control) of the high dose (30 mg/kg) dams of both the C-sectioned and rearing groups, after only the 4th day of treatment (day 20 of gestation). The only indication of toxicity to the offspring of the C-sectioned dams was a statistically significant decrease in mean fetal weight at the high dose. In the dams allowed to give birth, there was an increase in number of stillborn pups, and an apparent dose related increase in mortality of the newborn pups during the first week postpartum in the mid and high dose groups, but no statistical analysis was performed. The birth weight and the body weight gain of pups during lactation was reduced in the 30 mg/kg group compared to control.

In the supplemental Segment III study, where only the 30 mg/kg dose was tested, reduced maternal body weight gain was evident by day 20 of gestation (after only 4 days of treatment), with weights remaining below control weights through the first week of lactation. Gestation length was significantly prolonged, a finding that was interpreted by the sponsor as a "pharmacologically-induced tocolytic effect" (inhibition of uterine contractions), and there was a large increase in number of pup deaths at birth and during the first 2 weeks of lactation. Also, a decrease in pup weight was noted at birth and during the first week of lactation. These findings of maternal and fetal toxicity at 30 mg/kg confirm the observations noted for the 30 mg/kg group of the main Segment III study. Prolongation of gestation, which was seen in the supplemental Segment III study, had not been observed in the primary Segment III study but was observed in the Segment I study as well as in a Segment II study in another strain of rat (see table which follows and table on page 156).

DOSAGE THRESHOLDS FOR FERTILITY-REPRODUCTION STUDY AND PERINATAL-POSTNATAL STUDIES IN RATS

Report No.	T0002152	T1002153	T3008898
Study Type	Fertil-Reproduction	Peri- post-natal	Peri- post-natal
Strain	Wistar	Wistar	Wistar
Dose (mg/kg)	0, 3, 10, 30	0, 3, 10, 30	0, 30
Vehicle	glycerol-water-PEG	Glycerol-water-Lutrol	Glycerol-water-Lutrol
Days Administered	From 10 wks (males) or 3 wks (females) prior to mating to GD 7	GD 16 to PPD 21	GD 16 to PPD 14
C-Section Day (GD)	*20*	20	Not done
No. Fem/gp. C-Sectioned	23-27	25	N/A
No. Fem/gp. Littered	22-27	20-23	25
Maternal toxicity			
1. Decr. weight gain	>30 mg/kg	>10 ≤30 mg/kg#	≤30 mg/kg#
2. Decr. food intake	>30	>30	>30
3. Prolonged Gestation	>30	>30	≤30
Fetal (C-sect) toxicity			
1. Decr. survival	>30 mg/kg	>30 mg/kg	N/A
2. Decr. fetal weight	>30	>30	N/A
3. Decr. placental wt	>30	>30	N/A
4. Incr. malformation	>30	>30	N/A
Neonatal Toxicity			
1. Incr. stillborn	>30 mg/kg	>3 ≤10 mg/kg	≤30 mg/kg
2. Decr. survival	>30	>3 ≤10 (1st week)	≤30 (1st wk)
3. Decr. birth weight	>30	>10≤30	≤30
4. Decr. wt gain	>30	>10≤30	≤30

Limited to GD 16-20; effect was slight and barely significant in the first study, highly significant in the second.

Two Segment II studies were performed with rats, the first one with Long-Evans rats which received the drug in a polyethylene glycol-glycerol-water vehicle, and the second one with Sprague-Dawley rats which received the drug in an aqueous-Tylose vehicle. In both tests, the doses administered were 10, 30 and 100 mg/kg. In the second test with Sprague-Dawley rats (but not in the first one with Long-Evans rats), half the pregnant dams on test were allowed to litter and raise their young until 25 days postpartum; then selected males and females in each litter were monitored to sexual maturity. In both studies, a dose related decrease in body weight gain was noted for dams at the 2 highest doses. In the Long-Evans rat, there was no effect of nisoldipine on fetal weight, but in the Sprague-Dawley study, a dose related decrease was noted (significant at the 2 highest doses). There was an increase in postimplantation loss in the high dose group of the Sprague-Dawley study. There were indications of fetal immaturity in the 100 mg/kg group (more clearly noted with the Sprague-Dawley rat), as indicated by increased incidence of incomplete ossification of various bones and of stunted fetuses. Also in the Sprague-Dawley study (not shown in the following table), an increased number of fetuses with slightly increased (relative to normal control) dilatation of lateral ventricles and/or space between the body walls and organs occurred mainly in 2 litters of the 100 mg/kg group and was associated with low body weights of the fetuses in these two litters. Prolongation of gestation length was noted for the high dose group. Curiously, the birth weights of pups from the mid and high dose dams were slightly higher than control (the same observation was made for the 10 and 30 mg/kg groups of the Segment I study, but the opposite observation, i.e. a decrease in pup weight at birth, was made in the Segment III main and supplemental studies), and there was no increase in stillbirths and neonatal deaths, as had been observed in the Segment III studies. It is also pertinent to point out that in contrast to the Segment III studies where treatment was continued through the time of expected delivery, in the Segment II study with Sprague-Dawley rats, treatment was limited to days 7 to 17 of gestation. Thus, prolongation of pregnancy occurred a few days after treatment with nisoldipine had been discontinued.

DOSAGE THRESHOLDS FOR ADVERSE EFFECTS IN RAT DEVELOPMENTAL STUDIES

Report No.	7596	LSR 87 BAG0520/938
Strain	Long-Evans	Sprague-Dawley
Dose (mg/kg)	0, 10, 30, 100	0, 10, 30, 100
Vehicle	Glycerol-water-PEG	Aqueous tylose
Days Administered (GD)	6-15	7-17
Day of C-Section (GD)	20	20
No./Gp. C-Sectioned	20-21	21
No./Gp. Littered	N/A	11
Maternal Toxicity 1. Mortality 2 Decr. weight gain 3 Decr. food intake 4. Prolonged gestat	 >100 mg/kg >10 ≤30 Not measured N/A	 >100 mg/kg >1 ≤30 >10 ≤30 >30 ≤100
Fetal Toxicity 1. Incr. post-implant loss 2. Decr. fetal wt 3. Decr. placental wt	 >100 mg/kg >100 >30 ≤100	 >30 ≤100 mg/kg >10 ≤30 >100
Neonatal & postnatal toxicity (to time of breeding)	N/A	>100 mg/kg

In both Segment II rabbit studies, there was a compound related decrease in body weight gain of the pregnant does. In the first study, where the doses administered were 3, 10 and 30 mg/kg and the vehicle used was glycerol-water-PEG, a decrease in mean number of live male fetuses per doe ($P< 0.05$) resulted in a decrease in ratio of live male:female fetuses in the 30 mg/kg group. There was also a small increase in incidence of fetuses with anomalies (e.g. forelimb abnormalities and cleft palate) in the 30 mg/kg (high dose) group, but this was not associated with an increased incidence of any specific malformation. In the second study, where only the 30 mg/kg dose was tested and the vehicle used was aqueous tylose, mean fetal and placental weights were decreased and "underdeveloped forms" (defined as fetuses weighing $\leq$ 2.5 g), were increased vs control. The investigators attributed the increase in malformations in the first rabbit study to the increase in maternal stress. They suggested that the combination of the glycerol-water-PEG vehicle and the drug resulted in an increased incidence of diarrhea in the high dose dams, and that the diarrhea caused an increased incidence of does which aborted their entire litters (4 at high dose had diarrhea; 2 of these aborted and 1 died). However, diarrhea and abortion, also observed in 1 low dose dam and 1 mid dose dam, were considered "normal" for this strain of rabbit.

In both the rat and rabbit studies, increased incidence of malformations, increased fetal lethalities and/or depressed fetal weights were observed only with maternally toxic doses.

DOSAGE THRESHOLDS FOR ADVERSE EFFECTS IN RABBIT DEVELOPMENTAL STUDIES

Report No.	7595	7595 (Suppl)
Strain	Himalayan	Himalayan
Doses (mg/kg)	0, 3, 10, 30	0, 30
Vehicle	Glycerol-water-PEG	Aqueous tylose
Days Administered (GD)	6-18	6-18
Day of C-section (GD)	29	29
No./Group C-Sectioned	10-13	11
Maternal Toxicity		
1. Decr. body wt gain	>10 ≤30 mg/kg	≤30 mg/kg
2. Lethality	>10 ≤30*	>30
3. Diarrhea	>10 ≤30*	>30
4. Spontan. abortion	>10 ≤30*	>30
Fetal Toxicity		
1. Decr. survival	>10 ≤30 mg/kg#	>30 mg/kg
2. Decr. fetal wt.	>30	>10 ≤30
3. Decr. placental wt	>30	>10 ≤30
4. Incr. malformation	>10 ≤30**	>30

* In the first study with glycerol-water-polyethylene glycol vehicle, there were 4 high dose does with diarrhea, 2 of which aborted and 1 of which died. There was also 1 at low dose with diarrhea which aborted and one at mid dose which aborted but did not have diarrhea; none of the controls aborted. In the second test with aqueous tylose vehicle, there were no deaths and none of the treated animals aborted or had diarrhea. The high dose deaths and abortions in the first study were attributed by the sponsor to diarrhea, caused by an interaction of vehicle and compound, although the 2 vehicles were not directly compared in the same experiment.

The mean number of male live fetuses was significantly reduced in the 30 mg/kg group, resulting in a reduction in ratio of males:females in the first study. The sponsor considered the reduction in ratio of males:females a spontaneous occurrence and the overall decrease in number of live fetuses was attributed to the stress of diarrhea.

** Malformations in fetuses of the high dose group occurred in 3 dams. There was an increase in total number of fetuses with malformations and total number of dams that had fetuses with malformations (no statistical analysis), but no increase in any specific malformation. The investigators attribute the increase in malformation rate to increased stress due to diarrhea.

In the study with cynomolgus monkeys, clinical signs, which included heavy bleeding (followed by abortion), reduced food intake, diarrhea and vomiting, occurred during the treatment period in all the groups, including vehicle control. Although the symptoms were generally more frequent and of longer duration in the 100 mg/kg treated monkeys, they were still considered by the investigators to be due to treatment with the vehicle (polyethylene glycol-glycerin-water). In the opinion of this reviewer, the stress of handling these feral monkeys (rather than, or in addition to, the vehicle), contributed to the observed symptoms. Of the 10 or 11 pregnant monkeys on test in each group, 6 to 8 of them aborted in every group, and deaths occurred in 3 control, 1 mid-dose (30 mg/kg) and 5 high dose (100 mg/kg) monkeys. The single death at the mid dose, and 4 of the 5 deaths at the high dose, were associated with a volvulus (twisting or knotting of the intestine), which was not seen in control animals which died. Malformations were observed in the only surviving fetus of the 100 mg/kg group (left forelimb and tail anomalies, observed externally and by skeletal examination). In spite of the very few surviving fetuses that could be examined for malformations (3 controls, 2 low dose and 1 high dose) it is claimed, "The skeletal abnormalities in this one fetus are considered to be related to treatment wit. _ because similar defects were never observed in control fetuses of previous studies of the same type in Macaca fascicularis". No historical control data or details on how many previous studies or the number of control monkeys that were examined for teratogenicity are provided to support this statement. It should be pointed out that malformations occurred only at a dose level that was highly maternally toxic.

(nisoldipine) was tested for mutagenicity by five *in vitro* test systems (Salmonella/microsome, CHO HGPRT forward mutation assay, mouse hepatocyte primary culture DNA repair, a CHO test for clastogenicity, and by a test for inhibition of intercellular communication between two types of liver cells, 1) a primary rat culture ("wild type cells") and 2) the ARL14-TG^R cell line. It was further tested for mutagenicity in two *in vivo* systems (mouse micronucleus and mouse dominant-lethal). All the tests for mutagenicity appeared to be adequately performed, and positive controls in all of them confirmed the acceptability of the studies. Based on the outcome of these *in vitro* and *in vivo* studies, nisoldipine was not found to be mutagenic.

In conclusion, the preclinical studies summarized above have demonstrated the efficacy of the test drug as an antihypertensive and antiischemic agent. Adverse effects are seen only at high multiples of the maximum recommended human dose.

RECOMMENDATION

The NDA is approvable with suggested changes in labeling.

Sidney Stolzenberg, Ph.D

Xavier Joseph, DVM
August 29, 1994

ATTACHMENTS (3)

cc:
Orig.NDA
HFD-502
HFD-345/GJames
HFD-110
HFD-110/CSO
HFD-110/SStolzenberg
HFD-110/XJoseph
CAR 8/31/94

D.R.

NDA REVIEW
Clinical Pharmacology

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

NDA:	20-356
Name of Drug:	Nisoldipine, Coat Core Tabs
Sponsor:	Miles
Indications:	Hypertension
Submitted:	03/31/93
Received:	04/05/93
Assigned:	04/09/93
Reviewed:	08/25/93
Review Completed:	02/14/94
Reviewer:	Shaw T. Chen, M.D.,Ph.D.

FEB 16 1994

Overview of NDA (Clinical Pharmacology)

Nisoldipine is a calcium channel blocker of dihydropyridine derivative type being developed for the treatment of hypertension . In this initial application, approvals of a sustained release formulation (coat-core) for indications are requested. As a part of new parallel, team approach, this medical review covers only the areas related to clinical pharmacology.

The sections on clinical pharmacology (Section 8.1 of NDA) contain data of 17 studies, involving 393 patients/subjects, on sustained released formulation (coat-core, referred to as CC in this memo). Of these, 183 participated in 6 U.S. studies. In addition, the submission also includes results of 47 studies on the immediate release preparation (IR), most of which were conducted in foreign countries. Except for three small studies (total 12 normal subjects, copies of publications only), full reports of all studies listed in Section 8.12 were submitted.

Additional pharmacokinetic and bioavailability data were presented in Section 6 of the NDA, which include 129 foreign studies on IR or other non-CC formulations not repeated in the clinical sections (Section 8, as noted above). These studies will be reviewed by the biopharmaceutical group of the Agency and not commented in this report.

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PHARMACOKINETICS

Formulation Design

The new coat-core formulation, which has a slowly-dissolving coat and an immediate release core, was designed based on the observation that nisoldipine is readily absorbed in the upper gastrointestinal tract but cleared by a first pass rapidly and a marked decrease in the rate of absorption but lower first pass metabolism in the colon.

Absorption/Disposition

The absorption of radiolabeled oral nisoldipine solution was rapid (T_{max} 0.42 hr) and extensive (87%). Despite efficient absorption, absolute bioavailability of the parent drug was only 8.4%, due to a high first pass effect (Study 400). Measured by iv infusion, nisoldipine has a volume of distribution about 2.3 to 3.4 L/kg (Study 330).

In single dose studies (Studies 102-106), oral doses of nisoldipine 6-20 µg/kg administered as IR capsules resulted in C_{max} of 2.7-19.3 µg/L within 30 minutes[1] after dosing. Nisoldipine was detectable (>1 µg/L) 4 hrs later only in the high dose groups (12 µg/kg and above). Compared with the IR formulation, nisoldipine administered in the controlled release (CR) forms had reduced C_{max}, greater AUC, prolonged mean residence time (MRT), duration of plasma concentration above 0.3 ng/ml and T_{max} (6 subjects, Study 632):

Formulation	C_{max} ng/ml	AUC_{norm} g.hr/L	MRT hrs	$T_{c>0.3}$ hrs	T_{max} hrs
CR (E 029)	0.86	57.2	21.2	23.9	12
IR	1.55	31.3	4.2	4.4	2
CR/IR	0.55	1.82	5.02	5.42	
(95% Confidence)	(0.34-0.90)	(1.30-2.56)	(3.48-7.23)	(3.48-8.45)	

Time-courses of plasma concentrations after administration of the three CR formulations were compared with that of the IR dose in Figure 1. Based on these characteristics, the CR formulation E 029 was chosen from the three studied for further development. While the bioavailability was relatively higher than the IR form, absolute bioavailability of the CR formulation was still low (5.5%) in another study (Study 637).

Bioavailabilities (C_{max} and AUCs) of nisoldipine were dose-proportional for both the IR (at 2.5-20 mg, Studies 125, 339, D85-024-01) and CC (at 10-60 mg) formulation (Study D91-035)[2].

[1] There may be greater variability in T_{max}, which was longer (mean 2 hrs) for IR nisoldipine in another study (632).

[2] The 10 mg dose may not be as linear as other doses, but the deviation is non-significant.

Figure 1

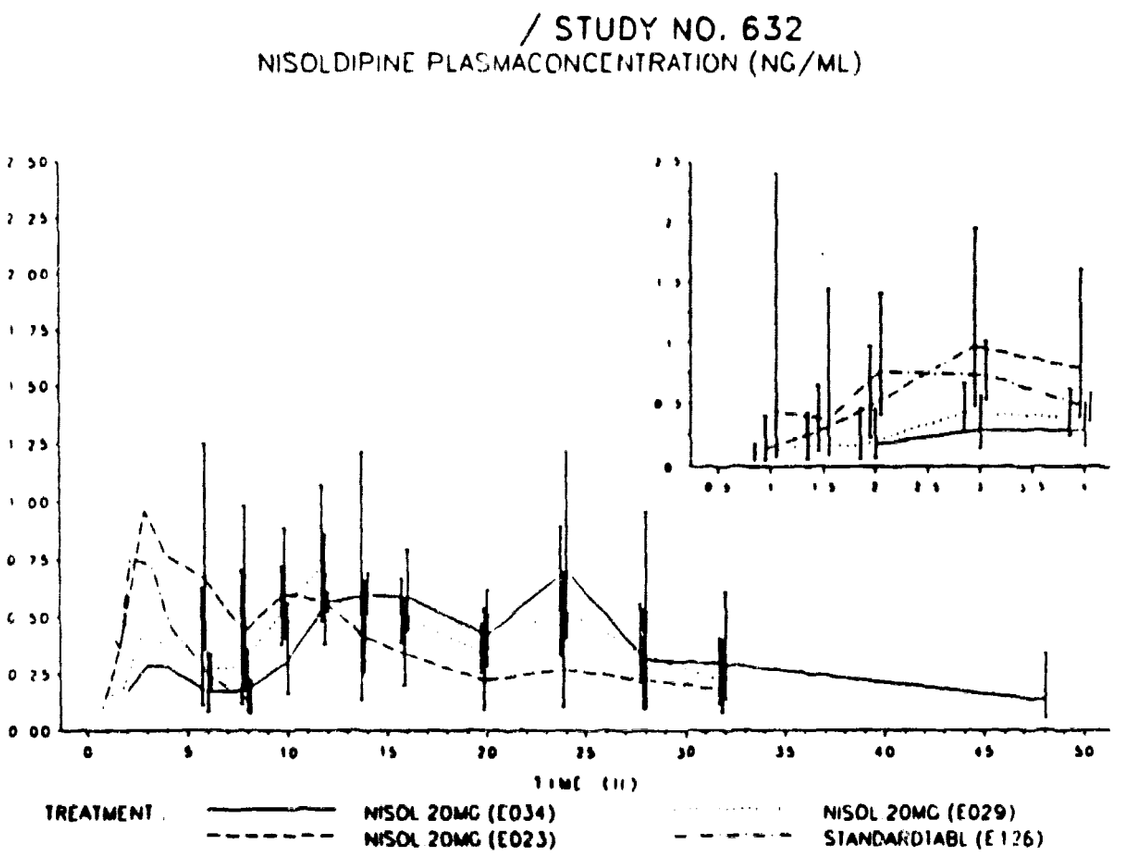


Fig. 1

Although there is no evidence of accumulation with **multiple doses** of IR formulation (10 mg bid), bioavailability of CC nisoldipine increased moderately after 7 days of daily 20 mg dosing (see Table below, Study 645). At these doses, fluctuations of plasma nisoldipine levels were lower for the CC formulation (113% vs 434% for IR).

Formulation		C_{max} ng/ml	AUC_{norm} g*h/L	$T_{c>0.3}$ hrs	T_{max} hrs
CC	Day 1	0.84	40.3	14.9	11.1
	Day 7	1.09	58.9	28.4	9.2
IR	Day 1	2.18	40.8	10.8	2.4
	Day 7	1.95	40.3	11.6	2.3
CC/IR	Day 7	0.56	1.46	2.45	
(95% Confidence)		(0.47-0.66)	(1.27-1.69)	(1.95-3.08)	

While the AUC_{norm} remain similar regardless of fed or fasted state, C_{max} was increased by 38-48% when 20 mg of CC nisoldipine was administered together with or 1 hour after breakfast (Study 666). **Dose-dumping by food** of the CC formulation (administered within 5 minutes after completion of a meal) was even more pronounced with 30 mg (65-236% increase in C_{max}, 11-42% decrease in AUC) and 40 mg (92-292% increases in C_{max}, 7-39% decrease in AUC) doses in Study D92-045-02 (range given are 90% CI's). This food effect was both dose and formulation dependent, since C_{max} and AUC_{norm} were increased only modestly (31 and 28% respectively) by food for the IR formulation (20mg, Study 323), and may have clinical implications in patients usually older than those in the food studies (see Comments on Individual Studies below).

At the concentrations 20 times or higher than that observed in kinetic studies, nisoldipine and its enantiomers are >99% **protein bound**. Partitioning between plasma and blood or erythrocytes were moderate (0.7 blood to plasma, 0.3 erythrocyte to plasma). (Study 339, Ref. 3 (PB19611))

Metabolism

Following oral administration of nisoldipine solution, eleven metabolites, but no unchanged parent drug, was detected in the urine (Study 400, Reference 15 (PB 16626)). The proposed biotransformation pathways of nisoldipine in humans are described in Fig 2. In man, hydroxylation of the isobutyl ester appears to be the major product. Of the three metabolites detected in human plasma (M9, M10, M13), M-10 [redacted] is most abundant (approx 10-20 times of parent drug) and M-9 [redacted] is the only one with biological activity (about 1/10 of parent drug, Study P1010947). The latter is present at approximately the same[3], dose-proportional concentration as the parent drug (Studies 339, D85-024-01).

[3] In another study (No. 125), a metabolite was present at higher C_{max} and AUC (2-4 times of parent drug), which was later identified as sum of M-9 + M-10 due to nonspecific assay (12/20/93 Amendment).

1 page
purged

Excretion

Excretion of nisoldipine metabolites was predominantly renal (70% urine, 12% feces with oral dose), and varies little with route of administration (80% urine, 14% feces with iv dose) (Study 400). Terminal elimination half lives of iv nisoldipine, as measured in 4 healthy subjects in Study 330, were 11-12 hrs, with systemic clearance of 544-768 ml/hr*kg. Nisoldipine is probably not dialyzable (Study 311).

Pharmacokinetics of Enantiomers

The bioavailability of nisoldipine is dominated by the (+) enantiomer, when administered as racemic mixture with only the (+) enantiomer labeled with radioisotope (Ref 43), which is also the one with higher cardiovascular activity (in vitro studies). Since all clinical pharmacology studies and efficacy/safety trials were conducted using the racemic mixture, the kinetic parameters for the two enantiomers have no practical relevancy.

Pharmacokinetics in Disease States

Pharmacokinetics of nisoldipine CC formulation in **hypertensive patients** was examined in two double blind, parallel placebo controlled studies (D90-022 & D88-059). While the CC formulation was not compared with the IR form in any hypertensive groups, kinetics of nisoldipine in both normotensive and hypertensive elderly subjects were described in a third study (Study 712).

In study D90-022, 23 patients (5 placebo, 18 nisoldipine) were randomized and treated for 22 days with nisoldipine dosage increased every 4 days[4] from 30 to 60 mg and every 7 days from 90 to 120 mg. As summarized below, the kinetic parameters measured at the end of dosing periods were dose-proportional at 30-90 mg, non-linearity of 120 mg was dismissed for small number of patients (3). In this study, higher C_{max} of nisoldipine in hypertensive patients were reached at approximately the same T_{max} as that in normotensive subjects, but cross-study comparison is difficult to interpret.

Dose (mg qd)	C_{max} ng/ml	AUC_{0-24} ng.hr/ml	T_{max} hrs
30	4.79±0.68	74.28± 7.96	7.22±0.93
60	8.48±0.81	129.76±12.74	9.08±1.97
90	13.02±1.20	199.31±16.45	6.78±2.30
120	14.92±2.01	226.58±12.41	4.00±1.00

[4] Since the bioavailability of CC nisoldipine increased moderately from Day 1 to Day 7 in a study on normotensive subjects (Study 645, see above), 4 days may not be sufficient for reaching steady state for the two low doses in this study. Also, the ascending doses were not separated by washout period (see comments on individual study).

In the second study in hypertensive patients (D88-059), total of 69 patients were randomized in parallel to receive placebo, 5, 10, 20, or 30 mg CC nisoldipine for 7 days. Plasma nisoldipine concentrations were dose-proportional within the range of these doses both on Day 1 and Day 7 (with 40-70% increases from Day 1 to Day 7). The least square mean kinetic parameters at the end of 7 day dosing period are shown below:

Dose (mg qd)	C_{max} ng/ml	AUC_{0-24} ng.hr/ml	T_{max} hrs
5	0.65	8.39	9.21
10	1.02	16.17	4.79
20	2.13	28.24	3.65
30	2.79	40.34	3.73

At least in the elderly (>65), bioavailability of nisoldipine CC was not influenced by the elevated blood pressure in hypertensive patients (Study 712):

Day 7	Normotensive	Hypertensive
C_{max} (ng/ml)	2.61	2.59
AUC_{0-24} (ng*h/ml)	36.9	38.7

Despite the fact that metabolites of nisoldipine are eliminated predominantly by renal excretion, patients with various degrees of **renal impairment** (but not requiring dialysis) had similar pharmacokinetic parameters for the parent drug when given nisoldipine 20 mg in CC formulation for 7 days (Study D92-001).

Cr clearance	>90	61-90	30-60	<30
Day 8, Mean (ml/min/1.73m^2)				
C_{max} (ng/ml)	3.33	3.21	2.54	2.97
AUC_{0-24} (ng*h/ml)	40.0	50.3	38.4	43.8

However, pharmacokinetic effects of renal function on nisoldipine were slightly greater (although not significantly) with initial doses (Day 1)

Cr clearance	>90	61-90	30-60	<30
Day 1, Mean (ml/min/1.73m^2)				
C_{max} (ng/ml)	1.77	2.37	2.71	2.57
AUC_{0-24} (ng*h/ml)	25.3	32.8	36.1	32.1

Thus the accumulations of nisoldipine with multiple doses appeared to be blunted somewhat by the decrease in renal function (Day 8 vs Day1). As expected, elimination of some metabolites was more affected by renal function than the parent drug, but like nisoldipine, the differences were noted mostly on the first day and diminished over multiple dosing (Study D92-001). While there is less pharmacodynamic concern because the only active metabolite, [redacted] was the least influenced by renal impairment, it not clear whether substantial increases (with the initial doses) of other more abundant metabolites by renal impairment has any long-term toxic effect. In this study the group with moderate renal impairment (Cr Cl 30-60 ml/min/1.73m^2) had higher mean age (63 vs 52-54 for other groups), but there is no clear trend suggesting that the conclusion was affected by such difference in age. Administered in the IR form, nisoldipine bioavailability was increased by about 40% when creatinine clearance decreased from >80 to <25 ml/min (Study 364). While nisoldipine was not detectable in the dialysate, thus probably not removed by hemodialysis, bioavailability of nisoldipine IR in patients on dialysis resembled that in subjects of normal renal function in the same study.

Hepatic failure increases bioavailability of nisoldipine administered as CC tablets. Compared with normal subjects, cirrhotic patients who received 10 mg nisoldipine CC had higher C_{max} and AUC_{0-24} (4-5 folds, Study D90-026). There appeared to be less effect of liver function on the bioavailability of nisoldipine in IR formulation, however, the studies were not controlled and the results were variable (Studies 294, 452).

Demographics Differences in Pharmacokinetics

While there is no significant difference with acute dosing (1 day), bioavailability of nisoldipine, administered as CC 20 mg daily for one week, was increased in the elderly normotensive subjects (65-84 years old), as compared with that in the younger subjects (Study 712):

Day 7	Young	Elderly
C_{max} (ng/ml)	1.41	2.61
AUC_{0-24} (ng*h/ml)	14.7	36.9

Bioavailability of IR nisoldipine was also higher (2-3 folds) in the elderly, but little accumulation was observed after one week dosing (Study 563).

Drug Interactions

The effects of other drugs on nisoldipine pharmacokinetics were evaluated in the following studies:

Immediate Release Formulation

Second Drug, Doses	Nisoldipine Doses	Interaction Observed	Study
Ranitidine, 300 mg qd X 3 days vs placebo	IR 20 mg one dose on Day 3	nisoldipine AUC increased 24%	385
Cimetidine, 400 mg one dose then 200 mg tid X 3 doses vs no treatment	oral & iv solution 10 mg po, 0.374 mg iv one dose each period	bioavailability of oral nisoldipine increased by 48%	399
Propranolol, 40 mg one dose vs placebo	IR 20 mg one dose	nisoldipine AUC, C_{max} increased by 30% & 57%	417

CC Formulation

Second Drug, Doses	Nisoldipine Doses	Interaction Observed	Study
Ranitidine, 150 mg bid X 6 days vs placebo	CC 20 mg one dose on Day 5	nisoldipine AUC, C_{max} decreased by 15-20%	738
Cimetidine, 400 mg bid X 6 days vs placebo	CC 20 mg one dose on Day 5	nisoldipine AUC, C_{max} increased by 30-45% t_{max} decreased by 4 hrs	738
Propranolol, 40 mg tid X 5 days vs no treatment	CC 20 mg qd X 5 days	nisoldipine AUC, C_{max} unchanged, $t_{1/2}$ decreased by propranolol (by 20%)	704
Quinidine, 648 mg bid x 2 doses vs no treatment	CC 20 mg qd x 1 dose	nisoldipine AUC reduced by 25%, C_{max} unchanged but at lower t_{max}	703

Bioequivalence of Various Formulations

Bioequivalence between clinical trial and market tablets and between various coat-core dosage forms have been determined in several studies. Details of the results are referred to the Biopharmaceutical Review.

Comments on Individual Pharmacokinetic Studies

In general, pharmacokinetic studies on nisoldipine, either CC or IR formulation, were well designed and properly conducted. Compared with the translated foreign reports, the U.S. studies (study numbers beginning with D) were better documented and probably more reliable. Minor deficiencies for a few studies and interpretation of the data different from that of sponsor have been pointed out, mostly as footnotes, in previous sections. Other than that, there are no study defects collectively serious enough to invalidate the conclusion on kinetic behavior of CC nisoldipine. In addition to the following comments, which are arranged below in the order of Study numbers, further detailed reviews on individual kinetic studies are referred to the Biopharmaceutical Review.

Studies 102-106

Based on a summary report (no detailed protocol), there is nothing remarkable in these placebo controlled, dose-escalating, kinetic studies using IR formulation in 12 normal subjects.

Studies 125, 339

These were placebo-controlled, double-blind, randomized, 3-4 sequence crossover studies on dose-proportionality of nisoldipine IR and metabolites in 12 normal subjects (6 actually treated in Study 125). While treatments were separated by at least one week in Study 339, they were given in three successive days in Study 125. Thus results of the latter study may be confounded by residual effect of preceding dose.

In vitro protein binding of nisoldipine at 20 ng/ml was also performed in Study 339, using each subject's pre-dose plasma.

Studies 294/452

These are two pharmacokinetic studies of IR nisoldipine and its metabolites in cirrhotic patients. The results were translated from foreign reports and no detailed protocols were submitted with the NDA. Subjects in Study 294 were hypertensive but the blood pressures were not described in Study 452. Neither was controlled with subjects of normal liver function.

Study 311

This is a kinetic and tolerability study in patients requiring regular hemodialysis. Seven patients were treated with nisoldipine 10 mg once daily, with dosage titrated up to 40 mg per day, for 3 months. The treatment effects on blood pressure and tolerability were not controlled.

Study 323

This is a food-pharmacokinetic study of IR nisoldipine 20 mg dose. Eight healthy, young male subjects were randomized to receive a single dose of the study drug either in a fasted (1.5 hrs pre-meal) or fed (20 minutes after start of meal) state and crossed over to the opposite food state two-weeks later. Small differences in heart rate response in fasted/fed states were noted (increased 10 bpm vs increase 15 bpm) , but probably of no clinical significance.

Studies 330, 400

These were two uncontrolled bioavailability studies using oral/iv solution in small groups of healthy volunteers. A few subjects were excluded from data analysis due to radioisotope overdose in 2 of 12 subjects in Study 400 and leakage of infusion system in 2 of 6 subjects in Study 330. Washout interval was adequate for the crossover study (28 days, Study 400).

Study 364

This is also a kinetic study in renally impaired patients (Cr Clearance >80 ml/min, <25 ml/min or uremic on dialysis, 29 patients), but treated with single dose IR formulation only. The sponsor concluded that a 40% increase in nisoldipine bioavailability in renal failure was not a significant effect (see discussion above).

Study 400 (PB 14514, Biotransformation)

This was part of study 400 (see above for comments on bioavailability study) which described metabolite profile in human urine after oral and iv administration of radioisotope labeled nisoldipine. Eleven metabolites were identified, but no test of biologic activity was performed and plasma metabolite profile was not investigated.

Study 563

This is a pharmacokinetic study of IR nisoldipine in 21 normotensive subjects, 9 were of 20-28 years of age and 12 were older than 65. All were treated with IR nisoldipine 10 mg for 8 days.

Study 632

Three controlled release formulations (CR) were evaluated in this non-blind, randomized, crossover, single dose study in six healthy volunteers. The kinetics of CR formulation was compared with that of IR nisoldipine administered in the fourth period to all subjects, the wash-out between treatment periods was 6 days. Treatments were given in fasted states.

Study 637

Bioavailability of a controlled release formulation (CR E 029) selected from Study 632 was evaluated further, relative to an iv solution of nisoldipine, in this non-blind, crossover study in 12 normal subjects. Washout out between treatments was adequate (6 days) and study drugs were administered in fasted states.

Study 645

Steady-state pharmacokinetics of nisoldipine CC 20 mg was compared with that of IR formulation given as 10 mg bid in this non-blind crossover study. The study drugs were given in fasted state for one week and the treatments were separated by 7-day washout periods. Total of 18 male subjects were treated and included in the data analysis.

Study 666

This is an open-label study of food effect on pharmacokinetics of nisoldipine 20 mg CC tablets. Twelve young male subjects (24-33 years old) were randomized to receive the a single dose of the study drug in fasted (2 hrs pre-meal), together with (within 7 minutes after start of meal), or one hour after an American breakfast. All subjects also receive another dose together with a Continental dinner in a non-randomized fourth period. Treatment periods were separated by one week washouts. Moderate increase in C_{max} were observed when nisoldipine 20 mg CC was given in fed states, but no difference in clinical adverse events was noted.

Study 712

Bioavailabilities of nisoldipine CC in normotensive and hypertensive elderly (65 and older) patients were compared in this open-label, non-randomized study. In addition, influence of age on pharmacokinetic was also assessed in young and old normal subjects in the same study. Total of 58 subjects (46 young/elderly normal subjects, 12 hypertensive elderly) were treated with daily doses of 20 mg for 7 days.

Study D85-024-01

This is another dose-proportionality study using an ascending-dose, uncontrolled, non-crossover design. Single doses of nisoldipine IR 2.5-20 mg were administered at weekly intervals to twenty subjects. Kinetics of major metabolites in plasma were also described.

Study D88-059

This is a double-blind, placebo-controlled, multiple-dose, kinetic and tolerability study of nisoldipine CC in 69 hypertensive patients. After a 3-week placebo run-in, patients with SDBP of 95-115 mmHg were randomized to receive placebo, 5, 10, 20 or 30 mg nisoldipine CC in five parallel groups and treated for 7 days. The study design was better than that of higher dose range (D90-022, above) and dose proportionality in hypertensive patients was clear over the range of doses in this study. However, quite a few patients were excluded from the analyses for various reasons, which included 6 disqualified for low baseline SDBP (but included in the kinetic data), 10 without steady state blood samples and data of another 10 were considered invalid because these patients took nisoldipine instead of placebo on Day 0. One additional drop-out was due to blood drawing discomfort.

Study D90-022

This is a double-blind, placebo-controlled multiple ascending dose, kinetic and tolerability study of nisoldipine CC in 23 hypertensive patients. Patients were randomized after a 3-week placebo run-in and the dosages were increased every 4 days for the two low doses (30 & 60 mg)

and every 7 days for higher doses (90 & 120 mg) without washout intervals (see above). The concern was that 4 day may not be adequate for reaching steady state and the carry over effects from previous lower dose can not be excluded. Thus the results of this study should be accepted with reservation and dose-proportionality is better supported by a parallel design (Study D88-059, but covered a lower dose range, see next).

Study D90-026

Pharmacokinetics of nisoldipine CC in patients with cirrhosis was evaluated and compared with that in normal subjects in this study. Sixteen, 8 in each group with well matched age, sex and weight, were treated with 10 mg daily for 7 days. Accumulation was observed in both groups.

Study D91-035

This was an open-label, four period crossover study on dose-proportionality of CC nisoldipine. Twenty-four healthy male subjects were randomized to one of four treatment sequences with single doses of 10-60 mgs. Treatments were separated by 7 day wash-outs and given in a fasted state.

Study D92-001

This is a unblinded pharmacokinetic study in patients with renal impairment, using the group with creatinine clearance of >90 ml/min/1.73m^2 as the control for renal function. In addition to the parent drug, kinetics of three major metabolites were also described. Of the 46 patients were treated with nisoldipine CC 20 mg once daily for 7 doses, 42 had valid kinetic data. While the overall recruitment had encountered some difficulty, two subjects were excluded because of "over-enrollment" in the mild renal impairment group. Two additional patients had indeterminate renal function and were excluded from the kinetic analysis. As noted in the above, age was not well matched in the four treatment groups, but no trend was discernible.

Study D92-045-02

This is the most important pharmacokinetic study on food effects, which described a significant dose-dumping phenomenon when nisoldipine CC was administered immediately (within 5 minutes) after a 20-minutes standard high-fat breakfast (see above for description of pharmacokinetic results). This was a randomized, open-label, two-way crossover study. Twenty-eight healthy, young (19-42 years), male subjects with near ideal weights were randomized to receive a single 30 or 40 mg CC tablet in a fasted (4 hour pre-meal) or fed state. After a one-week washout period, all subjects were crossed over at the same dose level to the opposite food state. Except for a higher heart rate in the fed state, the sponsor claimed that despite the marked change in kinetic behavior of nisoldipine CC, there were no pharmacodynamic consequences of dose-dumping by food. In 5 subjects with fed/fasted C_{max} ratio of ≥ 5, more complaints of headache were seen in the fasted state (3 vs 1) and one subjects reported dizziness under fed state only.

Study PB 16626

This was a study on biotransformation of nisoldipine in several animal species and in human. Metabolites detected in urine, but not in plasma, were identified. There were no qualitative differences in biotransformation of nisoldipine in rats, dogs, monkeys or man. Biological activities of metabolites were not described.

Study PB 19611

This is an *in vitro* human plasma protein binding and erythrocyte-plasma partitioning study of nisoldipine over the concentration range of 0.1 to 10 μg/ml. Results of protein-binding in this ^{14}C study were consistent with that of Study 339.

Study P1010947

Results of this foreign study (possibly animal) was cited in the NDA as the basis of the biological activities of various nisoldipine metabolites. However, there is no synopsis of the study anywhere in the application and despite repeated request by the Agency, the sponsor has had difficulty locating and submitting a full report as of the date of this memo.

Study (Reference 43)

This is a manuscript published by Frost et al in *Dose-Response Relationships of Drugs*, no original data were submitted.

Drug Interaction Studies

The results of studies on drug-interactions were separated into the following three groups:

Effect of other drugs on pharmacokinetics of nisoldipine.
Effect of other drugs on pharmacodynamics of nisoldipine.
Effect of nisoldipine on other drugs' pharmacokinetics/dynamics.

Only the first category was included in the Pharmacokinetic Sections, the remaining two will be discussed in the pharmacodynamic sections below. Limitations of individual studies, such as single/multiple doses, duration of treatment and use of control groups, have been described in tables summarizing the results.

Summary of Pharmacokinetic Issues

In summary, pharmacokinetic properties of nisoldipine administered as IR or CC formulations have been studied adequately and well-described in the submission. Bioavailability of nisoldipine CC was low due to high first pass effect and appeared to be linearly proportional to doses of 5-90 mg. Accumulation after 7 days of administration was modest and products of extensive metabolism were excreted renally. Relative to the young subjects, nisoldipine was slightly more bioavailable in the elderly after one week dosing. Bioavailability of nisoldipine was not significantly different in patients with hypertension, angina or renal impairment (except for the initial doses for the latest), but were markedly increased in hepatic failure subjects. Pharmacokinetics of nisoldipine was affected by concomitant administration of cimetidine, ranitidine and quinidine, other drug interactions may be possible through high degree of protein binding (> 99%).

While the kinetic data of CC nisoldipine support a longer dosing interval than the IR form, clinical effectiveness of once-daily dosing depends on the correlation with pharmacodynamic and efficacy findings. It should also be noted that the problem of dose-dumping when nisoldipine CC is administered in a non-fasted state can not be ignored and must be addressed appropriately in the labeling. This phenomenon is more pronounced than with the IR formulation and not exactly unexpected since similar problem has been observed in another approved drug formulated identically and developed by the same sponsor.

The differences in food effects between 20 mg (Study 666) and 30, 40 mg doses (Study D92-045-02) of nisoldipine CC may be due to variations in relative timings of drug administration and meal ingestion both in fasted and fed states. Both the absolute C_{max} and increase relative to fasted state were greatest when nisoldipine CC 30-40 mg was administered immediately after completion of a meal and compared to a prolonged fasted state (for additional 4 hrs post dose) (Study D92-045-02). While this sustained "fasted state" (4 hrs post dose) may not be a realistic simulation of large efficacy trials or practical settings, the dramatic increase in plasma nisoldipine concentration by food may result in excessive hypotension because nisoldipine plasma levels in efficacy trials resembled that of fasted state and there is a good kinetic-dynamic correlation (see below). Thus nisoldipine CC should not be administered concomitantly with meal, but instead after overnight fast and 1-2 hours before breakfast. Appropriate instruction to avoid dose administration in a fed state should be included in the labeling.

PHARMACODYNAMICS

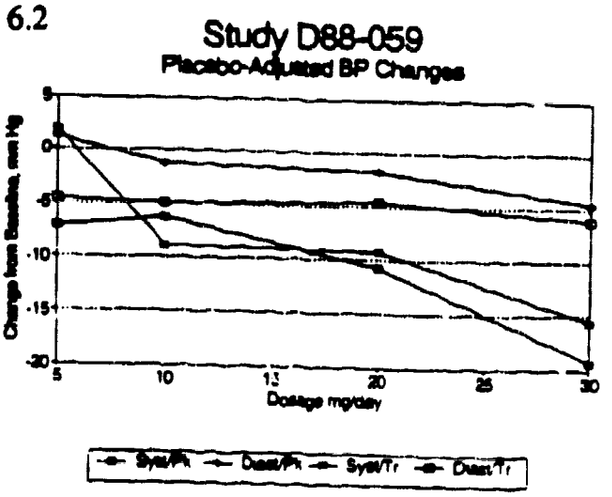
Pharmacodynamic data of nisoldipine are described in three groups in this review: **Principal cardiovascular effects, Non-cardiovascular pharmacological activities** and **Drug interactions**. Integrated summary of pharmacodynamic data as presented in the application and reviewed below were in general based on U.S. studies. Results of small scale foreign studies, mostly open and uncontrolled, were only commented briefly whenever appropriate. It should be noted that most dynamic data were obtained from studies using iv or IR oral form, only the effects on blood pressure and heart rate have been evaluated with the CC formulation. Issues related to tolerability of nisoldipine CC formulation in kinetic studies were also summarized at the end of this section.

Principal Cardiovascular Effects

In contrast with what the sponsor has stated in the NDA, **blood pressure and heart rate** changes were not small nor inconsistent in normal subjects treated with CC nisoldipine. Approximately 4-8 hours after a single dose of 10-60 mg, mean supine diastolic pressure decreased by a maximum of 5-7 mm Hg and mean heart rate increased by 6-11 bpm in a uncontrolled study (D91-035, Appendix 13.9.10). Similar but slightly greater effects (8-9 mm Hg) on SDBP were also seen with a single 60 mg dose in Study D90-020, before adjusted for a placebo response of about 4 mm Hg drop (Study Report Section 13.9.4). Mean Heart rate increases in the same study were 10-15 bpm (vs 2-4 bpm for placebo). The IR formulation produced more rapid but comparable degrees of heart rate and SDBP changes (Study 125, single dose). Relative to the normal subjects, the blood pressure responses of hypertensive patients were certainly more notable with multiple dosing at 5-40 mg/day (about 10 mm Hg drop in SDBP over placebo with IR form, Study 372). *Placebo-subtracted* blood pressure reductions in patients with mild to moderate hypertension were dose-related from 5 to 30 mg of CC nisoldipine (especially in systolic pressures, Study D88-059):

Dose	Changes in Systolic/Diastolic BPs, mm Hg, Day 7	
mg/d	8 hrs post dose	24 hrs post dose
5	- 7.1 / + 1.2	+ 1.9 / - 4.6
10	- 6.3 / - 1.2	- 8.8 / - 4.9
20	-10.7 / - 1.7	- 9.2 / - 4.6
30	-19.2 / - 4.6	-15.6 / - 6.2

On the right dose-response plot, 8 hrs data were used for peak effects. However, due to a large placebo response at 6-10 hours post dose in this study, the SDBP changes at 8 hrs did not appear to be the peak effects and greater antihypertensive activity was noted around 14 hours (-2.8 to -8.6 mm Hg over placebo, Study Report Section 13.9.4).



However, such dose-response relationship was not observed at higher dose range (30-90 mg/day) in another study of hypertensive patients (see Table on next page, D90-022)[5].

Study D90-022

Dose	Changes in Diastolic BPs, Placebo adjusted, mm Hg	
mg/d	8 hrs post dose	24 hrs post dose
30	- 9.4	- 6.4
60	- 9.2	- 6.7
90	- 9.1	- 6.6

Responses to 120 mg was distinctively higher (19.0 and 9.3 mm Hg at 8 and 24 hrs post dose) in the same study, but there were too few patients (3) received this maximum dose. As noted before, the dosages were forced-escalated in rapid sequence and not evaluated in parallel groups in this study, thus made the interpretation difficult.

Changes of heart rate in hypertensive patients due to nisoldipine appeared to be less consistent than that observed in normal subjects (Study 372, IR form). As noted in the NDA, heart rate increases were in the range of 5-10 bpm over baseline values for all nisoldipine groups in Study D90-022. However, it is not apparent whether the changes were caused by nisoldipine since the variation of heart rate in the placebo patients was in the same range and there is no clear trend in the difference between groups that suggests a treatment effect. Heart rate changes were not documented in Study D88-059.

With intravenous administration of nisoldipine at 3-13 μg/kg, changes in blood pressures (decreased by 11-16 mm Hg) and heart rates (increased by 10-30%) were dose-related and slightly more pronounced than that observed in the oral studies. Most of such studies were single-dose, uncontrolled, in small number of patients with underlying coronary artery disease (some were receiving concurrent beta blockers during measurements, Studies 344, 560, Ref 1-5)[6]. Directly compared with diltiazem in a published report (Ref 4)[6], nisoldipine (6 μg/kg) increased heart rate (by 14 bpm) but not diltiazem (500 μg/kg). Both drugs reduced peak systolic pressure by 24-28%.

[5] The sponsor claimed that blood pressure reductions not adjusted for placebo response were dose-related (see Summary of Clinical Pharmacology, Section 8.14), but admitted that the relationship did not exist when placebo responses were subtracted (see Study Report, Section 10.6).

[6] For reference cited, see List and Location of Publications, Section 8.14.3, NDA Vol 116. It should be noted that Ref 1-4 were reported by the same group of investigators.

Effects of nisoldipine on **other hemodynamic parameters** were summarized from 9 studies, three with full reports submitted with the NDA and 6 from published literatures (Ref 1-6, reprints only, no original data). Of these, hemodynamic effects were measured with intravenous administration of nisoldipine in 7 reports and with oral dosing in 2. In a double-blind, placebo controlled study (Study 372), nisoldipine IR titrated from 5 to 40 mg/day (bid) every 2 weeks were effective in blood pressure reduction for 36 hypertensive patients (see above) and reduced peripheral resistance as expected from a calcium channel blocker. Compared to placebo, cardiac index was increased either at rest or during exercise, but the effect of oral nisoldipine on LVEF was probably not meaningful. Changes in hemodynamic parameters as measured by radionuclide techniques are summarized below:

Changes/Baseline:	Resting		Exercise	
Parameters	Nisoldipine	Placebo	Nisoldipine	Placebo
Heart rate (bpm)	- 3.1/77	- 1.9/76	- 0.5/129	- 3.4/131
SBP (mm Hg)	- 24.1/172	- 8.5/171	- 20.4/219	- 13.3/217
DBP (mm Hg)	- 20.1/110	- 8.3/109	- 13.8/123	- 3.5/116
Total Peripheral Resistance (dynes/s/cm^{-5})	-302/1435	-132/1519	-165/953	- 32/940
Cardiac Index (L/min/m^2)	+ 0.31/4.1	+ 0.05/3.9	+ 0.37/7.4	- 0.01/7.4
Stroke Index (ml/m^2)	+ 5.6/53	+ 2.4/52	+ 2.7/58	+ 1.3/56
LVEF	+ 0.04/0.63	- 0.01/0.67	+ 0.02/0.75	+ 0.00/0.75
Double Product	- 23.9/134	- 9.2/130	- 27.4/283	- 24.7/288

A single oral dose of or 20 mg IR nisoldipine decreased systemic vascular resistance significantly, but maintained same cardiac output, as measured invasively in 12 patients undergoing electrophysiology evaluations (Study 178, uncontrolled):

	Baseline	2 hrs post dose	
Heart rate	64	73	bpm
arterial pressures, Syst/Diast	130/76	120/69	mm Hg
Right Atrial Pressure	4	2	mm Hg
Pulmonary Arterial Pressures S/D	19/8	20/8	mm Hg
Pulm Cap Wedge Pressure	8	7	mm Hg
Cardiac Index	3.75	3.71	L/min/m^2
Systemic Resistance	1307	1038	dynes/s/cm^{-5}
Stroke Index	50	53	ml/m^2

In another single oral dose study (Ref 6)[6], nisoldipine 10 mg attenuated both the drop in exercise LVEF in patients with coronary disease (-10% after vs -18% before treatment) and the decrease in LVEF during cold pressor test in patients with signs of ischemia but normal coronary artery .

Acute effects of intravenous nisoldipine on invasive hemodynamic factors are fairly consistent in Study 560 and several published reports (Ref 1-5)[6]. As noted above, most of these studies were single-dose (3-13 μg/kg)[7], uncontrolled, in small number of patients with coronary artery disease (some were on concurrent beta blockers during measurements). Similar to that observed with oral administration, total systemic vascular resistance was decreased (by about 30-35%) with iv nisoldipine in all studies described (Ref 1-3)[6]. Nisoldipine iv also decreased intra-aortic and left ventricular systolic pressure (by 15-30%), but not LV end diastolic pressure in these studies. Nisoldipine did not appear to have negative inotropic activities. Ejection fraction and stroke volume were increased by 16 and 21-24%, respectively, and cardiac out was up 26-36%.

The hemodynamic effects of nisoldipine on **coronary blood flow** have been examined in several uncontrolled studies using intravenous nisoldipine. In patients with coronary artery disease, nisoldipine administered intravenously increased coronary blood flow, but only in normal vessels or in area with collateral support (by 38-52%, 6 patients on background therapy of atenolol, Study 344). It also dilated the coronary arteries for up to 15 minutes after a 0.5 or 1.0 mg dose infused over 4 minutes, which was not plasma level related, however (Study 560). In several published reports, nisoldipine increased coronary blood flow by 17-50% (Ref 1-4)[6], thus reduced the calculated coronary vascular resistance by 40-50%. Myocardial oxygen consumption changes (decreased by 4-8%) were not significant in these studies. Similar to nicardipine, nisoldipine reduced myocardial lactate production in patients evaluated for angina (Ref 5)[6].

In patients with stable angina, nisoldipine IR given as a single oral dose of 20 mg increase exercise tolerance by 200 watts-min, as compared with 10 watts-min for placebo (Study 126). Nisoldipine also reduced ST-segment change more than that by placebo at the maximum stress (0.8 mm vs 0.1 mm) in the same study. Exercise duration was increased in a published report (Ref 6)[6], but the study was not controlled and no data on ST change were described. These **anti-ischemic effects**, however, were not observed in another single oral dose study of similar design (Study 648/649).

In an **electrophysiology** study using oral nisoldipine (baseline controlled, Study 178), sinus cycle length and AH intervals were shortened by 12% and 9%, respectively, about 120 minutes after a 10 or 20 mg single dose. Other ECG changes (QTc, QRS, HV, corrected sinus recovery time, effective ventricular and atrial refractory periods) were not significant. In another similar study using the same IR oral doses (Study 135), there were no significant changes in intra-cardiac conduction times and the effects of nisoldipine on sinus node were also mild (except for sinus node recovery time and SA conduction time, which were decreased by 11-15%, all other changes were less than 10%). However, electrophysiological measurements may not be performed at time of maximum effect (within 20-45 minutes of dosing) in the latter study.

[7] In Ref 3, hemodynamic measurements were performed after a 4.5 μg/kg bolus followed by a constant infusion of 0.2 μg/kg/min over 30 minutes.

With intravenous administration at single dose of 1.5 μg/kg, similar shortening of sinus period were noted at 15-40 minutes post dose, without other changes in refractory periods (Study 144). The effect of iv nisoldipine on sinus cycle length was blunted some what (to 7%) if the patients were pre-treated with beta-blockers (Study 257,.

Some ECG changes, usually seen as T-wave flattening or inversion, appeared to associated with nisoldipine in several tolerability studies. They occurred most frequently (65% of 17 treated patients vs none of placebo) in Study D90-022, a 3-week double blind, placebo-controlled, ascending dose study in hypertensive patients. The incidence is probably dose and plasma concentration related, since in this study, it increased from 22% to 80% with doses up from 30 mg to 120 mg, observed at peak but not trough drug level in some patients, and reversible when study drug was discontinued. Also, patients who reported the ECG changes had significantly higher C_{max} than those who did not (Study Report Section 10.8.4). These reactions were correlated with blood pressure drop and hemodynamic effects have been proposed as the mechanism in literatures for this and other calcium blockers. Higher rate of T-wave ECG changes in Study D90-022 was attributed to rapid and forced dose escalation. This issue has been discussed in more details by Dr. Dern in his Medical Review.

Based on one double-blind, crossover study comparing **neurohormonal effects** of nisoldipine IR 10 mg, nifedipine 20 mg, and placebo (Study 199), nisoldipine ha. no significant effects on the renin-angiotensin-aldosterone system or noradrenaline concentration in normal volunteers.

Nisoldipine IR at 20 mg daily increased **regional blood flows** in liver and kidney, but the effect was transient and no different from that of placebo by Day 4 (Ref 7)[8]. Similar time course of effects was noted for some renal function parameters (GFR and sodium excretion) in the same study. Nisoldipine, administered intravenously, increased blood flow in forearm more than nitrendipine or nifedipine (Ref 8)[8].

Non-cardiovascular Pharmacological Activities

Nisoldipine IR given at 10 mg bid for 4 weeks has no significant effect on **thyroid** function tests or **prolactin** level in young and healthy subjects (placebo controlled, Study 670). Similar to verapamil (160 mg), nisoldipine inhibit **platelet aggregation** after 4 days of treatment with 20 mg daily. However, nisoldipine plasma concentration at such dose did not have the same anti-platelet effect *in vitro* and unlike verapamil, nisoldipine did not displace yohimbine from specific platelet binding sites (Ref 9)[8]. The sponsor claimed that nisoldipine, at 5-20 mg daily for 4 weeks, induced favorable changes (increased HDL and apoprotein A) in plasma **lipoproteins** in a group of 15 hypertensive patients (Ref 10)[8]. The study, however, had no concurrent controls. In a double-blind, placebo-controlled 3-week trial in normal subjects (Study 479), there were no significant differences between placebo and nisoldipine (10 mg bid) in **psychomotor** per nance tests.

[8] For reference cited, see List and Location of Publications, Section 8.15.3, NDA Vol 116. References 7-10 cited in this review correspond to Publications 1-4 of the list on Page 08 15 00000[illegible], Section 8.15.3.

Drug Interactions

Pharmacodynamic interaction studies were conducted for nisoldipine and the following drugs:

a. Effect of Other Drugs on Nisoldipine Pharmacodynamics

Immediate Release Formulation

Second Drug, Doses	Nisoldipine Doses	Interaction Observed	Study
Ranitidine, 300 mg qd X 3 days vs placebo	IR 20 mg one dose Day 3	no differences in hemodynamics	385
Cimetidine, 400 mg one dose then 200 mg tid X 3 doses vs no treatment	oral & iv solution 10 mg po, 0.374 mg iv one dose each period	cimetidine had no additional hemodynamic effects[9]	399
Propranolol, 40 mg one dose vs placebo	IR 20 mg one dose	propranolol attenuates heart rate increase by nisoldipine	417

CC Formulation

Second Drug, Doses	Nisoldipine Doses	Interaction Observed	Study
Propranolol, 40 mg tid X 5 days vs no treatment	CC 20 mg qd X 5 days	no significant changes in hemodynamics	704

b. Effect of Nisoldipine on Other Drugs

Second Drug, Doses	Nisoldipine Doses	Interaction Observed	Study
Quinidine, 500 mg bid X 5 doses	IR 10 mg bid X 7 days vs placebo	quinidine AUC increased 17-26%, no ECG changes	384
Quinidine, 648 mg bid x 2 doses	CC 20 mg qd x 1 dose vs no treatment	no significant changes in quinidine kinetics	703
Warfarin, "steady state"	IR 10 mg bid X 21 days vs placebo	no change on warfarin level or anti-coagulation effect	349

[9] model dependent, see comments on individual studies.

Propranolol, 40 mg one dose	IR 20 mg one dose vs placebo	propranolol AUC, C_{max} increased by 43% & 68% no effect on beta blockade	417
Propranolol, 80 mg bid X 7 days	IR 10 mg bid for 7 days vs placebo	propranolol AUC, C_{max} unchanged, higher heart rate w/ nisoldipine	Ref 57
Propranolol, 160 mg qd X 2 weeks	IR 20 mg qd for 2nd week vs placebo	propranolol AUC, C_{max} increased by 30% & 50%, further BP reduction and higher heart rate by nisoldipine	382[10]
Propranolol, 40 mg tid X 5 days	CC 20 mg qd X 5 days vs no treatment	propranolol AUC, C_{max} decreased by 14-15%, $t_{1/2}$ increased by 25%, no effects on hemodynamics	704
Atenolol, 100 mg qd X 2 weeks	IR 20 mg qd for 2nd week vs placebo	atenolol C_{max} increased by 20% further BP reduction and higher heart rate by nisoldipine	382[10]
Digoxin, 0.6 mg qd X 2 days then 0.3 mg qd X 20 days	IR 10 mg bid Days 9-22 (no control)	digoxin plasma level increased 7% (95%CI 3-20%)[11] no dynamic interaction	413
Digoxin, 0.25 mg bid X 7 days (pre-treated 14 days)	IR 10 mg bid X 7 days vs placebo	digoxin plasma level increased 15% ($p<0.05$) no dynamic interaction[13]	Ref 58[12]

[10] see comments on individual studies for design problems.

[11] the sponsor has claim no kinetic interaction in this study, but the data are consistent with the results of Ref 58.

[12] heart failure patients

[13] the authors claimed interaction in pre-ejection periods, 139±11 ms with placebo vs 129±11 ms with nisoldipine.

Tolerability Findings

Tolerability findings in pharmacologic studies are described as follows, which are included as part of dynamic characterization and not to be relied on heavily for safety assessment. For complete assessment of adverse experiences and safety profile of nisoldipine, reference is made to the reviews on efficacy/safety trials by Drs. Dern and Stockbridge.

As noted in the above sections on pharmacokinetics, studies referred to as "dose-tolerability" in the NDA were of short term (mostly 7 days) and conducted in small number of subjects. In general, nisoldipine administered in CC formulation was well-tolerated in healthy volunteers (Studies D91-035, D90-020), hypertensive patients (D88-059, D90-022) and in subjects with hepatic (D90-026) or renal impairment (D92-001). Adverse experiences reported frequently in these small studies were roughly dose-related and not unexpected from those observed for other calcium channel antagonists, which included dizziness, edema, flushing, headache, nausea, postural hypotension and tachycardia. Somnolence was a frequent complaint in patients with hepatic dysfunction and overall frequency of adverse events was higher in the renally impaired patients. Abnormal ECG with T-wave changes (see above in Pharmacodynamics, Electrophysiology) was noted in at least three studies, one in normal subjects (D90-020), one in hypertensive patients (D90-022) and one in patients with renal impairment (D92-001). However, it was not clear whether the ECG changes were correlated with any clinically intolerable signs and symptoms. Similar ECG changes had not been as prominent in Phase III efficacy/Safety trials (see Dr Dern's Review).

Comments on Individual Pharmacodynamic Studies

While most pharmacodynamic studies of nisoldipine were of reasonable design and execution, some dynamic activities were not controlled and such results should be accepted with reservation. Again, the U.S. studies were better documented and probably more reliable than the translated foreign reports. Minor deficiencies and interpretation different from that of NDA have been noted in the discussion above. The following comments are arranged in the order of Study numbers for reference.

Studies 101-107, 109, 110, 115, and 116

These were all small tolerability studies in 4-6 normal subjects each. The adverse effects observed were similar to that of U.S. studies.

Study 125

In this single dose, placebo controlled crossover study, decreases in SDBP and increases in heart rate were dose-related in 6 normal subjects. However, there may not be adequate separation between doses to rule out carry-over effect (see Comments on Individual Pharmacokinetic Studies for study design).

Study 126

Effect of a single oral dose (20 mg) nisoldipine on exercise tolerance was evaluated in this placebo-controlled, double blind study in 12 patients with stable angina. While nisoldipine improved exercise capacity and ST-segment changes, single dose data are basically of little use for these endpoints.

Study 135

This a electrophysiologic study of single oral dose of nisoldipine (10-20 mg). No significant changes in intra-cardiac conduction were found, but the study was not controlled and the measurements may have been performed too soon after dosing (see description of data above).

Studies 144, 257

These were uncontrolled electrophysiology studies of similar design and same dosage (1.5 µg/kg iv). In Study 257, patients were pretreated with atenolol 75 mg/day or pindolol 15 mg/day for 3 days.

Study 178

This is an uncontrolled hemodynamic and electrophysiologic study of nisoldipine in 12 patients undergoing arrhythmia evaluation. The patients were randomized to receive a single dose of nisoldipine IR 10 or 20 mgs orally. Invasive hemodynamic and electrophysiologic data were collected before and 120 minutes after dosing. Electrophysiology data were not cited by the sponsor in the pharmacodynamic summary (see description of results above).

Study 199

In this double blind, single dose, crossover study, nisoldipine IR 10 mg was compared with nifedipine 20 mg and placebo in 9 young and healthy subjects. Effects on blood pressure, heart rate and neurohormonal system were measured. Separation of treatment periods was adequate (one week).

Study 201

This is a small study in 12 Japanese healthy subjects. Nisoldipine was well-tolerated at single doses of 2.5-20 mg. No kinetic data were available.

Study 344

This is an open label, uncontrolled study of iv nisoldipine (3 µg/kg/3 min) on regional myocardial blood flow in 6 patients with coronary heart disease. Background therapy with atenolol 100 mg/day was continued for all patients.

Study 372

This is a double blind, placebo-controlled, 8-week study in 72 hypertensive patients. After a 3 week washout, the dosage was forced-titrated from 5 mg qd to 20 mg bid every two weeks. Ergometric exercise was performed by every patient but hemodynamic measurements were done in only randomly selected half of the patients. Treatment groups were well-matched.

Study 382

This is a double blind, placebo controlled drug-interaction study to assess the effects of adding nisoldipine to established beta blocker (atenolol or propranolol) therapies in normotensive subjects. Eight young and healthy subjects were randomized to one of the following two treatment sequences (a or b, reproduced from NDA Vol 148, Page 08 17 0013029):

a.	Weeks	0-------1-------2-------3-------4-------5
	beta blocker (b) or placebo (p)	bbbbbbbbbbbb--------pppppppbbbbbb
	nisoldipine (n) or placebo (q)	----------nnnnnn----------qqqqqq
b.	Weeks	0-------1-------2-------3-------4-------5
	beta blocker (b) or placebo (p)	pppppppbbbbbb--------bbbbbbbbbbbb
	nisoldipine (n) or placebo (q)	---------qqqqqq----------nnnnnn

In the above scheme, the third week (between the end of Week 2 and beginning of Week 4) was a washout. With either sequence, nisoldipine (n) was administered after one week of beta blocker (b) therapy but the matching placebo (q) was given after a week of beta blocker matching placebo (p), although they were both concomitant with beta blockers during Week 2 or 5. Thus the nisoldipine treatment was not well controlled.

Study 399

This is another drug-interaction study to evaluate the effects of pretreatment with cimetidine on the pharmacokinetics and pharmacodynamics of nisoldipine. Eight normal subjects were given a single dose of nisoldipine as a 10 mg oral solution or 0.374 mg iv infusion over 40 minutes (two crossover periods separated by 5 days), without (no placebo) and with cimetidine treatment (400 mg x 1 followed by 200 mg tid the next day of measurement). Using a sigmoidal E_{max} model, it was calculated that the hemodynamic changes due to cimetidine pre-treatment can be attributed to a 48% increase in nisoldipine bioavailability, and the sponsor concluded that cimetidine has no additional dynamic interaction with nisoldipine.

Study 479

This a double-blind, parallel placebo controlled, 3 week study to evaluate the effect of nisoldipine (10 mg IR bid) on psychomotor functions in 30 normal healthy subjects.

Study 560

Changes in diameters of coronary arteries before and after iv nisoldipine (0.5 or 1.0 mg over 4 minutes) treatment were measured by angiography in 26 patients with coronary heart disease in this uncontrolled study. Plasma drug levels correlated with other hemodynamic activities but not vasodilating effects.

Study 648/649

This is another single oral dose study on the anti-ischemic effect of nisoldipine, but unlike Study 126 (see above), a CC tablet (20 mg) was tested. Despite a rigorous design (double blind, parallel placebo controlled, multicenter), nisoldipine had no effect on several angina endpoints measured. But again, not much has been shown with a single dose study.

Study 670

This study was double-blind, placebo controlled in young and healthy subjects. Effects of nisoldipine IR treatment at 10 mg bid for 4 weeks on thyroid function and prolactin were assessed.

Study D88-059

See Comments on Individual Pharmacokinetic Studies for study design and execution.

Study D90-020

This is a double blind, parallel placebo controlled, two single-dose crossover study designed to demonstrate bioequivalence of 3x20mg and 2x30mg nisoldipine CC tablets. Blood pressure and heart rate effects, as well as tolerability data, were also collected.

Study D90-022

See Comments on Individual Pharmacokinetic Studies for study design and execution.

Study D91-035

See Comments on Individual Pharmacokinetic Studies for study design and execution.

Ref 1-4

As noted above, these four studies were published by the same group of investigators, thus not to be considered as four independent reports. Invasive hemodynamics, both systemic and coronary, of nisoldipine were measured in patients with coronary heart disease. Except for Ref 4, which compared nisoldipine (6 µg/kg) with diltiazem (500 µg/kg), none of the other studies were controlled. For these studies, only publication reprints were submitted (no original data).

Ref 5

Effects of nisoldipine on myocardial metabolism were compared with that of nicardipine in this paper published by M.F. Rousseau et al. Thirty-two patients with angina pectoris were treated with nisoldipine 0.06-0.12 µg/kg infused intravenously over 10 minutes. Measurements were performed at basal state and during a cold pressor test.

Ref 6

This is a hemodynamic study of oral nisoldipine (10 mg IR single dose) on left ventricular functio.., using radionuclide angiographic techniques. Changes in left ventricular function were measured during exercise in 20 patients with chronic stable angina and coronary disease and responses to a cold pressor test was evaluated in additional 12 patients with ischemic pain and abnormal exercise test but normal coronary arteries. The study was not controlled. Reprint of publication only, no original data were presented in the NDA.

Ref 7, 8 (References 1, 2 of Section 8.15.3)

These are published reports of studies on regional blood flow (liver and kidney, Ref 7, forearm, Ref 8). Nisoldipine was given orally (20 mg/day x 4 days) in the former and intravenously (10 µg/kg) in the latter. The investigation was conducted in young, normal subjects in both studies. Reprints only, no original data reviewed.

Ref 9 (References 3 of Section 8.15.3)

Effects of nisoldipine (20 mg/day for 4 days) on platelet aggregation was compared with that of verapamil (160 mg/day for 4 days) in this published report. Reprint only, no original data.

Ref 10 (References 4 of Section 8.15.3)

Without a concurrent control, the claimed favorable effects of nisoldipine on lipoprotein profile can not be taken seriously and will not be described in the labeling. Also reprint of publication, no original data.

Drug Interaction Studies

As noted above in the kinetic sections, drug-interactions were separated into the following three groups:

Effect of other drugs on pharmacokinetics of nisoldipine.
Effect of other drugs on pharmacodynamics of nisoldipine
Effect of nisoldipine on other drugs' pharmacokinetics/dynamics.

The first category has been described in the Pharmacokinetic Sections. For the remaining two, limitations of individual studies, such as single/multiple doses, duration of treatment and use of control groups, have been described in tables summarizing the results. Studies 382 and 399 have been commented above in this section.

Summary of Pharmacodynamic Issues

Antihypertensive activity of nisoldipine has been demonstrated for both IR and CC formulations. Dosages ranging from 5 to 120 mg have been studied in at least 6 studies, however, dose-response relationship in these short-term studies on CC formulation was not consistent. While the placebo-adjusted blood pressure reductions were proportional to doses of 5-30 mg/day, the response was flat over higher doses of 30-90 mg/day in a less well-designed study (see comments above). Systemic vascular resistance was consistently decreased by nisoldipine, but heart rate changes appeared to be mild in the pharmacodynamic studies.

Nisoldipine did not appear to have significant negative inotropic activities and except for a modest decrease in sinus cycle length, had no appreciable chronotropic effects either. However, the changes in T wave as observed in a few pharmacologic studies need to be re-examined in large efficacy/safety trials. Nisoldipine may increase coronary blood flow in patients with coronary artery disease, but other measurements of anti-ischemic effect were not consistent.

There were no significant pharmacodynamic interactions between nisoldipine and ranitidine, cimetidine, or propranolol (with CC formulation of nisoldipine). Nisoldipine may increase bioavailability of quinidine, propranolol, atenolol and digoxin, but the extends were variable and of unclear dynamic consequences or clinical meaning.

Based on a somewhat limited experience, nisoldipine had no adverse pharmacologic effects on neurohormonal system, regional blood flow, thyroid and prolactin activity, lipoprotein profile or psychomotor functions. Nisoldipine may inhibit platelet aggregation and its clinical implication should be reviewed in the safety data. Nisoldipine CC appeared to be well-tolerated in the small scale clinical pharmacology studies, some of which were conducted in normotensive subjects. Adverse events were commonly seen in other calcium channel blockers.

PHARMACOKINETICS/DYNAMICS CORRELATIONS

As noted in the Summary of Pharmacodynamic Issues, placebo-adjusted blood pressure changes were dose-related for 5-30 mg (one week study on CC formulation, Study D88-059), correlation with plasma drug concentration and total bioavailability was also good:

Dose	C_{max}	AUC_{0-24}	Changes in Systolic/Diastolic BPs, mm Hg, Day 7	
mg/d	ng/ml	ng.hr/ml	8 hrs post dose	24 hrs post dose
5	0.65	8.39	- 7.1 / + 1.2	+ 1.9 / - 4.6
10	1.02	16.17	- 6.3 / - 1.2	- 8.8 / - 4.9
20	2.13	28.24	-10.7 / - 1.7	- 9.2 / - 4.6
30	2.79	40.34	-19.2 / - 4.6	-15.6 / - 6.2

Fit by linear regression was reasonable and suggested that plasma nisoldipine concentration of 2 ng/ml was required for a 5 mm Hg drop in diastolic BP over placebo. Such relationship was less clear at higher doses (30-90 mg/day, Study D90-022). When blood pressure responses and plasma drug concentrations were fitted with linear regression which included placebo data for plasma level of zero, estimated slopes were significant or nearly so for 30 mg and 60 mg. However, as noted before, placebo-corrected blood pressure reductions were not dose-related and the dosages were forced titrated rapidly in sequence in this study.

In four large hypertension efficacy trials (D88-054, D89-029, D89-039 and D90-019), which covered doses from 10 to 60 mgs/day, overall correlation between systolic/diastolic blood pressure reductions and plasma drug concentration was good for each study pooled over all dosages, and for 30-60 mg doses pooled over all four studies (Table on next page). The estimated slopes from linear regression analysis indicated that trough supine diastolic blood pressure decreased by 1.57 mm Hg per 1 ng/ml (overall pooled analysis), or 1.67-2.38 mm Hg per ng/ml for the three monotherapy studies.

In two Phase III angina trials (D88-060 and D90-015), placebo-subtracted changes in exercise durations from baseline were related to dose/plasma concentration as follows:

Dose	C_{max}	C_{min}	Changes in seconds, p for correlation with drug levels			
mg/d	ng/ml	ng/ml	Peak	p	Trough	p
10	-	0.79	-		1	0.037
20(D88-060)	-	1.25	-		20	0.905
20(D90-015)	2.10	1.57	29	0.012	34	0.184
30	-	2.16	-		32	0.054
40	3.70	2.56	6	0.108	7	0.017
60	5.70	4.07	34	0.577	37	0.777

Of these, the estimated slopes were significant or nearly so for 10, 30, 40 mgs at trough, and 20 mg at peak. Pooled over all dosages, the correlation was significant in Study D88-060 (10-30 mg, trough) only. Overall correlations between dose, plasma level and exercise tolerance in angina patients were poor.

Correlation of trough nisoldipine level and blood pressure responses:
(From NDA Section 8.13.8.2, Vol 116)

NISOLDIPINE/EFFICACY POOL
US CC HTN

TABLE
CORRELATION COEFFICIENTS OF TROUGH BLOOD LEVELS WITH TROUGH BLOOD PRESSURE
FOR ALL PATIENTS VALID FOR EFFICACY ANALYSIS

PROTOCOL	NIS CC QD DRUG GROUP	N	SUPINE SYSTOLIC BP R	SUPINE SYSTOLIC BP P	SLOPE	SUPINE DIASTOLIC BP R	SUPINE DIASTOLIC BP P	STANDING SYSTOLIC BP R	STANDING SYSTOLIC BP P	STANDING DIASTOLIC BP R	STANDING DIASTOLIC BP P
D88-054	ALL	87	-0.17893	0.0980	-2.11	-0.30898	0.0036	-0.11794	0.2788	-0.28230	0.0060
D89-028	ALL	158	-0.14107	0.0836	-1.03	-0.30298	0.0001	-0.22868	0.0044	-0.29461	0.0001
D89-039	ALL	114	-0.28121	0.0001	-2.38	-0.40179	0.0001	-0.27060	0.0001	-0.30104	0.0001
D90-018	ALL	118	-0.30830	0.0006	-1.67	-0.44982	0.0001	-0.28118	0.0015	-0.46786	0.0001
D88-054	10MG	30	0.04850	0.8031	---	-0.34381	0.0078	0.11873	0.5286	-0.23418	0.2139
D88-054	20MG	30	-0.47472	0.0060	---	-0.38234	0.0330	-0.38317	0.0316	-0.36930	0.0447
D88-054	30MG	27	-0.22186	0.2668	---	-0.16068	0.4233	-0.21617	0.2788	-0.34713	0.0761
D89-028	20MG	56	0.15689	0.2482	---	0.14361	-0.2810	0.12068	-0.3787	0.07007	0.6079
D89-028	40MG	49	-0.15621	0.2838	---	-0.33064	0.0147	-0.14883	0.3075	-0.33553	0.0001
D89-028	60MG	53	-0.09154	0.5145	---	-0.31901	0.0189	-0.17371	0.2135	-0.27782	0.1007
D89-039	20MG	59	-0.15072	0.2545	---	-0.26441	0.0430	-0.19165	0.1458	-0.28609	0.0280
D89-039	40MG	55	-0.36456	0.0062	---	-0.35004	0.0068	-0.41101	0.0018	-0.49788	0.0004
D90-018	30MG	66	-0.27069	0.0229	---	-0.32130	0.0081	-0.28486	0.0035	-0.42230	0.0004
D90-018	60MG	52	-0.27936	0.0438	---	-0.44891	0.0008	-0.22932	0.1048	-0.44382	0.0008
ALL	10MG	30	0.01800	0.9331	---	-0.34381	0.0838	0.11873	0.5286	-0.23418	0.2139
ALL	20MG	145	-0.08009	0.5496	---	-0.08741	0.2436	-0.18634	0.0804	-0.20408	0.0138
ALL	30MG	83	-0.26392	0.0106	---	-0.28817	0.0066	-0.21687	0.0070	-0.29867	0.0001
ALL	40MG	104	-0.22874	0.0190	---	-0.32601	0.0007	-0.25083	0.0102	-0.47381	0.0001
ALL	60MG	106	-0.17762	0.0685	---	-0.28736	0.0001	-0.18492	0.0483	-0.39788	0.0002
ALL	ALL	478	-0.28588	0.0001	-1.87	-0.38170	0.0001	-0.28818	0.0001	-0.45456	0.0001

CONCLUSIONS

It appears that pharmacokinetic properties of nisoldipine has been well-described for both the immediate release (IR) and the control release (CC) formulations. Variations in bioavailability of nisoldipine in patients of different concurrent diseases and demographic characteristics have been examined, which should be addressed in relevant sections of labeling, especially the issues of dose-dumping by food. Once daily use of nisoldipine CC tablets for hypertension is supported by the kinetic data.

While the pharmacodynamic profile of nisoldipine was studied mostly using intravenous and the IR formulations, it has been demonstrated that nisoldipine is a vasodilating antihypertensive with minor electrophysiologic effects and insignificant inotropic activities. There is no reason to expect significantly different behavior for the CC tablets. For major cardiovascular effects of nisoldipine, there is good correlation with dose (5-30 mg/day) and plasma drug concentration.

It is concluded that clinical pharmacology of nisoldipine CC has been adequately characterized for the patients to be treated that instructions on its clinical use for hypertension can be written for the labeling.

Shaw T. Chen, M.D.,Ph.D.

cc:
ORIG: NDA- 20-356
HFD-110
HFD-110/CSO ✓
HFD-426/PMarroum
HFD-110/PDern, CDuarte, NStockbridge
HFD-110/SChen/02/15/94

Statistical Review

STATISTICAL REVIEW AND EVALUATION

NDA #: 20-356 **Drug Class:** **Date:** JAN 4 1994

Applicant: Miles Pharmaceutical Division

Name of Drug: Nisoldipin Coat-core Tablets, 10, 20, 30, and 40 mg, q.d.

Indication: 1) Hypertension, alone or in combination with other antihypertensive agents;

Documents Reviewed: Volumes 1-5, 379, 388, and 400 of the NDA submission dated March 31, 1993. Also the data for the primary efficacy variable, supine diastolic blood pressure, was submitted on diskette for the double-blind portions of Studies D90-019, D90-029, and D90-039.

Medical Officer: The medical officer for this review is Dr. Cristobal Duarte.

I. INTRODUCTION

Nisoldipine is a dihydropyridine calcium channel blocker derived from nifedipine. The product is currently approved in 23 countries, although clinical development was discontinued in the United States because the brief duration of effect required multiple daily dosing. This application is for an extended-release formulation of nisoldipine, and studies once daily dosing for both hypertension The coat-core tablet consists of an inner core containing 20% of the nisoldipine dose in an immediate-release form, surrounded by an outer coat containing 80% of the nisoldipine dose in a slow-release form.

This submission consisted of the results of two Phase II and three Phase III trials for hypertension which were carried out in the United States, and one South African hypertension study. The submission also contained two Phase III trials for angina which were carried out in the United States, and

II. CONTROLLED CLINICAL TRIALS

II.A. PROTOCOL NO. D90-019

II.A.1 Study Description

Study D89-019 was a sixteen-center dose-response study designed to compare three fixed doses of diltiazem with placebo in patients with mild to moderate hypertension.

The plan called for patients to be randomized to one of four parallel groups, receiving either placebo, nisoldipine 30 mg qd, nisoldipine 60 mg qd, or nisoldipine 90 mg qd, over an six week double-blind period. The 90 mg nisoldipine arm was deleted from the protocol by amendment before any patients were randomized.

After a four week washout period, patients who had a supine diastolic blood pressure (DBP) between 100 and 114 mmHg on each of the last two pre-randomization visits were eligible for randomization to double-blind treatment. The two supine DBP readings were required to be within 7 mmHg of each other. Blood pressure measurements were taken at trough (24 hr ± 30 minutes post-dose).

A total of 309 patients were enrolled in the placebo run-in period, and 221 were randomized to treatment group, with 72 assigned to placebo, 76 to nisoldipine 30 mg qd, and 73 to nisoldipine 60 mg qd. Nine placebo patients dropped out of the study, as did three low dose and 12 higher dose nisoldipine patients, while 197 patients completed the study A total of 213 were considered valid for efficacy analyses.

Patients were instructed to take three tablets each morning prior to 11 a.m. All patients randomized to nisoldipine began at 30 mg once daily, and those randomized to the 60 mg dose were titrated after one week. The relationship of drug administration to meals was not specified. The double-blind treatment phase lasted a total of six weeks, with patients evaluated weekly for the first four weeks and then again at the end of the study (week 6).

The primary efficacy variable was change from baseline (mean of weeks 3 and 4 of the placebo run-in) to endpoint in supine diastolic blood pressure at trough, 24 hours post-dose. Secondary efficacy variables included change from baseline in trough standing DBP and supine and standing systolic blood pressure (SBP). Response rates at trough were also analyzed, with response define in four different ways, (1) supine DBP no more than 90 mmHg; (2) a fall in supine DBP of at least 10 mmHg, (3) supine DBP$\leq$ 90 mmHg or a fall in supine DBP $\geq$ 10 mmHg; and (4) supine DBP$\leq$ 90 mmHg and a fall in supine DBP $\geq$ 10 mmHg.

At eight of the 16 centers 24-hour ambulatory blood pressure monitoring (ABPM) was performed at week 3 of the placebo run-in and at week 5 of the double-blind treatment period. The sponsor used the ABPM data to analyze the peak/trough ratio. A total of 117 patients had 24-hour ABPM monitoring. In-clinic blood pressure measurements were also taken at for 12 hours post-dose at seven centers after 4 weeks of placebo run-in and after six weeks of double-blind treatment.

II.A.2 Sponsor's Analysis

The sponsor performed both an evaluable patient analysis, including those patients with at least two post-baseline evaluations who were not protocol violators, and an intent-to-treat analysis including all patients at least one post-baseline evaluation. A last-observation-carried-forward approach was used to include the available data from patients who dropped out of the study. The initial analysis of variance model for the

primary and secondary change from baseline endpoints included treatment group, investigator, and treatment by investigator interaction terms. The interaction term was dropped if it was not significant at the .05 level. The initial comparison was the average of the nisoldipine groups versus placebo If this was significant then pairwise comparisons were done to identify which nisoldipine doses were favored over placebo.

The sample size for this study was selected to give 90% power to detect a difference of at least 5 mmHg between the nisoldipine groups and the placebo group. This was calculated using an estimated standard deviation of 8 mmHg. The sponsor compared the demographics of the four groups at baseline using a Mantel-Haenszel test on sex, race, smoking status, and use of previous antihypertensive medications, and found no significant differences. The groups were also similar with respect to age, weight, height, and years of hypertension, which were compared using an analysis of variance model.

Change from Baseline Blood Pressure Measurements at Endpoint (LOCF) (Intent-to-Treat Data Set)

	Supine DBP	Supine SBP	Standing DBP	Standing SBP
Placebo (N=71)				
Baseline mean	103.64	155.31	102.91	151.22
Change from baseline	-4.68	-1.88	-2.60	-1.94
Nisoldipine 30 mg (N=76)				
Baseline mean	104.41	157.29	103.82	153.57
Change from baseline	-11.58	-12.10	-9.59	-13.36
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Nisoldipine 60 mg (N=73)				
Baseline mean	104.76	158.43	103.74	154.12
Change from baseline	-14.46	-16.67	-12.41	-15.94
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Interaction p-value	0.4675	0.1074	0.2651	0.4209

The results of the sponsor's analysis of their evaluable patient data set were similar, with all of the active drug groups demonstrating a highly statistically significant difference from the placebo group. The same was true of the analysis at each of the various time points after at least two weeks of double-blind therapy. Each active treatment group tended to reach a plateau in blood pressure response after two or three weeks of double-blind therapy, and the results for the group were very consistent beyond that point. The treatment by center interaction term was not significant at the .05 level.

The response variables also demonstrated results similar to the change from baseline blood pressure values, as can be seen below.

Response Rates

	Placebo N=71	Nisoldipine 30 mg qd N=76	Nisoldipine 60 mg qd N=66
A) DBP ≤ 90 mmHg	16 (23%)	26 (34%)	37 (56%)
B) DBP decrease ≥ 10 mmHg	22 (31%)	37 (49%)	50 (76%)
C) A) or B)	22 (31%)	37 (49%)	50 (76%)
D) A) and B)	16 (23%)	26 (34%)	37 (56%)

The ambulatory blood pressure data was analyzed by smoothing the hourly means from the eight centers using a Fourier transform. The mean trough and peak values and trough to peak ratios were obtained from the smoothed data. Peak effect for each nisoldipine group was defined as the greatest difference between the nisoldipine group and placebo in mean change from baseline. Trough effect was defined as the 24-hour post-dose difference from placebo.

The peak diastolic blood pressure response occurred at 13 hours post-dose for the nisoldipine 30 mg group and at 4 hours post-dose for the nisoldipine 60 mg group. The peak/trough ratios for the placebo subtracted ambulatory measurements were -12.1/-9.5 = 78% and -15.2/-14.2 = 93%, respectively. The corresponding systolic peak/trough ratios were 83% and 76%.

The number of adverse events experienced in the placebo group was statistically significantly different from both of the nisoldipine groups. The most frequently reported adverse events included headache and peripheral edema in the placebo group and both nisoldipine groups. Adverse events were most likely to occur early in the first two weeks of double-blind therapy, although they continued to occur at a reduced level throughout the study. One patient experienced a cardiac arrest and died while receiving Placebo during the double-blind portion of the study. No other patients died during the study.

Adverse Events

	Placebo N=72	Nisoldipine 30 mg qd N=76	Nisoldipine 60 mg qd N=73
Patients with ≥ one event	32 (44%)	47 (62%)	54 (74%)
Possibly drug related	19 (26%)	23 (30%)	33 (45%)
Serious adverse events	2 (3%)	4 (5%)	10 (14%)
Withdrew due to a. e.'s	3 (4%)	1 (1%)	11 (15%)

II.A.3. Reviewer's Comments

This study gives substantial evidence that nisoldipine, in doses of 30 and 60 mg qd, reduces blood pressure when compared to placebo. After establishing a drug effect by comparing the mean of the combined nisoldipine groups with placebo and reaching statistical significance, the sponsor compared each dose group directly with placebo, and both were highly statistically significant. The protocol and the study report do not mention any adjustments for multiple comparisons. However, the results of this study were so significant that even using the conservative Bonforonni adjustment for the multiple comparisons, they remain highly statistically significant for the primary and secondary blood pressure endpoints.

This reviewer performed several additional analyses on the data including analysis of covariance using baseline as a covariate for both change from baseline and for endpoint supine DBP. The results of these additional analyses did not differ substantially from the results submitted by the sponsor, and demonstrated the robustness of the efficacy results.

The results of this study demonstrate a dose-response relationship for the 30 mg qd and 60 mg qd doses of nisoldipine in both efficacy and safety. The higher dose group consistently showed a greater reduction in all four blood pressure measurements. This group also had more adverse events and more serious adverse events. This study involved a forced titration, and many of the patients in the high dose group possibly had an adequate response at the lower dose, and did not need the additional risk of adverse events which came with the additional blood pressure reduction. This reviewer feels that doses of nisoldipine lower than those studied here might be adequate for many patients.

The protocol stated that the original model for the analysis of the blood pressure variables would include an interaction term, but that the interaction term would be dropped if it was not significant at the .05 level. The test for interaction is a test with very low power and therefore interaction is usually tested at the .15 level. Using this level, one of the secondary endpoints, supine SBP, demonstrated a significant

treatment by center interaction in the endpoint analysis (p= 0.1074). The primary endpoint also had interaction terms with p-values which were greater than .05 but less than .15 at several time points, but not in the endpoint analysis. The inclusion of the interaction term in the analysis of variance model did not change the statistical significance of any of the primary or secondary endpoints. This reviewer calculated the results by center and found that while the response at the various centers was different in magnitude, they trended in the same direction in almost all centers. The interaction appears to be quantitative in nature, and probably a result of the variability of the blood pressure responses.

II.B. PROTOCOL NO. D89-029

II.B.1. Study Description

D89-029 was a sixteen-center dose-response parallel study comparing placebo with three once daily doses of nisoldipine on a background of atenolol 50 mg qd in patients with mild to moderate hypertension. Patients were randomized to one of four parallel groups, receiving either placebo or nisoldipine 20 mg qd, nisoldipine 40 mg qd, or nisoldipine 60 mg qd, over an six week double-blind period.

Following a two-week placebo run-in period, patients with supine diastolic blood pressure between 100 and 119 mmHg entered a single-blind four week run-in period where all received atenolol 50 mg qd, but no other hypertensive therapy. Patients who had a supine diastolic blood pressure between 95 and 114 mmHg after the four weeks of atenolol therapy were eligible for randomization to one of the four double-blind treatments, while continuing their atenolol therapy.

A total of 418 patients were enrolled in the placebo run-in, and 313 continued into the atenolol run-in period. Most of the patients who did not continue were not eligible because their blood pressure had dropped too much while receiving atenolol. A total of 251 patients were randomized to double-blind treatment group; 62 to placebo, 62 to nisoldipine 20 mg qd, 63 to nisoldipine 40 mg qd, and 64 to nisoldipine 60 mg qd. Three placebo patients dropped out of the study, as did one low dose, five mid-dose, and seven higher dose nisoldipine patients. A total of 238 patients were considered valid for the efficacy analyses.

Patients were instructed to take two tablets and one capsule each morning prior to 11 a.m. All patients randomized to nisoldipine began at 20 mg once daily, and those randomized to the higher dose groups were titrated weekly. The relationship of drug administration to meals was not specified. The double-blind treatment phase lasted a total of six weeks, with patients evaluated at weeks 1, 2, 4, and 6. The primary efficacy variable was change from baseline (mean supine DBP at week 4 of the single-blind atenolol phase) to endpoint (the last double-blind visit) supine diastolic blood pressure (DBP) at trough, 24 hours post-dose. Secondary efficacy variables included change from baseline in standing DBP and supine and standing SBP. The change

from baseline during the atenolol phase was also evaluated. Response rates at trough were also analyzed, with response define in four different ways, (1) supine DBP no more than 90 mmHg; (2) a fall in supine DBP of at least 10 mmHg, (3) supine DBP≤ 90 mmHg or a fall in supine DBP ≥ 10 mmHg; and (4) supine DBP≤ 90 mmHg and a fall in supine DBP ≥ 10 mmHg.

At eight of the 16 centers 24-hour ambulatory blood pressure monitoring (ABPM) was performed at week 3 of the atenolol run-in and at week 5 of the double-blind treatment period. The sponsor used the ABPM data to analyze the peak/trough ratio. A total of 141 patients (centers 01 through 08) had 24-hour ABPM monitoring, including 33 randomized to placebo, 34 to nisoldipine 20 mg, 35 to nisoldipine 40 mg, and 34 to nisoldipine 60 mg.

II.B.2. Sponsor's Analysis

The sample size for this study was selected to give 90% power to detect a difference of at least 5 mmHg between the nisoldipine 40 mg group and the placebo group. This was calculated using an estimated standard deviation of 8 mmHg The sponsor compared the demographics of the four groups at baseline using the Cochran-Mantel-Haenszel test (adjusting for center) on sex, race, smoking status, and several other factors. The analysis of the previous use of antihypertensive medications was marginally significant with $p=0.065$. Only three patients had previously been treated, and two of those three were randomized to the placebo group. The groups were also similar with respect to age, weight, height, and years of hypertension, which were compared using an analysis of variance model.

The sponsor performed both an evaluable patient analysis, including patients with at least 19 days of double-blind therapy, and an intent-to-treat analysis including all patients who had at least one post-baseline evaluation. A last-observation-carried-forward approach was used to include the available data from patients who dropped out of the study. The initial analysis of variance model for the primary and secondary change from baseline endpoints included treatment group, investigator, and treatment by investigator interaction terms. The interaction term was dropped if it was not significant at the .05 level. The initial comparison was the average of the nisoldipine groups versus placebo. If this was significant then pairwise comparisons were done to identify which nisoldipine doses were favored over placebo.

Change from Baseline Blood Pressure Measurements at Endpoint (LOCF) Intent-to-Treat Data Set

	Supine DBP	Supine SBP	Standing DBP	Standing SBP
Placebo + Atenolol (N=62)				
Baseline mean	100.74	158.30	102.10	154.20
Change from baseline	-3.90	-0.12	-1.66	+2.30
Nisoldipine 20 mg + Atenolol (N= 62)				
Baseline mean	100.58	158.19	102.13	153.81
Change from baseline	-10.00	-12.51	-8.85	-10.14
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Nisoldipine 40 mg + Atenolol (N= 62)				
Baseline mean	100.97	159.32	103.46	157.22
Change from baseline	-12.01	-19.03	-12.39	-21.75
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Nisoldipine 60 mg + Atenolol (N= 64)				
Baseline mean	100.81	160.90	102.32	155.72
Change from baseline	-13.73	-22.38	-14.30	-21.89
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Nisoldipine versus placebo	0.0001	0.0001	0.0001	0.0001
Interaction p-value	0.1107	0.3041	0.5361	0.4636

The results of the sponsor's analysis of their evaluable patient data set were similar, with all of the active drug groups demonstrating a highly statistically significant difference from the placebo group. The same was true of the analysis at each of the various time points after the first week of double-blind therapy. Each active treatment group tended to reach a plateau in blood pressure response after two or three weeks of double-blind therapy, and the results were very consistent beyond that point. The treatment by center interaction term was significant at the .05 level for the analysis of supine DBP at the first double-blind visit, but not for the secondary blood pressure variables at that visit, nor for any blood pressure variables at later visits or at endpoint.

The response variables also demonstrated results similar to the change from baseline blood pressure values, as can be seen below.

Response Rates

	Placebo N=59	Nisoldipine 20 mg qd N=61	Nisoldipine 40 mg qd N= 59	Nisoldipine 60 mg qd N= 59
A) DBP ≤ 90 mmHg	19 (32%)	34 (56%)	40 (68%)	39 (66%)
B) DBP decrease ≥ 10 mmHg	14 (24%)	31 (51%)	40 (68%)	44 (75%)

The ambulatory blood pressure data was analyzed by smoothing the hourly means from the eight centers using a Fourier transform. The mean trough and peak values and trough to peak ratios were obtained from the smoothed data. Peak effect for each nisoldipine group was defined as the greatest difference between the nisoldipine group and placebo in mean change from baseline. Trough effect was defined as the 24-hour post-dose difference from placebo.

The peak diastolic blood pressure response occurred at 3 hours post-dose for the nisoldipine 20 mg group, at 23 hours post-dose for the nisoldipine 40 mg group, and at one hour post-dose for the nisoldipine 60 mg group. The peak/trough ratios were -5.0/-9.4 = 53%, -12.8/-13.1= 97%, and -12.9/-13.0 = 99%, respectively. The corresponding systolic peak/trough ratios were 86%, 100%, and 94%.

Adverse Events

	Placebo N=62	Nisoldipine 20 mg qd N= 62	Nisoldipine 40 mg qd N= 63	Nisoldipine 60 mg qd N= 64
Patients with ≥ one event	28 (62%)	37 (60%)	44 (70%)	42 (66%)
Possibly drug related	14 (23%)	16 (26%)	28 (44%)	28 (44%)
Serious adverse events	2 (3%)	5 (8%)	2 (3%)	5 (8%)
Withdrew due to a. e.'s	1 (2%)	1 (2%)	4 (6%)	4 (6%)

The most frequently reported adverse events included headache and peripheral edema in the nisoldipine groups. The most frequently reported adverse events in the placebo group included rhinitis, peripheral edema, and headache. Adverse events were most likely to occur early in the study (prior to week 4), although they continued

to occur at a reduced level throughout the study. There were no deaths reported during this study.

II.B.3. Reviewer's Comments

This study demonstrated that nisoldipine treatment resulted in additional blood pressure reduction when used in the presence of atenolol. The primary and secondary endpoints all demonstrated statistically significant reductions in blood pressure during the six week study. This reviewer again performed several alternative analyses and found that the results were robust.

The results also demonstrate a dose-response trend for the 20 mg qd through 60 mg qd doses of nisoldipine in both efficacy and safety. The higher dose groups consistently showed a greater reduction in each of the blood pressure measurements, and also an increasing number of adverse events. This study again involved a forced titration, and many of the patients in the higher dose groups possibly had an adequate blood pressure response at lower doses and did not need the additional risk of adverse events which came with the additional blood pressure reduction. This reviewer feels that doses of nisoldipine lower than those studied here might be adequate for many patients.

The protocol stated that the original model for the analysis of the blood pressure variables would include an interaction term, but that the interaction term would be dropped if it was not significant at the .05 level. The test for interaction is a test with very low power and therefore interaction is usually tested at the .15 level. Using this level, the primary endpoint, supine DBP, demonstrated a significant treatment by center interaction in the endpoint analysis for both the evaluable patient data set ($p=0.1478$) and for the intent-to-treat data set ($p=0.1107$). This reviewer calculated the results by center and compared them. The centers vary substantially in the response of the various nisoldipine groups, but in no case did the placebo group have a greater response to therapy than did the treated groups.

II.C. PROTOCOL NO. D89-039

II.C.1. Study Description

D89-039 was a four-arm parallel study comparing placebo, two once daily doses of nisoldipine, 20 mg. and 40 mg, and verapamil 240 mg bid, in patients with mild to moderate hypertension. The protocol for the sixteen center study included a fifth group randomized to nisoldipine. 80 mg qd, but this group was dropped shortly after the beginning of the study (not prior to randomization) after the sponsor received information from another study that nisoldipine doses in excess of 60 mg qd were not well-tolerated..

After a four week washout period, patients who had an average supine DBP between 95 and 114 mmHg on each of the last two pre-randomization visits were eligible for

randomization to double-blind treatment. Blood pressure measurements were taken at trough (24 hr ± 30 minutes post-dose). The double-blind portion of the study lasted 12 weeks, but patients randomized to placebo were switched to verapamil 240 mg qd for the final four weeks of the study.

A total of 413 patients were enrolled in the placebo run-in period, and 320 were randomized to treatment group; 75 to placebo, 78 to verapamil, 76 to nisoldipine 20 mg qd, 76 to nisoldipine 40 mg qd, and 15 to nisoldipine 60 mg qd. Eleven patients randomized to the placebo group dropped out before the end of the study, as did five verapamil patients, 12 low dose nisoldipine patients, and 12 medium dose nisoldipine patients. The 15 patients who had been randomized to high dose nisoldipine were dropped from the study when that arm was deleted, so 265 patients completed the study A total of 290 patients were considered valid for efficacy analyses.

Patients were instructed to take two tablets and one capsule each morning prior to 11 a.m. and another capsule 12 hours later. All patients randomized to nisoldipine began at 20 mg once daily, and those randomized to the 40 mg dose were titrated after one week. The relationship of drug administration to meals was not specified. The double-blind treatment phase lasted a total of eight weeks at the original randomized dose groups, followed by four weeks where the placebo group received verapamil 240 mg qd and the other three groups remained on their randomized therapy. Patients were evaluated weekly for the first four weeks, and then biweekly for the rest of the study.

The primary efficacy variable was change from baseline (defined as the mean of six readings, three taken at week 3 and three at week 4 of the placebo run-in period) to endpoint (defined as week 8 of the double-blind portion of the study) in supine diastolic blood pressure (DBP) at trough, 24 hours post-dose. The primary efficacy comparison was between the 40 mg qd nisoldipine group and the placebo group. The comparison of the 20 mg qd nisoldipine group and the placebo group was of secondary importance. Secondary efficacy variables included trough standing DBP and supine and standing systolic blood pressure (SBP). Response rates at trough were also analyzed, with response define in four different ways, (1) supine DBP no more than 90 mmHg; (2) a fall in supine DBP of at least 10 mmHg, (3) supine DBP≤ 90 mmHg or a fall in supine DBP ≥ 10 mmHg; and (4) supine DBP≤ 90 mmHg and a fall in supine DBP ≥ 10 mmHg.

At eight of the 16 centers 24-hour ambulatory blood pressure monitoring (ABPM) was performed at week 3 of the placebo run-in and at week 7 of the double-blind treatment period. The sponsor used the ABPM data to analyze the peak/trough ratio.
A total of 141 patients (centers 01 through 08) had 24-hour ABPM monitoring, including 34 randomized to placebo, 36 to verapamil, 35 to nisoldipine 20 mg, and 36 to nisoldipine 40 mg. A total of 163 patients (centers 06 through 13) had 12-hour post-dose in-house blood pressure readings (every two hours) at week 4 of the placebo run-in and at week 8 of the double-blind treatment period.

II.C.2. Sponsor's Analysis

The sample size for this study was selected to give 90% power to detect a difference of at least 5 mmHg between the nisoldipine 40 mg group and the placebo group. This was calculated using an estimated standard deviation of 8 mmHg. The sponsor compared the demographics of the four groups at baseline using the Cochran-Mantel-Haenszel test (adjusting for center) on sex, race, smoking status, and use of previous antihypertensive medications, and no significant differences were found. The groups were also similar with respect to age, weight, height, and years of hypertension, which were compared using an analysis of variance model.

Change from Baseline Blood Pressure Measurements at Endpoint (LOCF) Intent-to-Treat Data Set

	Supine DBP	Supine SBP	Standing DBP	Standing SBP
Placebo (N=75)				
Baseline mean	99.76	154.69	100.58	151.18
Change from baseline	-4.30	-1.93	-2.09	-2.40
Nisoldipine 20 mg qd (N=75)				
Baseline mean	99.99	152.67	100.80	150.84
Change from baseline	-7.97	-10.17	-6.99	-11.42
p-value vs placebo	0.0004	0.0001	0.0001	0.0001
Nisoldipine 40 mg qd (N=76)				
Baseline mean	100.35	154.22	101.27	150.20
Change from baseline	-11.22	-15.50	-11.34	-14.90
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Verapamil 240 mg bid (N=78)				
Baseline mean	99.95	151.67	100.66	148.62
Change from baseline	-14.46	-14.79	-13.21	-14.95
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Interaction p-value	0.6287	0.1693	0.5060	0.6371

The sponsor performed both an evaluable patient analysis, including those patients with at least two post-baseline evaluations who were not protocol violators, and an intent-to-treat analysis including all patients who had at least one post-baseline evaluation. A last-observation-carried-forward approach was used to include the available data from patients who dropped out of the study. The initial analysis of variance model for the primary and secondary change from baseline endpoints included treatment group, investigator, and treatment by investigator interaction terms. The interaction term was dropped if it was not significant at the .05 level.

The results of the sponsor's analysis of their evaluable patient data set were similar, with all of the active drug groups demonstrating a highly statistically significant difference from the placebo group. The same was true of the analysis at each of the various time points after at least two weeks of double-blind therapy. Each active treatment group tended to reach a plateau in blood pressure response after two or three weeks of double-blind therapy, and the results were very consistent beyond that point. The treatment by center interaction term was significant for some visits, but not for the final two visits or for the endpoint values for either data set.

The response variables also demonstrated results similar to the change from baseline blood pressure values, as can be seen below.

Response Rates

	Placebo N=70	Nisoldipine 20 mg qd N=72	Nisoldipine 40 mg qd N=76	Verapamil 240 mg bid N=72
A) DBP ≤ 90 mmHg	19 (26%)	35 (50%)	50 (69%)	62 (82%)
B) DBP decrease ≥ 10 mmHg	10 (14%)	28 (40%)	47 (65%)	59 (78%)
C) A) or B)	20 (28%)	38 (54%)	53 (74%)	67 (88%)
D) A) and B)	9 (13%)	25 (36%)	44 (61%)	54 (71%)

The ambulatory blood pressure data was analyzed by smoothing the hourly means from the eight centers using a Fourier transform. The mean trough and peak values and trough to peak ratios were obtained from the smoothed data. Peak effect for each nisoldipine group was defined as the greatest difference between the nisoldipine group and placebo in mean change from baseline. Trough effect was defined as the 24-hour post-dose difference from placebo.

The peak diastolic blood pressure response occurred at 4 hours post-dose for the nisoldipine 20 mg group, at 24 hours post-dose for the nisoldipine 40 mg group, and at 4 hours after the morning dose for the verapamil 240 mg bid group. The peak/trough ratios were -6.7/-9.7 = 66%, -11.7/-11.7 = 100%, and -11.1/-12.9 = 86%, respectively. The corresponding systolic peak/trough ratios were 66%, 100%, and 78%.

Adverse Events

	Placebo N=75	Nisoldipine 20 mg qd N=76	Nisoldipine 40 mg qd N=76	Verapamil 240 mg bid N=78
Patients with ≥ one event	48 (64%)	49 (64%)	57 (75%)	55 (71%)
Possibly drug related	34 (45%)	33 (43%)	42 (55%)	39 (50%)
Serious adverse events	7 (9%)	9 (12%)	12 (16%)	3 (4%)
Withdrew due to a. e.'s	3 (4%)	10 (13%)	11 (14%)	4 (5%)

The overall incidence of adverse events was not statistically significantly different for the four treatment groups. The most frequently reported adverse events included headache and peripheral edema in the placebo group and both nisoldipine groups. The most frequently reported adverse events in the verapamil group included constipation and headache. Adverse events were most likely to occur early in the study (prior to week 4), although they continued to occur at a reduced level throughout the study. There were no deaths reported during this study.

II.C.3. Reviewer's Comments

This study clearly demonstrates the efficacy of nisoldipine when compared to placebo in the treatment of mild-to-moderate hypertension. A dose-response exists for both diastolic and systolic blood pressure, and for adverse events. The differential between the groups appeared within a few weeks of treatment, and was consistent for the rest of the study. This reviewer again analyzed the data using several other models, and found the results to be consistent.

The group randomized to verapamil consistently demonstrated results which were at least as good as the higher nisoldipine dose, and were often superior (although not often statistically significantly better). The response rates for the verapamil group were also higher than those of the nisoldipine groups. The sponsor stated that verapamil is often used as once-a-day therapy, and the twice-daily dosing used in this study could have given that group an advantage over the once-daily dosing of nisoldipine. Although the verapamil group did not usually achieve statistical significance when compared to the nisoldipine groups, the study was not powered as an equivalence trial and the results should not be interpreted as showing the treatments are the same.

II.D. OTHER HYPERTENSION STUDIES

II.D.1. Study Descriptions

The sponsor submitted the results of three additional placebo-controlled clinical trials, two which were carried out in the United States. These studies lend supportive

evidence of the efficacy and safety of nisoldipine in the treatment of mild to moderate hypertension.

Study D88-054 was a pilot parallel dose-ranging eight-center study comparing placebo with three once daily doses of nisoldipine, 10 mg. 20 mg. and 30 mg over a four week period. This was the first exploratory clinical trial carried out in the target population and approximately 30 patients were randomized to each dose. The 20 mg and 30 mg dose groups both achieved statistically significantly greater reductions in supine DBP, supine SBP, and standing SBP than did the placebo group.

Study D89-026 was a pilot parallel dose titration study comparing placebo with nisoldipine, 10 - 40 mg once daily over a nine week period. Patients randomized to nisoldipine received 10 mg qd for the first week and were titrated upward an additional 10 mg on a bi-weekly basis if their supine DBP remained ≥ 85 mmHg at trough. A total of 72 patients were randomized to nisoldipine and 34 to placebo, which was also titrated based on blood pressure response. At the end of the nine weeks of treatment 6% of the nisoldipine patients remained at 10 mg qd, 6% were receiving 20 mg qd, 30% were receiving 30 mg qd, and 57% had been titrated to 40 mg qd. The nisoldipine group achieved statistically significantly greater reductions in supine and standing DBP and SBP.

Study D90-006 was a parallel multicenter study comparing placebo with three once daily doses of nisoldipine, 10 mg. 20 mg. and 30 mg over a six week period. This study was carried out in South Africa. All patients randomized to nisoldipine began at 10 mg qd and were titrated to their assigned dose after one week. Approximately 50 patients were randomized to each group. In the endpoint analysis all three nisoldipine groups achieved statistically significantly greater reductions in supine and standing DBP and SBP than did the placebo group.

IV. OVERALL SUMMARY AND CONCLUSIONS

The sponsor submitted the results of six trial involving patients with mild-to-moderate hypertension, including three Phase III studies performed in the United States. Study D90-019 was a dose-response study comparing two doses of nisoldipine (30 mg and 60 mg) with placebo in once-daily dosing. Both nisoldipine dose groups had statistically significantly better reduction in blood pressure than did the placebo group, and the groups demonstrated a dose-response relationship for both efficacy and safety. Study D90-029 compared placebo with three once daily doses of nisoldipine (20 mg, 40 mg, and 60 mg) on a background of atenolol 50 mg qd. All three nisoldipine/atenolol groups had statistically significantly better reduction in blood pressure than did the placebo/atenolol group. The responses trended in a dose-response order for both blood pressure reduction and for adverse experiences. Study D90-039 was a four-arm placebo and active-controlled study comparing two doses of nisoldipine (20 mg and 40 mg) with verapamil 240 mg bid and placebo. The nisoldipine groups had statistically significantly better reduction in blood pressure than the placebo group. The verapamil group had a somewhat better response than

did the nisoldipine groups, although the results were not often statistically significant. However, this trial was not designed as an equivalence trial, and was under-powered to detect the differences seen in the study. The study could not detect a difference, but that does not imply that the treatments are the same.

Clearly the nisoldipine doses studied in this trial (20 mg to 60 mg, qd) are effective at lowering blood pressure. What is not clear is that the dose range has been adequately examined, especially at the lower end. The maximal dose appears to be limited by adverse reactions. This reviewer feels that doses of nisoldipine lower than those studied here might be adequate for many patients and should be examined.

There were two potential problems in the design of these studies. The original model for the primary analysis of variance included a treatment by center interaction term which was dropped if the p-value for interaction was less than .05. The test for interaction is a very low powered test, and interaction is usually tested at the .15 level. The results of these studies were robust whether or not an interaction term was included in the model, and the interactions which were statistically significant appeared to be qualitative rather than quantitative. These studies each involved multiple doses of nisoldipine, and none of the analysis plans included adjustments for multiple comparisons. The p-values that resulted from the analyses, however, were all less than 0.001, and thus could stand up to a Bonforonni adjustment.

The overall summary and conclusions section may be conveyed to the sponsor.

Nancy D Smith

Nancy D. Smith, Ph.D.
Mathematical Statistician

Concur: Dr. Chi Chi 1/4/94

for Dr. Dubey 1-4-94

Chemist Review

Koeder

SEP 16 1994

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-356 CHEM.REVIEW #: 4 REVIEW DATE: 09-Sep-94

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
ORIGINAL	31-Mar-93	05-Apr-93	05-Apr-93
AMENDMENT	20-Jun-94	21-Jun-94	24-Jun-94
	29-Jul-94	03-Aug-94	05-Aug-94
	29-Jul-94	03-Aug-94	05-Aug-94

NAME & ADDRESS OF APPLICANT: Miles Inc.
Pharmaceutical Division
400 Morgan Lane
West Haven, CT 06516-4175

DRUG PRODUCT NAME
- Proprietary: Not yet established
- Nonproprietary/USAN: Nisoldipine
- Code Name/#: CAS-63675-72-9
- Chem.Type/Ther.Class: 1 S

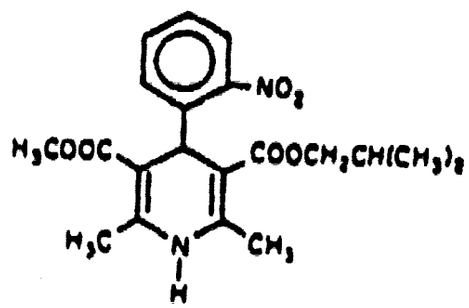
Patent Status: Patents which claim the drug, Nisoldipine, and its use are as follows:

U.S. Patent No. 4,154,836	Expires May 15, 1996 and covers the compound, pharmaceutical compositions for increasing coronary perfusion; and claims methods for increasing coronary perfusion.
U.S. Patent No. 4,892,741	Expires January 9, 2007, covers the coat-core tablet.
U.S. Patent No. 4,600,778	Expires July 15, 2003, covers the preferred process for providing Nisoldipine.

PHARMACOL.CATEGORY/INDICATION: Hypertension

DOSAGE FORM: Coat core (extended release) Tablets
STRENGTHS: 10, 20, 30 and 40 mg
ROUTE OF ADMINISTRATION: Oral
DISPENSED: __X__ Rx _____ OTC

STRUCTURAL FORMULA, CHEMICAL NAME, MOLECULAR FORMULA, MOLECULAR WEIGHT:



Chemical name(s):

3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-methyl-2-methylpropyl ester, (±)

(±)-Isobutyl methyl 1,4-dihydro-2,6-dimethyl-4-(o-nitrophenyl)-3,5-pyridinedicarboxylate

Molecular Formula: $C_{20}H_{24}N_2O_6$ Molecular Weight: 388.42

SUPPORTING DOCUMENTS:

RELATED DOCUMENTS (if applicable):

CONSULTS: EA was requested on 6/11/93, amended 8/4/93.

REMARKS/COMMENTS:

The nisoldipine CC tablets consist of a tablet core with a rapid active ingredient release in a compressed press-coating with controlled, delayed active ingredient release. To achieve protection from light, the tablets are film-coated.

Nisoldipine, racemate, will be used in the preparation of the drug product. Studies using enantiomers of nisoldipine were performed. (+)-Nisoldipine was found to be 10-20 times more potent than (-)-nisoldipine in hypertensive rats. There was no relevant difference in oral efficacy between (+)-nisoldipine and the racemate in either rats or dogs. (+)-Nisoldipine binds to isolated membranes with an affinity 100 times higher than (-)-nisoldipine.

In general, (+)-nisoldipine shows a spectrum of activities in standard safety pharmacology testing similar to the racemate, but at lower dose levels.

EER requested on 6/4/93. Acceptable on 12/16/93.

Methods validation - requested of DDA on 12/14/93. DO will be assigned when additional sample will be picked up. (Foreign manufacturing facility)

July 29, 1994 amendment - response to deficiencies.

July 29, 1994 amendment - change in dissolution specifications.

Proposed expiration date - 24 months.

Dissolution specifications (3 hours - 15 · · 6 hours - · 12 hours - nlt is acceptable (if Biopharm reviewer agrees).

CONCLUSIONS & RECOMMENDATIONS:

Responses to the deficiencies were satisfactory.

cc:
Orig. NDA 20-356
HFD-110/Division File
HFD-110/CunninghamD/9/9/94
District
HFD-110/CSO
HFD-102/CKumkumian (#1 only)
R/D Init by: SUPERVISOR

Danute G. Cunningham, Review Chemist
filename: 20356RO4.NDA

9-16-94

BIO Review

NDA: 20-356
Nisoldipine Coat-Core Tablets
10, 20, 30 and 40 mg
Sustained release tablets
Miles Pharmaceutical Division,
Priority: 1S

[illegible]
[illegible] 1993
September 24,1993.
October 14,1993.
November 04,1993.
November 23,1993.
December 29,1993.
March 14, 1994.
March 18, 1994.
June 27, 1994.
July 18, 1994.
July 29, 1994.

Type of submission: new molecular entity. Reviewer: Patrick J. Marroum.

Synopsis:

-The sponsor has adequately studied the pharmacokinetics (single and multiple dose) of nisoldipine coat-core tablet.
-Dosage form proportionality has been established between the 20 and 30 mg tablets only but not between the 20 and 40 mg strengths.
-Dose proportionality was established for AUC and CMAX in the dosing range of 20 to 60 mg. The 10 mg tablet strength gave more than dose proportional plasma levels when compared to the higher strengths.
-The effect of food on the rate and extent of nisoldipine absorption was investigated in 3 separate studies. Food increased CMAX by up to 300 % while decreasing AUC by up to 26 %.
-Adequate studies have been performed in elderly normal and hypertensive subjects.
-The effect of liver and renal disease has been adequately characterized in this NDA.
-Drug interaction studies between nisoldipine and warfarin, cimetidine, ranitidine, digoxin, quinidine, propranolol and atenolol have been performed.
-The sponsor failed to characterize the effect of gender on the pharmacokinetics of nisoldipine.
-The sponsor has adequately validated the gas chromatographic assay used in these studies.
-The sponsor attempted to correlate the in vitro dissolution with the in vivo performance of 3 different formulations of nisoldipine but failed to establish any relationship due to the fact that nisoldipine undergoes site specific gut wall metabolism.
-The dissolution method proposed by the sponsor for nisoldipine C.C. seems to be acceptable with the specifications recommended by the Division of Biopharmaceutics.

RECOMMENDATION:

The sponsor's NDA 20-356 appears to be acceptable for meeting the biopharmaceutics requirements provided that the comments on Pages 13 to 15 are adequately addressed by the sponsor.

TABLE OF CONTENTS

The following studies were not reviewed because they either pertain to the immediate release formulation which is not subject for approval under this NDA or were not deemed pertinent for the approval of nisoldipine C.C.

Study 339	Investigation of the relation between the systemic bioavailability and the dose of in healthy subjects.
Study 115	Plasma concentrations after oral administration of capsules and tablets (micronized substance and coprecipitate) to healthy volunteers.
Study 297	Investigation into the bioequivalence of various oral formulations of nisoldipine in healthy volunteers by a crossover trial.

Study D85-038	Bioequivalence study of formulations of nisoldipine 2.5 mg, 5 mg and 10 mg tablets in normal subjects.
Study D85-037	Bioequivalence study of formulations of nisoldipine 20 mg tablets in normal subjects.
Study 116	Plasma concentrations after the oral administration of 5 and 10 mg tablets. Comparison of micronized and ground active substance.
Study 330	Steady-state pharmacokinetics of nisoldipine in healthy male volunteers.
Study 102-106	Plasma concentrations after oral administration of different doses (6 to 20 μg/kg) of to healthy test subjects.
Study 125	Plasma levels in volunteers after oral administration of 10 and 20 mg of in the form of tablets.
Study 294	Plasma concentrations during treatment with of hypertensive patients with impaired liver function.
Study 452	Pharmacokinetics and hemodynamic effects of nisoldipine in patients with liver cirrhosis after PO and IV administration.
Study 364	Nisoldipine pK in renal dysfunction.
Study 399	Pharmacokinetics and hemodynamic effects of nisoldipine and its interaction with cimetidine in healthy volunteers.
Study D88-054	Comparative double blind pilot study of the safety and efficacy of once daily doses of nisoldipine 10, 20, 30 mg CC tablets vs placebo in hypertensive patients.
Study D89-029	Double blind randomized study of safety and efficacy of once daily doses of nisoldipine 20, 40 and 60 mg (2x30 mg) CC tablets vs placebo in combination with atenolol 50 mg in hypertensive patients.
Study D89-039	Comparative double blind study of safety and efficacy of once daily doses of nisoldipine 20, 40 and 80 mg CC tablets vs a twice daily doses of verapamil SR 240 mg caplets vs placebo in hypertensive patients.
Study	A double blind randomized study of the safety and efficacy

D90-019 [redacted]

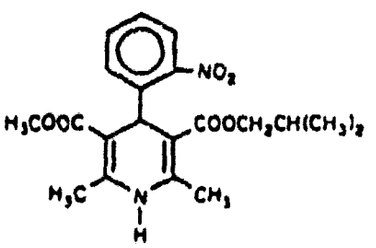
Study D88-060 Efficacy and safety of CC nisoldipine 10, 20 and 30 mg qd vs placebo in patients with stable exertional angina pectoris.

Study 0417 Acute pK/pD interaction of nisoldipine and propranolol.

Appendix II:

Background:

Nisoldipine is a dihydropyridine calcium channel blocker. It is 3,5-pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-methyl 2-methylpropyl ester. It has the following structural formula:



Nisoldipine is a yellow crystalline substance, practically insoluble in water but soluble in ethanol. No information about the octanol/water partition coefficient was submitted in this NDA. It has a molecular weight of 388.4. Nisoldipine coat core tablets consist of an external coat and internal core. Both contain nisoldipine, the coat in a slow-release formulation and the core in a fast-release formulation. Nisoldipine CC tablets contain either 10, 20, 30 or 40 mg of nisoldipine for once a day oral administration.

Nisoldipine C.C. is indicated for the treatment of hypertension and may be used alone or in combination with other antihypertensive agents. In general, therapy should be initiated with 10 mg orally once daily. The usual maintenance dosage is 20 or 30 mg once daily. Doses beyond 40 mg are not recommended.

It is to be noted that this NDA was first submitted on March 31, 1993. A non approval letter was issued on March 25, 1994 based on deficiencies in both the Chemistry and the Clinical sections of the application. It was resubmitted to the Agency on August 3, 1994. The user fee goal for this application is February 3, 1995.

SUMMARY OF BIOAVAILABILITY/PHARMACOKINETICS/PHARMACODYNAMICS:

I-BIOAVAILABILITY/BIOEQUIVALENCE:

A-Absolute Bioavailability:

The absolute bioavailability of 20 mg nisoldipine coat core compared to the dose corrected IV dose infused over 1 hour was 5.5 % with a 95 % CI ranging from 4.8 % to 6.4 %. (Study 0637).

B-Food Effects:

The effect of food on the pharmacokinetics of nisoldipine C.C. was investigated in 2 separate studies. Study 666 where a 20 mg nisoldipine C.C. tablet was given fasted, together with a high fat breakfast, 1 hour after a high fat breakfast and together with dinner showed that the coadministration with meals remarkably increased CMAX from 26 %(with dinner) up to 48% (together with breakfast) with a corresponding shortening of TMAX by about 2 to 3 hours. However food did not seem to have any effect on the extent of bioavailability of nisoldipine since there was no difference between the AUCs in fed and fasted states.

Study D92-045-02 showed that the effect of food was even more pronounced on the 30 and 40 mg C.C. tablets. Food increased the CMAX for nisoldipine on the average by 250 to 300 % (CMAX increased from 1.9 to 4.5 ng/ml for the 30 mg and from 2.7 to 7.5 ng/ml for the 40 mg strength) while decreasing the AUC by 26 % in the fed state as compared to the fasted state (AUC decreased from 49.2 to 35.4 ng*hr/ml for the 30 mg and from 70.4 to 53 ng*hr/ml for the 40 mg strength).

Similar results as far as CMAX is concerned were observed with the 20 mg IR tablet of nisoldipine. Food increased the AUC by 28 % and CMAX by 31 % compared to the fasting state (Study 323).

C-Bioequivalence:

Study 5678 showed that 1x40 mg was bioequivalent to 2x20 mg C.C. tablets as far as AUC was concerned since the 90 % CI of the log transformed AUC was 85.26 to 112.24 %. The same could not be said for CMAX because the 90 % CI of the log transformed CMAX was 94.62 to 140.03 %. Thus, 1x40 mg C.C. tablet is considered not bioequivalent to 2x20 mg tablets.

[redacted]

On the other hand study [redacted] 5x20 mg tablets, the AUC ratio (2x10/5x20) was [redacted] with log transformed 90 % CI of 95 to 115 while the CMAX ratio was 0.93 % with corresponding log transformed 90 % CI for 83.64 to 109.87 %.

Study KF 715 established the bioequivalence between the old 20 mg and the new 20 mg C.C. formulation as far as AUC was concerned. As for CMAX, none of the three strengths of the new formulation were bioequivalent to the old 20 mg formulation. The 5, 10 and 20 mg strengths were higher on the upper limit of the 90 % CI compared to the old 20 mg strength.

II.PHARMACOKINETICS:

The terminal half-life of nisoldipine was estimated to be about 7 hours after an infusion of 0.08 mg/kg for 20 hours. This corresponded to a systemic clearance of 544 to 768 ml/hr/kg. The volume of distribution was estimated to be between 2.3 and 3.4 l/kg (Study 330).
Following the administration of nisoldipine C.C. 20 mg once a day for 7 days, the CMAX was 0.84 ng/ml on day 1 and 1.09 ng/ml on day 7. The AUCnorm was 40.3 g*hr/l on day 1 and 58.9 g*hr/l for day 7 giving an accumulation ratio based on AUC of 1.46. CMAX following administration of an immediate release 10 mg tablet CMAX was 2.18 ng/ml on day 1 and 1.95 ng/ml on day 7 indicating that there is no accumulation of nisoldipine with the immediate release formulation. The fluctuation index for the IR tablet given bid was 439 % as compared to 113 % following the controlled release formulation (Study 645).
Study 606/618 showed that after IV administration similar plasma concentrations were obtained for both enantiomers. However, after oral administration the concentration of the (+) nisoldipine (which is pharmacodynamically more active than the (-)) was about 6 times higher than the (-) nisoldipine. The CMAX for the (+) hydroxylated dihydropyridine (M9) was 7.6 times higher than the (-) enantiomer.

III-METABOLISM:

In man, hydroxylation of the isobutyl ester appears to be the major biotransformation pathway. 70 to 80 % of the urinary metabolites in the first 12 hours after administration are the metabolites M4, M5 and M12 (Study 400),(see metabolic scheme in Appendix II). Metabolites M1 and M2 represent about 10 % of the urine metabolites in man (Study 16626). Only metabolite M12 was hydrolyzable with beta glucuronidase yielding M5 as the aglycon. The only metabolite with known activity is (M9) with 10 % of the activity exhibited by nisoldipine and is present in approximately equal concentrations in the plasma as nisoldipine. Study 600 provided some evidence that nisoldipine undergoes some degree of gut wall metabolism which is decreasing from the proximal to the distal parts of the intestine with no metabolism occurring in the colon.
It is to be noted that even though nisoldipine seems to be extensively metabolized, the sponsor did not identify the enzymes responsible for its biotransformation.

IV-DOSE PROPORTIONALITY:

The dose proportionality of immediate [redacted] established between the doses of 2.5 and 20 mg [redacted] both AUC and CMAX [redacted] dose proportional manner (Study D85-024-01).

As for the coat core formulation, the dose proportionality was established for doses in the range of 20 to 60 mg (using the 20 mg tablet except for the 40 mg dose where the 10 mg tablet was used). However, the dose normalized values for CMAX and AUC for the 10 mg dose were somewhat higher and statistically different as compared to the values for the 20, 40 and 60 mg doses (Study D91-035). These differences in results were not explained by the sponsor.

V SPECIAL POPULATIONS:

A.Renal Impairment:

In patients with severe renal impairment (creatinine clearance less than 30 ml/min), nisoldipine plasma concentrations on day 1 were higher by as much as 2 fold compared to subjects with normal renal function. However, this difference seemed to have subsided by day 7. Even though renal impairment does not seem to alter significantly the pharmacokinetics of nisoldipine C.C. and its metabolites. Caution should be exercised in dosing and titrating these patients. (Study D92-001).

B.Hepatic Impairment:

Study D90-026-01 shows that liver impairment has a pronounced effect on the pharmacokinetics of nisoldipine C.C. since both AUC and CMAX were increased four fold as compared to normal volunteers. Therefore extra care should be exercised when giving this drug to patients with impaired liver function.

C.Elderly:

Study 563 shows that the plasma concentrations of nisoldipine following the administration of 10 mg IR for 8 days tended to be higher in the elderly as compared to the young. CMAX was 1.76 (+/-0.84)ng/ml compared to 4.96 (+/-3.22) ng/ml in the elderly. AUC in the young was 7 (+/-3.12) ng/hr/ml compared to 15.04 (+/-9.33) ng/hr/ml in the elderly. However, there was no difference in the single dose and multiple dose of nisoldipine in both the young and the elderly and there was no accumulation upon multiple dose administration.
On the other hand, study 712 where 20 mg nisoldipine C.C. was administered for 7 days showed that after single dose the elderly normal and hypertensive patients tended to have about 50 % higher AUCs than young healthy subjects. CMAX in the elderly hypertensives was also about 50 % higher than either the healthy young or elderly volunteers. Moreover, upon multiple administration, there was a greater tendency for increase in AUC and CMAX for both the elderly healthy and hypertensive subjects compared to the young. It is to note that there was essentially no accumulation in the young in this study but in the elderly healthy and hypertensives, the accumulation ratio was around 2.

D-Gender:

The effect of gender on the pharmacokinetics of nisoldipine [redacted] investigated by the sponsor. An attempt by this reviewer to correlate gender with AUC and CMAX from data obtained from Study 712 was inconclusive due to insufficient data.

V-DISEASE STATES:

A-Hypertension:

Studies D90-022 and D88-059 showed that hypertension does not have any effects on the pharmacokinetics of nisoldipine C.C. since the plasma levels obtained from these studies were similar to those obtained in healthy subjects.

B-Stable Exertional Angina Pectoris:

Study 734 showed that the pharmacokinetics of nisoldipine C.C. does not appear to be affected by stable exertional angina pectoris since the plasma levels obtained in these patients was similar to the plasma levels obtained in healthy and hypertensive subjects.

VI-DRUG INTERACTIONS:

A-Ranitidine:

Coadministration of ranitidine with nisoldipine C.C. did not have any effect on the pharmacokinetics of nisoldipine (Study 738).

B-Cimetidine:

Cimetidine seems to have a pronounced effect on nisoldipine C.C. pharmacokinetic parameters since there was more than 50 % increase in some parameters of interest. Multiple dose administration of 400 mg of cimetidine increase nisoldipine CMAX from 1.05 to 1.74 ng/ml and its AUC from 14.97 to 23.2 mcg*hr/l. Therefore, great caution should be exercised when both these drugs are administered concomitantly, the patients should be monitored and dose adjustments made as necessary (Study 738).

C-Warfarin:

Study 349 showed that coadministration of steady state doses between 3 and 10 mg of warfarin with 10 mg IR nisoldipine did not have any effect on the pharmacokinetics of nisoldipine. Moreover, nisoldipine did not affect the prothrombin times of patients that were on warfarin.

D-Quinidine:

Coadministration [redacted] increased quinidine [redacted] pharmacokinetics could [redacted] measured in this study.

E-Propranolol:

Coadministration of 20 mg nisoldipine capsules either acutely or chronically caused a significant increase in both AUC and CMAX for propranolol. The propranolol AUC increased from 1556 (+/-) 1135) to 2098 (+/- 1501) with single dose nisoldipine and up to 2482 (+/- 2099) ng.hr/ml with multiple dosing of nisoldipine. CMAX increased from 143 (+/- 44) ng/ml to 222 (+/- 67) ng/ml with either single dose or multiple dose administration of the calcium channel blocker (Study 3982).
However Study 704 showed that coadministration of 20 mg nisoldipine C.C. with 40 mg propranolol tid did not have any significant effects on the plasma concentrations of either drugs.

F-Atenolol:

Coadministration of 20 mg nisoldipine capsules either acutely or chronically caused a significant increase in both AUC and CMAX of atenolol. The CMAX for atenolol increased from 455 (+/- 135) ng/ml to 540 (+/- 146) ng/ml while the AUC for the beta blocker increased from 5854 (+/- 2291) ng*hr/ml to 6987 (+/- 2269) ng*hr/ml. These results are very similar to what was seen when nisoldipine was coadministered with propranolol (Study 3982).

G-Beta Acetyl Digoxin:

Coadministration of 10 mg of nisoldipine IR tablets bid with 0.6 mg/day of beta acetyl digoxin did not seem to have any effect on the pharmacokinetics of beta acetyl digoxin (Study 413).
(Note: In the labelling, the results of this study appear as lack of interaction with Digoxin and not acetyldigoxin. This issue was discussed with Dr Chen supervisory Medical Officer HFD 110 who thought that labelling the results of this study as no interaction with digoxin was appropriate.)

VII-PHARMACOKINETIC/PHARMACODYNAMIC RELATIONSHIPS:

The sponsor attempted to establish a pharmacokinetic/pharmacodynamic model in hypertensive patients using the results of study D90-022. This pharmacodynamic model was established using the program Attract which is based on linear system analysis and methods utilizing hysterisis minimization. The sponsor reported that the pharmacodynamic responses (mainly drop in blood pressure) follow a sigmoid EMAX model.
The modelling method used by the sponsor is not valid due to the fact that it is very difficult to see hysterisis with this formulation of this drug (see also the comments following Study D90-022).
The Division of Biopharmaceutics attempted to model the pharmacodynamics with the

pharmacokinetics of Nisoldipine C.C. using [redacted] data from study D90-022 and study D88-059 were used in this attempt. However, due to the fact that both studies for the same 30 mg dose gave totally different plasma concentrations (study D90-022 gave almost double the plasma concentrations of study D88-059), these studies could not be combined and the final model was established using data from the same study that the sponsor model. The best model that describes this set of data was found to be an EMAX model with a maximal reduction of diastolic blood pressure of -23.9 mm of Hg. The EC50 was estimated to be 3.94 ng/ml.

VIII-FORMULATION:

The coat-core tablet consists of a slowly dissolving coat surrounding a more rapidly dissolving core. Within the coat the active ingredient is finely distributed in a matrix of a hydrophilic gel-forming polymer. On contact with water a swelling process begins at the tablet surface and the soft material formed is continuously eroded. The active ingredient contained in the eroded material can then be dissolved and absorbed. Initially, the diameter of the tablet and thus its surface area change very little resulting in a constant release of active ingredient over a period of about 6 to 8 hours (i.e. zero order dissolution kinetics). When the erosion of the coat has advanced, the dissolution of the fast-release core causes an increase in the release rate over a period of about 2 hours. Thus, the decreasing release rate of drug from the tablet coat (due to diminishing tablet surface area) is countered by the rapid dissolution of the core.
The composition of the different strengths tablets of nisoldipine Coat Core is summarized in Appendix II.
It is to be noted that all the pivotal clinical trials were done using the to be marketed formulation.

IX-PROTEIN BINDING:

The plasma protein binding of nisoldipine is very high since less than 1 % is unbound at a concentration range between 100 ng/ml and 10 mcg/ml. The binding is primarily to albumin. There was no stereoselectivity in binding since both enantiomers had similar degree of binding as observed with the racemate. (study 19611)

X-RED BLOOD CELL PARTITIONING:

The erythrocyte/plasma partition coefficients are about 0.3 mostly independent of the concentration in the range studied 0.1 to 10 mcg/ml. However, the partition between erythrocytes and plasma water is very high in the order of 56 indicating a high affinity of the blood cells and other tissues to nisoldipine. There was no difference in partitioning between the racemate and its enantiomers. (Study 19611).

XI-DISSOLUTION:

The proposed dissolution method for the coat-core tablet formulation was the USP method II (paddle method) at a paddle speed of 50 rpm. The medium was 900 ml of phosphate buffer with

1% sodium lauryl sulfate. The proposed dissolution specifications are:
-3 hours
-6 hours
-12 hours: Not less than
*For the evaluation after stage 2 of the USP acceptance table, single tablets and mean value are specified by the limits % and % respectively.
Based on the performance of the bio lots submitted in this NDA, the following dissolution specifications are recommended:
-3 hours %.
-6 hours. %.
-12 hours: not less than

XII-IN VIVO IN VITRO CORRELATION:

An attempt was made by the sponsor to correlate the in vitro and in vivo performance of the nisoldipine coat-core tablets. However, the sponsor could not establish any level of correlation (A,B or C) due to the fact that nisoldipine undergoes variable gut wall metabolism which is dependent on the site in the gastro-intestinal tract.

XIII-ASSAY:

Concentrations of nisoldipine and its metabolites from biological fluids were determined using . Overall, the assay methodology as well as its validation were satisfactory.

Comments to be Sent to the Firm:

1-Nisoldipine appears to exhibit stereospecific first pass metabolism. After P.O. administration the (+) nisoldipine plasma levels are 6 times higher. Since the (+) enantiomer of nisoldipine is responsible for most if not all the activity of this drug, the sponsor should have used a stereospecific assay for all pk studies.

2-The sponsor did not determine the ratio of the two enantiomers in special populations. The sponsor is asked to submit any available data on the ratio of the enantiomers of the parent compound as well as any relevant metabolites in dose proportionality, drug interaction studies and special populations (such as liver impairment patients, elderly etc...) as compared to healthy volunteers.

3-The sponsor failed to identify the specific enzymes that are responsible for the metabolism of nisoldipine. The sponsor is requested to identify the enzyme systems responsible for the metabolism of nisoldipine even though the sponsor believes that there is no need to conduct such studies because it is believed that the same enzyme that is responsible for the metabolism of nifedipine (i.e. 3A4) is also responsible for the metabolism of nisoldipine. Nevertheless, it is necessary to confirm this by conducting an in vitro metabolic study.

4-The dissolution data submitted in this NDA showed a great deal of variability specially [illegible] the 9 hour time point. Some of the higher strengths lots i.e. the 20 and 30 mg tablet strength seem to give different results when the same lot studied under the same conditions were tested within the same day and also on different days. The firm is requested to give an explanation for this variability.

5-Based on the performance of the bio lots submitted in this NDA, the following dissolution test and specifications are recommended:
USP paddle at a speed of 50 rpm in 900 ml of phosphate buffer pH 6.8 with 1 % sodium lauryl sulfate
-3 hours: %.
-6 hours: %.
-12 hours: not less than %.

6-Studies D90-022 and D88-059 do not give the same plasma levels for the 30 mg dose. In the first study (D90-022) the plasma levels obtained with the 30 mg dose are almost double than what was obtained with the same dose in Study D88-059. AUC was 74.28 +/- 7.96 compared to 33.097 +/- 6.077 ng*hr/ml while CMAX was 4.79 +/- 0.68 compared to 2.473 +/- 0.458 ng/ml. The sponsor is asked to explain the observed discrepancy between the 2 studies.

7-In several of the studies, the sponsor did not include any assay validation. It is the Division of Biopharmaceutics policy to recommend that a full description of the analytical assay be included in the study reports. Data on the linearity, specificity, sensitivity and on the accuracy and precision of the analytical methodology should be included in each study report.

8-The modelling approach taken by the sponsor to analyze the relationship between the pK of nisoldipine and its pd was found to be inadequate by the reviewer due to the reasons outlined in the Comments following Study D90-022 on page 189.

9-In Studies D90-022 and D88-059, CMAX and AUC (only in Study D90-022) increased in a less than dose proportional manner with dose. Yet, the results of Study D91-035 indicate that the pharmacokinetics of nisoldipine from the coat-core formulation should be linear between 20 and 60 mg. The sponsor is asked to explain the discrepency in these results.

10-Quinidine is a known inhibitor of the cytochrome P450 isoenzyme DII6. In the drug interaction study between quinidine and IR nisoldipine, the sponsor only measured the plasma concentrations of quinidine. The sponsor should have also measured the nisoldipine plasma concentrations to see whether quinidine had any effect on the metabolic pathways responsible for the metabolism of this dihydropyridine compound.

11-In the renal impairment study, the sponsor should have determined the protein binding of nisoldipine. It is not uncommon to see significant protein binding changes which will affect the plasma levels of the drug under study.

12-The sponsor did not investigate either the effect of gender or race on the pharmacokinetics of nisoldipine. The sponsor is asked to submit any additional data that might be available that would address this issue.

13-The Pharmacokinetics and metabolism section of the package insert should be rewritten as follows:

Pharmacokinetics:

Nisoldipine activity is primarily due to the (+) enantiomer. Studies with radiolabelled drug have demonstrated that administered nisoldipine is relatively well absorbed into the systemic circulation with 87 % of the radiolabel recovered in urine and faeces. Elimination of nisoldipine is exclusively by metabolism with no unchanged nisoldipine recovered in the urine. Nisoldipine pharmacokinetics are independent of dose in the range of 20 to 60 mg. Upon multiple dosing, nisoldipine accumulation is predictable from a single dose. The bioequivalency between 2x30 mg and 3x20 mg nisoldipine has been established. However 1x20 vs 2x10 and 2x20 mg vs 1x40 mg tablets were inequivalent with respect to CMAX.

Absorption: The absolute bioavailability of nisoldipine was found to be 5.5 %. Nisoldipine's bioavailability is due to presystemic metabolism with evidence of gut wall metabolism which decreases from the proximal to the distal parts of the intestine with no metabolism occurring in the colon.

Food has a pronounced effect on the release of nisoldipine from the coat-core formulation. CMAX increased by up to 300 % and AUC decreased by up to 26 %. However, the food effect was not as pronounced on the immediate release capsule since AUC increased by 28% and CMAX by 31 %. Concomitant intake of food with nisoldipine Coat-Core is contraindicated.

The volume of distribution of nisoldipine after IV administration was estimated to be between 2.3 and 3.4 l/kg. The plasma protein binding is very high since less than 1 % is unbound over the plasma concentrations of 100 ng/ml to 10 mcg/ml. Nisoldipine poorly penetrates into red blood cells with a blood/plasma ratio of 0.3 mostly independent of concentration over the range of 0.1 to 10 mcg/ml.

Maximal plasma concentrations of nisoldipine are reached 6 to 12 hours after dosing. The terminal elimination half-life ranges from 7 to 12 hours. With a 40 mg tablet of nisoldipine Coat-Core, CMAX was 3.1 ng/ml and the AUC_{0-48} was 54.3 ng*hr/ml. After oral administration, the concentration of the (+) nisoldipine was about 6 times higher than the (-) isomer.

Metabolism: 11 metabolites have been identified in the urine. In man the major biotransformation pathway appears to be the hydroxylation of the isobutyl ester. Metabolite M9, which is the hydroxylated derivative of the side chain of nisoldipine, is the only one that appears to have any activity (10 % of the parent compound) and is present in equal amounts as nisoldipine in plasma. Cytochrome P450 is believed to play a major role in the metabolism of nisoldipine, however, the particular isoenzyme system that is responsible for its metabolism has not been identified.

Excretion: No unchanged nisoldipine is eliminated in the urine.

Special Populations:

Geriatric: Elderly patients have been found to have 2 to 3 fold higher plasma concentrations than young subjects.

Renal dysfunction: Because renal elimination is not a significant pathway, dosing adjustments in patients with mild to moderate renal impairment is not necessary.

Hepatic Insufficiency: In patients with liver cirrhosis given 10 mg nisoldipine C.C., plasma concentrations of the parent compound were found to be 4 to 5 times higher than healthy young subjects. Thus lower maintenance doses may be required in both cirrhotic patients and in the elderly.

Gender and Race: The effect of gender or race on the pharmacokinetics of nisoldipine has not been investigated.

Disease States: Neither hypertension nor stable exertional angina pectoris alter the pharmacokinetics of nisoldipine.

Drug-Drug Interactions: No significant interactions were found between nisoldipine immediate release (IR) and warfarin or beta acetyl-digoxin. However, IR nisoldipine increased plasma quinidine concentrations by about 20 %.
A 30 to 40 % increase in AUC and CMAX of nisoldipine was observed with concomitant administration of 400 mg cimetidine twice daily. There was no interaction with ranitidine 150 mg twice daily..
Coadministration of 20 mg nisoldipine IR with 160 mg propranolol once daily caused a 35 % increase in propranolol AUC and 55 % increase in CMAX. The interaction with nisoldipine C.C. was negligible. Atenolol's AUC and CMAX were increased by 20 % when coadministered either acutely or chronically with 20 mg IR nisoldipine capsules.

9-In the Dosage and Administration section of the package insert, a statement should be included that nisoldipine coat-core should be taken on an empty stomach.

P Marroum 11/18/1994
Patrick J. Marroum Ph.D.

Biopharm Day October 4 1994 (Collins, Ludden, Malinowski, Fleischer, Chen, Gillespie, Parekh, Marroum).
RD/ FT initialed by A Parekh Ameeta Parekh 11/21/94

cc: NDA 20-356, HFD 110, HFD 426 (Marroum, Fleischer), Chron, Drug, FOI (HFD 19), HFD 340 (Vishwanathan), F, CR, A, DI, Pk/PD, RI, A, CD.

NISOLDIPINE-BIOTRANSFORMATION IN MAN.

Study No. 400 Volume: 37 Pages: 06-00-3018- 06-00-3078

INVESTIGATORS:

OBJECTIVE:

To investigate and compare the renal metabolite profiles of nisoldipine (administered orally and intravenously) to human volunteers.

MATERIAL AND METHODS:

Racemic ^{14}C nisoldipine was synthesized with chemical purity of 99.1% and specific activity of 53 µci/mg.
Lot # 959633

Route of administration: oral
Dosage: 12 mg per volunteer
Radioactive dose: $1.443*10^9$ dpm per volunteer.

Route of administration: intravenous
Dosage: 0.8 mg per volunteer
Radioactive dose: $1.0101*10^8$ dpm per volunteer.

12 volunteers were given 14C-labelled nisoldipine orally and intravenously. The urine was collected over four intervals (0-4, 4-8, 8-12, and 12-24) to determine the metabolic profile. The degrees of excretion of metabolites in the last two collections intervals were below 4% of the initial dose. Six of at least eleven metabolites were identified and compared with authentic material by different spectroscopic methods.

RESULTS

The majority of the metabolites were carboxylic acids. The unchanged active substance was absent. The two main metabolites, M-4, and M-5 (figure 1), accounted for about 80% of the renally excreted activity. These two metabolites were also among the main renally excreted metabolites in the rat, dog, and monkey. M-4 and M-5 were observed in urine samples at all times, but to a smaller extent in each successive collection period. together they account for 40% of the renally excreted activity in the 12-24 h urine. The quantitative differences between the two metabolite profiles after IV or PO administration were not significant. M-4 and M-5 accounted for 28.2 and 43.3% for IV administration Vs 32 and 48.8% after oral administration, which indicates that the two main metabolites were balanced. Hydroxylation of the isobutyl ester appears to be the preferred biotransformation pathway (M-1, M-2, and M-12) since they account for 70-80% of the renally eliminated activity. Ester cleavage account for about 10% of the activity (table 10, and 11).

COMMENT:

Although faeces were collected over 96h. (iv) and 144h (po), no qualitative or quantitative analysis of the drug or its metabolites in faeces was reported. So the study does not account for the fate and distribution of the total dose.

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Table 10: biotransformation pathway via hydroxylation of the isobutyl moiety [2]

metabolite	rat 0-24 h	dog 0-24 h	monkey 0-7h	monkey 7-11 h	monkey 11-24 h	human 0-4 h	human 4-8 h	human 8-12 h	human 12-24 h urine
M-4	11.0	9.4	10.2	6.2	7.5	32.0/30.1	21.1/26.2	16.1/18.2	9.7/10.1
M-5	34.9	44.9	27.2	23.7	26.7	48.8/48.9	44.7/46.9	38.1/36.9	29.8/27.4
M-12	3.1	11.7	30.8	31.1	15.5	3.9/ 2.7	8.0/ 8.7	13.0/13.3	6.3/ 7.0
total	49.0	66.0	68.2	61.0	49.7	84.7/81.7	73.8/81.8	67.2/68.4	45.8/44.5

Table 11: biotransformation pathway via ester cleavage of the isobutyl moiety [2]

metabolite	rat 0-24 h	dog 0-24 h	monkey 0-7h	monkey 7-11 h	monkey 11-24 h	human 0-4 h	human 4-8 h	human 8-12 h	human 12-24 h urine
M-1	14.6	4.9	4.5	4.7	6.3	4.1/3.6	10.7/ 6.8		
M-2	7.4	4.4	3.1	4.1	5.6	4.2/4.2	3.5/ 3.8	3.9/4.4	2.8/3.3
M-3	11.1	4.7	9.6	6.2	5.5				
M-6	1.2	1.2	0.5	-	2.6				
R-7	3.6								
total	37.9	15.2	17.7	15.0	20.0	8.3/7.8	14.2/10.6	3.9/4.4	2.8/3.3

Biotransformation of nisoldipine in rat, dog, monkey and man.

Study: 16626 Volume: 37 Pages: 3079-3105.

Investigators:

Objectives:

The objective of this study was to determine the metabolic fate of nisoldipine in the rat, dog, monkey and cat.

Formulation

-[6-^{14}C] nisoldipine CC with a specific activity of 45 μCi/mg and 53 μCi/mg with a radiochemical purity of 98.5 %.

Study Design:

12 healthy male volunteers were given 0.8 mg ^{14}C in 16 ml by IV infusion as an aqueous solution containing ethanol and polyethylene glycol. The same dose of nisoldipine was given orally as an aqueous solution (12 mg/1.2 ml containing ethanol and Cremophor RH 40). The study design was an open study with crossover from IV to PO administration. Plasma, urine and stool were collected sequentially for 4 (IV) and 6 days (PO).

Assay:

Results:

In man, the mean (SD) excretion after IV administration was 82.2 % (7.4) in the urine and 14.4 % (9.5) in the faeces 4 days post administration. After oral administration, the mean excretion

Table 1. Metabolites identified in rats, dogs, monkeys and man

	rat			perfused rat liver		dog		monkey	man
metabolite	urine 0-24h	plasma 0.5h	bile 0-6h	perfusate 3h	bile 3h	urine 0-24h	plasma 1.5h	urine 0-24h	urine 0-24h
M- 1	14.6	-	-	-	-	4.9	-	4.7	4.5
M- 2	7.4	23.0	1.2	2.0	0.7	4.4	6.3	3.5	3.5
M- 3	11.1	5.8	5.7	-	1.9	4.7	3.9	8.7	-
M- 4	11.0	5.3	7.3	16.5	1.2	9.4	9.6	8.6	27.2
M- 5	34.9	13.3	24.2	43.7	11.2	44.9	46.5	23.7	46.3
M- 6	1.2	2.3	1.4	-	0.8	1.2	-	0.7	-
R- 7	3.6	-	n.b.	n.b.	n.b.	-	-	n.b.	n.b.
R- 8	n.b.	-	-	-	-	-	-	-	-
M- 9	-	7.9	-	-	-	-	-	-	-
M-10	-	3.3	6.8	4.3	4.7	-	-	-	-
M-11	-	6.2	-	-	-	-	-	-	-
M-12	3.1	-	10.1	2.7	22.3	11.7	-	29.2	5.4
M-13	-	-	3.7	1.5	1.7	-	-	-	-
M-14	-	-	}	-	}	-	-	-	-
M-15	-	-	} 19.1	-	} 18.7	-	-	-	-
M-16	-	-	}	-	}	-	-	-	-
R- 3	-	12.2	-	-	-	-	-	-	-
R- 5	-	11.7	-	-	-	-	-	-	-
nisoldipine	-	2.2	-	-	-	-	-	-	-
total	86.9	93.2	79.5	70.7	63.2	81.2	66.3	79.1	86.9
% of dose	27.8		54.1	17.6	35.5	18.7		57.7	64.0

n.b. not balanced
- not detectable by TLC

Results are given in % of the radioactivity recovered in urine, bile, serum and perfusate, resp.

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oinding to human plasma proteins and erythrocyt: plasma partitioning in vitro for the racemate and its enantiomers.

Study: 19611. Volume: 1.31 Pages: 454-472

Investigators:

Objectives:

The objective of the study was:
To investigate in vitro the human plasma protein binding and red blood cell partitioning for the racemic drug and its enantiomers using the carbon 14 labelled compounds.

Formulation:

radiochemical purity 99 %.

Study Procedure:

Protein binding was determined by equilibrium dialysis and ultrafiltration.
The red blood cell partitioning was determined by incubation of the racemate and the enantiomers in 3 ml of carbogen aerated blood. The hematocrit and the radioactivity were determined directly after incubation in triplicate. The plasma was obtained from the remaining blood by centrifugation . Radioactivity concentration in plasma is measured in triplicate.

Assay:

Results:

The results of the in vitro determinations of protein binding are given in Table 1. Table 2 gives the results of the erythrocyte/plasma partitioning experiments.
It can be seen from the results that both methods gave comparable results and that nisoldipine is greater than 99 % bound and that there was no stereoselectivity in binding since both enantiomers had similar degree of binding as observed with the racemate.
The erythrocyte/plasma partition coefficients are about 0.3 mostly independent of the

concentration in the range studied 0.1 to 10 mcg/ml. However, the partition between erythrocytes and plasma water is very high in the order of 56 indicating a high affinity of the blood cells and other tissues for . There was no difference in partitioning between the racemate and its enantiomers.

Table 2: [^{14}C]- and enantiomers

In vitro partitioning of radioactivity between plasma and erythrocytes in freshly prepared heparinized blood samples of male and female healthy volunteers. Incubation time was 10 min. Arithmetic means and standard deviations of n=3 are given.

substance	sex	HK	C_B [µg·ml^{-1}]	C_P [µg·ml^{-1}]	C_E [µg·ml^{-1}]	$P_{E/P}$	f_u [%]	$P_{E/PW}$
[^{14}	male	0.48±0.02	0.102±0.008	0.152±0.012	0.049±0.009	0.32±0.05	0.68	47.1
			0.932±0.039	1.39 ±0.06	0.439±0.047	0.32±0.04		47.1
			9.87 ±0.39	13.44 ±0.45	5.80 ±0.41	0.43±0.03		63.2
	female	0.42±0.04	0.112±0.015	0.157±0.025	0.052±0.009	0.33±0.03	0.59	55.9
			0.948±0.018	1.33 ±0.07	0.431±0.070	0.33±0.07		55.9
			10.58 ±0.57	14.11 ±0.43	5.79 ±0.45	0.41±0.04		69.5
[^{14}	male	0.48±0.02	0.138	0.199	0.071	0.36*	0.68	52.9
			0.929±0.136	1.40 ±0.24	0.396±0.042	0.29±0.04		42.6
			8.87 ±1.62	10.57 ±2.63	4.87 ±1.00	0.48±0.17		70.6
	female	0.42±0.04	0.128±0.012	0.181±0.011	0.056±0.013	0.31±0.06	0.67	46.3
			1.00 ±0.01	1.40 ±0.07	0.471±0.018	0.34±0.02		50.7
			9.20 ±0.39	12.12 ±0.77	5.14 ±0.51	0.42±0.02		62.7
[^{14}	male	0.48±0.02	0.099±0.012	0.151±0.024	0.041±0.005	0.28±0.08	0.65	43.1
			0.808±0.068	1.21 ±0.10	0.366±0.043	0.30±0.02		46.2
			8.73 ±0.47	12.88 ±0.75	4.09 ±0.43	0.32±0.04		49.2
	female	0.42±0.04	0.096±0.012	0.136±0.014	0.043±0.008	0.31±0.03	0.56	55.4
			0.823±0.046	1.17 ±0.10	0.360±0.049	0.31±0.07		55.4
			8.70 ±0.24	12.38 ±0.63	3.80 ±0.46	0.31±0.06		55.4

* n=2

Table 1: and enantiomers

In vitro binding to human plasma and plasma protein fractions at different concentrations as determined by equilibrium dialysis and ultrafiltration. Binding data are expressed as free (unbound) fraction f_u in percent (arithm. means and standard deviation). Pooled plasma of at least 3 male or female healthy volunteers was used for determination in triplicate. Human serum albumin (HSA: 40 $g \cdot l^{-1}$) and acidic alpha$_1$-glycoprotein (AAG: 0.7 $g \cdot l^{-1}$) were dissolved in the ultrafiltrate of heparinized human plasma.

substance	sex/ binding protein	equilibrium dialysis total conc. [$\mu g \cdot ml^{-1}$]	equilibrium dialysis fraction unbound [%]	ultrafiltration total conc. [$\mu g \cdot ml^{-1}$]	ultrafiltration fraction unbound [%]
[^{14}C]	male	0.11	<	0.09	<
		1.05	0.52±0.06	1.07	0.29±0.09
		9.79	0.75±0.06	9.55	0.49±0.01
	female	0.10	<	0.10	<
		1.04	0.56±0.04	1.29	0.27±0.03
		10.[illegible]	0.62±0.03	9.86	0.40±0.02
[^{14}C	male	0.08	<	0.09	<
		1.02	0.60±0.03	1.22	<
		10.5	0.75±0.02	10.9	0.46±0.01
	female	0.09	<	0.10	<
		0.98	0.54±0.07	1.23	<
		10.1	0.79±0.19	10.5	0.40±0.02
[^{14}C	male	0.11	[illegible]	0.10	<
		1.00	0.[illegible].06	1.00	<
		10.9	0.71±0.01	11.2	0.43±0.03
	female	0.10	<	0.11	<
		1.00	0.48±0.05	1.31	<
		10.4	0.63±0.06	11.0	0.29±0.01
[^{14}	HSA	0.99	1.96±0.14	0.90	1.33±0.03
	AAG	0.99	30.3 ±5.4	0.97	26.5 ±0.7

below detection limit (< 7 ng/ml)

Estimation of the bioavailability of nisoldipine in man.

STUDY: 3647 VOLUME: 1-36 PAGES: 2737-3017.

INVESTIGATOR:

OBJECTIVES:

1-To determine the absolute bioavailability of ^{14}C nisoldipine on the basis of plasma and urine data.
2-to determine the absolute bioavailability of an oral solution of nisoldipine on the basis of plasma data.
3-to measure the excretion of nisoldipine in urine and faeces.

FORMULATIONS:

-^{14}C nisoldipine intravenous solution batch # 131184-140, 0.8 mg in 16 ml (45.5 mcCi).
-^{14}C 1 % nisoldipine oral solution batch # 141184-141, 12 mg in 1.2 ml (110 mcCi).

STUDY DESIGN:

12 healthy male Caucasian volunteers from 18 to 24 years of age participated in this open study with crossover from intravenous to oral administration. Intravenous administration was given over a 15 minutes period. Blood samples were taken at 0, 5, 10, 15, 17.5, 20, 22.5, 25, 30, 35, 60, 80, 105, 135 minutes and at 3, 4, 6, 8, 12, 24, 32, 48, 72 and 96 hours after the IV infusion.
The blood sampling after oral administration was at -10, 0, 3, 5, 7.5, 10, 15, 20, 30, 40, 60, 80, 100 minutes and at 2, 3, 4, 6, 8, 12, 24, 32, 48, 72, 96, 120 and 144 hours.
Urine was collected before drug administration and at 0-4, 4-8, 8-12, 12-24, 24-48, 48-72 and 72-96 hours after medication.
Stools were collected in light protected containers according to the following time schedule: 0-24, 24-48, 48-72 and 72-96 hours after drug administration.

ASSAY:

RESULTS:

Figure 1 shows the geometric mean nisoldipine plasma concentrations after IV and PO administration while Figure 2 shows the mean total radioactivity concentrations for the same treatments. Table 1 summarizes the main pharmacokinetic parameters for nisoldipine after IV and PO calculated from plasma levels while Table 2 gives the same pharmacokinetic parameters calculated from measurement of total radioactivity. Figure 3 shows the mean cumulative urinary nisoldipine excretion after IV and PO administration while Table 3 gives the mean nisoldipine recovery after IV and PO administration both in the urine and faeces.

The results show that 80.3 % of the IV dose was recovered in urine but only 70 % after PO administration giving an absorption from the gut of about 87 % based on cumulative urinary excretion of the parent compound and its metabolites. However the absolute bioavailability calculated from AUC of the parent compound is 0.084 or 8.4 % which is indicative of a large first pass effect for nisoldipine.

13.8 % and 11.8 % of the radioactive nisoldipine dose was recovered in faeces after IV and PO administration respectively. These results may suggest that nisoldipine and its metabolites might be undergoing biliary excretion.

FIGURE 1

STUDY 400 (UOFS 7/85)

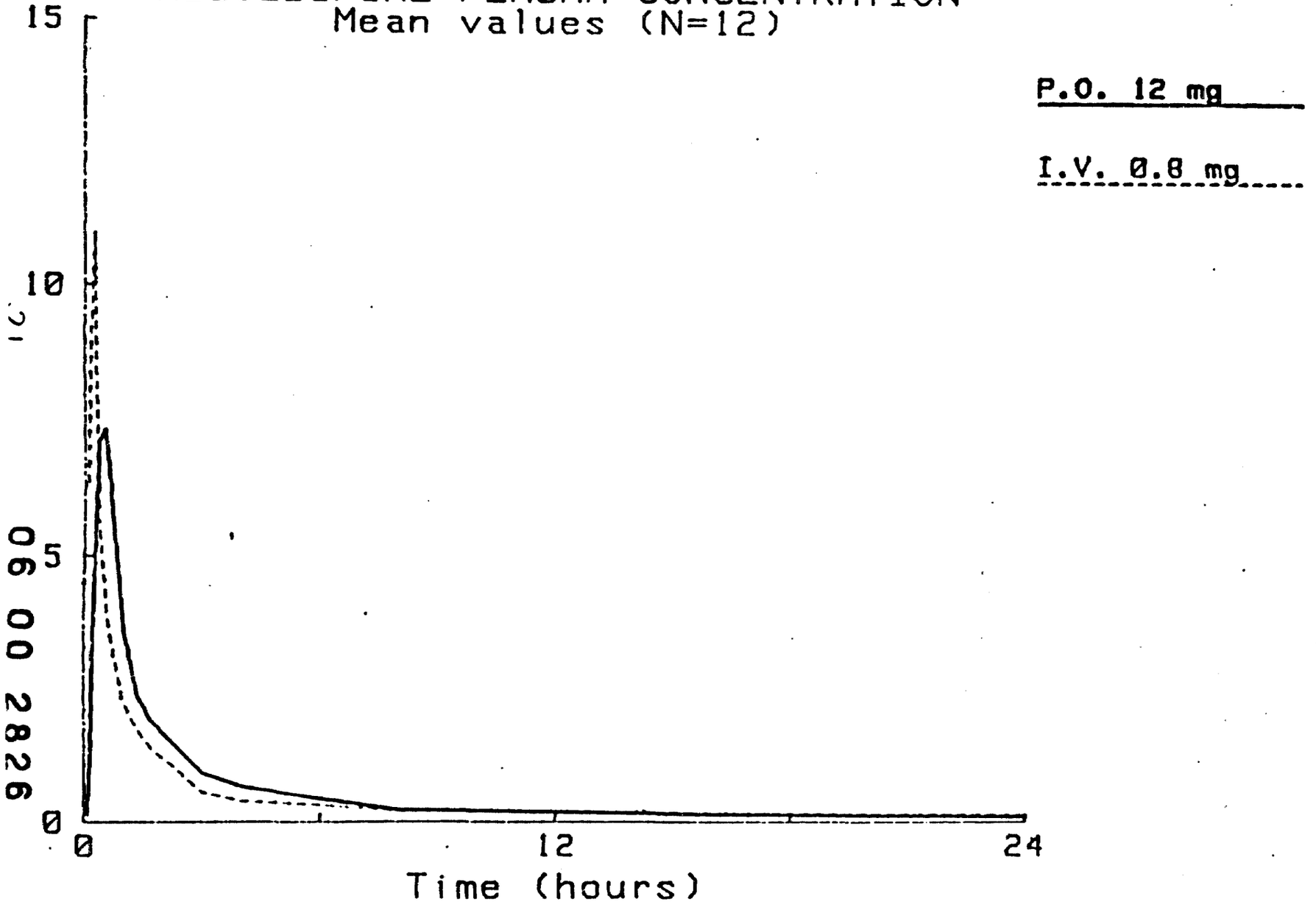

FIGURE 2

STUDY 400 (UOFS 7/85)

^{14}C NISOLDIPINE PLASMA DETERMINATION

Mean values (N=12)

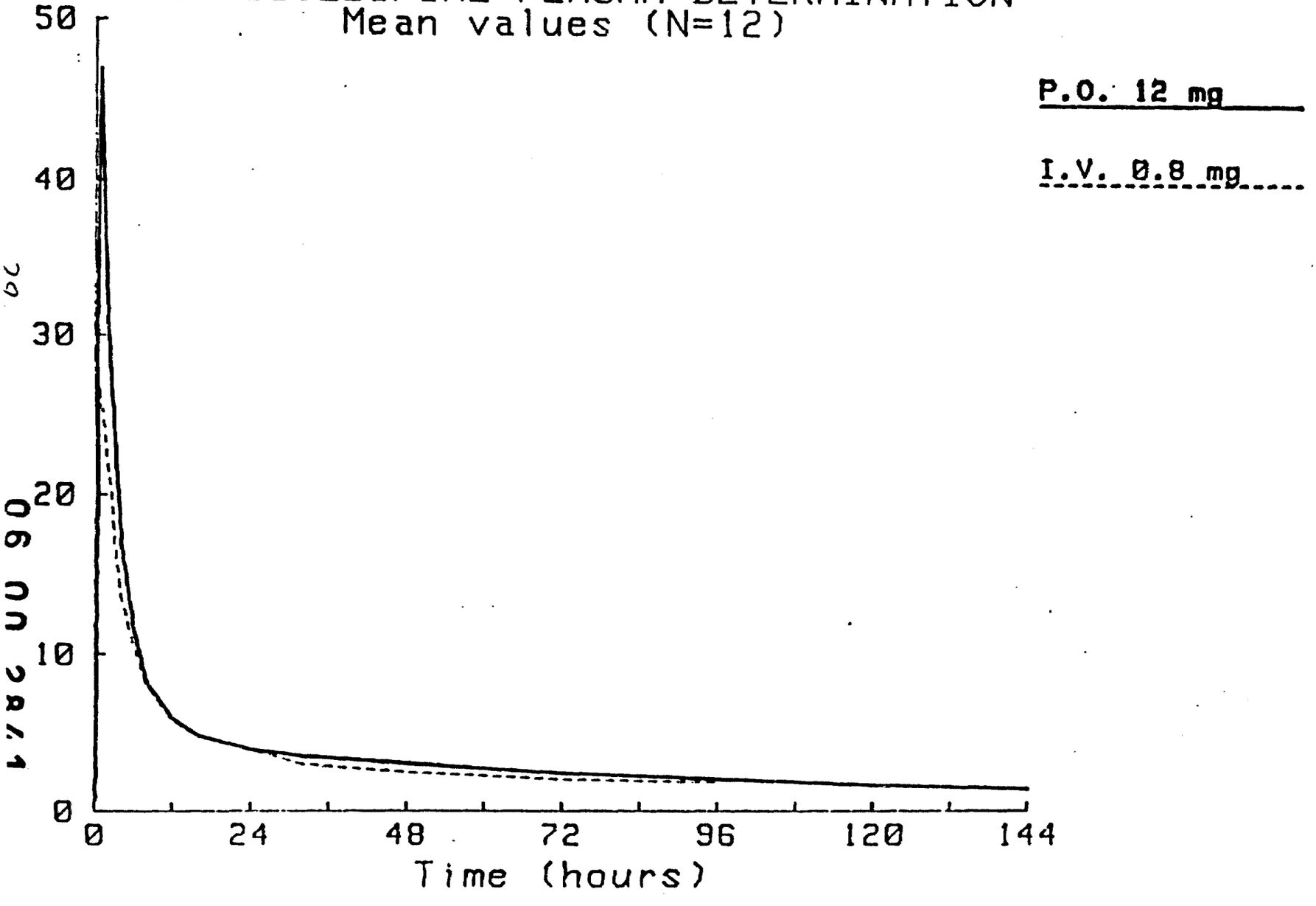

FIGURE 3

STUDY 400 (UOFS 7/85)

CUMULATIVE NISOLDIPINE URINARY EXCRETION
Mean values (N=12)

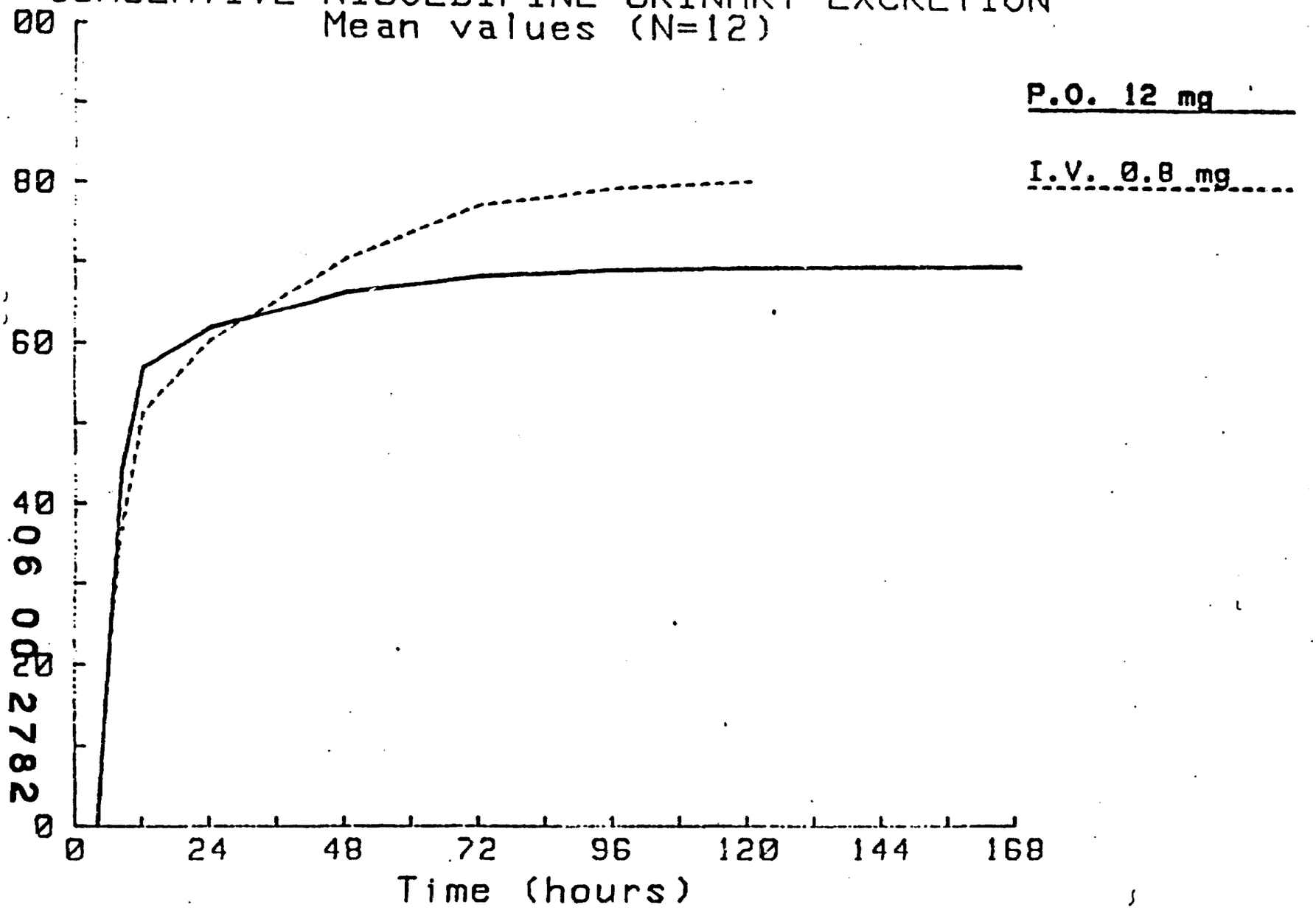

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TABLE 1

MEANS AND STANDARD DEVIATIONS

STUDY 400 (UOFS 7/85)
WHOLE BLOOD: APPARENT NISOLDIPINE PHARMACOKINETIC PARAMETERS
1 :TREATMENT : ORAL SOLUTION
2 :TREATMENT : INTRAVENOUS INFUSION

C MAX
(NG/ML)

	MEAN	S.D.	N
1 :	310.40	79.61	12
2 :	17.17	2.24	12

T MAX
(H)

	MEAN	S.D.	N
1 :	.78	.49	12
2 :	.79	.47	12

AUC
(NG/ML.H)

	MEAN	S.D.	N
1 :	4051.86	843.45	12
2 :	380.66	43.51	12

MRT
(H)

	MEAN	S.D.	N
1 :	46.83	1.67	12
2 :	43.70	3.22	12

TABLE 2

MEANS AND STANDARD DEVIATIONS

STUDY 400 (UOFS 7/85)
PLASMA: APPARENT NISOLDIPINE PHARMACOKINETIC PARAMETERS
1 :TREATMENT : ORAL SOLUTION
2 :TREATMENT : INTRAVENOUS INFUSION

C MAX (NG/ML)

	MEAN	S.D.	N
1 :	578.50	87.90	12
2 :	22.52	2.04	12

T MAX (H)

	MEAN	S.D.	N
1 :	.77	.41	12
2 :	.77	.35	12

AUC (NG/ML.H)

	MEAN	S.D.	N
1 :	6251.18	527.52	12
2 :	522.53	42.61	12

MRT (H)

	MEAN	S.D.	N
1 :	42.29	2.39	12
2 :	43.55	1.35	12

TABLE 3

MEANS AND STANDARD DEVIATIONS

STUDY 400 (UOFS 7/85)
CUMULATIVE NISOLDIPINE EXCRETION (%)
A :

URINE
(IV)

	ARITH MEAN	GEOM MEAN	S.D.	N
A :	80.331	79.374	11.813	12

URINE
(PO)

	ARITH MEAN	GEOM MEAN	S.D.	N
A :	69.945	69.184	10.113	12

FAECES
(IV)

	ARITH MEAN	GEOM MEAN	S.D.	N
A :	13.624	11.899	8.764	12

FAECES
(PO)

	ARITH MEAN	GEOM MEAN	S.D.	N
A :	11.787	11.556	2.458	12

TOTAL
(IV)

	ARITH MEAN	GEOM MEAN	S.D.	N
A :	93.955	92.847	13.899	12

TOTAL
(PO)

	ARITH MEAN	GEOM MEAN	S.D.	N

Study to investigate the absolute bioavailability of controlled release formulation of nisoldipine.

Study: 0637. Volume: 112. Pages: 06-04-7222-7234

Investigators:

Objectives:

1- to assess the tolerability and the pharmacokinetics of the controlled release formulations of nisoldipine in comparison to an intravenous application with special regard for the absolute bioavailability.

Formulation:

-20 mg nisoldipine C.C. tablets batch # 523081E029.
-Nisoldipine solution for IV administration (0.005 %) batch # 520219E501.

Study Design:

12 healthy male subjects between the ages of 18 and 40 years participated in this non-blind randomized crossover study with washout phases of 6 days.
Nisoldipine was administered in single oral doses of 20 mg C.C. tablet with 100 ml of water or 1 mg as 20 ml of the 0.005 % solution during a period of 1 hour.
Plasma concentrations after oral administration were determined at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16, 20, 23, 24, 28, 32 and 48 hours post medication.
Plasma concentrations after IV administration were determined at 0, 5, 10, 20, 45, 60, 70, 80, 90, 105, 120, 150, 180 minutes and at 4, 6, 8, 10, 12 and 24 hours after the start of the infusion. Also, measurement of blood pressure and heart rate was performed prior to blood sampling until 24 hrs after administration.

Assay:

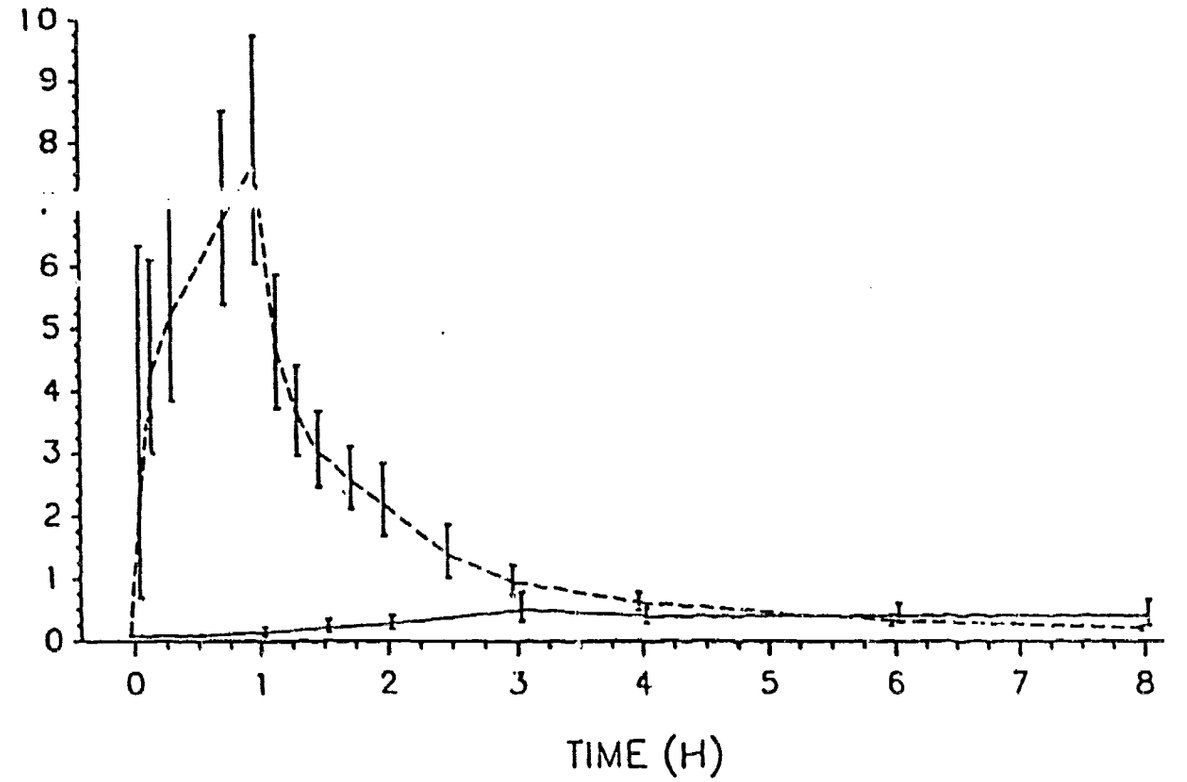
Results:

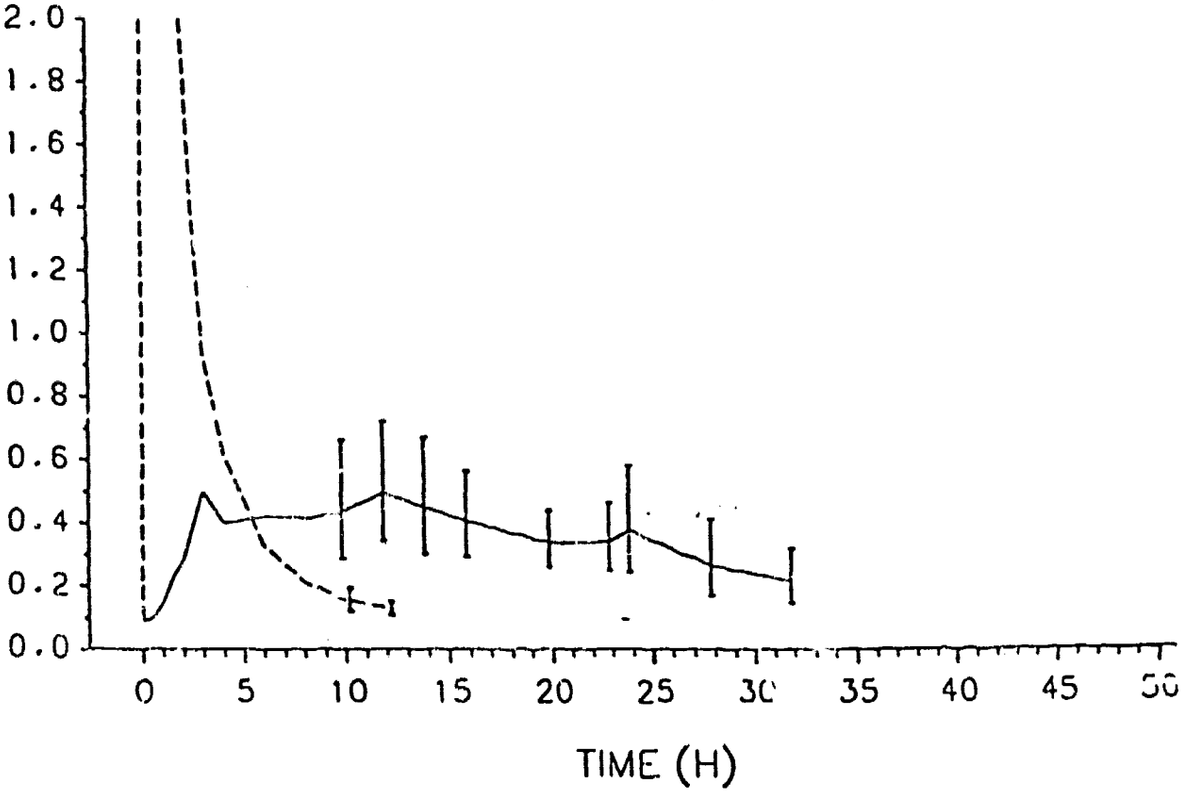
Figure 1 shows the mean plasma concentrations for nisoldipine after both IV and oral administration while Table 1 summarizes the most important pharmacokinetic parameters. From the results of the study, it can be seen that administration of the same dose IV as a controlled release tablet gave much higher plasma concentrations indicating that nisoldipine when given by the oral route of administration undergoes extensive first pass metabolism. The absolute bioavailability calculated based upon the ratio of the geometric mean AUC_{norm} PO/AUC_{norm}IV was only 5.5 % with a 95 % CI from 4.8 % to 6.4 %. The MRT of the IV infusion of 3.7 hours is prolonged to 20.6 hours for the C.C. formulation.

Fig. 1

/ STUDY NO. 637

NISOLDIPINE PLASMACONCENTRATION (NG/ML)





TREATMENT : ------- NISOLD. I.V. —— NISOLD. TABL.

06 04 7054

TABLE 1

/ STUDY 0637
TABLE 3.1: ESTIMATES OF PHARMACOKINETIC PARAMETERS
MEDIAN, MINIMUM, MAXIMUM BY DOSAGE

PARAMETER	NISOLD. I.V.	NISOLD. TABL.
AUC NORM (G*H/L)	1244.990 890.000 - 1480.000	67.414 36.800 - 119.000
CMAX (NG/ML)	8.355 4.560 - 9.520	0.745 0.450 - 1.030
MRT (H)	4.039 1.890 - 9.620	610 14.360 - 28.790
T 1/2 (H)	7.310 2.140 - 13.960	9.547 5 050 - 18.330

/ STUDY 0637
TABLE 3.1: ESTIMATES OF PHARMACOKINETIC PARAMETERS
GEOMETRIC MEAN, GEOMETRIC SD BY DOSAGE

PARAMETER	NISOLD. I.V.		NISOLD. TABL.	
AUC NORM (G*H/L)	1214.899	1.13	67.117	1.33
CMAX (NG/ML)	7.652	1.27	0.688	1.34
MRT (H)	4.191	1.56	20.635	1.24
T 1/2 (H)	6.930	1.70	9.906	1.60

EVIDENCE FOR DIFFERENCES IN GUT WALL METABOLISM OF NISOLDIPINE DEPENDING ON ABSORPTION SITE

Introduction

Nisoldipine, a calcium antagonist of the DHP type, is used in the treatment of hypertension and coronary heart disease. Even almost completly absorbed, NIS exhibits An low bioavailability (f) due to an extensive metabolisation during first pass. This first pass effect was considered to be mainly based on the high hepatic clearance, support by experiments performed in a perfused rat liver model (1).
Recently some evidence was found for relevant extra-hepatic contributions by gut wall metabolism to the first pass effect of DHPs (2, 3, 4).

In dog after intraportal administration the relative bioavailability of nisoldipine was 2–3 times higher as compared to intraduodenal administration. No indication was found on relevant differences in the formation of metabolites in the gut wall and the liver. The partial elimination process contribute with bioavailabilities of 42% (gut wall and/or intestinal lumen) and 29% (liver) to the resulting bioavailability of 12%. It has been concluded that from an oral dose of nisoldipine given to dogs, about 60% is metabolized prehepatically in the gut wall and only 30% in the liver.

Thus for the development of a controlled release formulation information on gut wall metabolism in man may be required.

Methods

Technique and Function of the High-Frequency Capsule (HF-Capsule)
For the convenient, stress-free application of substances at any location of the GI-tract a remote controlled capsule was developed 5. This capsule has no built-in electrical energy source. The energy needed for starting the drug release is supplied from an external high frequency transmitter. Due to the extract resonance tuning of the receiver integrated in the capsule, the trigger pulse for the opening mechanism can be kept at a very low energy level. The principle of the HF-capsule is illustrated in Fig. 1. The capsule consists of a smooth plastic case with a lenght of 28 mm and a diameter of 12 mm, which remains unchanged during the transportation through the GI-tract. In one half of the capsule the drug-device is localized. This part consists of a small latex balloon, which can be filled with the aid of normal hypodermic needles or injection syringes via an adapter. The maximum filling volume is 1 ml; solutions, suspensions and solids are suitable as fillings. In the other half of the capsule the release mechanism is located. The capsule is activated when a high frequency field is applied from outside the body and an oscillating circuit (coil and capacitor) within the capsule absorbs energy. This absorbed energy heats up a heating wire which melts a nylon thread holding a spring with a small needle under tension. Disengaging the spring, the water-resistant central part of the capsule is pierced, the balloon gets punctured and thus releases immediately the drug. The release mechanism is triggered by a commercial 27 MHz high frequency generator and a special designed ring antenna.

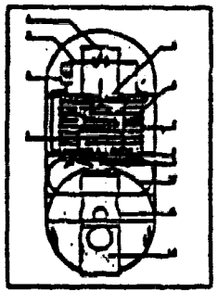


Fig. 1
Scheme of the HF-capsule
1 oscillating circuit
2 capacitor
3 heating wire
4 nylon thread
5 spring
6 plunger
7 cylinder liner
8 separation wall
9 small steel needle
10 latex container
11 holder for 10
12 plug

Study design
In a cross over study 4 healthy male volunteers (age to years; weight 70 – 92 kg) received 8.69 to 9.96 mg Nisoldipine in a HF capsule at four occasions and 10 mg solution at four occasions (A1, A2). The HF capsules solutions were released in stomach (B), jejunum (C), colon ascendens (D1) and colon descendens (D2).

Analytical Procedure and Calculation
Determination of Nisoldipine and the metabolites M 9 and M 10 has been performed by specific GC/ECD method.
Pharmacokinetic parameters of Nisoldipine, M 9 and M 10 were calculated by moment analysis. The individual means of A1/A2 were used as an intraindividual reference value (100%) in each run.
Non parametric tests were used to compare the geometric means in each pat of the study.

Results

Fig. 2
Localization of capsule at release and transmit times until release of nisoldipine at target segment (means, s.d.).

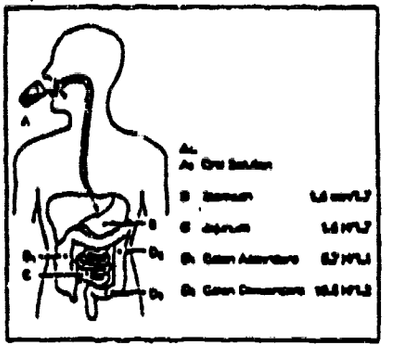
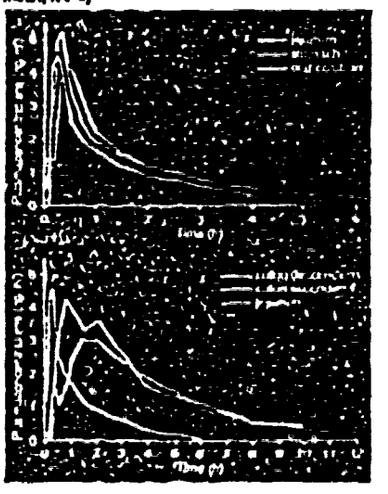


Fig. 3
Plasmaconcentration time profile of Nisoldipine after release in different segments of GI-tract (geometric mean; n = 4)



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G. Ahr[1], H. Stalb[2], J. Kuhlmann[1]

[1]Institute of Clinical Pharmacology, Bayer AG, Wuppertal,
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Frankfurt/M., Germany

Table 1
Pharmacokinetic data (geometric mean; s.d.) after capsule release at different segments of GI tract (B, C, D1) compared to solution (A)

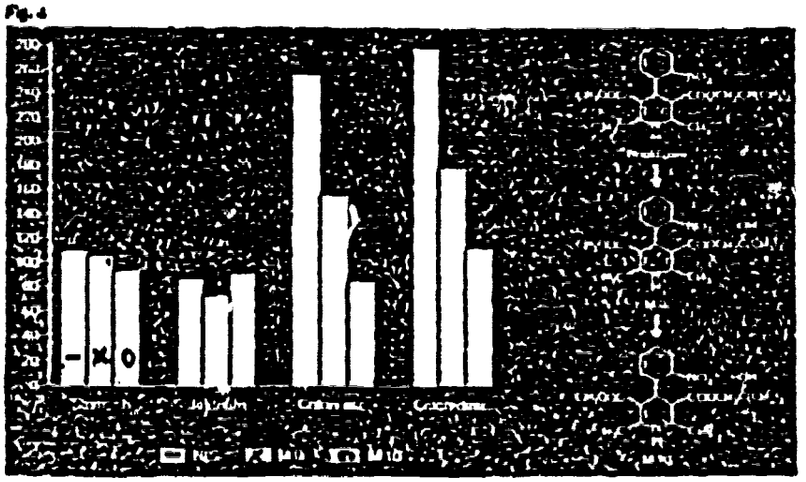
AUC norm [g*h/l]	A1 (s.d.)	A2 (s.d.)	B (s.d.)	C (s.d.)	D1 (s.d.)	D2 (s.d.)
NIS	66.7 1.55	69.4 1.50	61.6 1.57	46.8 1.60	143 1.76	155 2.46
M 9	53.1 1.15	56.4 1.23	54.7 1.29	49.8 1.35	126 1.47	146 1.79
M 10	773 1.46	87.4 [illegible]2	697 1.30	654 2.36	879 1.35	796 1.59

AUC norm [%]	A	B	C	D1	D2
NIS	100	111	84	257	279
M 9	100	107	75	158	1 [illegible]
M 10	100	90	83	94	113

Cmax norm [g/l]	A1 (s.d.)	A2 (s.d.)	B (s.d.)	C (s.d.)	D1 (s.d.)	D2 (s.d.)
NIS	41.9 1.79	35.3 2.32	39.0 2.42	41.5 2.61	36.9 1.87	43.0 2.32
M 9	39.4 1.33	37.8 1.99	44.3 1.16	37.7 1.74	34.2 1.34	38.2 1.64
M 10	483 1.34	541 1.45	436 1.22	405 2.23	784 1.96	788 1.96

t max [h]	A1 (s.d.)	A2 (s.d.)	B (s.d.)	C (s.d.)	D1 (s.d.)	D2 (s.d.)
NIS	0.34 1.79	0.49 1.49	0.37 1.36	0.19 1.74	1.42 2.87	1.84 1.96
M 9	0.46 1.96	0.49 1.39	0.36 1.65	0.39 1.88	2.87 1.56	1.84 1.96
M 10	0.35 1.36	0.35 1.42	0.37 1.36	0.21 1.80	2.81 176	1.49 1.59

Fig. 4



Discussion

In a study on the absorption behaviour of NIS in 4 healthy volunteers by using the high frequency capsule technique evidence was found that gut wall metabolism in man is de pendent on the absorption site. The relative bioavailability was unchanged with site of liberation, in extent and rate, in the upper gastrointestinal tract as oral solution (A1, A2) and as HF-capsule in stomach (B) and jejunum (C). The relative bioavailability increase after liberation in colon ascendens (D1) and descendens (D2) for NIS and a primary DHP metabolite (M 9), whereas a pyridine metabolite (M 10) remained almost unchanged.

The data on metabolites do not support the theory of a bypassing the liver as explanation of the increase in f. In case of bypassing the liver – administration comparable to IV dosing – a reduction in amount metabolized during first pass measured as a reduction of AUC as well as $C_{max,\ norm}$ for M 9 and M 10 has to be expected. The dependence of f on the absorption site for NIS and M 9 give indirect evidence that also in man gut wall metabolism contributes to the first pass effect in man.

This contribution seems to decrease from the proximal to the distal parts of the intestine and even may be abolished in the colon.

References

1. H.J. Ahr Pharmacol. 329, R 7 (1985)
2. S.X. Wang et al. Br. J. Pharmacol. Exp. Ther. 250, 832 (1989)
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5. A.H. Stalb et al. In; Novel Drug Delivery and its Therapeutic Application. Eds. Prescott, Nimmo. Wiley & Sons (1989)
6. A.H. Stalb et al. Arch. Pharmacol. 337, R 125 (1988)

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Nisoldipine Enantiomers: Assessment of Pharmacokinetics by stable isotope technique.

Study: 606/618 Volume: 61 Pages: 06-02-5788-5793 and

Investigators:

Objectives:

- to assess the tolerability and the pharmacokinetic properties of the racemate and the enantiomers of nisoldipine after IV and oral administration using a pseudo racemate containing a 4x13C-labelled (+)N and the non-labelled (-).

Formulation:

-1 mg nisoldipine pseudoracemate solution (20 ml as an IV infusion over a time period of 1 hour.
-20 mg nisoldipine pseudoracemate solution orally (2.5 ml as a 0.8 % solution diluted in 20 ml of water.

Study Design:

5 healthy male subjects between the ages of 25 and 35 years participated in either the IV or oral study. Three of them took part in both studies. Plasma samples were taken at defined times (not specified in the report) to measure the kinetics of nisoldipine and its enantiomers. Heart rate was recorded continuously for 90 minutes, blood pressure was measured automatically for 60 and 90 minutes every three minutes. Thereafter, heart rate and blood pressure were assessed 4, 8, 12 and 24 hours after drug application.

Assay:

Data Analysis:

Results:

Figure 1 shows the mean plasma concentrations for nisoldipine and its enantiomers for both the IV and PO administration. Figure 2 shows the plasma concentrations of (+) and (-) nisoldipine and its corresponding metabolites after single oral administration of the pseudoracemate. Table 1 shows the main pharmacokinetic parameters for nisoldipine and its enantiomers after both IV and PO administration while Table 2 the same PK parameters for nisoldipine and its major metabolites after PO administration only. As expected there was a drop in blood pressure and an increase in heart rate in all the subjects. The results also show that after IV administration similar plasma concentrations and pharmacokinetic parameters are obtained for both enantiomers. After oral administration, the CMAX values for the M9 metabolite (the hydroxylated dihydropyridine) were different by a factor of 7.6 whereas for the M3 metabolite (the pyridine metabolite) varied only by a factor of 0.84. These results might indicate that the pyridine formation might be less stereoselective than the side chain oxidation.
It can also be seen that the plasma concentrations as well as the AUC are consistently higher for the (+)N enantiomer. Therefore, the plasma concentrations of the non enantiospecific analysis mostly reflects the concentration of the active enantiomer. After IV administration, the concentration of the (+)N is about half the total concentration.

FIGURE 1

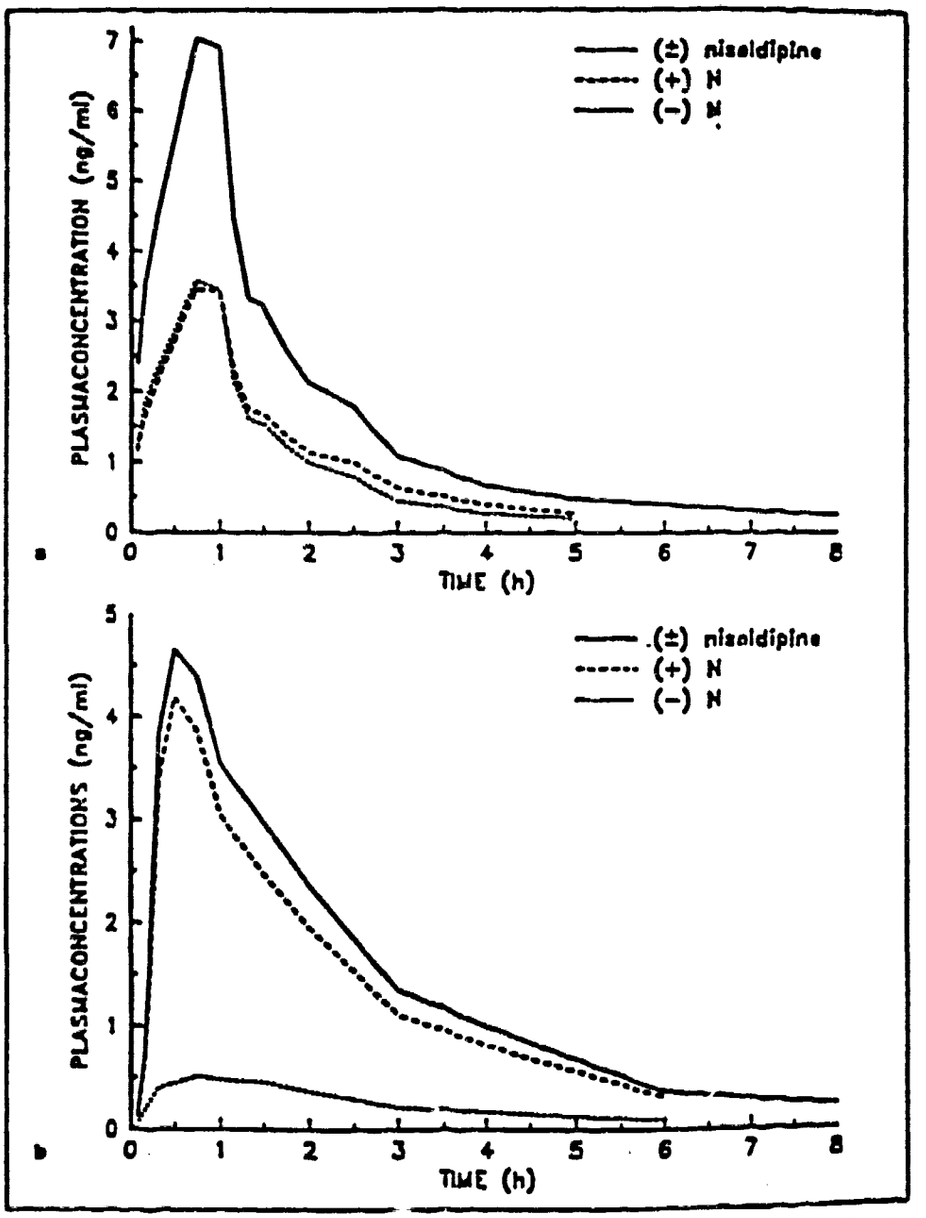

Figure 1. Plasma concentration of nisoldipine and its enantiomers after single application of stable isotope labelled pseudoracemate (mean of 4 volunteers). a) 1 h infusion of 1 mg nisoldipine; b) 20 mg oral solution of nisoldipine.

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FIGURE 2

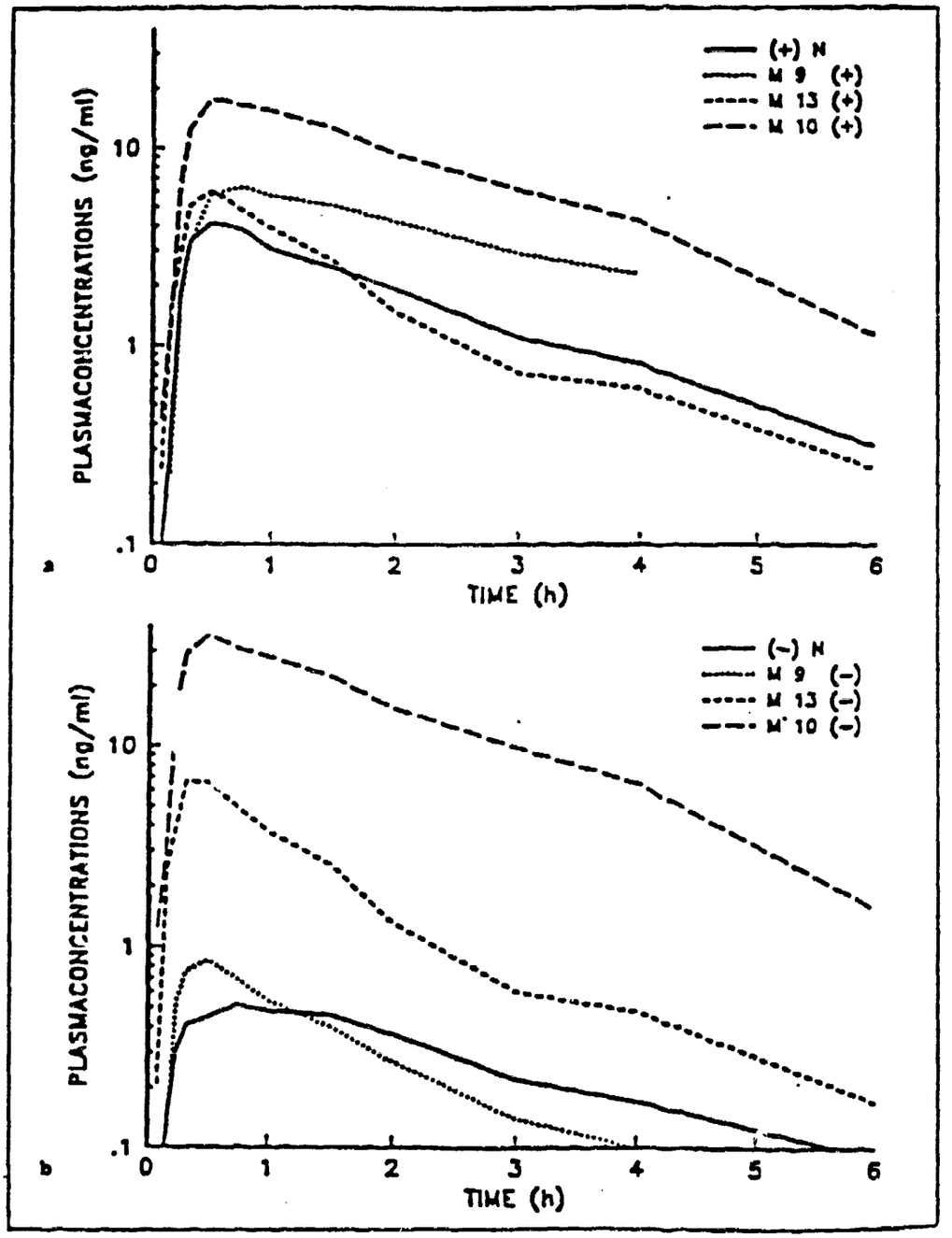

Figure 2. Plasma concentration of nisoldipine and M 9, M 10, M 13 after single oral application of stable isotope labelled pseudoracemate (mean of 4 volunteers). a) (+)N and its corresponding metabolites; b) (−)N and its corresponding metabolites.

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Table II. Pharmacokinetic parameters of (±)N, (+)N and (−)N after intravenous and oral dosing of (±)nisoldipine (geometric mean, geometric standard deviation).

	AUC_{∞} iv (kg·h/l) mean (sd)	AUC_{∞} oral (g·h/l) mean (sd)	f (%) mean	C_{max} iv (ng/ml) mean (sd)	C_{max} oral (ng/ml) mean (sd)	V_{ss} iv (l/kg) mean (sd)
(±)N	1.02 (1.21)	48.38 (1.27)	4.74	7.12 (1.26)	5.20 (1.10)	2.52 (1.20)
(+)N	1.08 (1.18)	81.16 (1.31)	7.51	3.55 (1.28)	4.67 (1.10)	2.07 (1.11)
(−)N	.88 (1.19)	12.97 (1.25)	1.47	3.59 (1.25)	.55 (1.11)	2.23 (1.15)

Table III. Pharmacokinetic parameters of (+)N and (−)N and their corresponding metabolites after oral dosing of (±) nisoldipine (geometric mean, geometric standard deviation).

	(+) enantiomer and its metabolites		(−) enantiomer and its metabolites	
	C_{max} (ng/ml) mean (sd)	t_{max} (h) mean (sd)	C_{max} (ng/ml) mean (sd)	t_{max} (h) mean (sd)
Parent drug	4.67 (1.10)	.55 (1.48)	.55 (1.11)	.57 (1.75)
M9	7.48 (1.59)	.87 (1.59)	.98 (1.21)	.38 (1.40)
M10	18.70 (1.20)	.66 (1.40)	38.00 (1.19)	.45 (1.48)
M13	6.79 (1.32)	.45 (1.48)	8.10 (1.42)	.41 (1.27)

PHARMACOKINETIC DOSE-PROPORTIONALITY STUDY OF NISOLDIPINE (BAY K 5552) 2, 5, 10, 20 MG TABLETS IN NORMAL SUBJECTS.

Study No. D85-024-01 Volume: 40 Pages: 06-00-4163 - 06-00-4355

INVESTIGATORS:

OBJECTIVE:

To determine the dose proportionality and single dose pharmacokinetics of nisoldipine over the range of 2.5 to 20 mg in healthy volunteers.

STUDY DESIGN

A non-randomized ascending-dose design, in which twenty healthy males sequentially received single doses of 2.5, 5, 10, and 20 mg of nisoldipine at weekly intervals. Blood samples were collected at 0.0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 5, 8, 11, 14, 24, and 48 h (only after 20 mg dose).

Dosage Forms

Nisoldipine tablets were administered in the following doses:

Tablet strength	Miles Formula #	batch#
2.5 mg	200-024	524525
5 mg	200-015	524652
10 mg	200-023	524652
20 mg	200-011	524652

Assay:

1 page
purged

DISCUSSION & CONCLUSION:

Nisoldipine and its active metabolite showed an increase of their AUC and Cmax values that were proportional to the increase of the oral doses.

Tmax, (which reflects rate of absorption) for nisoldipine and its active metabolite , did not change significantly with increasing dose. On the other hand, $t_{1/2}$ was not determined for the two lower doses and was highly variable for the other two doses. So determination of elimination kinetics from this study is not conclusive.

Comments

Sampling times were not the same for different doses (10, 20 mg) which may, in part, explain the differences observed in $t_{1/2}$ estimation.

The precision of the assay exceeded 20% CV at the LOQ, which is above the acceptable level.

NISOLDIPINE (F X 5552)
PHARMACOKINETIC DOSE-PROPORTI .TY STUDY OF NISOLDIPINE
2.5 MG, 5 MG, 10 MG and 20 MG TABLETS IN NORMAL SUBJECTS
STUDY D85-024-01 (LASSETER)

APPENDIX I
TABLE 2

Pharmacokinetic Parameters of Nisoldipine
Following Oral Doses of 2.5, 5, 10 and 20 mg
(Mean ± SD)

DOSE (mg)	Cmax (ng/ml)	Tmax (hr)	AUC (0-24) (ng-hr/ml)	AUC (0-inf) (ng-hr/ml)	Cl/f (L/hr)	$t_{1/2}$ (hr)
2.5	0.43 ± 0.25	1.27 ± 0.41	1.26 ± 1.06*	-	-	-
5	0.85 ± 0.39	1.46 ± 0.64	2.89 ± 1.38*	-	-	-
10	1.44 ± 0.83	1.43 ± 1.01	6.52 ± 2.98	6.69 ± 3.10	1887 ± 1030	9.3 ± 8.1
20	3.42 ± 1.94	1.62 ± 1.08	14.45 ± 6.11	18.25 ± 8.60	1377 ± 749	12.3 ± 5.3

*not extrapolated

APPENDIX I
TABLE 3

Pharmacokinetic Parameters of Nisoldipine Metabolite
Following Oral Doses of Nisoldipine
(Mean ± SD)

DOSE (mg)	Cmax (ng/ml)	Tmax (hr)	AUC (0-24) (ng-hr/ml)	AUC (0-inf) (ng-hr/nl)	$t_{1/2}$ (hr)
2.5	1.09 ± 0.41	1.21 ± 0.30	-	-	-
5	1.19 ± 0.51	1.72 ± 0.69	5.35 ± 2.43	-	-
10	2.01 ± 1.04	1.90 ± 1.00	10.77 ± 5.45	12.18 ± 6.48	6.8 ± 7.5
20	4.09 ± 1.84	1.79 ± 1.05	21.31 ± 9.08	27.79 ± 14.48	13.2 ± 11.3

NISOLDIPINE
PHARMACOKINETIC DOSE-PROPORTIONALITY STUDY OF NISOLDIPINE
2.5 MG, 5 MG, 10 MG and 20 MG TABLETS IN NORMAL SUBJECTS
STUDY D85-024-01 (LASSETER)

APPENDIX I
TABLE 4

Pharmacokinetic Parameters of Nisoldipine
Metabolites Following Oral
Doses of Nisoldipine
(Mean ± SD)

METABOLITE

DOSE (mg)	C_{max} (ng/ml)	T_{max} (hrs)	AUC (0-24) (ng-hr/ml)	$t_{1/2}$ (hrs)
2.5	2.64 ± 1.46	0.90 ± 0.33	4.10 ± 1.97	-
5	4.25 ± 2.36	1.31 ± 0.72	8.73 ± 4.16	-
10	6.55 ± 4.91	1.21 ± 0.73	15.10 ± 6.19	12.1*
20	12.16 ± 7.90	1.12 ± 0.64	33.32 ± 16.10	16.8**

* Calculated from mean 8-24 hour data
** Calculated from mean 11-24 hour data

METABOLITE

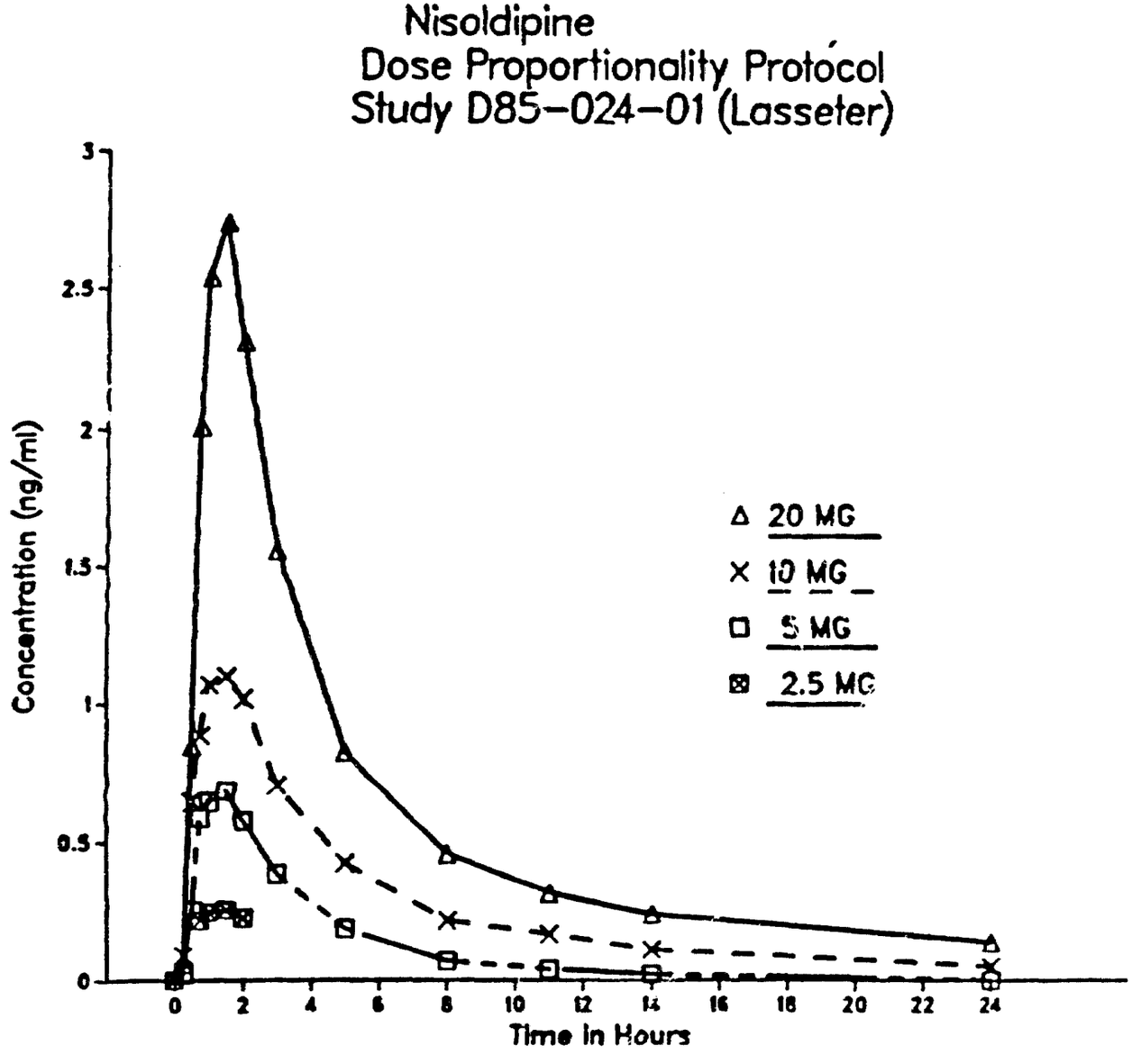
DOSE (mg)	C_{max} (ng/ml)	T_{max} (hrs)	AUC (0-24) (ng-hr/ml)	$t_{1/2}$ (hrs)
2.5	10.71 ± 4.99	1.15 ± 0.56	23.21 ± 10.86	
5	28.20 ± 12.90	1.45 ± 0.70	81.00 ± 38.39	
10	34.51 ± 17.47	1.31 ± 0.69	106.6 ± 47.74	9.2*
20	75.21 ± 30.99	1.54 ± 1.11	240.7 ± 100.00	12.1**

* Calculated from mean 11-24 hour data
** Calculated from mean 11-48 hour data

APPENDIX I

FIGURE 1

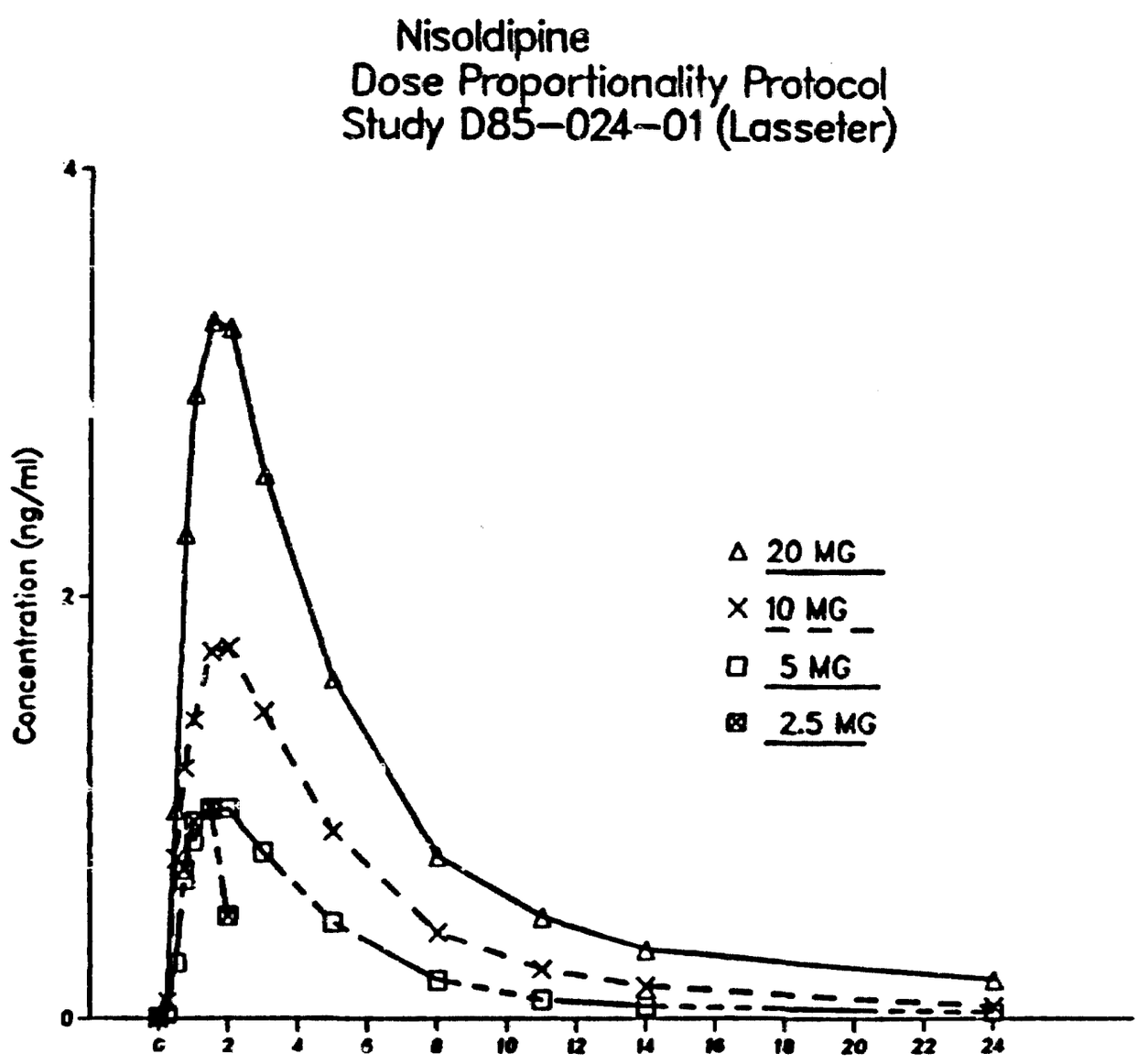
MEAN PLASMA CONCENTRATIONS OF NISOLDIPINE



APPENDIX I

FIGURE 2

MEAN PLASMA CONCENTRATIONS OF METABOLITE



A RANDOMIZED, SINGLE DOSE, 4-WAY CROSSOVER STUDY OF THE DOSE PROPORTIONALITY AND TOLERABILITY OF 10 MG, 20 MG, 40 MG, AND 60 MG NISOLDIPINE COAT-CORE TABLETS IN HEALTHY VOLUNTEERS.

Study No. D91-035 Volume: 39 Pages: 06-00-3614 - 06-00-4000

INVESTIGATORS:

OBJECTIVE:

To determine dose proportionality and single dose pharmacokinetics of 10, 20, 40, and 60 mg of nisoldipine coat-core tablets in healthy volunteers.

BACKGROUND:

A new formulation of nisoldipine that consists of a core of immediate release nisoldipine, surrounded by an outer coat of slowly-released nisoldipine. The firm claims, this formulation produces sustained plasma concentrations for at least 24 hours after dosing. The present study used 60 mg of this formulation (3X20 mg) as the highest dose.

STUDY DESIGN

A randomized, open label, (not blinded) four-way crossover, single center evaluation of 4 doses of nisoldpine. Twenty-four healthy male volunteers were randomly assigned to one of four sequence groups. Four single oral doses of 10, 20, 40, and 60 mg nisoldpine were given (fasting condition), with a washout period of 7 days. Blood samples were collected at 0.0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 24, 28, 32, 36, and 48 h post-dose.

Test drug

Nisoldipine coat-core tablets were administered in the following doses:

Dose	sequence	batch#
1 x 10 mg	A	524525
1 x 20 mg	B	524652
2 x 20 mg	C	524652
3 x 20 mg	D	524652

DISCUSSION AND CONCLUSION:

Dose proportionality was observed for 20, 40, and 60 mg doses. For the 10 mg doses, there was a departure from dose proportionality for AUC and Cmax. That departure was more pronounced for the Cmax values where the lower confidence limits for the 10 mg doses were below 80% ($p<0.05$). For AUC, at the 10 mg dose proportionality, the magnitude of departure was close to the acceptance levels.

there is no apparent nonlinearity in the PK parameters studied and the study, though flawed by sampling design, is acceptable.

Comments

Sampling period was not long enough to allow $t_{1/2}$ estimation.

Arithmetic Mean (% CV) of Pharmacokinetic Parameters for,
Nisoldipine Following a Single Dose of
10, 20, 40 or 60 mg Coat-core Tablet

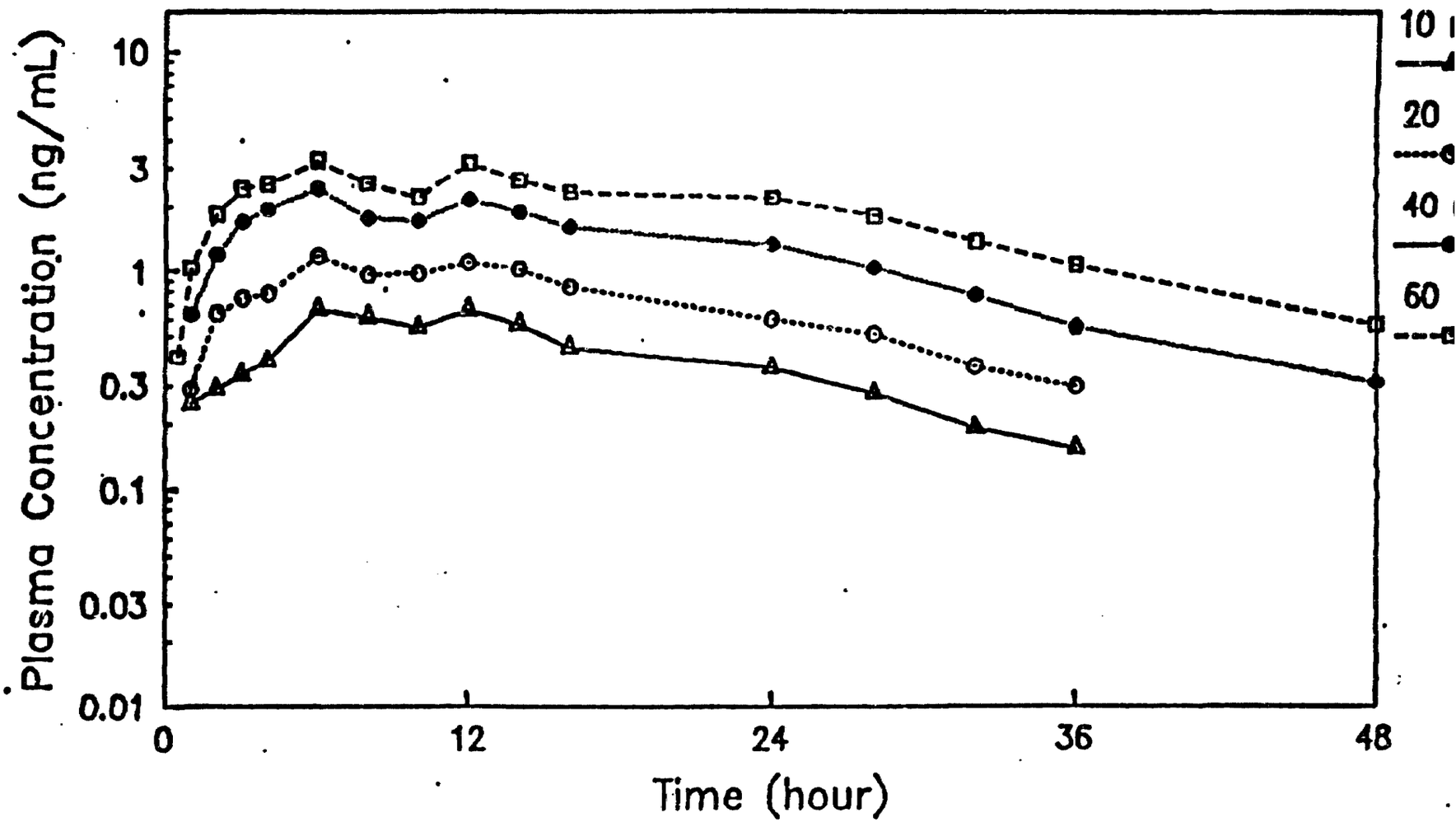
Dose (mg)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{0-48} (ng · hr/ml)	Dose-normalized C_{max}	Dose-normalized AUC_{0-48}
10	0.90 (43)	8.6 (45)	15.2 (33)	0.090	1.52
20	1.45 (39)	8.3 (44)	27.1 (37)	0.073	1.36
40	3.07 (49)	6.8 (45)	54.3 (47)	0.077	1.36
60	4.28 (52)	10.8 (62)	83.3 (43)	0.071	1.39

Ratios of Geometric Least Squares Means with 90% Confidence Intervals
and P-values from the Two-sided Test of Equality Between Doses

Variable	Comparison	Ratio (%)	90% Confidence Interval	p value
AUC_{0-48h}	20 mg/10 mg	88.4	80.0 - 97.8	0.046
	40 mg/10 mg	85.3	78.1 - 95.5	0.018
	60 mg/10 mg	88.6	80.1 - 98.0	0.048
	40 mg/20 mg	97.6	88.3 - 108.0	0.690
	60 mg/20 mg	100.1	90.5 - 110.7	0.983
	60 mg/40 mg	102.6	92.8 - 113.4	0.675
C_{max}	20 mg/10 mg	82.4	72.1 - 94.3	0.019
	40 mg/10 mg	85.0	74.3 - 97.2	0.047
	60 mg/10 mg	78.4	68.5 - 89.6	0.003
	40 mg/20 mg	103.1	90.2 - 117.9	0.706
	60 mg/20 mg	95.0	83.1 - 108.7	0.528
	60 mg/40 mg	92.2	80.6 - 105.4	0.314

Miles Inc.
Pharmaceutical Division
400 Morgan Lane
West Haven, CT 06516

Plasma Nisoldipine Concentrations Following Single Doses of Coat-Core Tablets in Healthy Subjects



Mean Plot

Study D91-035-

INVESTIGATION OF THE PHARMACOKINETICS AND TOLERABILITY OF A CONTROLLED RELEASE FORMULATION OF NISOLDIPINE UNDER STEADY-STATE CONDITIONS IN COMPARISON WITH THE IMMEDIATE RELEASE TABLET.

Study No. 645 Volume: 41 Pages: 06-00-4356- 06-00-4801

INVESTIGATORS:

OBJECTIVE:

a- Investigation of tolerability and pharmacokinetics of nisoldipine CC under steady-state (SS) conditions compared to the immediate release tablet.

b- Pharmacodynamic evaluation of cardiac performance.

MATERIAL AND METHODS:

I- Drug and dosage:
A: Nisoldipine CC, controlled release tablets (20 mg)
Pt. No. 523 081; Dev. No. 029
B: Nisoldipine immediate release tablets (10 mg)
Pt. No. 315 552; Dev. No. 126

II- Study Design:
18 healthy, male volunteers participated in the study. A randomized nonblind cross-over study of nisoldipine 20 mg controlled release (CR) compared to 10 mg immediate release (IR). The CR formulation was administered once daily while, the immediate release was administered twice daily. Both treatments were administered for 7 days and were separated by a wash-out phase of at least 7 days. Tablets were given after breakfast and 12 h later.
Blood sampling was done on day 1 and 7 before and 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 12.5*, 13*, 14, 16, 20, 23, and 24 h after morning administration. (* only for IR tablets)
Days 3-6: morning trough concentrations (before administration).
Days 8-9: 28, 32, 48, 52 and 56 h after the last administration.

RESULTS:

I- Pharmacokinetics:
For IR formulation, the mean CMAX (1.17 ng/ml) was reached 3 hours after dosing. Trough concentrations were mostly not estimated either on the first day or at SS as they were below detection limit.

For CR formulation, the mean CMAX (0.72 ng/ml) was reached after 12 hours after dosing. At SS, plasma concentrations higher than 0.1 ng/ml were maintained during the whole day. Trough concentrations were almost constant from day 3- day 7.

The plasma concentration time profile for the active metabolite (BAY r 9425) behave parallel to nisoldpine.
PK evaluation:
Comparing IR to CR formulation, the AUC_{norm} has increased from 40.3 to 58.9 g.h/l. The relative bioavailability increased to 146% with a 90% CI ranging from 126 to 169. CMAX was reduced by 56% going from IR to CR formulation with a 90% CI ranging from 47 to 66%.

Comparing day 1 to day 7, the bioavailability parameters (AUC, CMAX) for the IR formulation were numerically indistinguishable. On the other hand, AUC_{norm} CMAX for the CR formulation increased by 46% and 30% respectively.

Tolerability: at SS, both formulations were comparable but less side effects were reported after the CR dosing.

COMMENTS:
The assay method was not sufficiently senstive and precise to accurately determine trough concentrations and accumulation for both formulations.

DISCUSSION & CONCLUSIONS:

The plasma concentration time profiles of the IR and CR formulations were different; CR formulation caused a decrease in CMAX and an increase in CMIN so decreasing the degree of fluctuation.

Accumulation during the seven-day treatment was more pronounced for the CR formulation.

14SEP92

LPT 7
TABLE 5.3 ESTIMATES OF PHARMACOKINETIC PARAMETERS
QUOTIENTS TO RESULT OF 2*10 MG STD.TAB IN %
GEOMETRIC MEAN, GEOMETRIC SD, 90%-CONF. INTERV. ACCORDING TO MODEL
REFERRING TO ANALYSIS OF VARIANCE AND DUNNETT-TESTS.

PARAMETER	N SD(MODEL)		QUOTIENT (%) 1*20 MG NIS.CC /2*10 MG STD.TAB.	
AUC NORM (H*MG/ML)	18 1.42	MEAN, SD 90% C.I.	146.3 126.6 -	1.41 169.0
CMAX (NG/ML)	18 1.51	MEAN, SD 90% C.I.	55.6 47.0 -	1.49 65.9
FLUCTUATION	18 1.81	MEAN, SD 90% C.I.	26.0 20.4 -	1.79 33.3
DURATION C>=0.3 (H)	18 1.74	MEAN, SD 90% C.I.	244.7 194.5 -	1.75 307.8

study no. 645

table 5.4: comparison day 7/day 1

variable	treatment	day 1 x_{geom}	day 1 sd_{geom}	day 7 $\bar{x}_{geom}$	day 7 sd_{geom}	ratio (%) day 7/day 1 $\bar{x}_{geom}$	ratio (%) day 7/day 1 sd_{geom}	ratio (%) day 7/day 1 90%-confidence interval
AUC_{norm}	standard	40.84	1.55	40.26	1.51	98.6%	1.40	81.1, 115.6
	CC	40.34	1.65	58.88	1.47	146.0%	1.61	120.0, 177.5
C_{max}	standard	2.18	1.75	1.95	1.54	89.5%	1.31	70.0, 114.0
	CC	0.84	1.81	1.09	1.47	128.8%	1.64	105.1, 157,7

49

120

06 00 4476

Figure 1 mean plasma concentration of

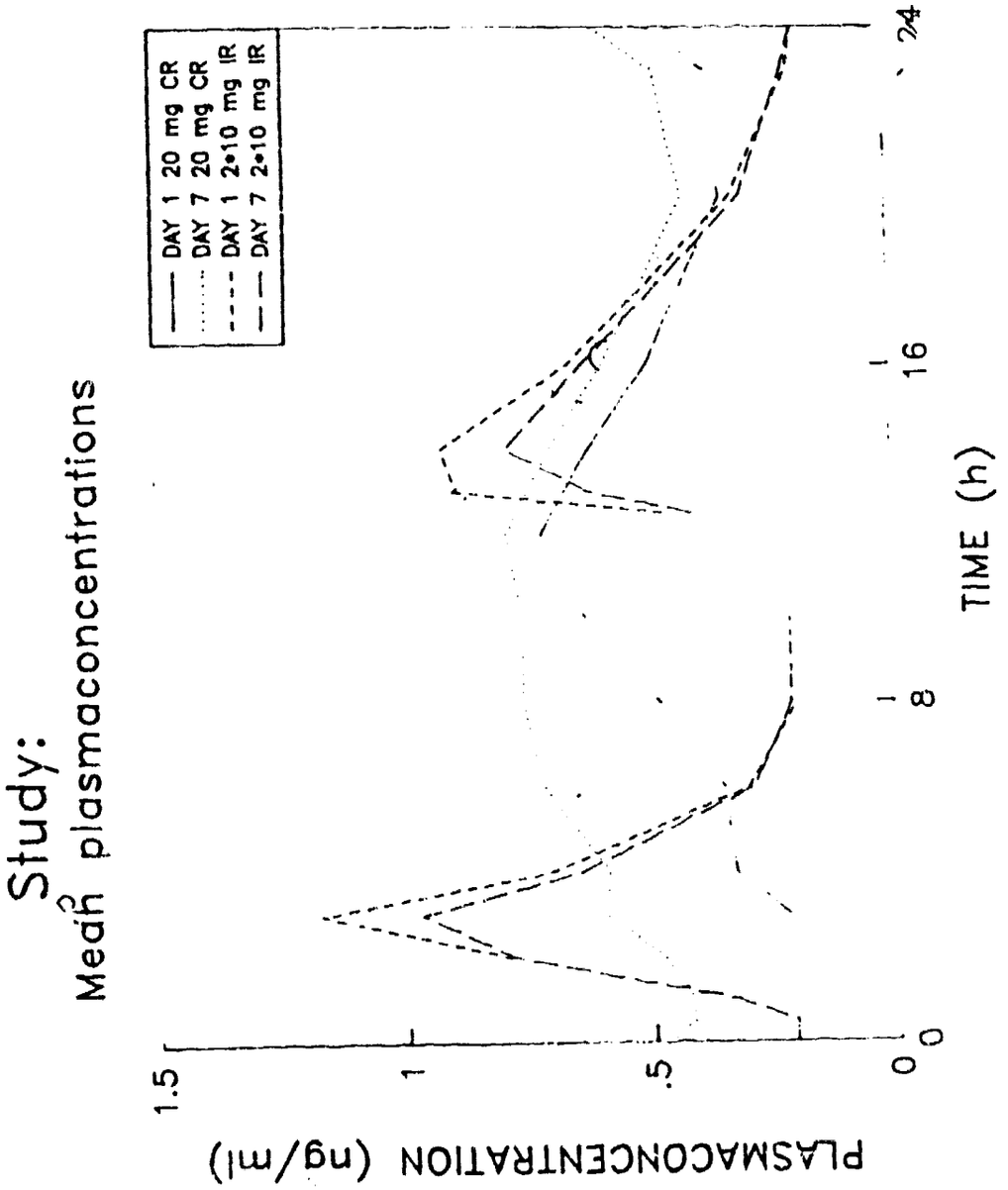


Figure 24

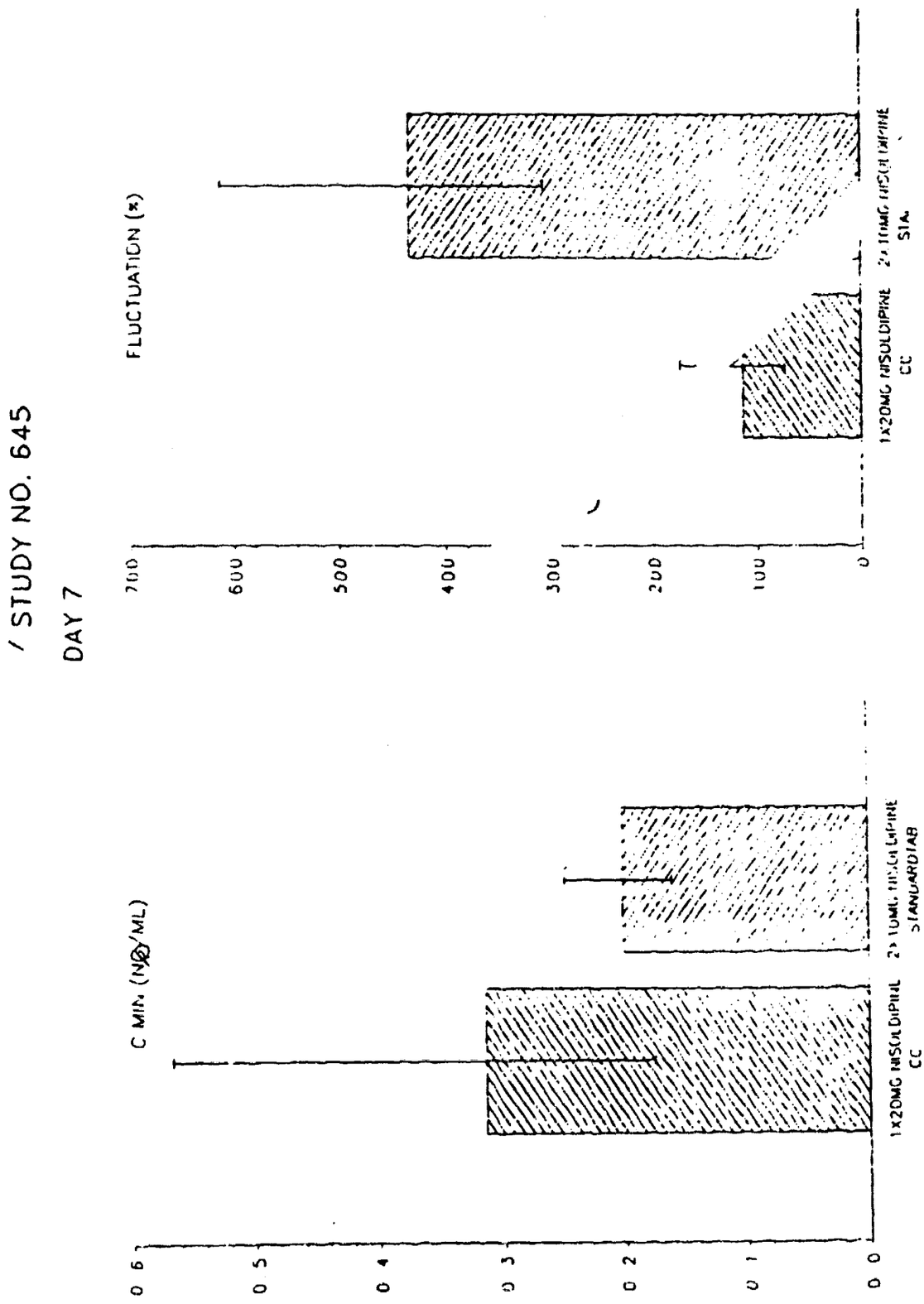

STUDY NO. 645
DAY 7
FLUCTUATION (%)
C MIN. (NG/ML)
1X20MG NISOLDIPINE CC
1X20MG NISOLDIPINE STANDARD TAB

Figure 22

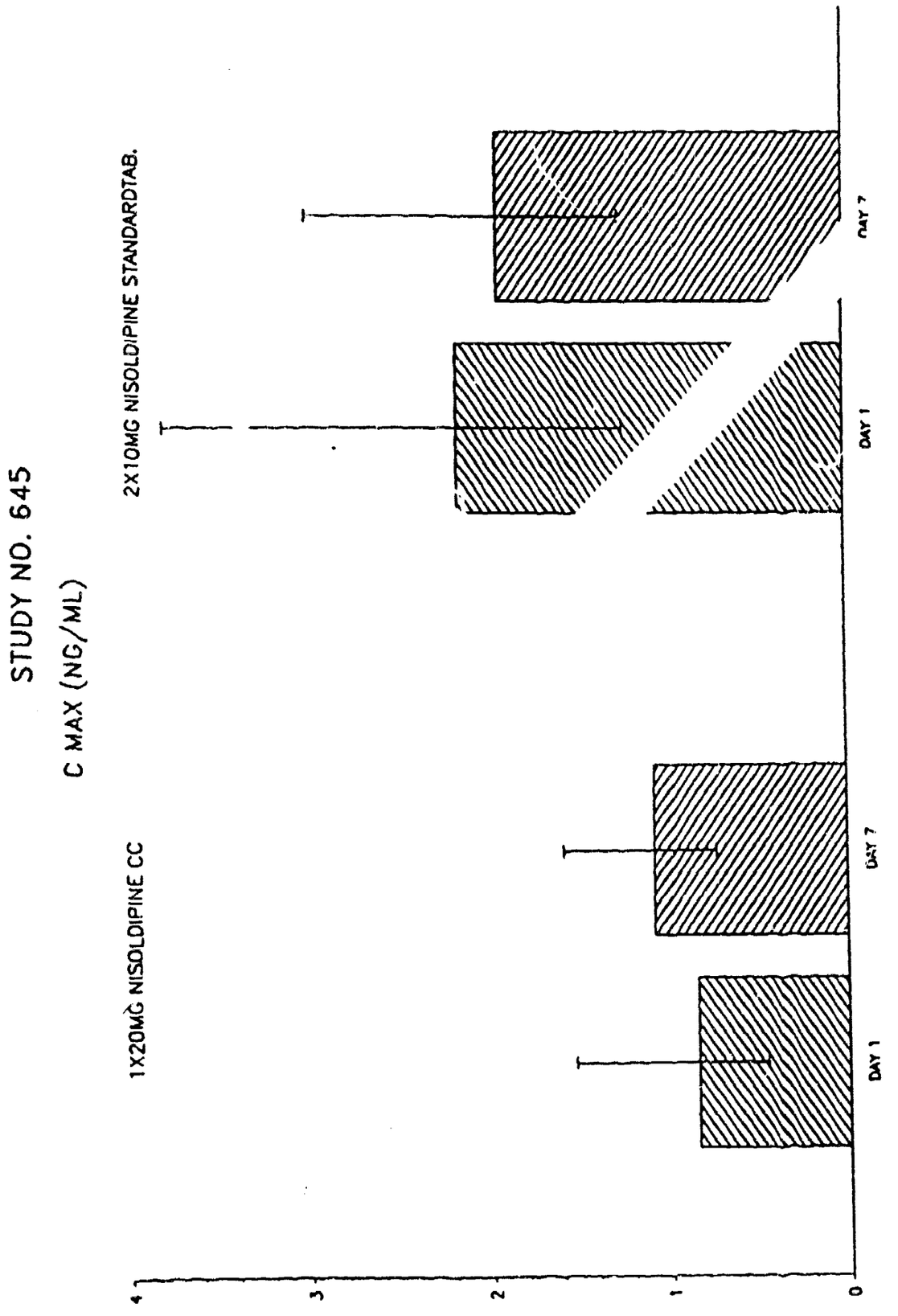
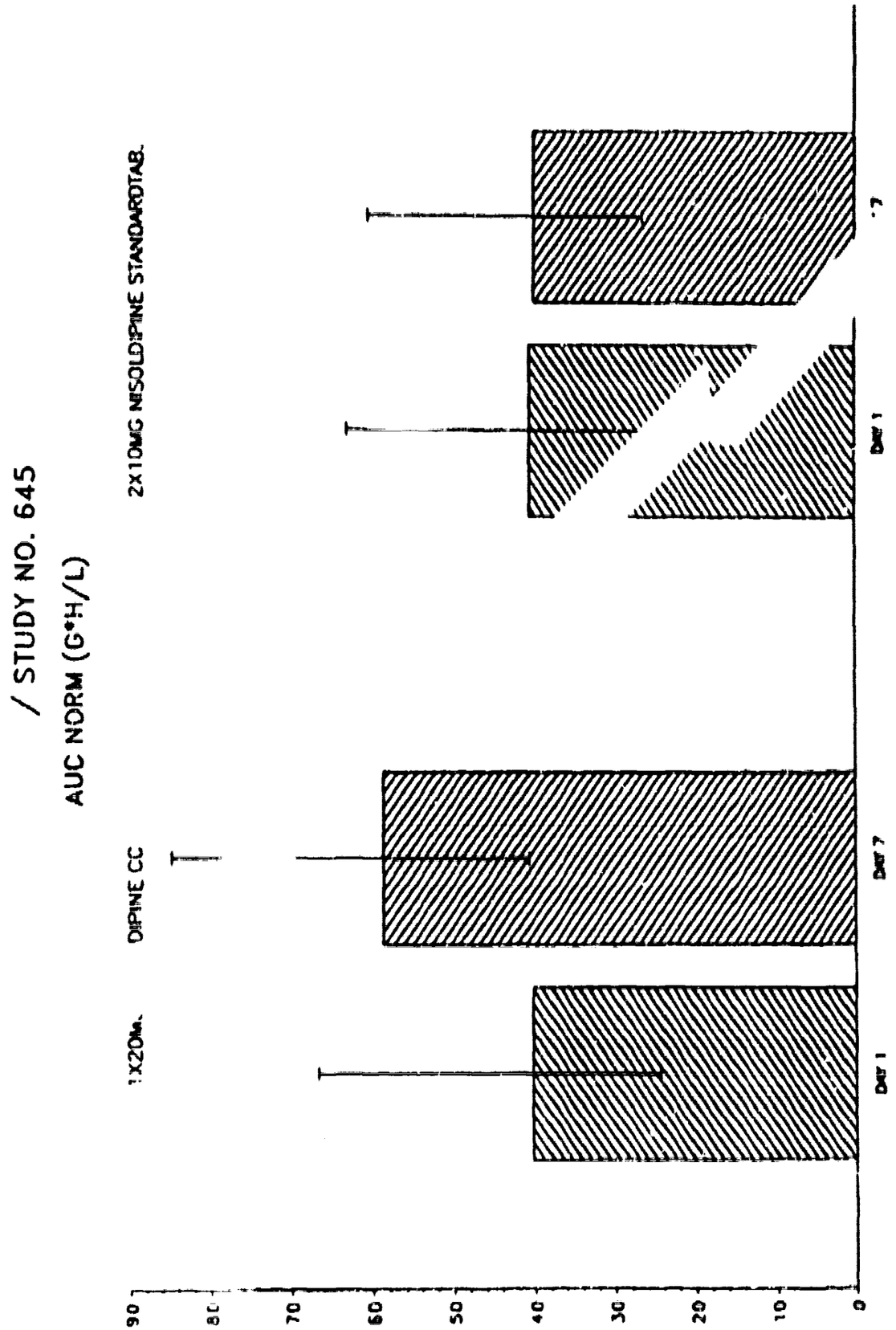

STUDY NO. 645
C MAX (NG/ML)
2X10MG NISOLDIPINE STANDARDTAB.
1X20MG NISOLDIPINE CC
DAY 7
DAY 1
DAY 7
DAY 1
4
3
2
1
0

Figure 20


/ STUDY NO. 645
AUC NORM (G*H/L)
2X10MG NISOLDIPINE STANDARDTAB.
DIPINE CC
1X20M.
DAY 1
DAY 7
DAY 1
DAY 7
90
80
70
60
50
40
30
20
10
0

Comparison of pharmacokinetics and tolerability of 2x20 and 1x40 mg nisoldipine controlled release formulation.

Study #: 5678 Volume: 1-31-32 Pages: 614-902.

Investigator:

Objectives:

The aim of the study is to investigate:
1-the pharmacokinetics and the tolerability of 2x20 and 1x40 mg nisoldipine C.C controlled release formulation.
2-to investigate the bioequivalence of 2x20 and 1x40 mg nisoldipine C.C. controlled release formulation.

Formulation:

-Nisoldipine 20 mg coat-core tablets batch # 524526 manufactured on june 1 1989, expiration date may 5, 1991.
-Nisoldipine 40 mg C.C. tablets batch # 526002 manufactured on january 31, 1990, expiration date september 30 1991.

Study Design:

24 fasting male healthy volunteers between the ages of 18 and 40 years participated in this three factorial cross over design with repeated measurements on both levels of factors period and treatment and randomized allocation of subjects to the third factor sequence of administration with 12 subjects per sequence study. The washout period was 1 week between treatments.
Each subject received each of the 2 treatments as a single dose of 40 mg nisoldipine (1x40 mg and 2x20 mg). Each subjects remained fasting until 2 hours post dose administration.
6 ml blood samples were collected according to the following schedule: 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 14, 16, 20, 24, 32, 36 and 48 hours after administration.
Faeces were sampled before administration and up to 72 hours post administration.

Assay:

Results:

Figure 1 shows the geometric means for the plasma concentration for both the 1x40 and 2x20 mg treatments. Table 1 and table 2 give a summary of the most important pharmacokinetic parameters for both treatments respectively. Table 3 gives the cumulative amounts of nisoldipine eliminated in the faeces following administration of the 2 different formulations. However, due to loss of some other fecal samples, this data was deemed irrelevant to the outcome of the study and was not evaluated any further.

Conclusion:

From the results of this study as shown in Table 4, the 40 mg nisoldipine tablet was considered bioequivalent to 2x20 mg tablets as far as AUC was concerned (90 % CI was 85.26 to 112.24 %) but it failed the 90 % confidence interval for CMAX (90 % CI was between 94.62 and 140.03 %).

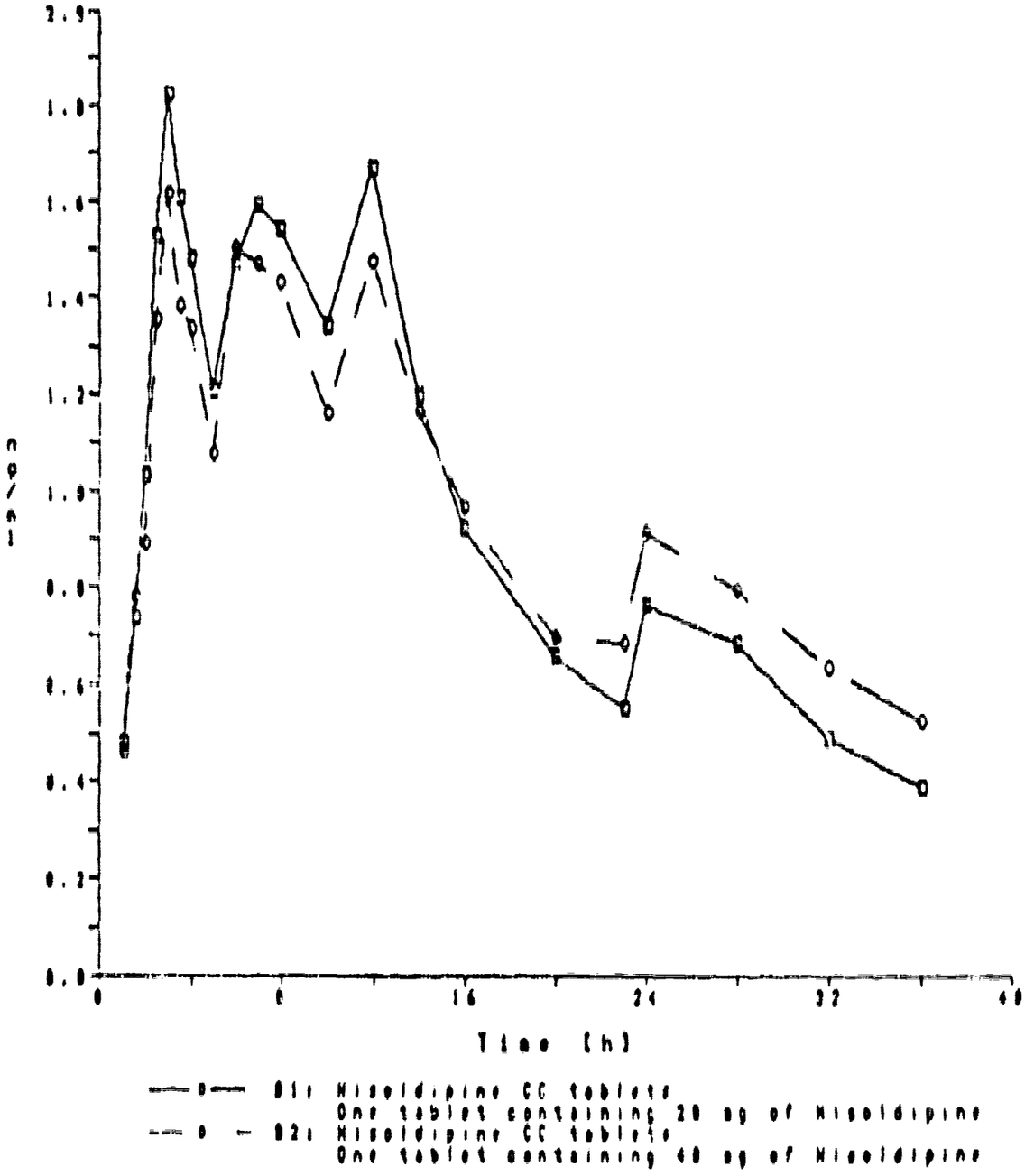


Figure 1

Synoptic plot of geometric mean concentrations of Nisoldipine [ng/ml] vs time [h].

Geometric mean not calculated if more than 1/3 single concentrations are <0.2 or no sample. Concentration <0.2 calculated as 0.1.

NONCOMPARTMENTAL PHARMACOKINETIC PARAMETERS OF NISOLDIPINE

Subj. No.	t 1/2 (h)	Cmax (ng/ml)	tmax (h)	AUC(0-ta) (ng*h/ml)	AUC(0-∞) (ng*h/ml)	MRT (h)	Cmax,norm (g/l)	AUC(0-ta,norm) (g*h/l)	AUC(0-∞,norm) (g*h/l)
MEAN	12.61	3.58	8.57	41.49	51.44	21.48	6.73	82.65	91.96
SDEV	5.85	2.73	8.36	16.18	18.41	8.06	4.94	33.28	38.22
GEO MEAN	11.50	2.84	6.16	39.27	47.47	22.30	5.35	74.77	89.25
GEO SDEV	1.56	2.02	2.19	1.41	1.36	1.38	2.04	1.45	1.42
LOW CON	9.64	2.15	4.50	32.89	39.69	19.58	4.02	61.21	73.67
UPP CON	13.73	3.77	8.43	48.38	56.77	25.39	7.10	91.34	108.21
MEDIAN	11.18	3.02	6.50	44.76	52.63	21.66	5.56	85.40	100.27
MIN	4.51	0.57	2.50	10.57	12.25	12.08	0.94	16.59	20.18
MAX	28.28	13.18	36.00	69.20	83.29	41.29	22.18	152.41	183.46
COUNT	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00

Table 1

Noncompartmental pharmacokinetic parameters of Nisoldipine following p.o. adm. of 2 x 20 mg of Nisoldipine CC tablets, one tablet containing 20 mg of Nisoldipine (treatment B1)

Single Values, Arithmetic Means, Standard Deviations, Geometric Means, Geometric Standard Deviations, Upper and Lower 95% Confidence Limits, Medians, Minimum, Maximum and Number of Cases.

NONCOMPARTMENTAL PHARMACOKINETIC PARAMETERS OF
NISOLDIPINE

Subj. No	t 1/2 [h]	Cmax [ng/ml]	tmax [h]	AUC(0-tz) [ng*h/ml]	AUC(0-∞) [ng*h/ml]	MRT [h]	Cmax,norm [g/l]	AUC(0-tz,norm) [g*h/l]	AUC(0-∞,norm) [g*h/l]
MEAN	12.10	[illegible]	8.36	45.42	52.92	24.81	5.91	85.27	99.77
SDEV	6.03	[illegible]	[illegible]	18.51	20.91	7.08	5.58	35.04	40.12
GEO MEAN	10.56	[illegible]	6.26	41.22	48.55	23.53	4.64	77.46	91.24
GEO SDEV	1.77	[illegible]	2.24	1.51	1.56	1.36	1.89	1.61	1.57
LOW CON	8.36	[illegible]	4.53	34.28	40.68	21.21	3.60	64.22	76.21
UPP CON	13.33	[illegible]	8.83	49.85	57.94	26.33	5.99	91.52	109.22
MEDIAN	11.42	[illegible]	6.52	44.96	48.11	23.41	4.57	87.23	93.53
MIN	3.34	[illegible]	1.55	15.25	21.44	10.86	1.64	27.29	41.70
MAX	26.65	[illegible]	32.02	76.39	85.26	38.02	27.10	145.46	159.32
COUNT	24.00	[illegible]	24.00	24.00	24.00	24.00	24.00	24.00	24.00

Table 9

Noncompartmental pharmacokinetic parameters of Nisoldipine following p.o. adm. of
1 x 40 mg of Nisoldipine CC tablets, one tablet containing 40 mg of Nisoldipine (treatment B2).

Single Values, Arithmetic Means, Standard Deviations, Geometric Means, Geometric Standard Deviations, Upper and Lower 95% Confidence Limits, Medians, Minimum, Maximum and Number of Cases.

Subj. No.	Ae B1 [ug]	Ae B1 [%]	Ae B2 [ug]	Ae B2 [%]
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
MEAN	3437.79	8.594	.	.
SDEV	3962.24	9.906	.	.
CV [%]	1.15	1.153	.	.
MEDIAN	1203.74	3.009	.	.
MIN	64.21	0.161	.	.
MAX	11002.43	27.506	.	.
COUNT	7.00	7.000	2.00	2.000

B1: p.o. adm. of 2 x 20 mg Nisoldipine CC tablets
One tablet containing 20 mg of Nisoldipine.
B2: p.o. adm. of 1 x 40 mg Nisoldipine CC tablets
One tablet containing 40 mg of Nisoldipine.

Table 3

Feces: Excreted amount of Nisoldipine [ug] and percentage of dose.

Single Values, Arithmetic Means, Standard Deviations, Coefficients of Variation, Medians, Minimum, Maximum and Number of Cases.

TABLE 4

90% CONFIDENCE LIMITS AND TEST OF BIOEQUIVALENCE

Parameter	MS Error (ANOVA)	geo.mean 100*B1/B2	lower limit	upper limit	bioeq. decision
Cmax,norm	0.156329	115.109332	94.623699	140.030019	false
AUC(0-tn,norm)	0.081927	96.526425	83.759172	111.239766	true
AUC(0-∞,norm)	0.076920	97.826364	85.261817	112.242477	true

Acceptable range of bioequivalence: 80 to 125

Student's t for a=0.05 (one tailed) and DF=22 : 1.717
24 subjects

B1 : Nisoldipine, following p.o. adm. of 2 x 20 mg
Nisoldipine CC tablets
One tablet containing 20 mg of Nisoldipine
B2 : Nisoldipine, following p.o. adm. of 1 x 40 mg
Nisoldipine CC tablets
One tablet containing 40 mg of Nisoldipine

Table 4

A controlled, double blind, two way single dose crossover study of the bioequivalence and tolerability of 3x20 mg vs 2x30 mg nisoldipine C.C. tablets in healthy volunteers.

STUDY #: D90-020-01 VOLUME: 1 32-1-33 PAGES: 902-1838.

INVESTIGATOR:

OBJECTIVES:

To determine the bioequivalence and single dose pharmacokinetics of 3x20 vs 2x30 mg nisoldipine C.C. tablets and to examine the safety and tolerability of a 60 mg dose of nisoldipine C.C.

FORMULATIONS:

20 mg nisoldipine C.C tablets batch # 523272.
30 mg nisoldipine C.C tablets batch # 523274.
Placebo tablets batch # 523167.

STUDY DESIGN:

30 healthy male volunteers between the ages of 18 and 40 years weighing between 140 and 220 lbs participated in this randomized, placebo controlled double blind crossover study. 24 subjects were assigned to the active treatment group while 6 subjects were assigned to the placebo group. The nisoldipine group was to be given, in a random order, either 3x20 mg or 2x30 mg dose of the C.C. at period 1, then crossed to the alternate dose at period 2. The placebo group was to be given nisoldipine placebo tablets at both periods. There was a one week washout period between treatments. 5 mls blood samples were collected predose and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 16, 23, 24, 28, 32, 36, 48 and 72 hours post-dose.
A pre-dose and up to 72 hours post dose urine and feces collections were to be performed.

RESULTS:

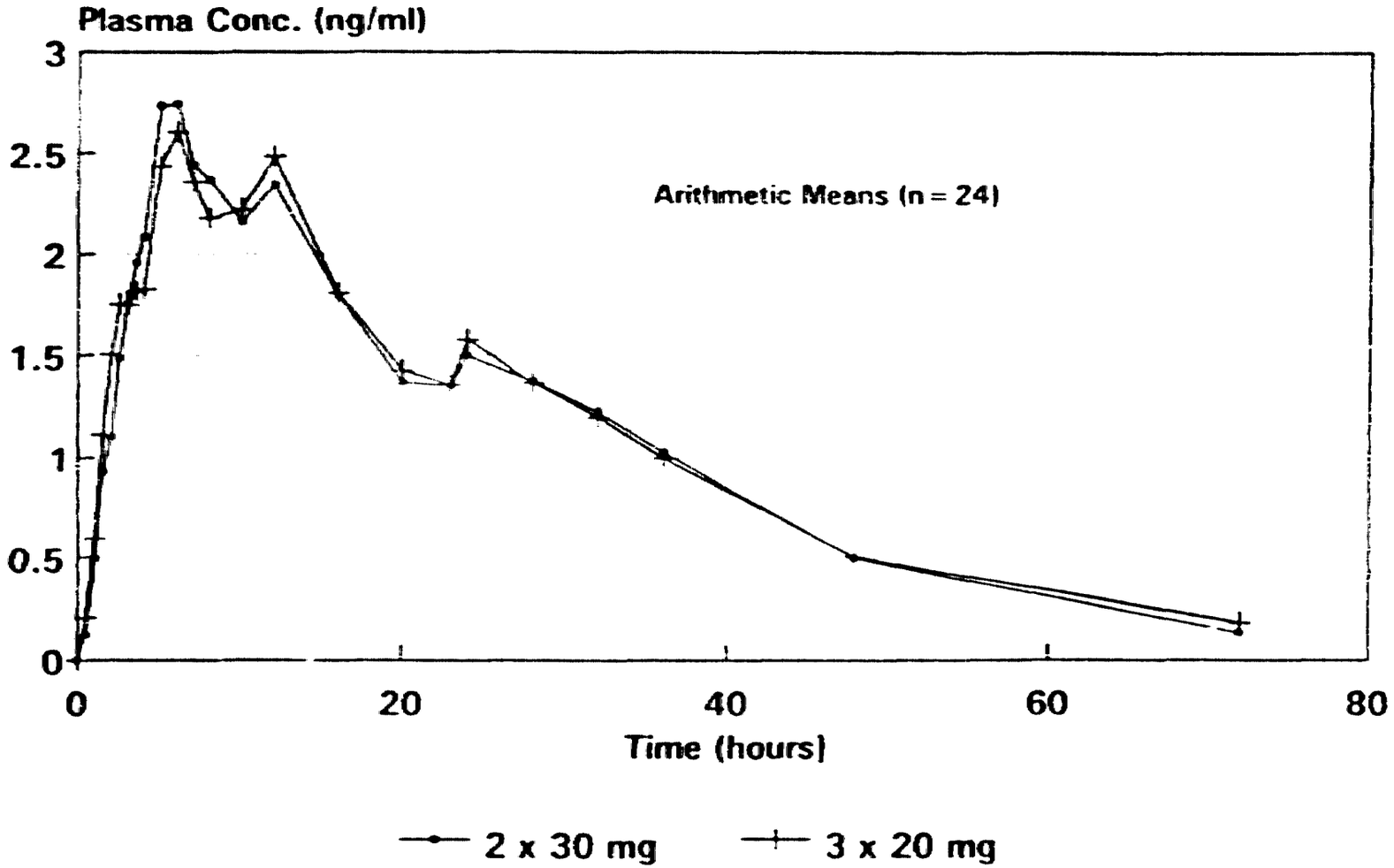
Mean plasma profiles for both treatments are presented in Figure 1.The 90 % confidence intervals for AUC and CMAX ratios are presented in Table 1.

	AUC (ng.hr/ml)	Ratio	90 % CI	CMAX (ng/ml)	Ratio	90 % CI
2x30 mg	81.98	1.0482	95-115	3.4	0.9587	83.64-109.87
3x20 mg	80.39			3.66		

Conclusion:

2x30 mg nisoldipine C.C. tablets are bioequivalent to 3x20 mg C.C. tablets.

Figure 1. Ni..ldipine Concentrations After 60 mg Given as Either 2x30 mg of 3x20 mg CC Tablets



Study D90-020

Pharmacokinetics and tolerability of nisoldipine after single administration of retard tablets with the old and new core composition.

STUDY # KF 715 VOLUME: 1-31 PAGES: 489-613.

INVESTIGATOR:

OBJECTIVES:

The objectives of the present study is to evaluate the bioequivalence of the new C.C formulation in doses of 1x20, 2x10 and 4x5 mg with the 20 mg old C.C. formulation after single administration.

FORMULATION:

Nisoldipine coat core (C.C.) tablets 20 mg (old formulation) batch # 523081, release date october 7th 1988, expiration date: december 12, 19889.

Nisoldipine C.C. tablets 10 mg (new formulation), batch # 523230, release date october 7 1988, expiration date: december 3, 1989.

Nisoldipine C.C. tablets 5 mg (new formulation), batch # 523228, release date october 7, 1988, expiration date: december 3, 1989.

Nisoldipine C.C. tablets 20 mg (new formulation), batch # 523232, release date october 7, 1988, expiration date: december 31, 1989.

STUDY DESIGN:

16 healthy male volunteers (average age 35.3 years, weight 75.2 Kg) participated in this randomized, non-blind four way cross-over study. A washout period of at least six days was allowed between individual treatments.
The test substances were administered to the fasting volunteers in the morning with 100 ml of water on an empty stomach. Food was administered 2 hours post drug administration.
Each subjects received each of the following treatments:
-20 mg tablet of the old formulation of nisoldipine C.C. tablet.
-4x5 mg tablets of the new formulation of nisoldipine C.C. tablet.
-2x10 mg tablets of the new formulation of nisoldipine C.C. tablet.
-20 mg tablet of the new formulation of nisoldipine C.C. tablet.
6 ml blood samples were taken at the following times: 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 20, 24, 36 and 48 hours post administration.
Urine was collected over the periods 0-24 hours and 24-48 hours after drug administration for

the determination of nisoldipine metabolites if needed.

RESULTS:

Table 1 shows the pharmacokinetic parameters after administration of the 4 different treatments while Table 2 gives the 90 % confidence for the parameters of interest in relation to the old 20 mg C.C. formulation. Figure 1 shows the mean pharmacokinetic profile for all the 4 different treatments. It can be seen that the 4x5 mg tablets of the new C.C formulation gave higher plasma levels than the three remaining formulations. The plasma levels for the 4x5 mg tablets were on the average 20 % higher resulting in bioinequivalence (as can be seen from table 2). Moreover, the 2x10 mg tablets of the new formulation passed the 90 % confidence interval as far as AUC is concerned, however, it failed in terms of CMAX since the 90 % CI was 91 to 132 %.

Conclusion:

Only the 20 mg new C.C formulation was considered to be bioequivalent to the old the 20 mg formulations. 2x10 and 4x5 mg of the new formulation are not **bioequivalent** to the 20 mg old formulation.

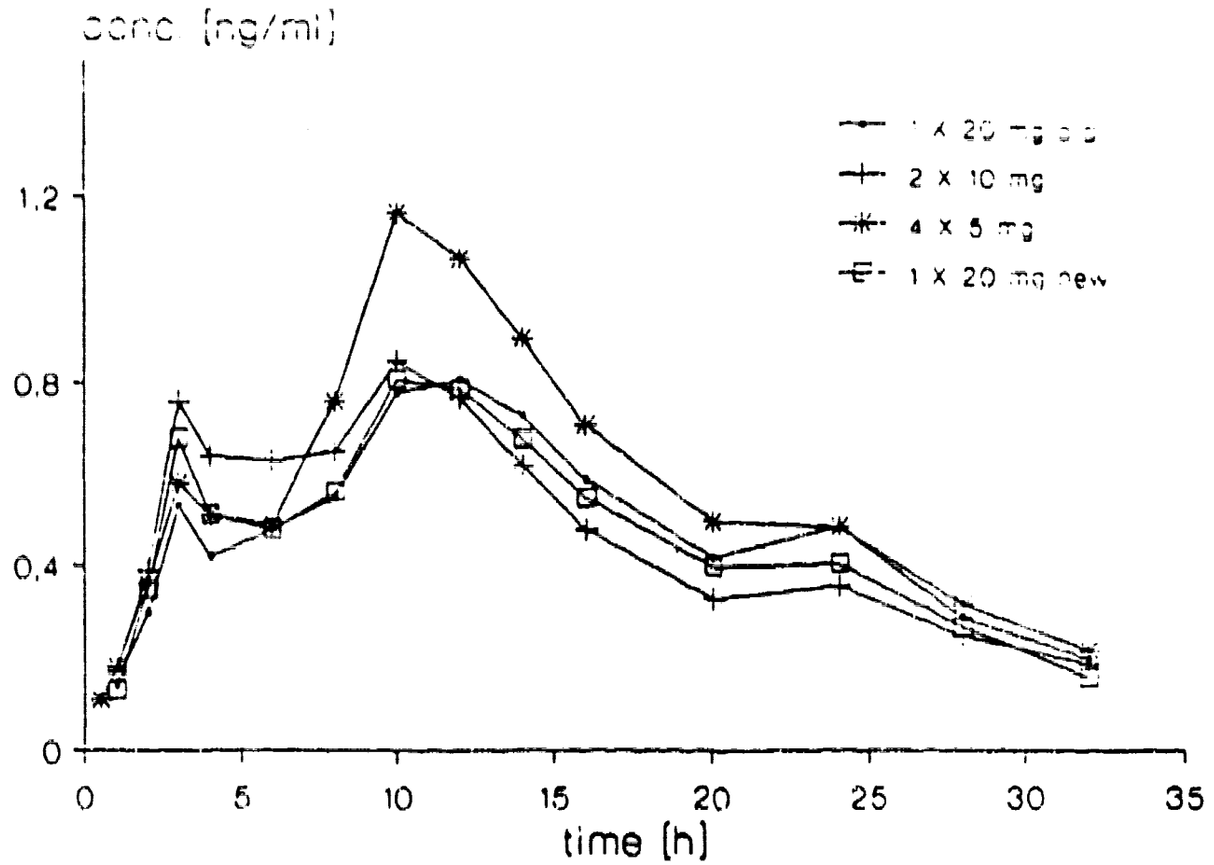


Fig. 1: Mean plasma concentrations after single administration of 20 mg nisoldipine as different retard tablets (1 x 20 mg, 2 x 10 mg and 4 x 5 mg with new core composition and 1 x 20 mg with old core composition); means without volunteer no. 4

Table 8: Comparison of pharmacokinetic parameters after single administration of 20 mg nisoldipine as different retard tablets (1 x 20 mg, 2 x 10 mg and 4 x 5 mg with new core composition and 1 x 20 mg with old core composition); geometric means and S.D. without volunteer no. 4.

Parameter	1 x 20 mg (old)		2 x 10 mg		4 x 5 mg		1 x 20 mg (new)	
AUC(0-48)	17.26	1.54	16.85	1.61	20.18	1.60	16.84	1.50
AUC-norm	64.46	1.51	62.90	1.55	75.34	1.62	62.89	1.46
MRT(0-48)	16.20	1.21	14.74	1.28	15.13	1.23	15.49	1.28
Cmax	1.04	1.62	1.08	1.58	1.31	1.50	0.99	1.79
Cmax-norm	3.90	1.57	4.04	1.54	4.89	1.47	3.69	1.74
C24h	0.49	1.56	0.36	2.27	0.49	1.89	0.41	1.52
Cmax/C24h	2.13	1.75	3.00	2.34	2.65	1.76	2.41	1.82
tmax	8.67	1.78	8.31	2.03	9.50	1.42	7.01	1.88
f	102.49	1.29	100.01	1.34	119.80	1.37		

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/ STUDY 656 (BF715)

METHOD VOL.MD.4

TABLE 6: ESTIMATES OF PHARMACOKINETIC PARAMETERS
COMPARISONS TO RESULT OF 1x20 MG TABL.MEM IN %
GEOMETRIC MEAN, GEOMETRIC SD, 90%-CONFIDENCE INTERVALS
REFERRING TO ANALYSIS OF VARIANCE AND DUNNETT-TESTS

PARAMETER		QUOTIENT (%) 1x20 MG TABL.BID /1x20 MG TABL.MEM		2x10 MG TABL. /1x20 MG TABL.MEM		4x5 MG TABL /1x20 MG TABL.MEM	
AUC (0-96HR)NG/ML	MEAN, SD 90% CONFID.INTERV.	102.5 87.7 -	1.29 119.7	100.8 85.6 -	1.34 116.8	119.8 102.6 -	1.37 139.9
AUC NORM (0-96HR)NG/ML	MEAN, SD 90% CONFID.INTERV.	102.5 87.7 -	1.29 119.7	100.8 85.6 -	1.34 116.8	119.8 102.6 -	1.37 139.9
CMAX (NG/ML)	MEAN, SD 90% CONFID.INTERV.	105.8 87.8 -	1.40 127.5	109.7 91.8 -	1.37 132.2	132.7 110.1 -	1.54 160.8
CMAX NORM (NG/ML)	MEAN, SD 90% CONFID.INTERV.	105.8 87.8 -	1.40 127.5	109.7 91.8 -	1.37 132.2	132.7 110.1 -	1.54 160.8
MRT (H)	MEAN, SD 90% CONFID.INTERV.	104.6 95.2 -	1.19 117.3	95.2 84.9 -	1.25 106.8	97.7 87.1 -	1.24 109.6
PLASM.CONC.24H NG/ML	MEAN, SD 90% CONFID.INTERV.	120.8 87.6 -	1.39 164.7	88.5 64.3 -	1.87 121.2	120.7 87.9 -	1.50 165.7
CMAX / CONC.24 H	MEAN, SD 90% CONFID.INTERV.	88.2 61.8 -	1.46 125.7	124.3 87.2 -	2.02 177.2	110.0 77.1 -	1.58 156.8

83

Pilot study on the pharmacokinetics and tolerability of three different controlled release formulations of nisoldipine in comparison to the IR tablet after single oral administration.

STUDY # 632. VOLUME: 1-38 PAGES: 3250-3560.

INVESTIGATOR:

OBJECTIVES:

The objective of the present study is to assess the pharmacokinetics and tolerability of three controlled release formulations of nisoldipine in comparison to the immediate release tablet.

FORMULATION:

-Nisoldipine coat core (C.C.) tablets 20 mg dev #034, pt # 523082.

-Nisoldipine C.C. tablets 20 mg dev #029, pt# 523081.

-Nisoldipine C.C. tablets 20 mg dev # 023, pt # 523028.

-Nisoldipine IR tablets 10 mg dev # E126, pt # 520210.

STUDY DESIGN:

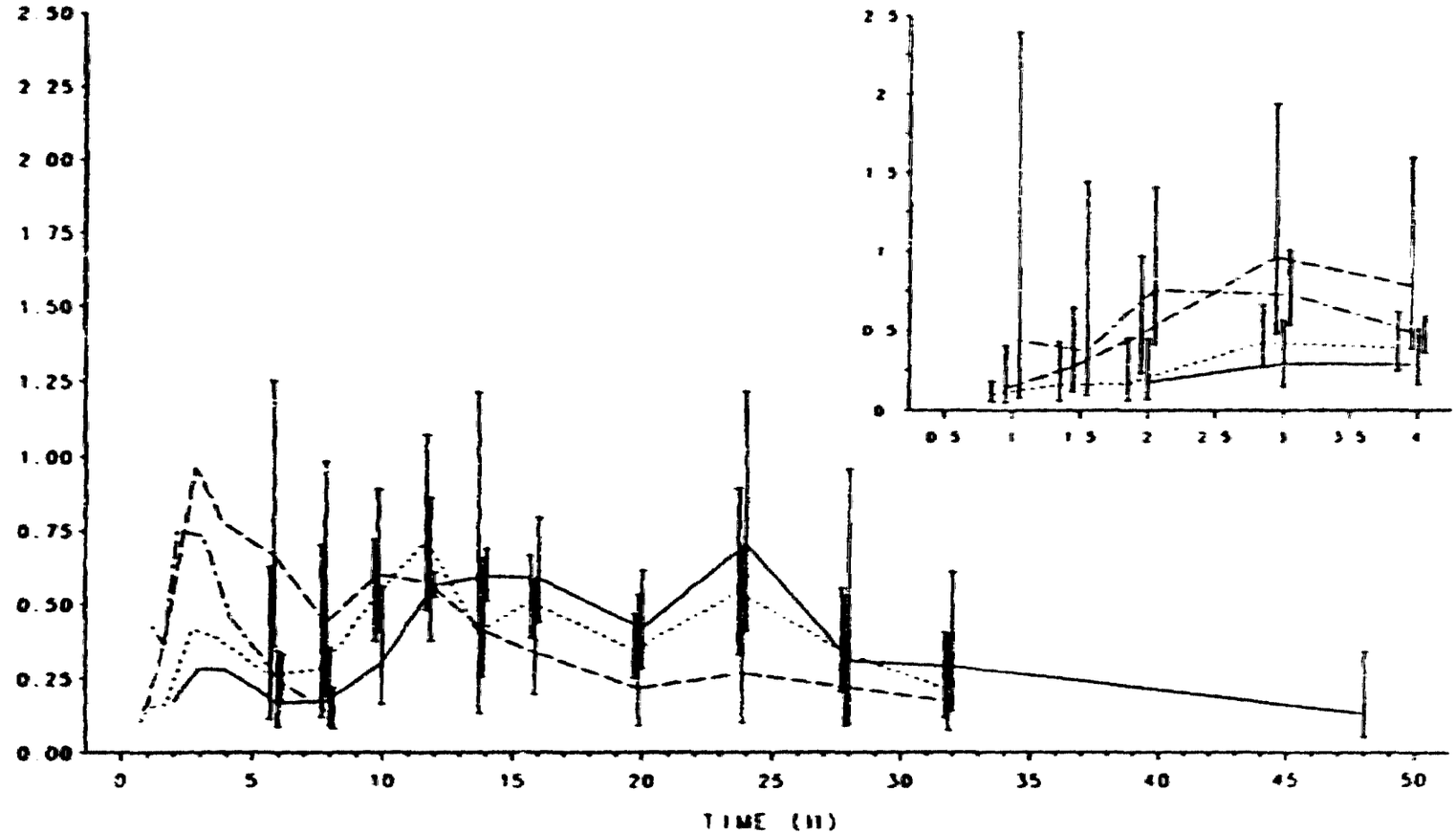
6 healthy male volunteers between the ages of 18 and 45 years participated in this non blind randomized triple crossover study for the C.C. tablet followed by a single dose administration of the immediate release formulation with washout phases of 6 days.
Plasma samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16, 20, 24, 28, 32 and 48 hours (56 hrs at period 2 and 3) post drug administration. Heart rate and blood pressure measurements were taken prior to blood sampling.

RESULTS:

Figure 1 shows the mean plasma concentration time profile for the 4 treatments while table 1 shows the corresponding pharmacokinetic parameters while Table 2 gives the 95 % confidence for the parameters of interest in relation to the 10 mg IR tablet.
The sponsor concluded from this study that slowing the release rate of nisoldipine increases its bioavailability as reflected by an increase in AUC, MRT and the duration during which the plasma concentration was above 0.3 ng/ml. This increase in bioavailability was accompanied by a significant decrease in CMAX.
Based on the result of this study, the sponsor thought that formulation E 029 gave the most favorable results and chose this formulation for further development.

/ STUDY NO. 632

NISOLDIPINE PLASMACONCENTRATION (NG/ML)



98

FIG. 1

26NOV92

/ STUDY 0632

TABLE 1 ESTIMATES OF PHARMACOKINETIC PARAMETERS
GEOMETRIC MEAN, GEOMETRIC SD BY TREATMENT

PARAMETER	N	NISOL 20MG (34)		NISOL.20MG (29)		NISOL.20MG (23)		NISOL.10MG (STD)	
AUC NORM (G*H/L)	6	68.673	1.78	57.153	1.40	52.949	1.72	31.347	1.36
CMAX (NG/ML)	6	0.800	1.56	0.857	1.51	1.060	1.80	1.547	2.12
MRT (H)	6	27.332	1.27	21.196	1.11	16.565	1.38	4.226	1.48
DURATION C>=0.3 (H)	6	24.008	1.65	23.912	1.29	17.961	1.92	4.489	1.24

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26NOV92

/ STUDY 0632

TABLE 2 ESTIMATES OF PHARMACOKINETIC PARAMETERS
QUOTIENTS TO RESULT OF NISOL.10MG (STD) IN %
GEOMETRIC MEAN, GEOMETRIC SD, 95%-CONF.INTERV. ACCORDING TO MODEL
REFERRING TO ANALYSIS OF VARIANCE WITHOUT ALPHA ADJUSTMENT

PARAMETER	N SD(MODEL)		QUOTIENT (%) NISOL.20MG (34) /NISOL.10MG (STD)	QUOTIENT (%) NISOL.20MG (29) /NISOL.10MG (STD)	QUOTIENT (%) NISOL.20MG (23) /NISOL.10MG (STD)
AUC NORM (G*H/L)	6 1.48	MEAN, SD 95% C.I.	219.1 1.52 156.9 - 307.7	182.3 1.43 129.8 - 256.1	168.9 1.59 120.3 - 237.2
CMAX (NG/ML)	6 1.74	MEAN, SD 95% C.I.	51.7 1.66 31.9 - 83.7	55.4 2.22 34.2 - 89.7	68.5 1.67 42.3 - 111.0
MRT (H)	6 1.52	MEAN, SD 95% C.I.	646.7 1.59 448.4 - 932.8	501.5 1.61 347.7 - 723.3	392.0 1.87 271.7 - 565.3
DURATION C>=0.3 (H)	6 1.66	MEAN, SD 95% C.I.	544.5 1.64 349.6 - 848.2	542.3 1.48 348.2 - 844.8	407.4 2.13 261.5 - 634.6

126

Clinical study on the influence of food and time of administration on the pharmacokinetics of controlled release nisoldipine.

Study: 666 Volume: 42-43 Pages: 4802-5361.

Investigators:

Clinical:

Objectives:

The objective of this study was the evaluation of the influence of food and time of administration on the pharmacokinetics of nisoldipine in a controlled release formulation.

Formulation

20 mg nisoldipine C.C. tablets batch # 52332.

Study Design:

Twelve healthy male volunteers between the ages of 24 and 33 years participated in this study. A three factorial 3x3x6 latin square design with repeated measurements on Factor A, Period B Treatment and randomization on Factor C sequence with 2 subjects per sequence was applied. Single doses of 20 mg nisoldipine C.C. tablets were applied at 8 AM in fasted state (2 h before an american standard breakfast), together with an american standard breakfast and in fed state (1 hr after an american standard breakfast) during the first three periods.
During the additionally performed 4th period, all volunteers received single doses of 20 mg nisoldipine C.C. tablets at 8 PM together with a continental dinner.
There was a washout period of at least one week between the four treatments. The 4 treatments were as follows:
b_1: administration in fasted state (2 hr before an American standard breakfast).
b_2: administration together with an American standard breakfast.
b_3: administration in a fed state (1 hr after an American standard breakfast).
b_4: administration together with a continental dinner.
The American standard breakfast with an average calorie content of 575 Kcal consisted of:
-2 cups (0.2 l) of decaffeinated coffee.
-1 glass(0.2 l) of orange juice.
-20 ml of evaporated milk (7.5 % fat).
-2 slices of toast.

-2 slices of boiled ham (40 g).
-20 g butter.
-20 g jam.

The continental dinner consisted of:
-1 glass (0.2 l) of orange juice.
-4-5 slices of toast.
-50 g butter.
-boiled ham, cheese and sausages.
-mineral water ad libitum.
12 healthy male volunteers between the ages of 18 and 35 participated in this study.
For Periods I-III blood samples were withdrawn at the following time points: -0.17, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 14, 16, 20, 24, 28, 32, 36, and 48 hr after administration.
For Period IV blood samples were withdrawn at -.17, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 11, 12, 14, 16, 20, 24, 28, 36 and 48 hours after administration.

Results:

Figure 1 shows the mean plasma profile for all the 4 different treatments while Table 1 gives the mean pharmacokinetic parameters.
It can be seen that AUC normalized for dose and weight were as follows:
-fasted state: 61.69 g*h/l
-together with breakfast: 61.03 g*h/l (ratio 99% with 90 % CI= 78-126 %).
-1 hr after breakfast: 50.3 g*hr/l (ratio 82 % with 90 % CI= 64-104 %).
-together with dinner: 59.22 g*hr/l (ratio 96 % with 90 % CI= 77-120 %).

CMAX following administration together with meals are remarkably higher as compared to CMAX following administration in a fasted state (1.35 ng/ml).
The geometric means, quotients and the 90 % CI compared to the fasted state were as follows:
-together with breakfast: 2 ng/ml 148 % with a 90 % CI of 103 to 212 %.
-1 hr after breakfast: 1.87 ng/ml 138 % with a 90 % CI of 97 to 198 %.

-together with dinner: 1.7 ng/ml 126 % with a 90 % CI of 93 to 171 %.

Conclusion:

1-The lowest bioavailability related to AUCnorm was found following administration 1 hr after breakfast.

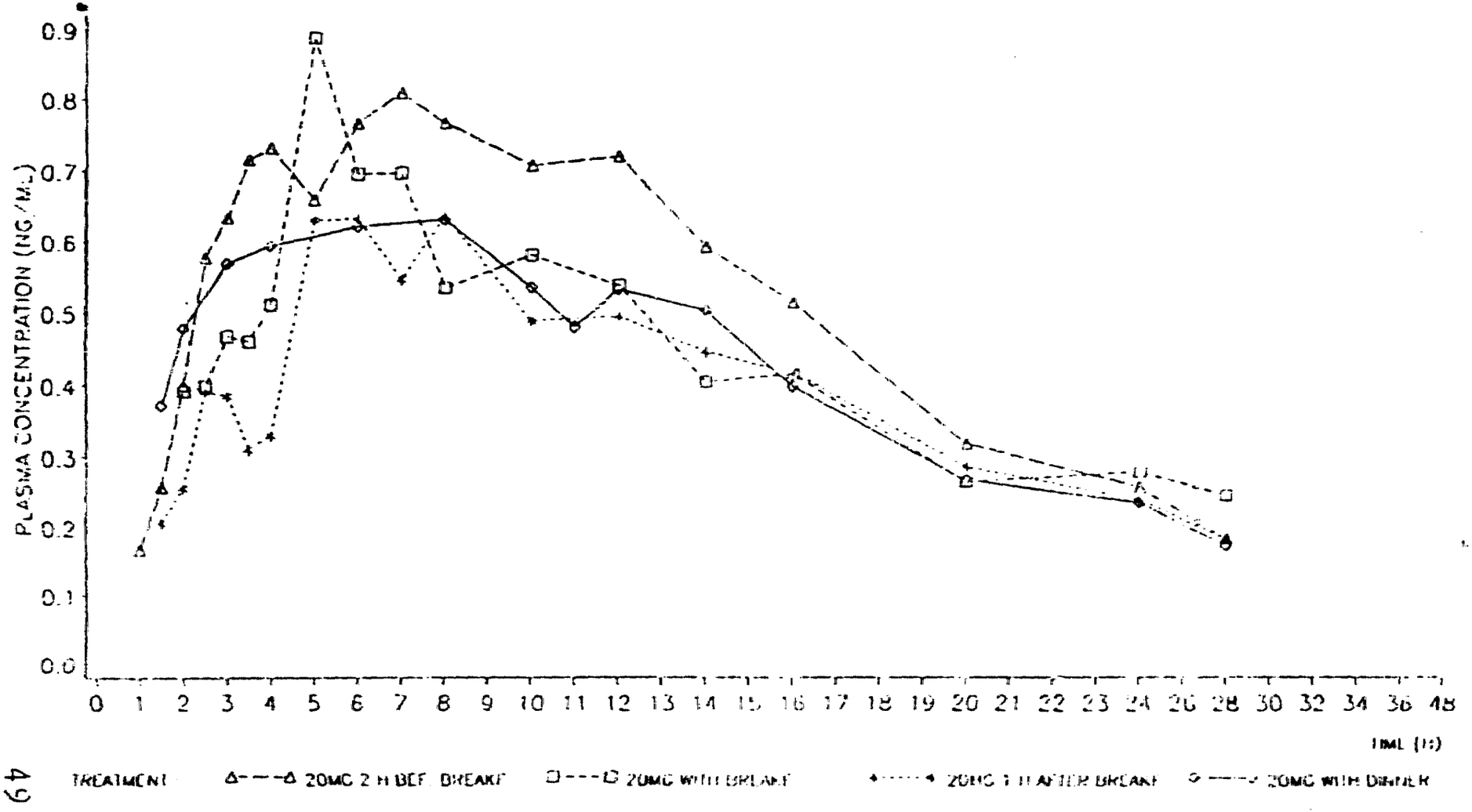
2- CMAX was higher by about 26 to 48 % higher when nisoldipine was administered with food.

3-Intake of food shortened TMAX by about 2 to 3 hours.

STUDY NO. 0666

PLASMA CONCENTRATIONS (NG/ML).GEOMETRIC MEANS

FIGURE 1:



STUDY NO. 666

TABLE 1: ESTIMATES OF PHARMACOKINETIC PARAMETERS
GEOMETRIC MEAN, GEOMETRIC SD BY TREATMENT

PARAMETER	N	FASTING		WITH BREAKFAST		1 H AFTER BREAKFAST		WITH DINNER	
AUD0-t (NG•H/ML)	12	15.65	1.45	15.48	1.74	12.76	2.30	15.02	1.95
AUC0-∞ (NG•H/ML)	12	18.40	1.39	18.35	1.56	15.44	1.91	17.81	1.92
AUDNORM (G•H/L)	12	61.69	1.49	61.03	1.80	50.30	2.37	59.22	2.01
CMAX (NG/ML)	12	1.35	1.67	2.00	1.72	1.87	1.95	1.70	2.01
CMAX,NORM (G/L)	12	5.33	1.67	7.87	1.78	7.37	1.98	-	-
T1/2 (H)	12	7.46	1.66	7.03	1.72	6.75	1.68	9.81	1.86
TMAX (H)	12	6.87	1.74	3.86	1.56	3.24	1.97	4.89	1.93
Duration C ≥ 0.3 NG/ML	12	20.43	1.45	16.97	1.77	15.00	2.52	-	-

Pharmacokinetics and tolerability of nisoldipine taken after breakfast and on an empty stomach.

Study: 0323 Volume: 43 Pages: 5362-5515.

Investigators:

Clinical:

Objectives:

1-To examine the effect of the timing of intake of a meal on the plasma concentration/time curve for nisoldipine in order to establish guidelines for intake where appropriate.

2-Examine in healthy subjects whether the intake of a meal has an effect on the relative bioavailability of nisoldipine.

Formulation:

-20 mg nisoldipine tablets batch # 929490.

Study Design:

8 healthy male volunteers participated in 2 way randomized crossover study. The 2 treatments were 20 mg nisoldipine either before or 1.5 hours after administration of a standardized breakfast. Blood samples were withdrawn at 0, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 24 hours after drug administration. Urine samples were collected for the following time intervals: 0-4 hr, 4-8 hrs, 8-24 hrs and 24 to 48 hours.
Blood pressure and heart rate were measured at the same time points when blood samples were withdrawn.
The standard breakfast consisted of one egg, 80 g of boiled ham, three rolls with butter and jam and 2 cups of caffeine free coffee.

Results:

Figure 1 shows the mean plasma levels after the fasted and fed treatments while Table 1 shows the main pharmacokinetic parameters with the corresponding 95 % Confidence intervals.
The results show that coadministration with food results in 28 % increase in AUC and 31 % increase in CMAX.
Table 2 shows a summary of the changes in systolic and diastolic blood pressure and heart rate. It can be seen from the results that when nisoldipine was taken with breakfast, the change in heart rate took place at a later time point but was more pronounced.

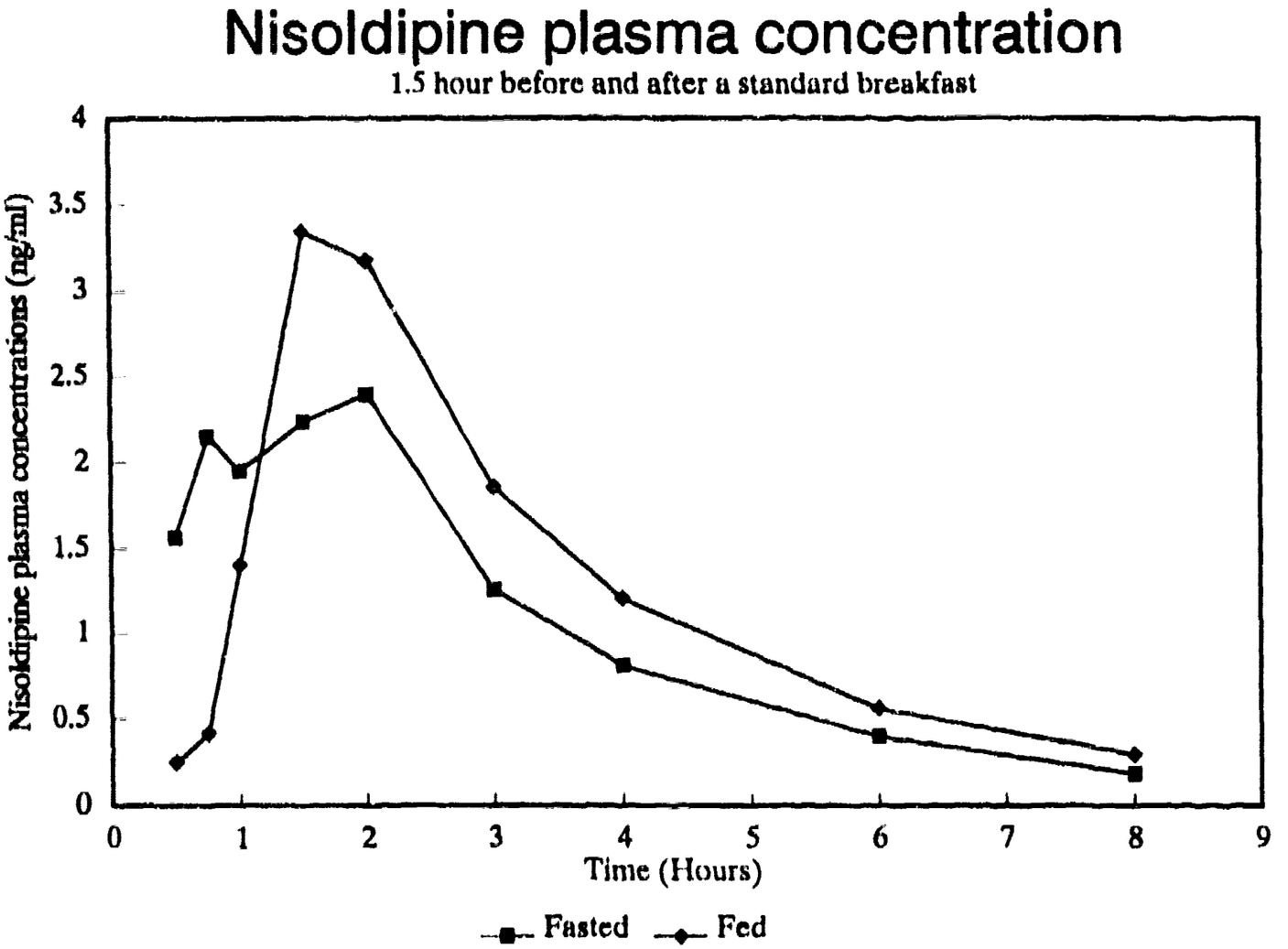
Conclusion:

1-Coadministration of food with nisoldipine resulted in both an increase in AUC and CMAX most probably due to a decrease in liver blood flow resulting in decreased first pass metabolism.

2-The results show that there was a great deal of inter-individual variability in the effect of food as can be observed in the very wide confidence intervals for the pharmacokinetic parameters.

3-The increase in plasma concentration of nisoldipine resulted in a more pronounced effect in heart rate since the change was more pronounced compared to when nisoldipine was given in a fasted state even though there was no difference in the effect on either the systolic or diastolic blood pressure between the fed and fasted state.

FIGURE 1


Nisoldipine plasma concentration
1.5 hour before and after a standard breakfast
Nisoldipine plasma concentrations (ng/ml)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
6
7
8
9
Time (Hours)
Fasted
Fed

table]:

variable	geometric	fasting	after meal	MQ · 1000 error	treatm. F period F	p	after meal / fasting mean (%)	95%-conf.limit (%)
AUC_{norm} (kg·h/1000 l)	mean	41.841	53.602	30.618	1.51	0.27	128.1	76.3, 215.1
	sd	1.644	1.318		0.82	0.40		
C_{max} (ng/ml)	mean	4.211	5.520	32.578	1.69	0.24	131.1	76.7, 223.8
	sd	1.539	1.614		3.97	0.10		
MRT	mean	4.049	4.159	19.681	0.02	0.88	102.7	67.7, 155.6
	sd	1.476	1.211		1.63	0.91		

STUDY NO.323
DIFFERENCES TO PREVALUES

TREATMENT=STAND.MEAL/DRUG VARIABLE=BP SYSTOLIC (MM HG)

TIME	N	MEAN	SD	MINIMUM	MEDIAN	MAXIMUM
AFT. 30 MIN - PRE	8	3.375	9.9562	-16	3.0	18
AFT. 45 MIN - PRE	8	0.625	11.7951	-26	2.0	12
AFT. 60 MIN - PRE	8	4.000	9.7980	-9	1.5	20
AFT. 90 MIN - PRE	8	3.125	11.7527	-15	2.0	23
AFT. 2 H - PRE	8	3.625	10.7429	-17	9.0	16
AFT. 3 H - PRE	8	-0.750	16.0424	-26	-3.0	22
AFT. 4 H - PRE	8	-5.125	13.3269	-24	-4.0	17
AFT. 6 H - PRE	8	7.125	5.1964	1	6.5	15
AFT. 8 H - PRE	8	3.500	10.0860	-15	6.0	14
AFT. 24 H - PRE	8	3.625	9.8116	-14	7.5	13

TREATMENT=STAND.MEAL/DRUG VARIABLE=BP DIASTOLIC (MM HG) - SITTING

TIME	N	MEAN	SD	MINIMUM	MEDIAN	MAXIMUM
AFT. 30 MIN - PRE	8	-0.750	8.2071	-16	2.5	7
AFT. 45 MIN - PRE	8	2.500	9.2273	-13	2.0	16
AFT. 60 MIN - PRE	8	-0.625	8.6510	-12	-0.5	16
AFT. 90 MIN - PRE	8	-3.125	3.4821	-7	-4.0	2
AFT. 2 H - PRE	8	0.625	7.9810	-10	-2.0	12
AFT. 3 H - PRE	8	-0.375	5.7802	-9	-0.5	8
AFT. 4 H - PRE	8	-2.250	11.3484	-21	3.0	8
AFT. 6 H - PRE	8	8.125	11.4447	-5	6.0	30
AFT. 8 H - PRE	8	5.125	7.1001	-2	3.0	17
AFT. 24 H - PRE	8	2.500	6.6762	-5	0.5	13

TREATMENT=STAND.MEAL/DRUG VARIABLE=HEART RATE (/MIN)

TIME	N	MEAN	SD	MINIMUM	MEDIAN	MAXIMUM
AFT. 30 MIN - PRE	8	5.125	7.3957	-3	5.0	18
AFT. 45 MIN - PRE	8	8.875	10.2461	-6	9.0	22
AFT. 60 MIN - PRE	8	14.875	12.5861	1	13.5	38
AFT. 90 MIN - PRE	8	14.750	11.0551	2	12.5	38
AFT. 2 H - PRE	8	11.875	12.1824	-1	9.5	37
AFT. 3 H - PRE	8	-0.125	8.9990	-13	-0.5	16
AFT. 4 H - PRE	8	-4.500	7.6996	-13	-5.5	13
AFT. 6 H - PRE	8	10.000	10.0854	-3	10.5	30
AFT. 8 H - PRE	8	9.250	13.7087	-6	7.5	35
AFT. 24 H - PRE	8	-4.125	8.3911	-16	-5.5	8

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STUDY NO.323
DIFFERENCES TO PREVALUES

TREATMENT=DRUG /STAND.MEAL VARIABLE=BP SYSTOLIC (MM HG)

TIME	N	MEAN	SD	MINIMUM	MEDIAN	MAXIMUM
AFT. 30 MIN - PRE	8	-4.250	11.8894	-25	-2.5	9
AFT. 45 MIN - PRE	8	-2.750	12.4757	-17	-4.5	13
AFT. 60 MIN - PRE	8	-1.750	12.9367	-28	2.5	12
AFT. 90 MIN - PRE	8	0.000	12.6829	-23	0.5	19
AFT. 2 H - PRE	8	3.625	8.2451	-8	7.5	12
AFT. 3 H - PRE	8	2.875	16.4789	-18	3.0	26
AFT. 4 H - PRE	8	1.375	11.5504	-13	0.0	21
AFT. 6 H - PRE	8	12.750	10.0250	-6	16.0	24
AFT. 8 H - PRE	8	6.125	16.9068	-26	6.0	29
AFT. 24 H - PRE	8	5.500	11.4268	-14	7.5	15

TREATMENT= DRUG/STAND.MEAL VARIABLE=BP DIASTOLIC (MM HG) - SITTING

TIME	N	MEAN	SD	MINIMUM	MEDIAN	MAXIMUM
AFT. 30 MIN - PRE	8	-0.500	9.5169	-20	-0.5	10
AFT. 45 MIN - PRE	8	-2.875	11.2686	-26	-1.0	8
AFT. 60 MIN - PRE	8	-4.750	9.3618	-18	-7.0	10
AFT. 90 MIN - PRE	8	-2.125	11.0897	-20	-2.5	18
AFT. 2 H - PRE	8	-1.250	8.4134	-16	2.5	7
AFT. 3 H - PRE	8	-3.375	10.9013	-20	-2.5	12
AFT. 4 H - PRE	8	2.250	11.6466	-20	1.0	16
AFT. 6 H - PRE	8	4.750	9.6325	-13	6.5	15
AFT. 8 H - PRE	8	2.750	5.9782	-5	4.0	12
AFT. 24 H - PRE	8	4.500	8.7831	-9	6.0	16

TREATMENT= DRUG/STAND.MEAL VARIABLE=HEART RATE (/MIN)

TIME	N	MEAN	SD	MINIMUM	MEDIAN	MAXIMUM
AFT. 30 MIN - PRE	8	9.375	7.9451	1	6.5	22
AFT. 45 MIN - PRE	8	7.000	9.8125	-4	5.0	21
AFT. 60 MIN - PRE	8	6.250	7.8513	-7	8.5	16
AFT. 90 MIN - PRE	8	5.875	8.1141	-4	4.5	21
AFT. 2 H - PRE	8	13.125	12.1707	-5	11.0	31
AFT. 3 H - PRE	8	13.875	12.9553	-3	15.0	32
AFT. 4 H - PRE	8	6.750	11.7565	-7	8.5	22
AFT. 6 H - PRE	8	13.000	10.3233	-6	17.0	23
AFT. 8 H - PRE	8	13.375	11.4385	-1	12.5	28
AFT. 24 H - PRE	8	-0.750	13.1230	-27	1.5	15

The effect of food on the pharmacokinetics of 30 and 40 mg nisoldipine C.C. tablets in healthy male volunteers.

Study: D92-045-02 Volume: 2 Pages: 1-671.

Investigators:

Clinical:

Objectives:

The aim of the study is to evaluate the effect of food on the pharmacokinetics of 30 and 40 mg Nisoldipine C.C. tablets.

Formulation:

Coat-Core tablet: 30 mg nisoldipine batch # 526245.
Coat-Core tablet: 40 mg nisoldipine batch # 526069.

Study Design:

28 healthy male volunteers between the ages 18 and 45 years participated in this open, randomized, 2 period crossover study. Two dose levels 30 and 40 mg were studied concurrently, each in a 2 way crossover design in a different set of 14 subjects.

At each dose level, the subjects received a single dose (30 or 40 mg) of nisoldipine C.C. either in the fasting state or immediately after a standardized breakfast. There was a 1 week washout period between each single dose at each dose level.
Blood samples were collected according to the following time table:0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 24, 30, 36, 48, 60 and 72 hours post-administration.
Breakfast was consumed in 20 minutes and the drug was given immediately (within 5 minutes) after the end of the meal.

The breakfast consisted of 1 cup of caffeinated or decaffeinated coffee (optional), 2 slices of buttered toast; jelly optional, 2 eggs fried in butter, 2 strips bacon, 4 ounces of hash brown potatoes and 8 ounces of whole milk.

Results:

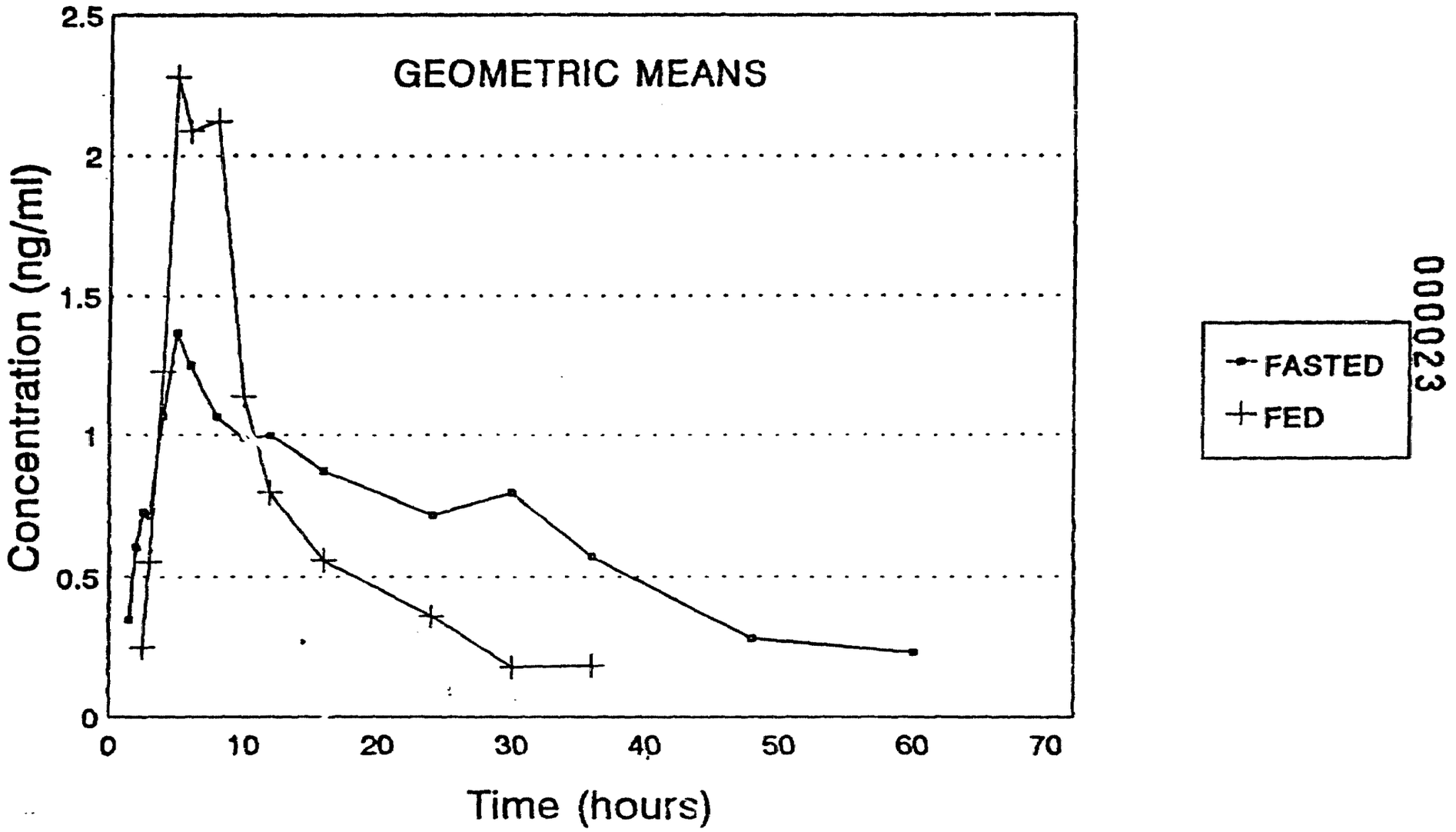
Figure 1 shows the geometric mean plasma profile for the 30 mg nisoldipine CC under fed and fasted conditions while Figure 2 shows the corresponding profile for the 40 mg tablet. Table 1 gives a comparison of the CMAX geometric means for all the 4 different treatments while Table 2 gives the same comparison for AUC.

Conclusion:

Administration of nisoldipine C.C. tablets immediately following a high fast breakfast resulted in greater peak plasma drug concentrations than were seen when the same dose was given on an empty stomach. CMAX on the average increased 250 to 300 %. However AUC was decreased on the average by 26 % in the fed state as compared to the fasted state.

SINGLE 30 MG DOSE GIV. .J FED & FASTED
(STUDY D92-045-02)

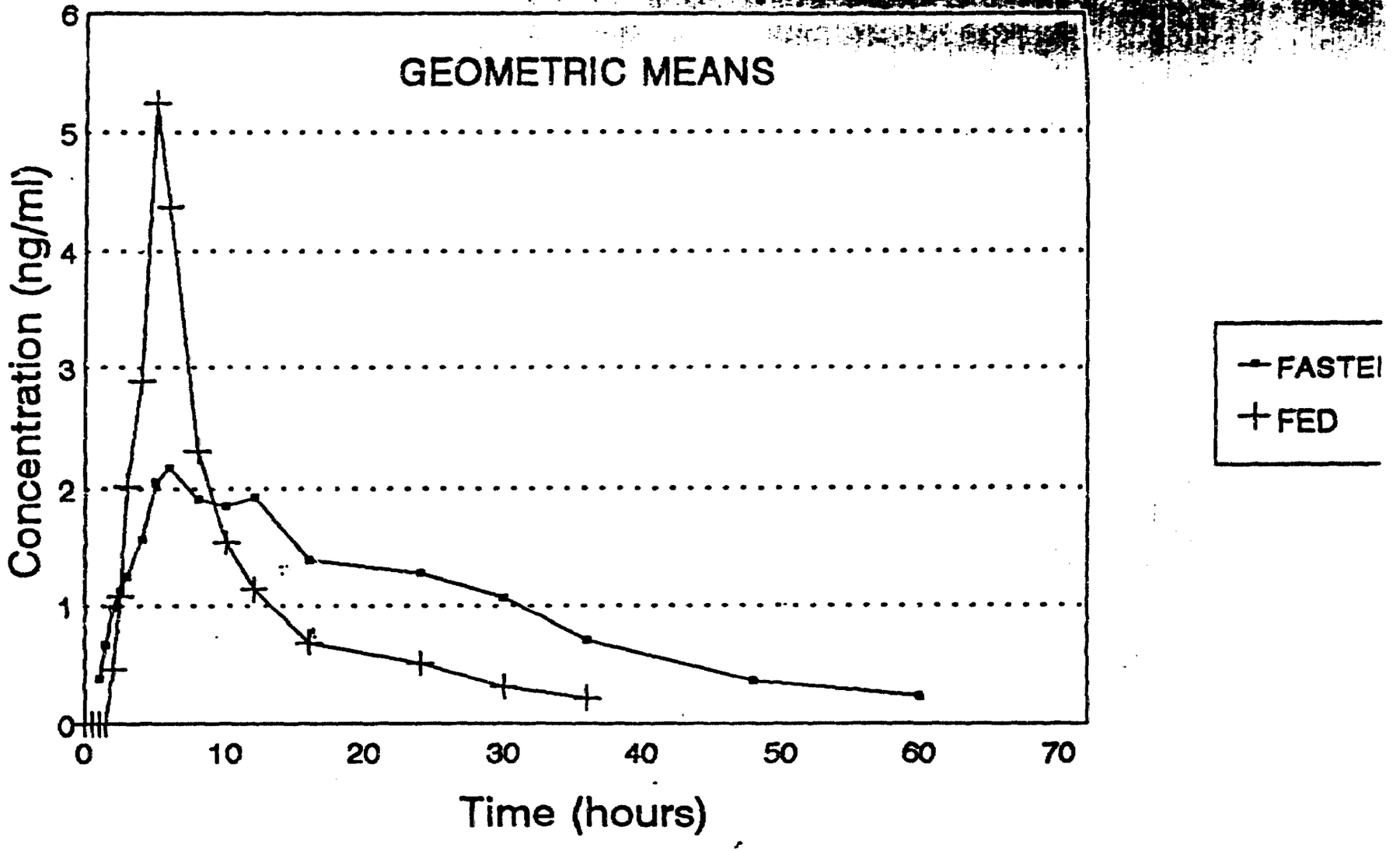
FIGURE 1



N = 14

Fig. 12

(STUDY D92-045-02)



N = 14

TABLE 1

Geometric Mean (approximate CV) C_{max}

	30 mg	40 mg
C_{max} (ng/ml) Fasted	1.9 (62%)	2.7 (53%)
C_{max} (ng/ml) Fed	4.5 (74%)	7.5 (72%)
Ratio (Fed/Fasted)	2.35	2.75
90% CI	1.65 - 3.36	1.92 - 3.92

TABLE 2

Geometric Mean (approximate CV) $AUC_{0-\infty}$

	30 mg	40 mg
$AUC_{0-\infty}$ (ng*h/ml) Fasted	49.2 (42%)	70.4 (50%)
$AUC_{0-\infty}$ (ng*h/ml) Fed	35.4 (46%)	53.0 (51%)
Ratio (Fed/Fasted)	0.72	0.75
90% CI	0.58 - 0.89	0.61 - 0.93

A study to determine the single dose and steady-state pharmacokinetics of nisoldipine coat-core tablet 20 mg in elderly and young volunteers and in elderly hypertensives.

Study #: 712. VOLUME: 1-44-47 PAGES: 6-01-0001-1337

INVESTIGATOR:

OBJECTIVES:

To determine and compare the acute and steady-state pharmacokinetic parameters of the nisoldipine C.C. tablet in healthy young and elderly volunteers and in elderly hypertensive patients and to evaluate the safety of nisoldipine in these subjects.

FORMULATIONS:

Nisoldipine 20 mg C.C. tablets batch # 524526 expiration date June 30 1992.

STUDY DESIGN:

This was an open, multiple dose, non randomized study. The study population was selected according to the following criteria:
-healthy young male volunteers between the ages of 18 and 30 years.
-healthy elderly male and female volunteers aged 65 years or more.
-male and female elderly patients with a history of mild to moderate essential hypertension aged 65 years or more.
20 mg nisoldipine C.C. tablet was administered at 8 AM on days 1, 3, 4, 5, 6 and 7. Days 1 and 7 being the profile days.
5 ml blood samples were collected according to the following schedule: 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 14, 16, 20, 24, 28, 32, 36 and 48 hours after drug administration. Blood samples for trough level assessment were collected on days 5 and 6 before drug administration. Measurements of systolic and diastolic blood pressure and heart rate with volunteers in the supine position having rested for at least ten minutes were made just prior to the drawing of each blood sample.
21 healthy elderly volunteers, 23 healthy young volunteers and 11 hypertensive elderly patients of both sexes completed the study.

RESULTS:

Figure 1 shows the geometric mean nisoldipine plasma concentrations after single dose (day 1) and multiple dose (day 7) of a 20 mg nisoldipine C.C. in young healthy volunteers, while Figure 2 and 3 show the same plasma concentrations obtained in healthy elderly volunteers and elderly hypertensive patients. Figure 4 gives the comparative mean plasma profiles after single dose administration in the three different populations while Figure 5 gives the comparative profiles after multiple dose administration. Table 1 to 3 give a comparison of the pharmacokinetic parameters among the three different populations while Table 4 to 6 gives the same comparison for the pharmacokinetic parameters after multiple dose administration. Figure 6 gives the mean trough plasma nisoldipine concentrations.

The results show that after single dose administration of 20 mg nisoldipine C.C., the elderly normal and hypertensive patients tended to have higher AUC than young healthy volunteers by about 50 %. CMAX in the elderly hypertensives was also 50 % higher than in either the healthy young or elderly volunteers.

However upon multiple dose administration, there was a greater tendency for increase in AUC and CMAX in both the healthy and hypertensive subjects as compared to the young subjects. This resulted in a greater degree of accumulation in the elderly compared to the young. This is most probably due to lower clearance rates in the elderly as compared to the young.

It is to note that there was essentially no accumulation in the young however in the elderly healthy and hypertensives, the accumulation ratio was around 2.

FIGURE 1

/ 0712

PLASMA NISOLDIPINE CONCENTRATIONS

Dose : 20 mg nisoldipine cc tablet

1 : Healthy young volunteers
(n = 20)

GEOMETRIC MEAN VALUES

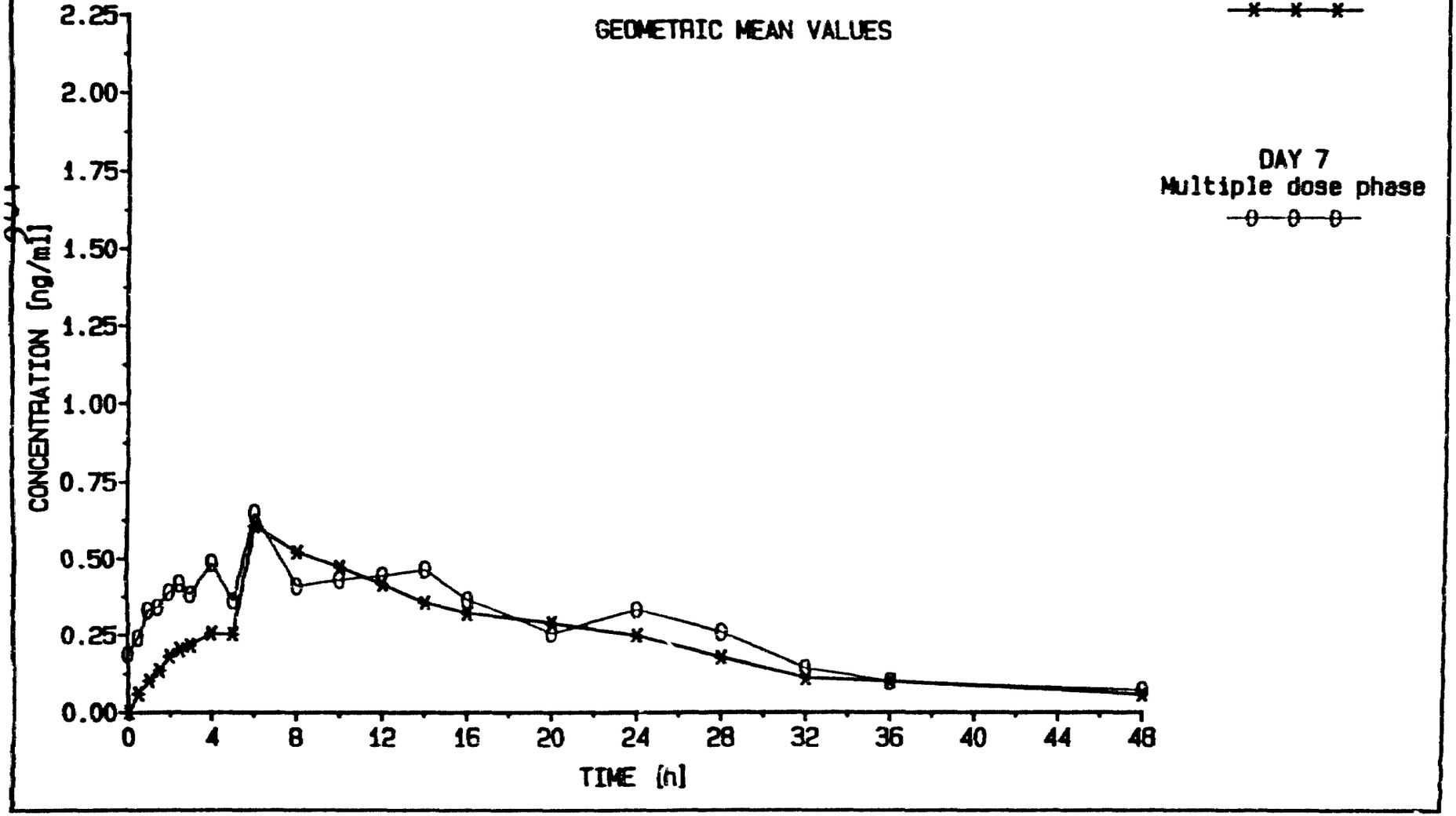


FIGURE 9

/ 0712

PLASMA NISOLDIPINE CONCENTRATIONS

Dose : 20 mg nisoldipine cc tablet

2 : Healthy elderly volunteers
(n = 21)

GEOMETRIC MEAN VALUES

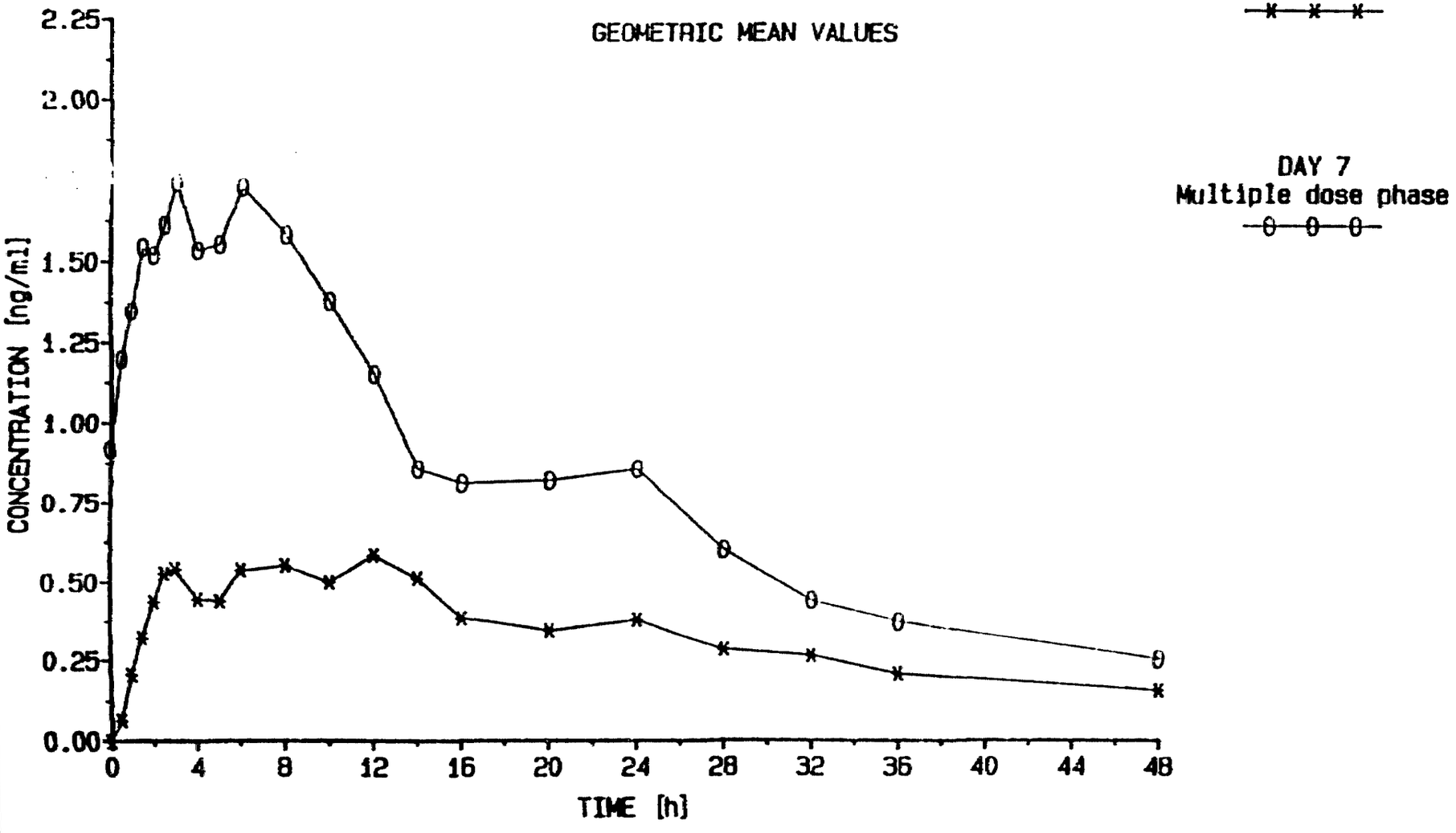


FIGURE 3

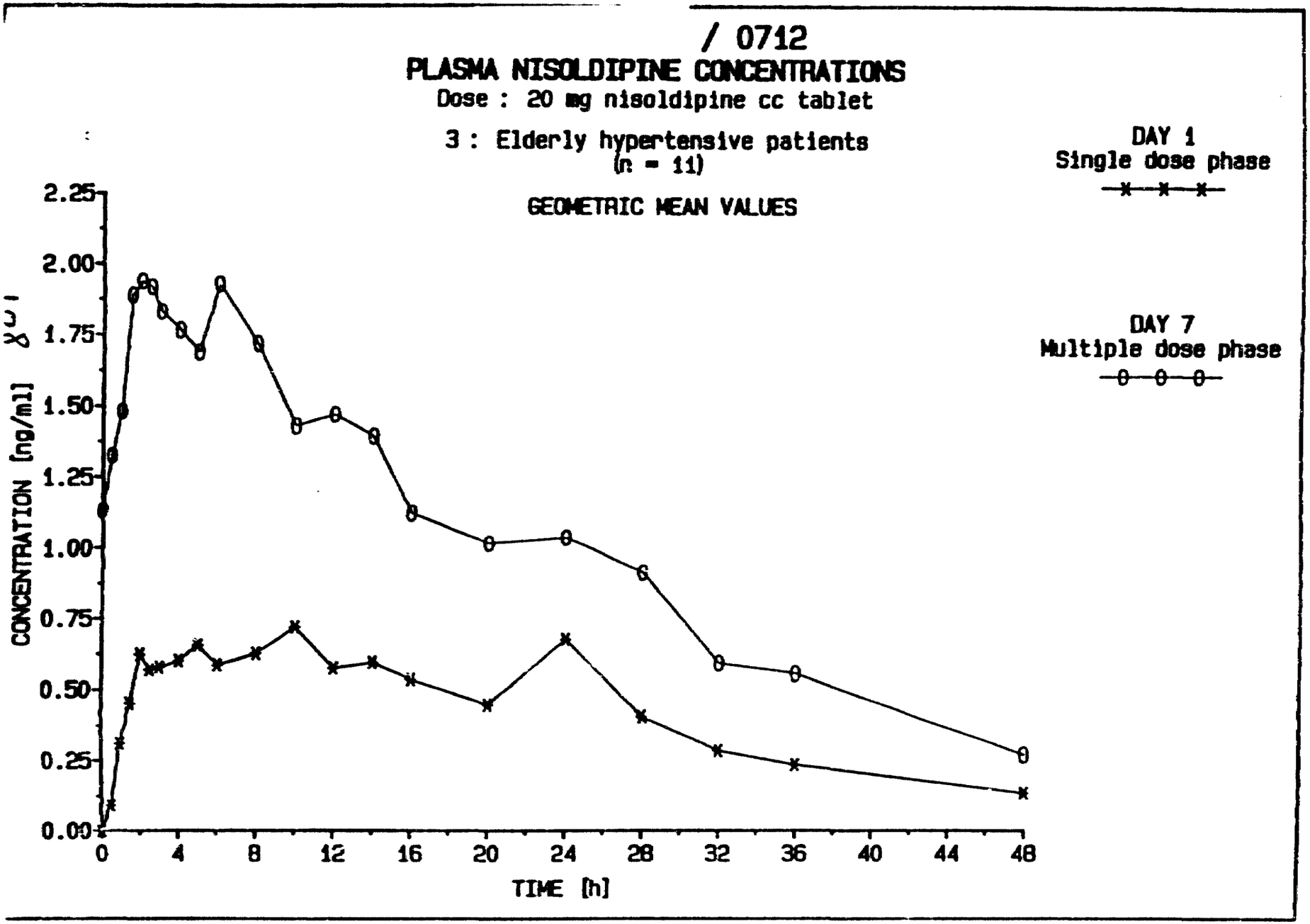


FIGURE 4

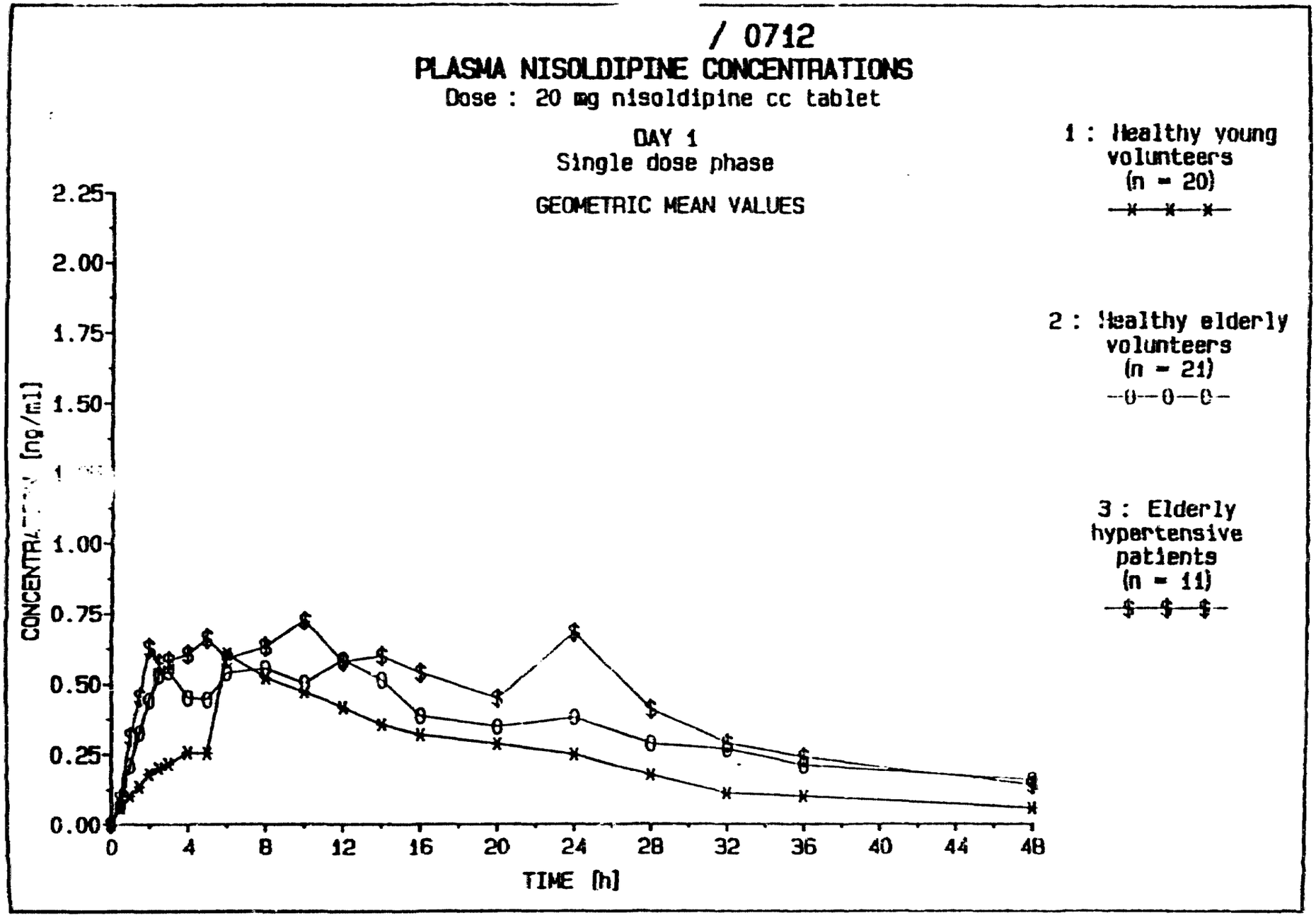


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FIGURE 5

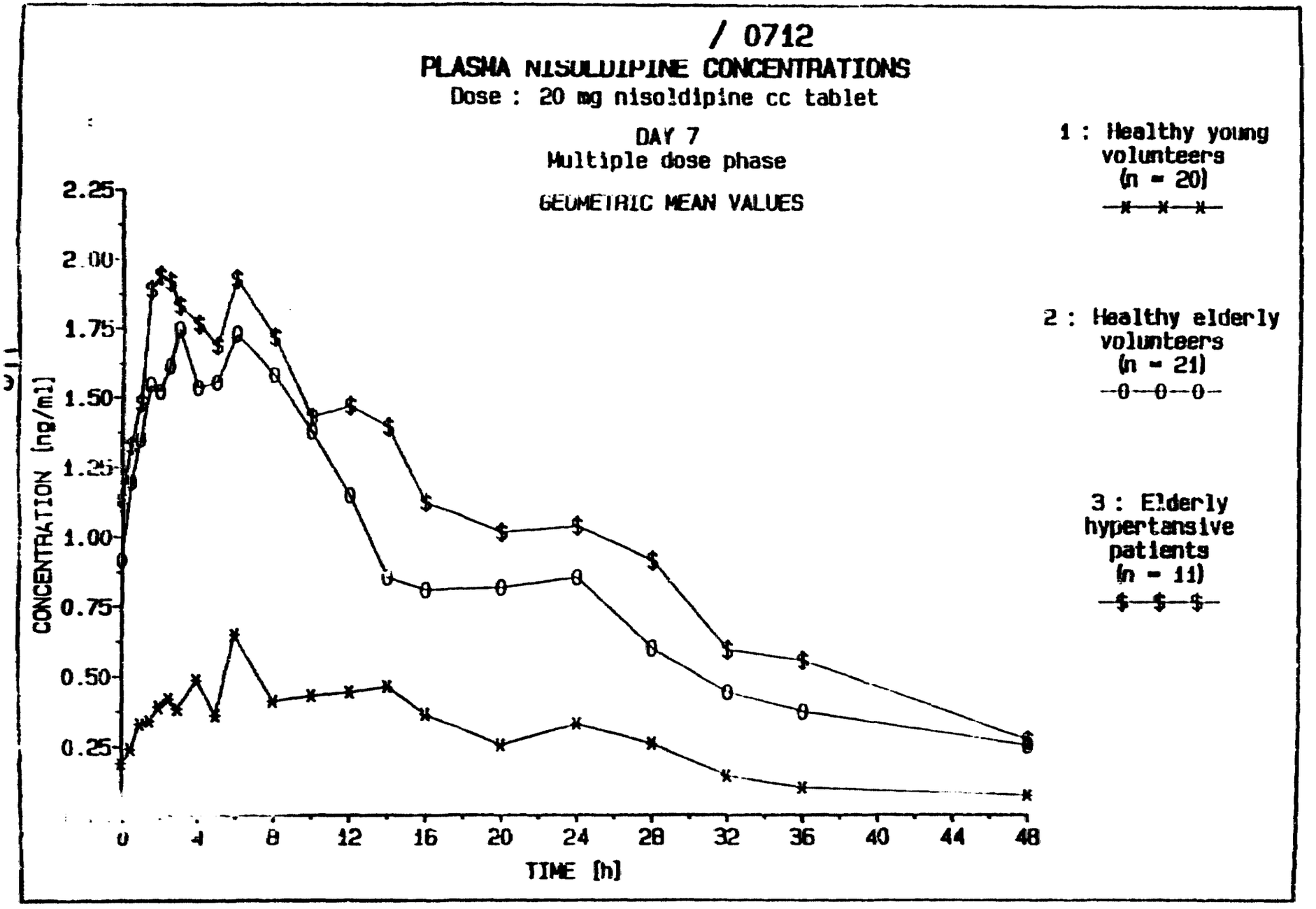

FIGURE 6

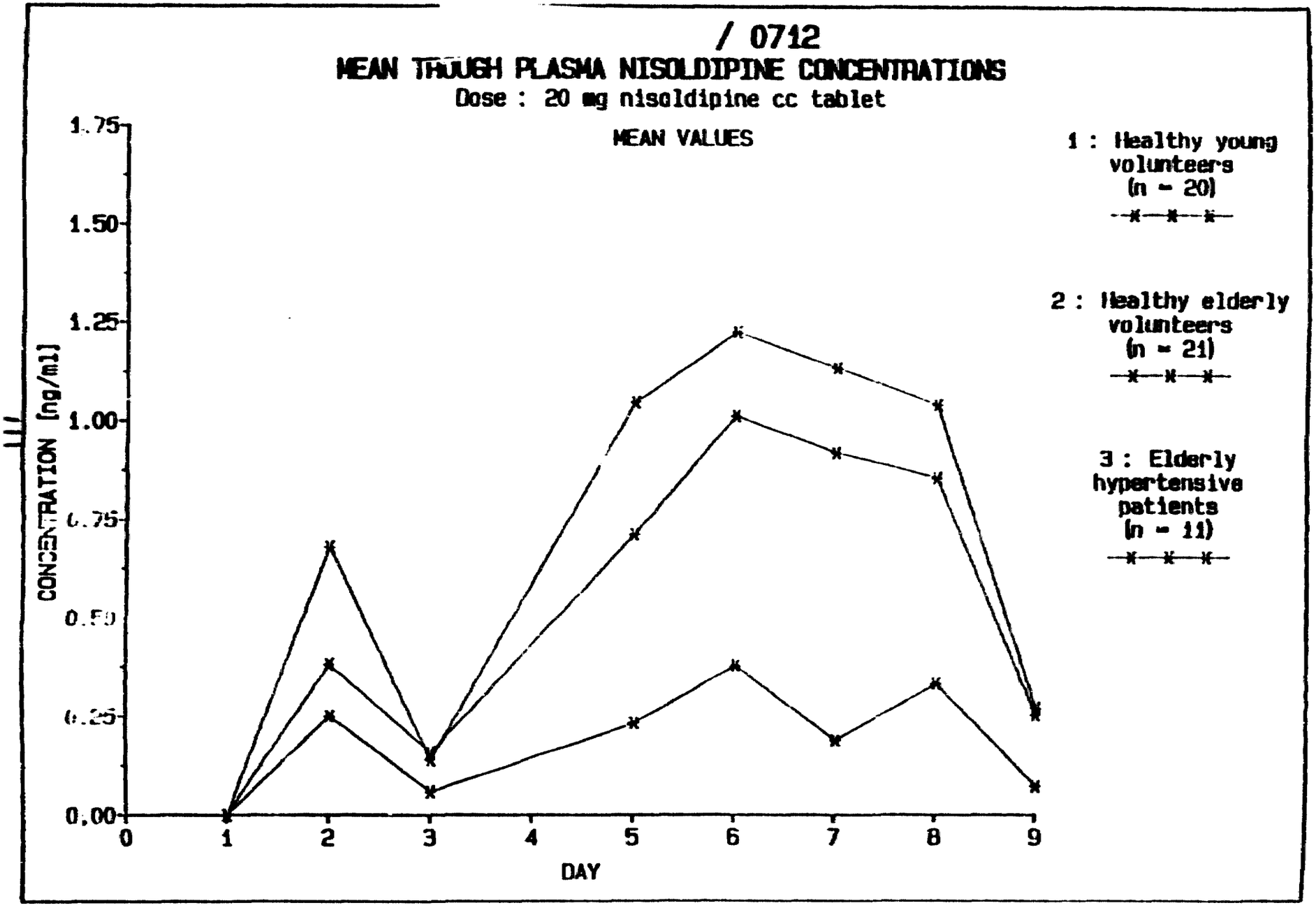

TABLE 3

Table 1: SUMMARY OF PHARMACOKINETIC DATA FOR NISOLDIPINE

Day 1 - Single dose phase (Dose : 1 x 20 mg nisoldipine cc tablet)

		Healthy young volunteers			Healthy elderly volunteers				
VARIABLE	UNIT	n	MEDIAN	RANGE	n	MEDIAN	RANGE	MEAN RATIO (%)*	90% CONFIDENCE INTERVAL (%)**
C_{max}	(ng/ml)	20	0.92		21	1.07			
t_{max}	(h)	19	6.00		21	6.00		10.0	-3.0-2.5]#
AUC(0-t_z)	(ng.h/ml)	20	15.0		21	23.3			
AUC(0-24h)	(ng.h/ml)	20	11.0		21	15.5			
AUC(0-∞)	(ng.h/ml)	20	18.1		21	26.0			
C_{max_norm}+	(mg/ml)	20	3.34		21	3.65		97	71-135
AUC(0-24h)$_{norm}$+	(mg.h/ml)	20	40.0		21	56.6		131	85-186
AUC(0-∞)$_{norm}$+	(mg.h/ml)	20	68.9		21	93.5		158	100-252
$t_{½}$	(h)	13	10.5		17	13.6		150	106-212
MRT	(h)	13	19.7		17	28.5			
CL/f	(l/h)	20	1105		21	770			
CL/f$_{norm}$++	(l/h/kg)	20	14.6		21	10.7			
V_{ss}/f	(l)	13	21186		17	17651			
V_{ss}/f$_{norm}$++	(l/kg)	13	323		17	259			

*: Nonparametric estimate of "elderly/young" mean ratio

**: Nonparametric 90% confidence interval for the "elderly/young" mean ratio

#: Nonparametric estimate of "elderly-young" mean difference and 90% confidence interval for the "elderly-young" mean difference

+: Normalized for dose per body mass.

++: Normalized for body mass.

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TABLE 4

Table 2: SUMMARY OF PHARMACOKINETIC DATA FOR NISOLDIPINE

Day 1 - Single dose phase (Dose : 1 x 20 mg nisoldipine cc tablet)

		Healthy young volunteers			Elderly hypertensive patients				
VARIABLE	UNIT	n	MEDIAN	RANGE	n	MEDIAN	RANGE	MEAN RATIO (%)*	90% CONFIDENCE INTERVAL (%)**
C_{max}	(ng/ml)	20	0.92		11	1.40			
t_{max}	(h)	19	6.00		11	6.00		10.0	-2.0-3.0[#]
AUC(0-t_n)	(ng.h/ml)	20	15.0		11	19.5			
AUC(0-24h)	(ng.h/ml)	20	11.0		11	18.1			
AUC(0-∞)	(ng.h/ml)	20	18.1		11	24.9			
$C_{max,norm}$+	(ng/ml)	20	3.34		11	5.88		152	86-228
AUC(0-24h)$_{norm}$+	(ng.h/ml)	20	40.0		11	75.1		174	102-286
AUC(0-∞)$_{norm}$+	(ng.h/ml)	20	68.9		11	95.8		202	117-360
$t_{½}$	(h)	13	10.5		10	10.4		107	81-189
MRT	(h)	13	19.7		10	21.8			
	(l/h)	20	1105		11	805			
CL/f$_{norm}$++	(l/h/kg)	20	14.6		11	10.4			
V_{ss}/f	(l)	13	21186		10	14291			
V_{ss}/f$_{norm}$++	(l/kg)	13	323		10	174			

*: Nonparametric estimate of "elderly/young" mean ratio

**: Nonparametric 90% confidence interval for the "elderly/young" mean ratio

#: Nonparametric estimate of "elderly-young" mean difference and 90% confidence interval for the "elderly-young" mean difference

+: Normalized for dose per body mass.

++: Normalized for body mass.

TABLE 5

Table 3: SUMMARY OF PHARMACOKINETIC DATA FOR NISOLDIPINE

Day 1 - Single dose phase (Dose : 1 x 20 mg nisoldipine cc tablet)

		Healthy elderly volunteers			Elderly hypertensive patients				
VARIABLE	UNIT	n	MEDIAN	RANGE	n	MEDIAN	RANGE	MEAN RATIO (%)*	90% CONFIDENCE INTERVAL (%)**
C_{max}	(ng/ml)	21	1.07		11	1.40			
t_{max}	(h)	21	6.00		11	6.00		0.5	-2.0-4.0]#
AUC(0-t_a)	(ng.h/ml)	21	23.3		11	19.5			
AUC(0-24h)	(ng.h/ml)	21	15.5		11	18.1			
AUC(0-∞)	(ng.h/ml)	21	26.0		11	24.9			
$C_{max,norm}$+	(mg/ml)	21	3.65		11	5.88		152	95-227
AUC(0-24h)$_{norm}$+	(mg.h/ml)	21	56.6		11	75.1		135	89-204
AUC(0-∞)$_{norm}$+	(mg.h/ml)	21	93.5		11	95.8		126	79-231
$t_{½}$	(h)	17	13.6		10	10.4		78	52-127
MRT	(h)	17	28.5		10	21.8			
CL/f	(l/h)	21	770		11	805			
CL/f_{norm}++	(l/h/kg)	21	10.7		11	10.4			
V_{ss}/f	(l)	17	17651		10	14291			
V_{ss}/f_{norm}++	(l/kg)	17	259		10	174			

*: Nonparametric estimate of "elderly patients/elderly volunteers" mean ratio

**: Nonparametric 90% confidence interval for the "elderly patients/elderly volunteers" mean ratio

#: Nonparametric estimate of "elderly patients-elderly volunteers" mean difference and 90% confidence interval for the "elderly patients-elderly volunteers" mean difference

+: Normalized for dose per body mass.

++: Normalized for body mass.

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TABLE 1

SUMMARY OF PHARMACOKINETIC DATA FOR NISOLDIPINE

Day 7- **Multiple dose phase** (Dose : 20 mg nisoldipine cc tablet once daily for 5 days)

		Healthy young volunteers			Healthy elderly volunteers				
VARIABLE	**UNIT**	**n**	**MEDIAN**	**RANGE**	**n**	**MEDIAN**	**RANGE**	**MEAN RATIO (%)***	**90% CONFIDENCE INTERVAL (%)****
$C_{ss,max}$	(ng/ml)	20	1.06		21	2.07			
$C_{ss,min}$	(ng/ml)	20	L.D.		21	0.33			
$t_{ss,max}$	(h)	20	6.00		21	3.00		[-3.0	-4.0 0][#]
AUC_{ss}	(ng.h/ml)	20	12.5		21	32.0			
AR		20	1.12		21	2.29		206	156-274
$C_{ss,max,norm}$[+]	(mg/ml)	20	3.74		21	7.25		193	144-261
$AUC_{ss,norm}$[+]	(mg.h/ml)	20	47.0		21	122		265	178-383
$t_{1/2}$	(h)	13	10.4		20	13.8		160	106-238
CL/f	(l/h)	20	1597		21	624			
CL/f_{norm}[++]	(l/h/kg)	20	21.3		21	8.22			

*: Nonparametric estimate of "elderly/young" mean ratio

**: Nonparametric 90% confidence interval for the "elderly/young" mean ratio

#: Nonparametric estimate of "elderly-young" mean difference and 90% confidence interval for the "elderly-young" mean difference

+: Normalized for dose per body mass.

++: Normalized for body mass.

TABLE 2

Table 6: SUMMARY OF PHARMACOKINETIC DATA FOR NISOLDIPINE

Day 7- Multiple dose phase (Dose : 20 mg nisoldipine cc tablet once daily for 5 days)

		Healthy elderly volunteers			Elderly hypertensive patients				
VARIABLE	**UNIT**	**n**	**MEDIAN**	**RANGE**	**n**	**MEDIAN**	**RANGE**	**MEAN RATIO (%)***	**90% CONFIDENCE INTERVAL (%)****
$C_{ss,max}$	(ng/ml)	21	2.07		11	2.42			
$C_{ss,min}$	(ng/ml)	21	0.33.		11	0.32			
$t_{ss,max}$	(h)	21	3.00		11	2.50		[-0.5	-3.0-0.5]#
AUC_{ss}	(ng.h/ml)	21	32.0		11	38.0			
AR		21	2.29		11	1.92		84	70-117
$C_{ss,max,norm}$+	(mg/ml)	21	7.25		11	11.0		139	102-188
$AUC_{ss,norm}$+	(mg.h/ml)	21	122		11	135		139	94-200
$t_{½}$	(h)	20	13.8		11	14.1		97	62-142
CL/f	(l/h)	21	6.24		11	526			
CL/f_{norm}++	(l/h/kg)	21	8.22		11	7.42			

*: Nonparametric estimate of "elderly patients/elderly volunteers" mean ratio

**: Nonparametric 90% confidence interval for the "elderly patients/elderly volunteers" *mean ratio*

#: Nonparametric estimate of "elderly patients-elderly volunteers" mean difference and 90% confidence interval for the "elderly patients-elderly volunteers" mean difference

+: Normalized for dose per body mass.

++: Normalized for body mass.

TABLE 6

Table 5: SUMMARY OF PHARMACOKINETIC DATA FOR NISOLDIPINE

Day 7- Multiple dose phase (Dose : 20 mg nisoldipine cc tablet once daily for 5 days)

		Healthy young volunteers			Elderly hypertensive patients				
VARIABLE	UNIT	n	MEDIAN	RANGE	n	MEDIAN	RANGE	MEAN RATIO (%)*	90% CONFIDENCE INTERVAL (%)**
$C_{ss,max}$	(ng/ml)	20	1.06		11	2.42			
$C_{ss,min}$	(ng/ml)	20	L.D.		11	0.32			
$t_{ss,max}$	(h)	20	6.00		11	2.50		13.5	-5.0 - 0.5]#
AUC_{ss}	(ng.h/ml)	20	12.5		11	38.0			
AR		20	1.12		11	1.92		186	138-288
$C_{ss,max,norm}$+	(mg/ml)	20	3.74		11	11.0		275	202-264
$AUC_{ss,norm}$+	(mg.h/ml)	20	47.0		11	135		347	254-549
$t_{1/2}$	(h)	13	10.4		11	14.1		149	104-220
CL/f	(l/h)	20	1597		11	526			
CL/f_{norm}++	(l/h/kg)	20	21.3		11	7.42			

*: Nonparametric estimate of "elderly/young" mean ratio

**: Nonparametric 90% confidence interval for the "elderly/young" mean ratio

#: Nonparametric estimate of "elderly-young" mean difference and 90% confidence interval for the "elderly-young" mean difference

+: Normalized for dose per body mass.

++: Normalized for body mass.

A study to investigate the acute and short term pharmacokinetic profiles of nisoldipine in elderly and young normotensive volunteers.

STUDY #: 563. VOLUME: 1-48 PAGES: 6-02-001-0219

INVESTIGATORS:

OBJECTIVES:

To investigate the pharmacokinetics of nisoldipine 10 mg PO in one group of young normotensive volunteers and one group of elderly volunteers after acute (1 day) and chronic (7-8 days treatments).

FORMULATION:

Nisoldipine immediate release 10 mg tablets (batch #: 929488).

STUDY DESIGN:

12 elderly (age greater than 65 years) healthy volunteers and 9 young healthy volunteers (age 20-28 years) participated in this open non-randomized study. Subjects took a 10 mg nisoldipine tablet for 8 days. On day 1 and day 8 plasma samples were collected at 0, 1, 1.5, 2, 3, 5, 7 and 24 hours after dosing. Blood pressure and heart rate measurements were taken at the same time schedule as for the plasma samples.

ASSAY: No description of the assay was presented in the study.

1

RESULTS:

Figure 1 shows the plasma concentration for day 1 in the young and elderly volunteers while Figure 2 shows the corresponding profile for day 8. Table 1 summarizes the mean pharmacokinetic parameters for both types of population.
It can be seen from the results that the plasma concentrations of nisoldipine tended to be somewhat higher in the elderly as compared to the young. However, there was no difference in the single dose and multiple dose pharmacokinetics of nisoldipine in both the young and the elderly. Moreover, no accumulation was observed upon multiple dose administration.

FIGURE 2

FIGURE 2 : MEAN SEMILOGARITHMIC PLOT OF PLASMA NISOLDIPINE IN YOUNG AND ELDERLY SUBJECTS - DAY 8

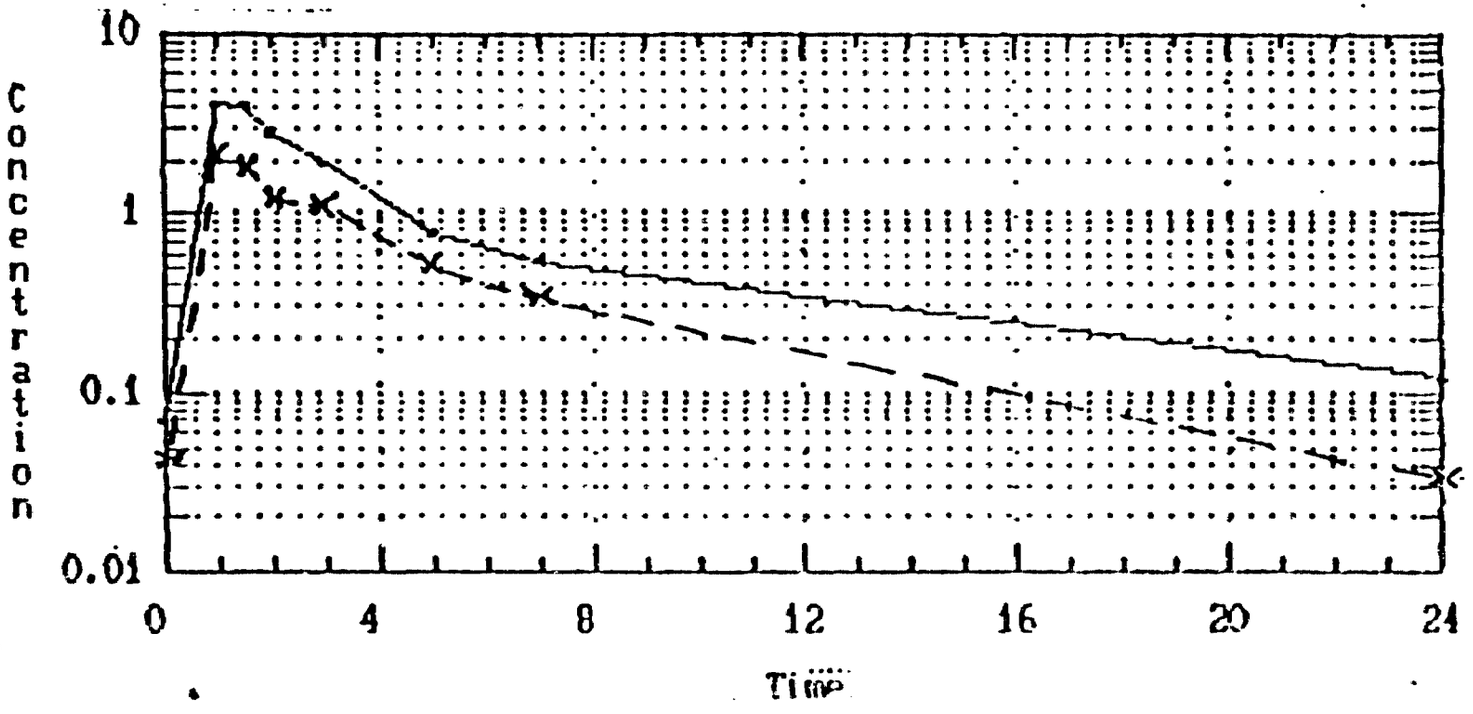
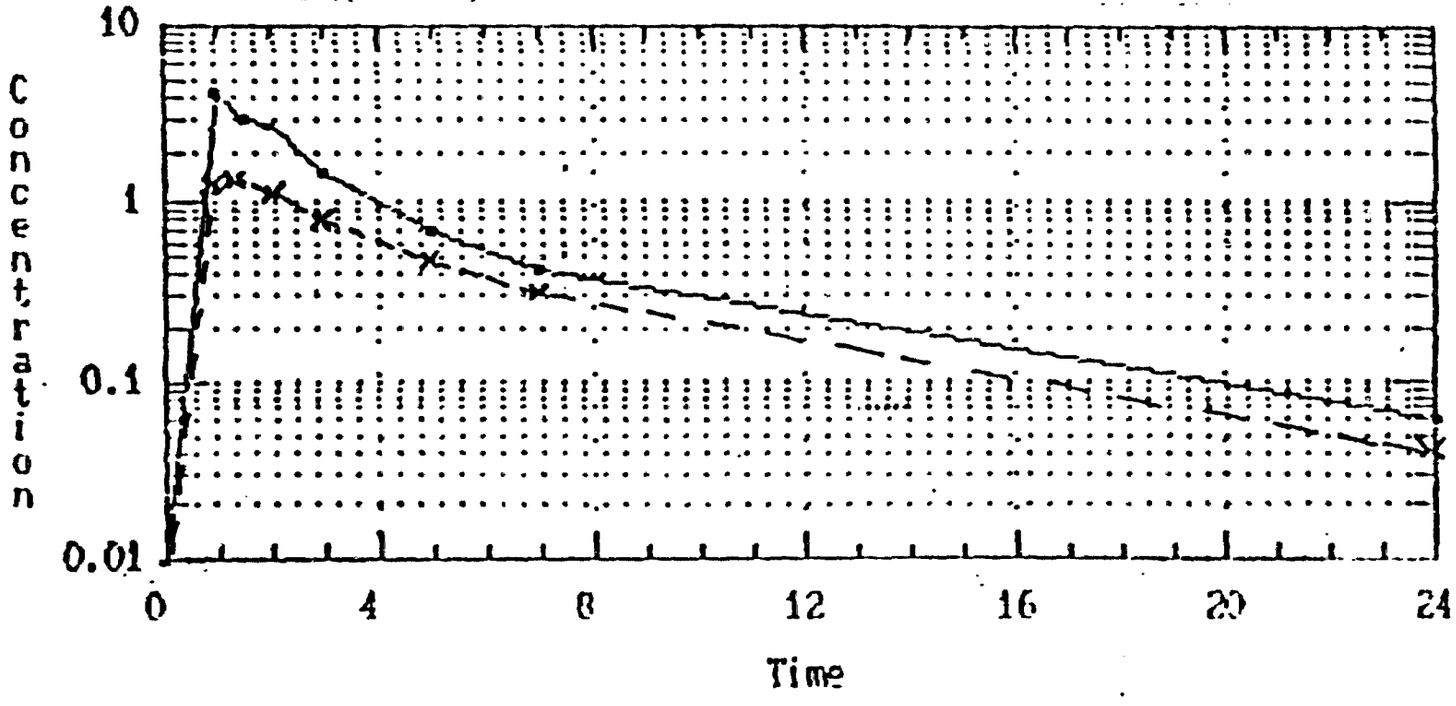


FIGURE1

FIGURE 1 : MEAN SEMILOGARITHMIC PLOT OF PLASMA NISOLDIPINE IN YOUNG AND ELDERLY SUBJECTS - DAY 1



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The effect of cirrhosis on the steady state pharmacokinetics of nisoldipine coat-core sustained release tablets.

Study #: D90-026-01 Volume: 1-49. Pages: 06-02-0220-0704.

Investigator:

Objectives:

The aim of the study is to:

1-to compare the pharmacokinetic profile of nisoldipine C.C. following a single dose 10 mg and multiple doses (10 mg qd for 7 days) in cirrhotic subjects vs a control group of healthy subjects.

2-to assess the general safety and tolerability of the above dose regimens in cirrhotic subjects vs healthy subjects.

Formulation:

-Nisoldipine 10 mg coat-core tablets batch # 526022.

Study Design:

This was a single center non randomized, non blinded study in which a total of 16 subjects participated (8 healthy subjects and 8 with mild to moderate hepatic impairment, 4 males and 4 females in each group). The study was conducted in 2 stages with a period of 8 days between stage I and stage II. In the first stage all subjects received a single 10 mg nisoldipine C.C. tablet. In stage II, each subject received 10 mg nisoldipine C.C. once a day for 7 days. Each healthy subject was to be matched to a subject in the hepatically impaired group with respect to sex age and weight (less than 10 % deviation was allowed).
Blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 hours post dose administration in stage I day 1 and stage II day 7.
In stage II day 1 blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 10, 12 and 16 hours. On days 7 of stage II an additional blood sample was collected at 72 hours.
Blood pressure and heart rate measurements were taken at the following times relative to dose: Stage I day 1 and stage II day 1 and day 7: predose, 1, 2, 3, 4, 6, 8, 10, 12, 16 and 24 hours. stage II, days 2-6, pre-dose, 4 and 8 hours.

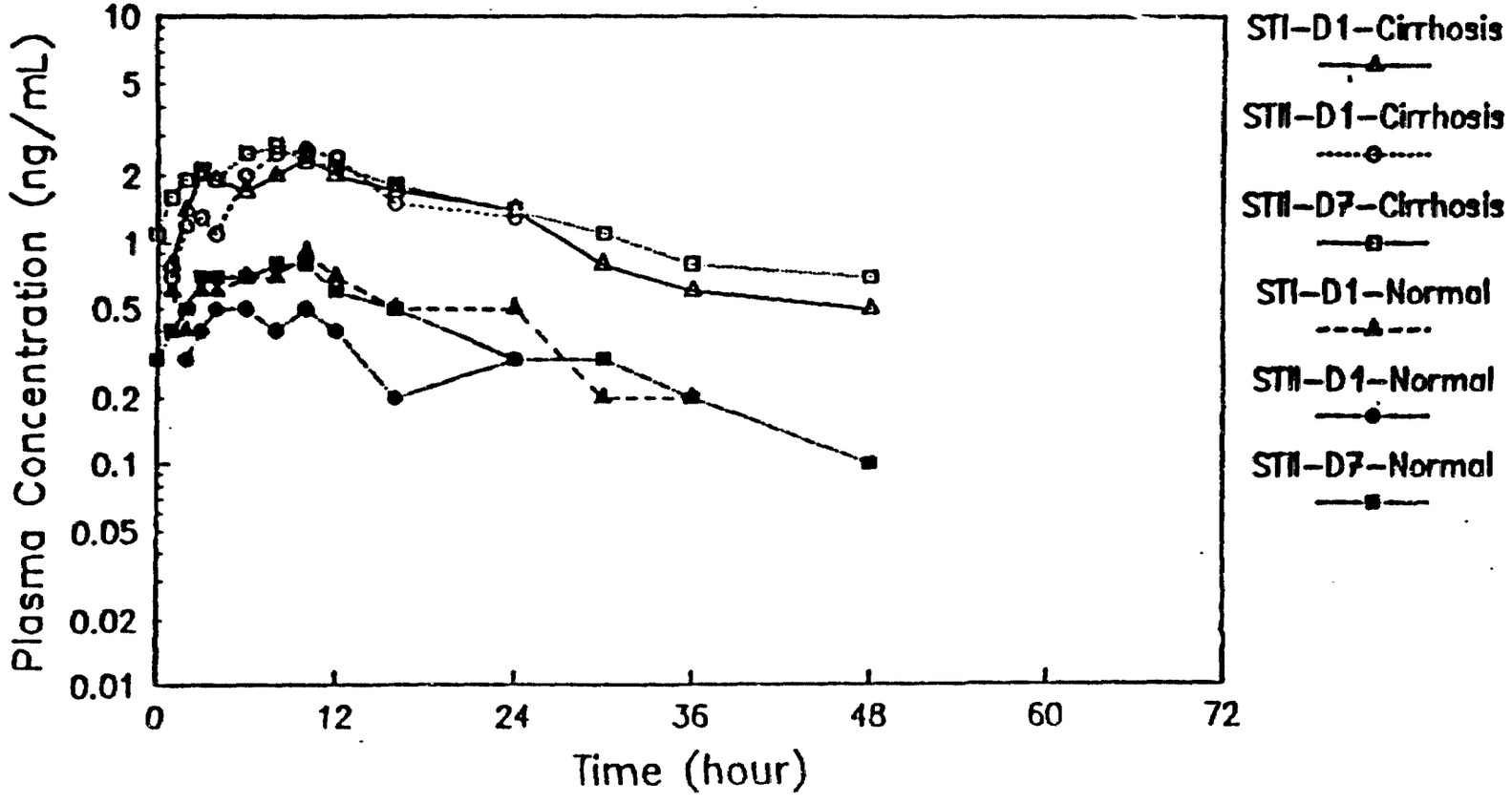
Results:

Figure 1 shows the means for the plasma concentration for both single and multiple dose administration of 10 mg nisoldipine C.C. in both the healthy volunteers and the liver impaired patients while Table 1 and 2 give a summary of the most important pharmacokinetic parameters for both stage I and II of this study.
The results show that there is an increase in both AUC and CMAX in the liver cirrhosis patients as compared to the healthy volunteers. This increase was almost three to four fold. Additionally, there was a slight nisoldipine accumulation in both the cirrhotic patients and the healthy volunteers since the accumulation ratio was 1.1 and 1.3 respectively.

Conclusion:

From the results of this study as shown in Table 1 and 2, liver impairment seems to have a pronounced effect on the pharmacokinetics of nisoldipine since both AUC and CMAX were increased four fold as compared to normal volunteers. Therefore extra care should be exercised when giving this drug to patients with impairment liver function, the initial dose should be lowered and the patient monitored to avoid any unwarranted side effects or toxicities.

Mean Plasma Nisoldipine Concentrations Following Single or Multiple Dose of 10 mg Coat Core Tablets in Normal and Cirrhotic Subjects



Mean Plot

Study D90-026-01

Table 1
Pharmacokinetic Parameters for Nisoldipine Following
A Single Dose of 10 mg Coat-Core Tablet-Stage I
[Arithmetic Mean (Coefficient of Variation)]

PARAMETERS (UNITS)	NORMAL (N=8)	CIRRHOTIC (N=8)
C_{max} (ng/mL)	1.03 (76)	2.89 (51)
T_{max} (hr)	8.13 (91)	7.25 (48)
AUC_{0-48} (ng·hr/mL)	19.0 (72)	58.2 (63)

Table 2
Pharmacokinetic Parameters Following Multiple Doses of
Nisoldipine 10 mg Coat-Core Given Once Daily for Seven Days
[Arithmetic Mean (Coefficient of Variation)]

PARAMETER (UNITS)	DAY	NORMAL (N=8)	CIRRHOTIC (N=8)
C_{max} (ng/mL)	1 7	0.73 (48) 0.94 (58)	2.98 (69) 3.62 (91)
T_{max} (hr)	1 7	6.5 (55) 4.6 (42)	10.0 (59) 6.9 (71)
AUC_{0-24} (ng·hr/mL)	1 7	7.55 (48) 11.9 (77)	39.9 (71) 46.7 (82)
AUC_{0-72} (ng·hr/mL)	7	17.7 (80)	76.3 (89)

Influence of renal function on the pharmacokinetics of nisoldipine C.C. tablets after single and multiple dosing.

STUDY #: D92-001 VOLUME: 1-50-52 PAGES: 06-02-797-2134.

INVESTIGATOR:

OBJECTIVES:

To determine the effect of impaired renal function on the pharmacokinetics of nisoldipine C.C. after single and multiple dosing.

FORMULATIONS:

-20 mg nisoldipine C.C. tablets batch # 524652.

STUDY DESIGN:

This was a multi-center, non-blinded, non randomized comparative study among four groups. 3 centers were to enrol 12 subjects each for a study total of 36 subjects. There were to have been 3 subjects per center in each of the 4 renal function groups. 27 seven subjects were to have renal impairment and 9 subjects were to serve as the control group. The 4 renal function groups were as follows:
-Group 1 (normal): 9 subjects with creatinine clearance of > 90 ml/min.
-Group 2 (mild): 9 subjects with creatinine clearance between 61 and 90 ml/min.
-Group 3 (moderate): 9 subjects with creatinine clearance between 30 and 60 ml/min.
-Group 4 (severe): 9 subjects with creatinine clearance of < 30 ml/min.
Patients on hemodialysis were not allowed to participate in the study.
On day 1 of the study, following an overnight fast, subjects were given a 20 mg dose of nisoldipine C.C. one hour prior to breakfast. Beginning on day 3, subjects were dosed once a day with 20 mg nisoldipine C.C. for 6 days. The final dose of nisoldipine was given the morning of day 8.
On day 1 and 8, blood samples were collected at: 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 16 hours. On day 2, samples were to be drawn at 20, 24, 28, 32 and 36 hours after the first dose of study drug. On day 3, a 48 hour post dose sample was drawn prior to the Day 3 dose. On day 5, a predose sample was drawn immediately before that morning dose. On day 9, samples were to be drawn at 20, 24, 28, 32 and 36 hours. On day 10, a 48 hour post dose sample was drawn. On day 1 and 2, urine was collected 0-12, 12-24, and 24-36 hours post-dose. Beginning on day 8, urine was collected 0-12, 12-24 and 24-36 hours post dose.

DATA ANALYSIS:

Data analysis was performed using standard pharmacokinetic techniques.

RESULTS:

Table 1 summarizes some of the pharmacokinetic parameters of nisoldipine after single and multiple dose administration in the 4 groups of patients while Figure 1 and Figure 2 show the corresponding plasma profiles for all 4 groups on day 1 and day 8 respectively. It can be seen from the results that the plasma concentrations of nisoldipine in patients with severe renal impairment (group 4) are slightly higher than the control group. Figure 3 and 4 give the corresponding plasma concentrations for . These plots indicate that the plasma concentrations on day 1 of this metabolite were significantly higher in the subjects with moderate and severe renal failure. However on day 8 there were no significant differences between the 4 groups even though the levels were still higher in the moderate to severe renal impairment group.

Figure 5 and 6 shows the plasma concentrations of metabolite I which is the most abundant metabolite with plasma concentrations ten times the parent compound while table 2 summarizes the most important pharmacokinetic parameters. The same trend of results was observed as with metabolite with the moderate to severe renal impairment patients showing much higher plasma concentrations on day 1 that the control group with the differences being less pronounced on day 8.

Figure 7 and 8 show the mean plasma concentration of metabolite the only active metabolite of nisoldipine (10 times less active than the parent drug) and is the least abundant

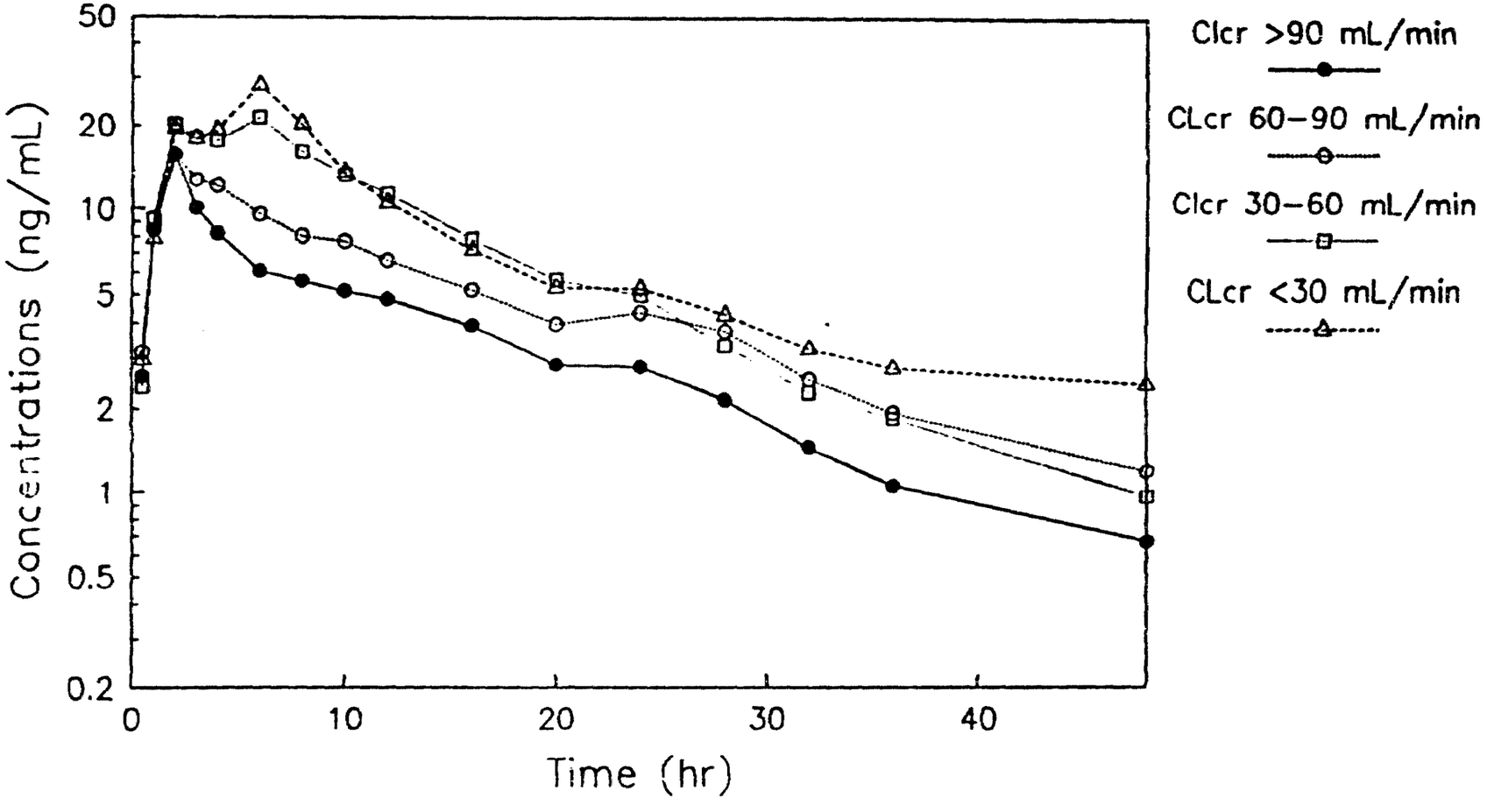
among the metabolites. Table 3 summarizes the major pharmacokinetic parameters for this metabolite. The results show that the moderately impaired patients but not the severely impaired, had modestly higher AUC and CMAX relative to the normal subjects. However these differences tended to disappear on day 8.

Conclusion:

In patients with severe renal impairment (creatinine clearance less than 30 ml/min, the nisoldipine plasma concentrations were higher by as much as 2 fold than the control group, however this difference seem to have subsided by day 8. Therefore, renal impairment does not seem to significantly alter the pharmacokinetics of nisoldipine and its metabolites. Patients should be closely monitored and titration to higher doses should be based on clinical response.

FIGURE 5

Plasma Concentrations For Following Single Dose of 20 mg Nisoldipine Coat-Core Tablet

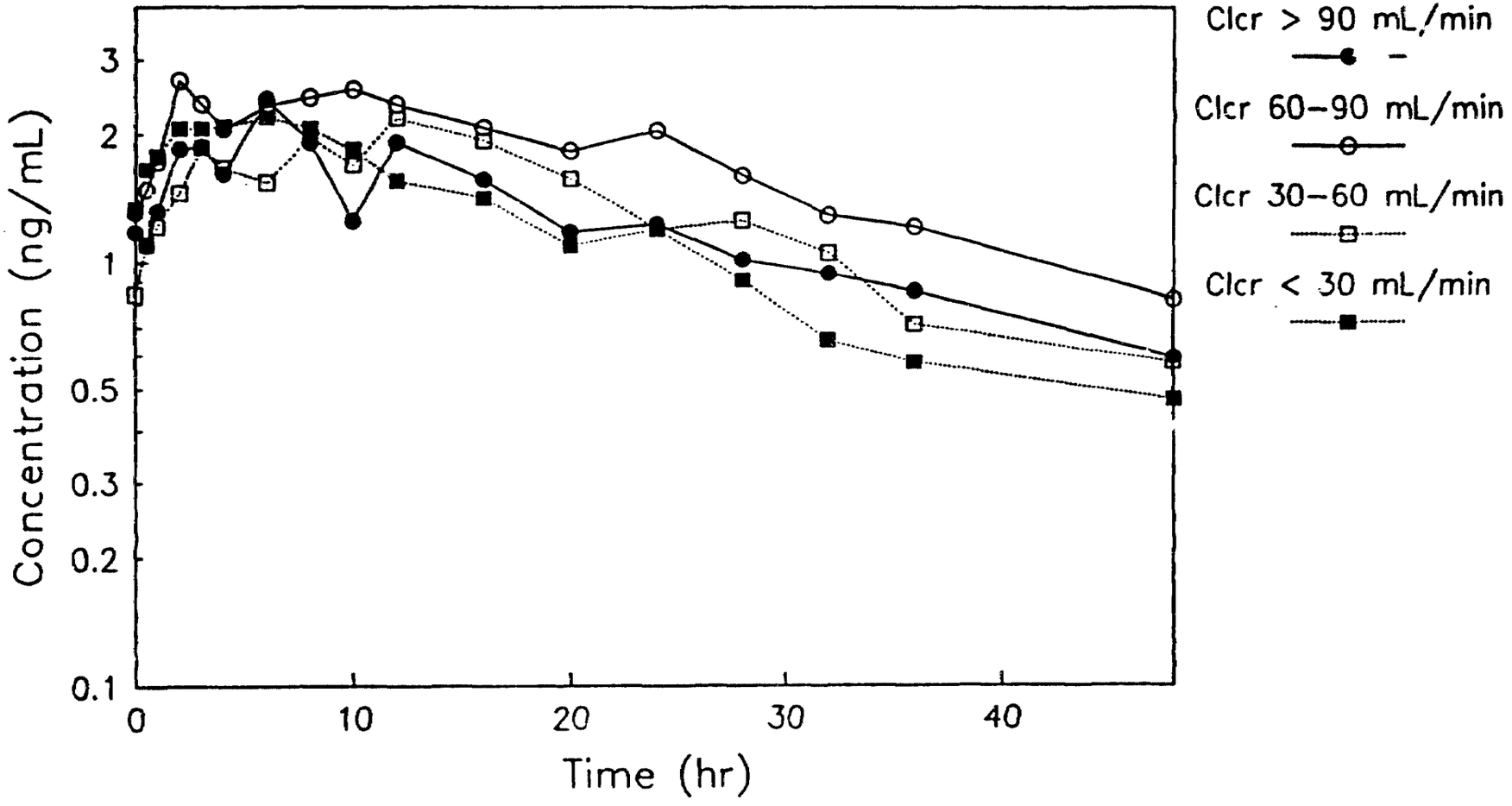


D92-001
FIGURE 5

Mean Plot
Day 1

FIGURE 2

Mean Plasma Concentrations For Nisoldipine Following a 20 mg Coat-Core Tablet Once Daily For Eight Days



Mean Plot
Day 8

Study D92-001
FIGURE 2

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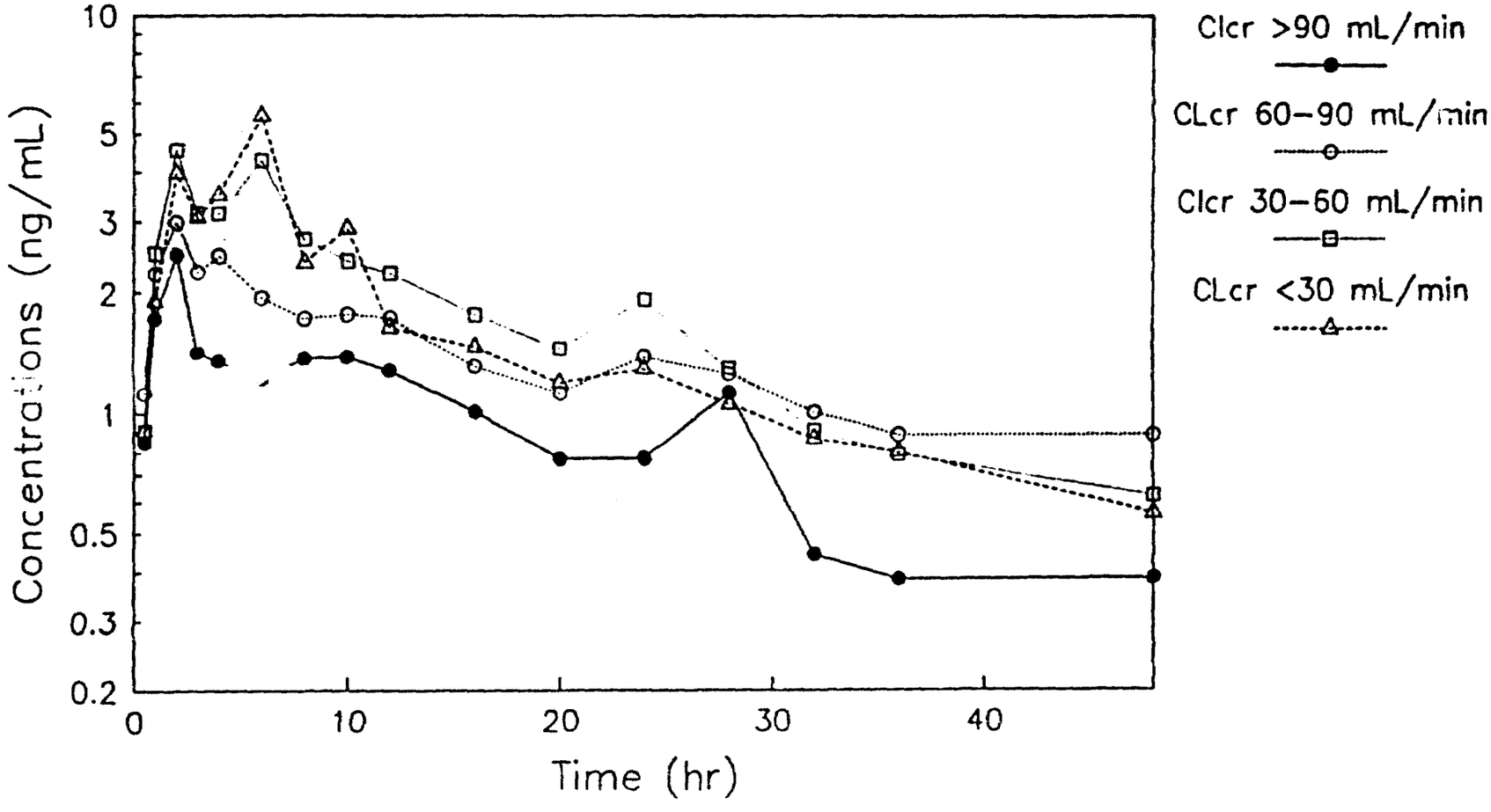
FIGURE 3

Plasma Concentrations For Following a Single Dose of 20 mg Nisoldipine Coat-Core Tablet

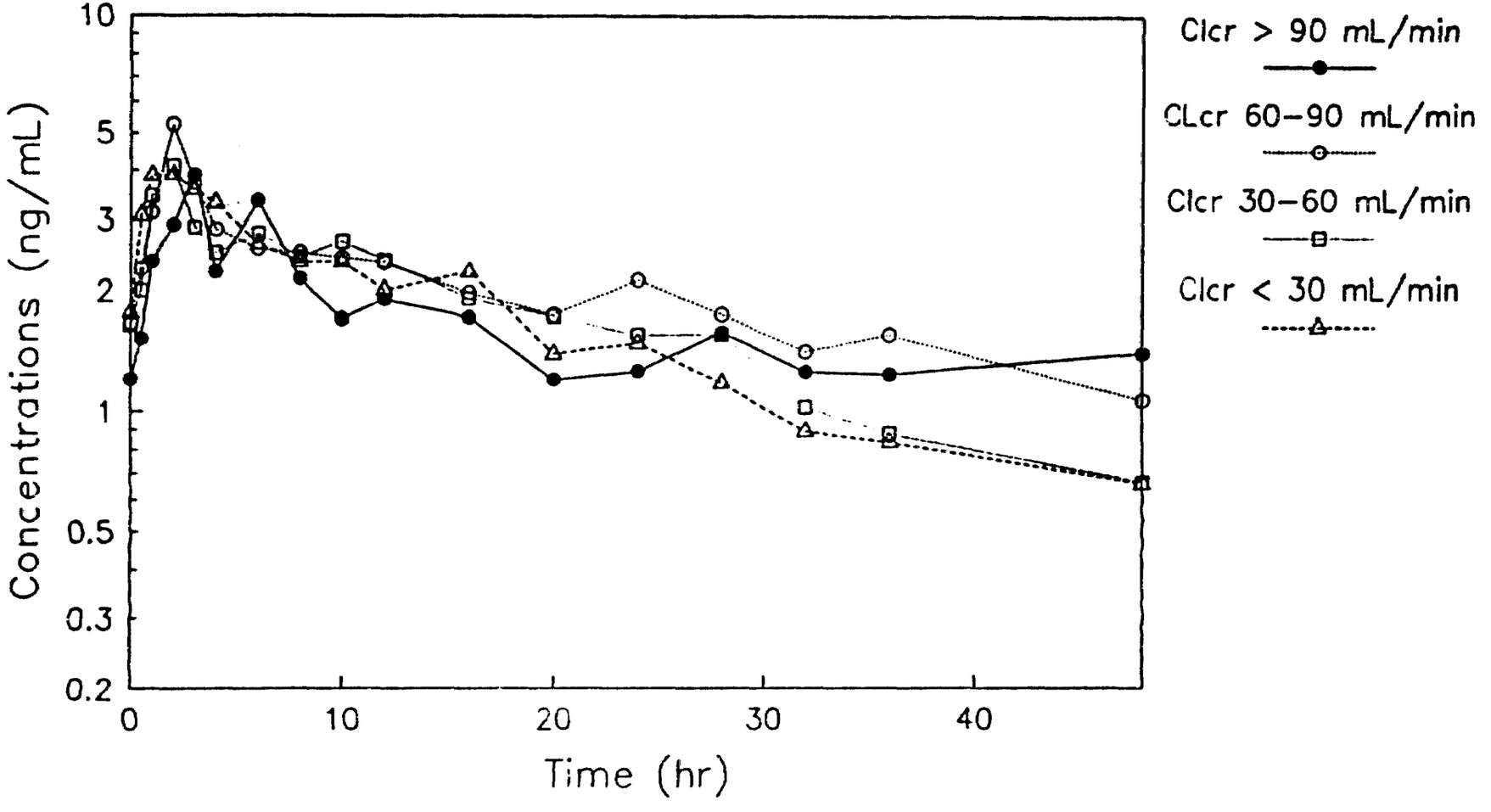


Mean Plot
Day 1

D92-001
FIGURE 3

FIGURE 4

Plasma Concentrations For Following a Single Daily Dose of 20 mg Nisoldipine Coat-Core Tablet For Eight Days

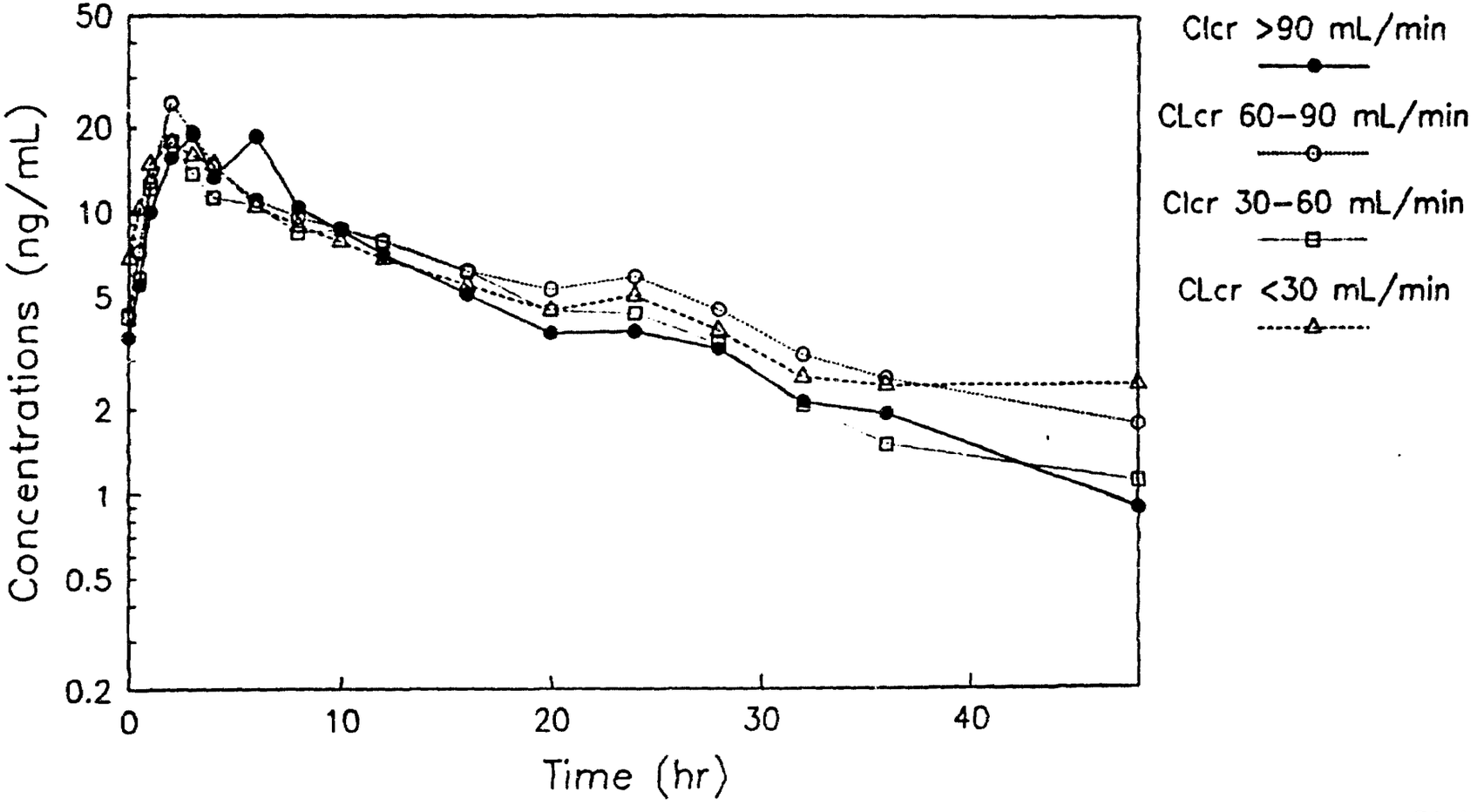


Mean Plot
Day 8

D92-001
FIGURE 4

FIGURE 5

Plasma Concentrations For Following
Single Dose of 20 mg Nisoldipine
Coat-Core Tablet

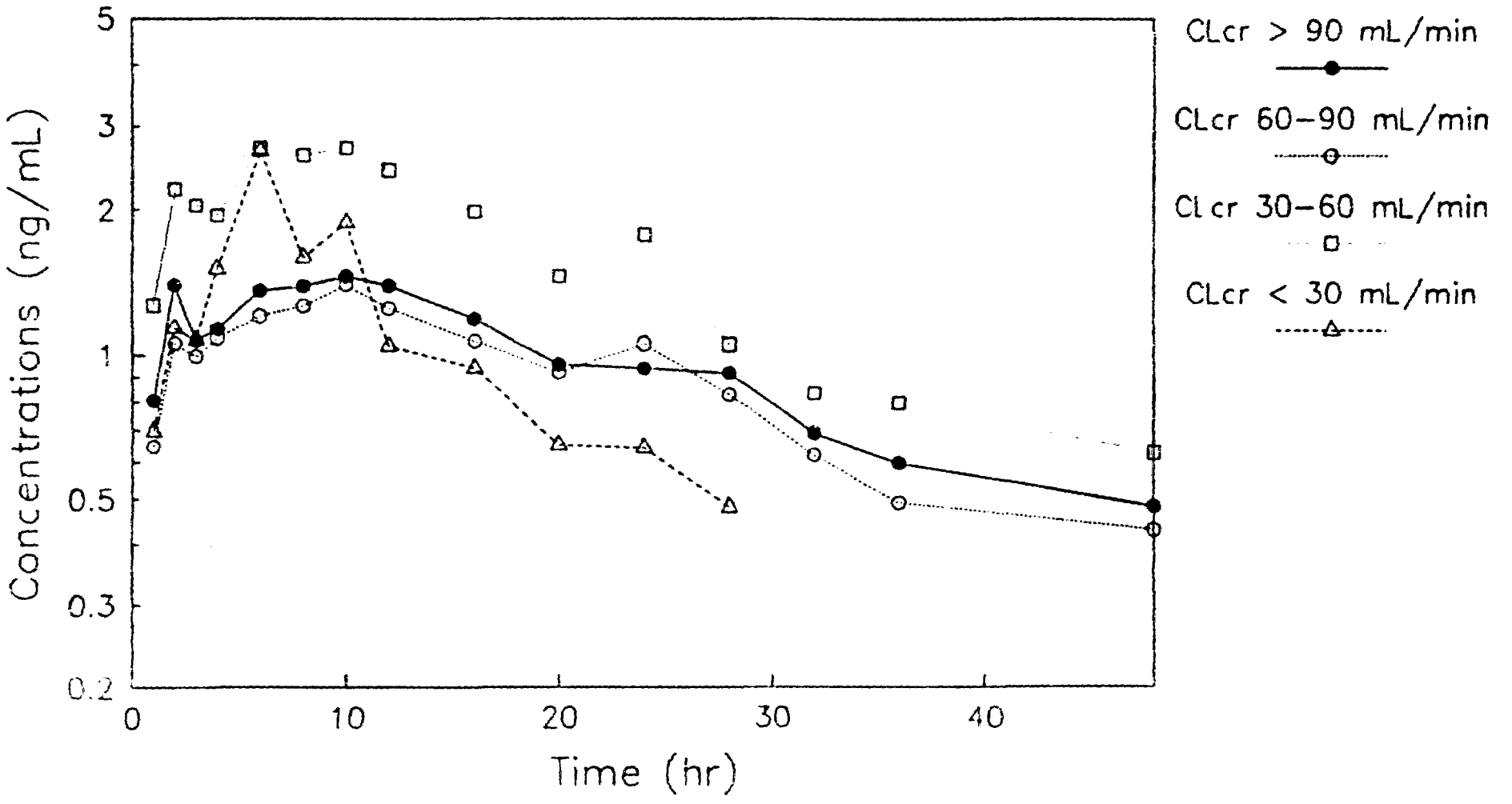


Mean Plot
Day 8

D92-001
FIGURE 6

FIGURE 6

Plasma Concentrations For Following a Single Dose of 20 mg Nisoldipine Coat-Core Tablet



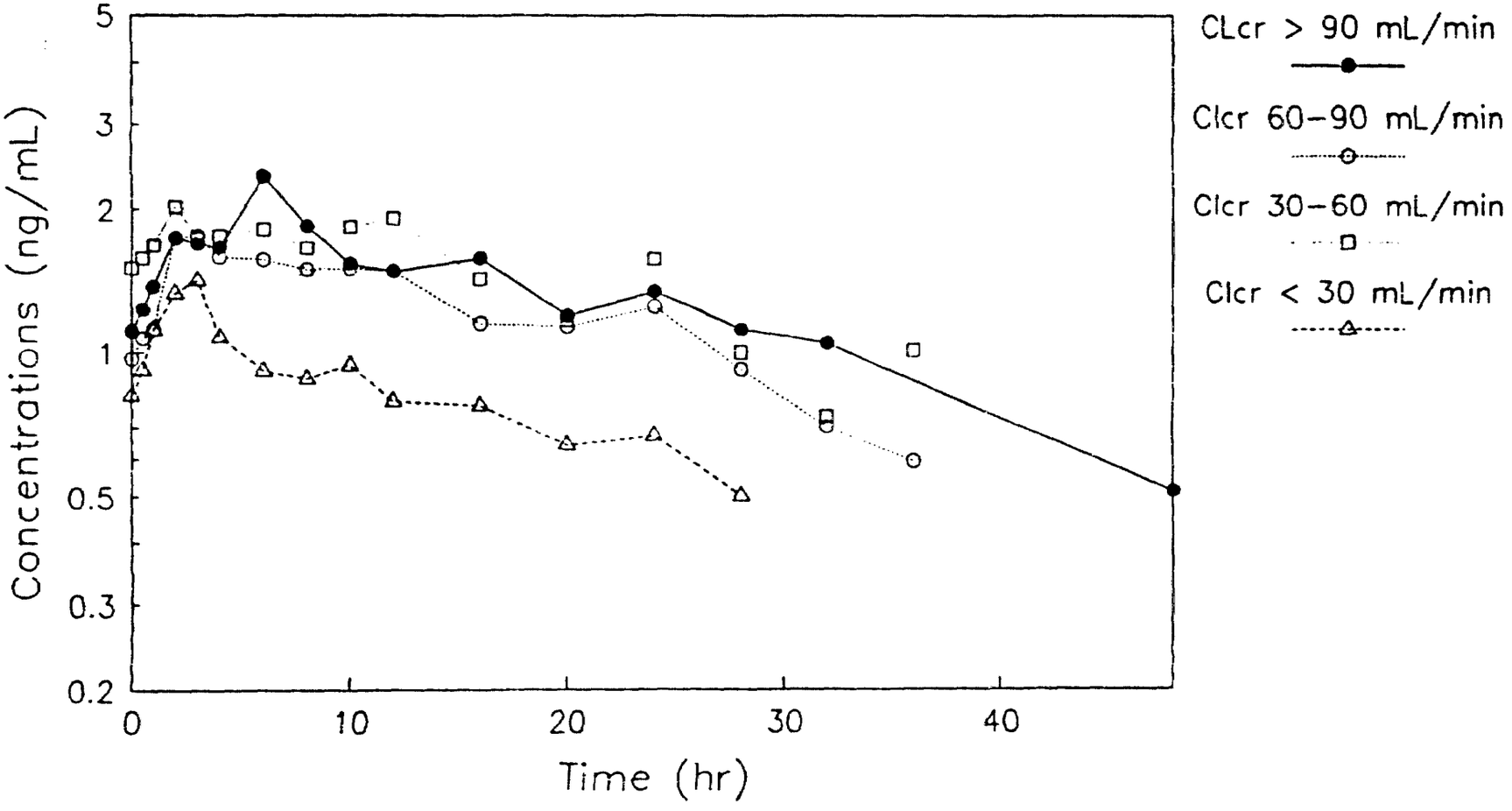
Mean Plot
Day 1

D92-001

FIGURE 7

FIGURE 7

Plasma Concentrations For Following a Single Daily Dose of 20 mg Nisoldipine Coat-Core Tablet For Eight Days



Mean Plot
Day 8

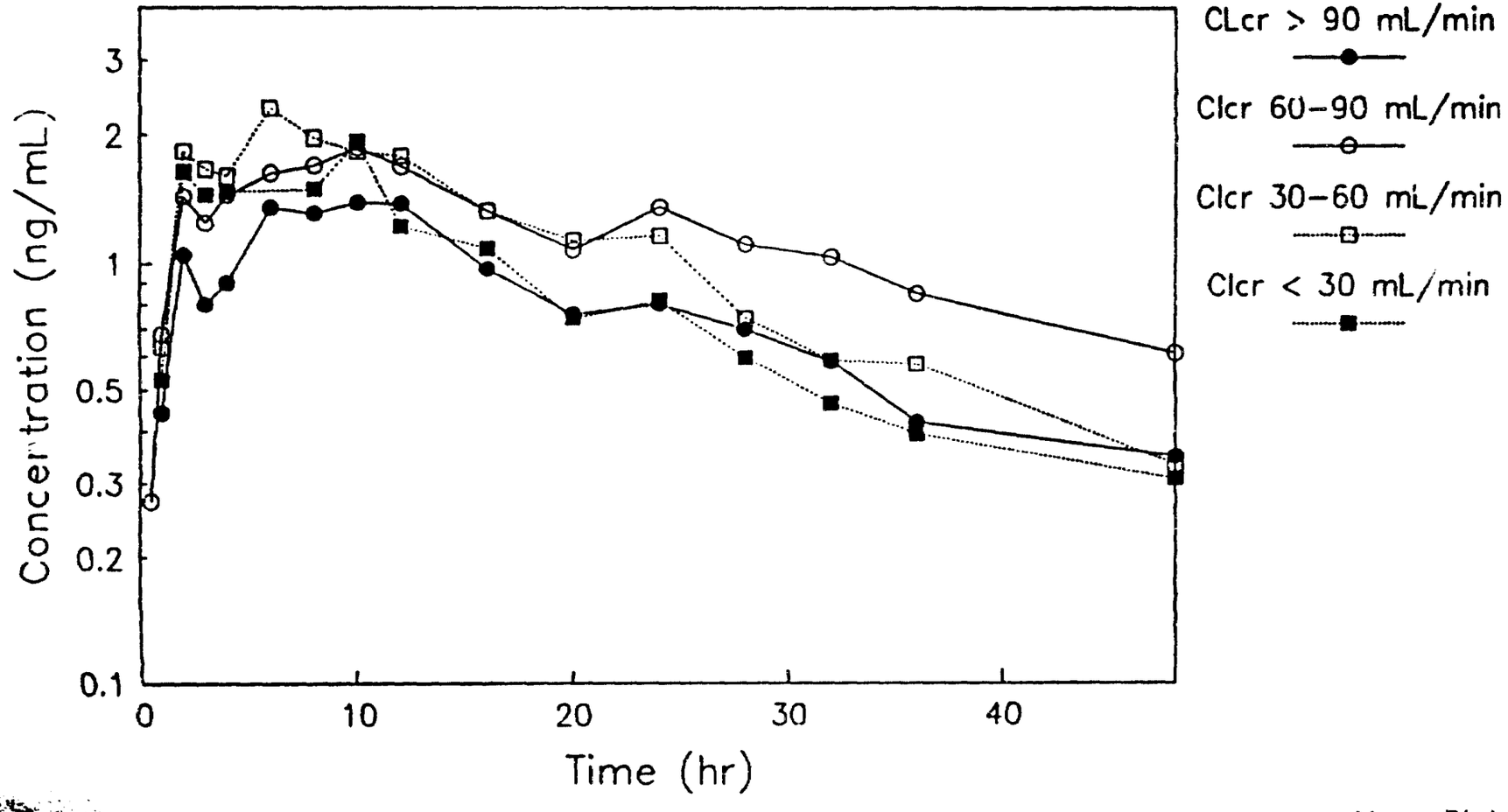
92-001
GURE 8

135

000711

FIGURE 8

Mean Plasma Concentrations For Nisoldipine Following a Single 20 mg Coat-Core Tablet



Mean Plot
Day 1

Study D92-001
FIGURE 1

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TABLE 1

Geometric Means (Geometric Standard Deviations)

	GROUP 1	GROUP 2	GROUP 3	GROUP 4
DAY 1 AUC (ng-h/ml)	20.7 (1.95)	29.0 (1.74)	30.1 (2.00)	25.0 (2.19)
DAY 8 AUC (ng-h/ml)	30.4 (2.28)	41.7 (2.00)	34.6 (1.63)	38.6 (1.74)
DAY 1 C_{max} (ng/ml)	1.5 (1.75)	2.0 (1.94)	2.2 (2.07)	2.0 (2.28)
DAY 8 C_{max} (ng/ml)	2.4 (2.36)	2.7 (1.83)	2.2 (1.71)	2.6 (1.84)
DAY 1 C_{av}* (ng/ml)	0.86 (1.95)	1.21 (1.74)	1.25 (2.00)	1.04 (2.19)
DAY 8 C_{av} (ng/ml)	1.27 (2.28)	1.74 (2.00)	1.44 (1.63)	1.61 (1.74)
24HR ACCUMULATION RATIO**	1.47 (1.76)	1.49 (1.46)	1.15 (1.83)	1.54 (1.27)
AUC Linear Accumulation+	1.08 (1.77)	0.95 (1.34)	0.80 (1.76)	1.07 (1.19)

* C_{av} = AUC/24 hours

** $AUC_{DAY\ 8}/AUC_{DAY\ 1}$

\+ $AUC_{0\text{-}24,\ DAY\ 8}/AUC_{0\text{-}tn,\ DAY\ 1}$; tn = time of last observed concentration

TABLE 2

VARIABLES	GROUP 1	GROUP 2	GROUP 3	GROUP 4
DAY 1 C_{max}*	16.7	15.8	25.1	28.2
DAY 8 C_{max}*	19.8	22.3	22.6	26.6
DAY 1 C_{max} ratio**		0.94 (0.59-1.49)	1.50 (0.90-2.49)	1.68 (0.95-2.98)
DAY 8 C_{max} ratio**		1.13 (0.76-1.68)	1.14 (0.74-1.78)	1.35 (0.82-2.20)
DAY 1 AUC_{norm}*	590.9	573.3	987.2	1029.8
DAY 8 AUC_{norm}*	758.0	701.6	759.6	999.9
DAY 1 AUC ratio**		0.97 (0.64-1.48)	1.67 (1.05-2.66)	1.74 (1.03-2.94)
DAY 8 AUC ratio**		0.93 (0.61-1.41)	1.00 (0.63-1.59)	1.32 (0.79-2.20)

* Geometric least squares means

** Ratios are values for a given group divided by Group 1 value; parentheses contain 90% Conf. interval

TABLE 3

9.4.3

This metabolite, the only active metabolite of nisoldipine, is approximately 10 times less active than the parent drug.[8] the least abundant of the metabolites, also appeared to be the least influenced by renal impairment. The data are presented in Appendix 13.8.4 and summarized below.

VARIABLES	GROUP 1	GROUP 2	GROUP 3	GROUP 4
DAY 1 C_{max}*	1.73	1.56	3.16	1.60
DAY 8 C_{max}*	2.63	1.81	2.64	1.51
DAY 1 C_{max} ratio**		0.90 (0.57-1.41)	1.82 (1.10-3.01)	1.04 (0.59-1.82)
DAY 8 C_{max} ratio**		0.69 (0.46-1.03)	1.00 (0.64-1.58)	0.58 (0.35-0.95)
DAY 1 AUC_{norm}*	117.4	87.6	172.7	85.1
DAY 8 AUC_{norm}*	143.7	102.5	143.6	79.3
DAY 1 AUC ratio**		0.75 (0.47-1.18)	1.47 (0.89-2.44)	0.72 (0.41-1.28)
DAY 8 AUC ratio**		0.71 (0.44-1.16)	1.00 (0.58-1.71)	0.55 (0.30-1.00)

* Geometric least squares means

** Ratios are values for a given group divided by Group 1 value; parentheses contain 90% Conf. interval

A randomized double blind, placebo controlled study to investigate the possible influence of nisoldipine on quinidine plasma levels.

STUDY: 384. VOLUME: 1-53 PAGES: 06-02-2199-2264.

INVESTIGATOR:

OBJECTIVES:

1-To assess the possible influence of nisoldipine on quinidine plasma levels in African and Caucasian volunteers.

FORMULATIONS:

-nisoldipine 10 mg tablets, batch # 929488, expiration date 11-18 19[illegible]
-nisoldipine placebo tablets, batch # 929270, expiration date 09-15 1986.
-quinidine bisulphate 250 mg tablets, Astra batch # KA1089, expiration date 2-1989.

STUDY DESIGN:

6 healthy male volunteers between the ages of 21 and 65 years (3 Caucasians and 3 Africans) participated in this randomized, double-blind, placebo controlled cross-over study, comparing the pharmacokinetic profile of quinidine bisulphate when on nisoldipine therapy 10 mg bid for seven days, with the pharmacokinetic profile when on placebo. Each subject received 2x250 mg quinidine twice a day on days 5 and 6 and once on day 7 of the study. There was a one week washout period between treatments.
Blood samples were collected at 0, 30, 60 and 90 minutes and 2, 3, 4, 6, 8, 10 and 24 hours after drug administration.

RESULTS:

Figure 1 shows the mean quinidine plasma concentrations with and without nisoldipine

coadministration while Table 1 and 2 summarize the most important pharmacokinetic parameters. The results show that only quinidine's AUC was increased upon coadministration of nisoldipine. This increase was in the order of 25 % and was statistically significant. No significant differences on quinidine CMAX, TMAX and MRT were observed.

CONCLUSION:

Coadministration of nisoldipine with quinidine, seems to increase quinidine plasma levels since the AUC increased by 25 %. However, the effect of quinidine on nisoldipine pharmacokinetics could not be measured since the dihydropyridine plasma concentrations were not measured.

TABLE 1

PHARMACOKINETIC DATA

AUC (0-24 hr) ng/ml hr

Volunteer No	PLACEBO		NISOLDIPINE
1			
2			
3			
4			
5			
6			
$\bar{X}$	17940.46		22637.19
SD	4823.01		6091.82
SE	1968.98		2486.99
t		2.66	
p		p<0.05	

AUC (0 - 10 hr) ng/ml hr

Volunteer No	PLACEBO		NISOLDIPINE
1			
2			
3			
4			
5			
6			
$\bar{X}$	14801.25		17348.21
SD	3339.94		5384.49
SE	1363.53		2198.21
t		1.13	
p		ns	

AUC (0-∞) ng/ml hr

1			
2			
3			
4			
5			
6			
$\bar{X}$	22420.59		28033.68
SD	6667.34		9546.75
SE	2731.93		3897.45
t		1.92	
p		ns	

TABLE 2

PHARMACOKINETIC DATA

MRT (mean resonance time) hr

1			
2			
3			
4			
5			
6			
$\bar{X}$	14.32		14.86
SD	4.54		3.67
SE	1.85		1.50
t		0.27	
p		ns	

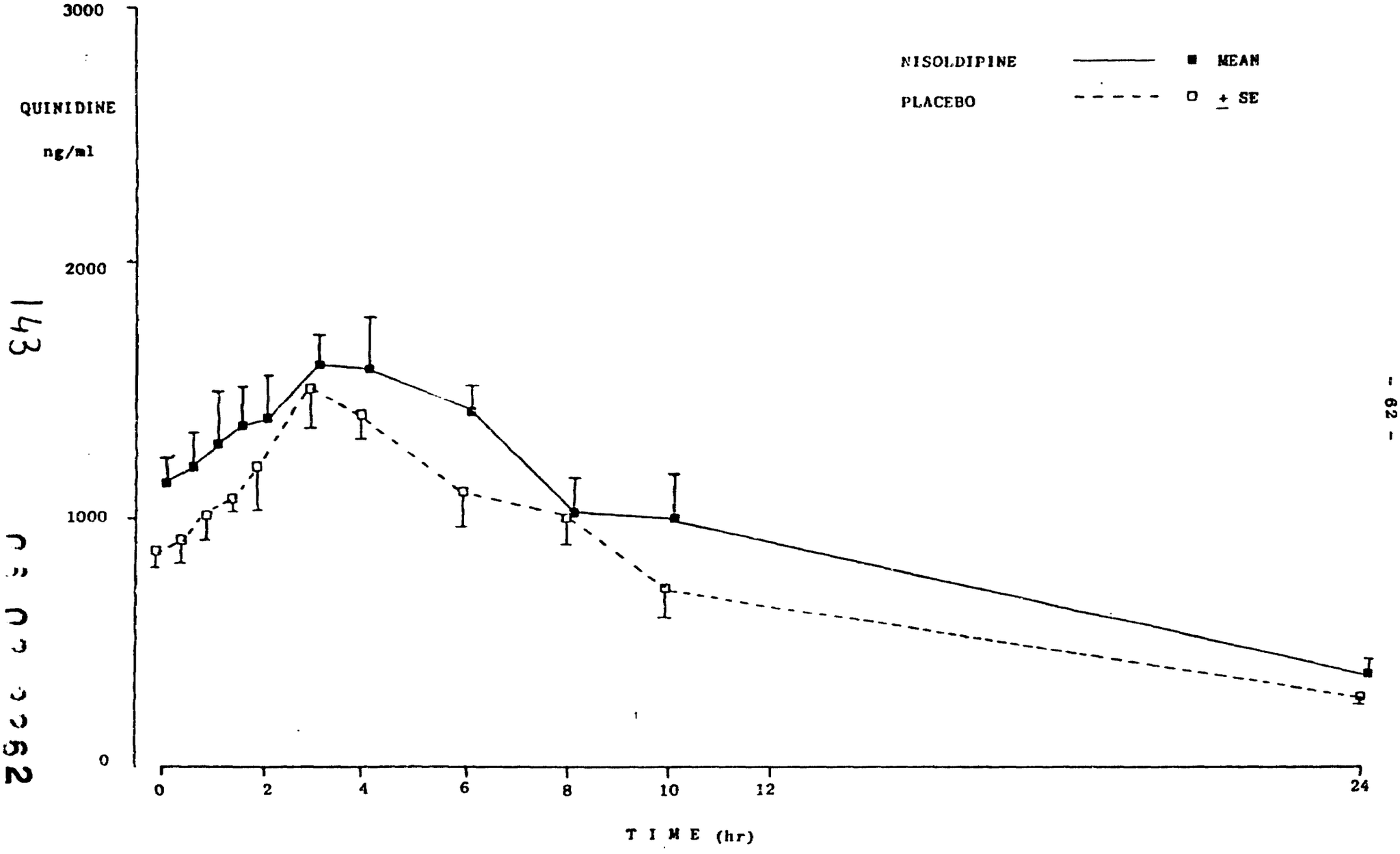
C MAX ng

1			
2			
3			
4			
5			
6			
$\bar{X}$	1703.67		1814.00
SD	459.64		362.75
SE	187.65		148.09
t		0.78	
p		ns	

T MAX hr

1			
2			
3			
4			
5			
6			
$\bar{X}$	3.33		3.17
SD	0.82		1.17
SE	0.33		0.48
t		-0.31	
p		ns	

FIG 1


3000
2000
1000
0
QUINIDINE
ng/ml
NISOLDIPINE
PLACEBO
MEAN
± SE
0
2
4
6
8
10
12
24
T I M E (hr)

To investigate the existence of a possible interaction between nisoldipine and warfarin.

Study #: 349. VOLUME: 1-53 PAGES: 6-02-2265-2367.

INVESTIGATOR:

OBJECTIVES:

To investigate whether nisoldipine alters the steady state total plasma levels of warfarin and to examine the effects of nisoldipine on the clotting profile of warfarin treated patients.

FORMULATIONS:

-Nisoldipine 10 mg tablets batch # 929488 expiration date November 11, 1986.
-Nisoldipine placebo.
-Warfarin (Marevan^{R}) batch # b/225.

STUDY DESIGN:

This was a placebo-controlled, randomized, double blind group comparison study in which 16 patients between the ages of 21 and 65 years who were receiving 3 to 10 mg warfarin following myocardial infarction or valve replacement.
After a placebo run in period of 7 days, the group of 16 patients was divided into 2 groups of 8 each. 1 group received 10 mg nisoldipine bid and the other group received placebo tablets bid for 21 days. Warfarin was continued at the steady-state throughout the trial.
10 ml blood samples were collected before and two hours after medication.
Clotting profiles were obtained on the following days: -7, -5, -3, 1, 3, 7, 14 and 21.

RESULTS:

Figure 1 shows the warfarin plasma concentrations for the 2 groups while Figure 2 shows the clotting profile for the nisoldipine and control group. Table 1 summarizes the mean clotting parameters as well as the mean of the 2 plasma warfarin concentrations (b1 which was before drug administration and b2 which was 2 hours post dose).

Conclusion:

The results show that the anticoagulant effect as well as the steady-state warfarin plasma concentrations were not affected by concomitant administration of a 10 mg nisoldipine IR tablet administered bid for 21 days.

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TABLE I

Warfarin Treatment

Parameter	Placebo Mean	Nisoldipine Mean	
PA(%)	12,8	8,7	NS
PT (Sec)	19,7	21,3	NS
PTT (Sec)	40,1	46,2	*S
Warfarin (B1) (ug/mℓ)	1,38	1,29	NS
Warfarin (B2) (ug/mℓ)	1,75	1,67	NS

* After baseline correction the difference for PTT was also not significant)

11.2.2 Analysis of variance to compare the mean baseline values with each of the mean values obtained as from Day 1 in a within-treatment comparison for all variables described in 11.1.1.

Clotting Profile

Warfarin-Placebo Treatment

Time	PA% Mean		PT(sec) Mean		PTT(sec) Mean	
Baseline	11,3	-	20,7	-	42,3	-
Day 1	12,9	NS	20,1	NS	40,3	NS
Day 3	12,0	NS	20,5	NS	39,5	NS
Day 7	11,8	NS	20,1	NS	43,5	NS
Day 14	13,1	NS	19,3	S	40,3	NS
Day 21	14,3	S	18,5	S	37,0	S

NS : Not Significant

S : Significant

FIGURE 1

UOFS 5/84

Annexure 6 (a)

WARFÁRIN PLASMA CONCENTRATION

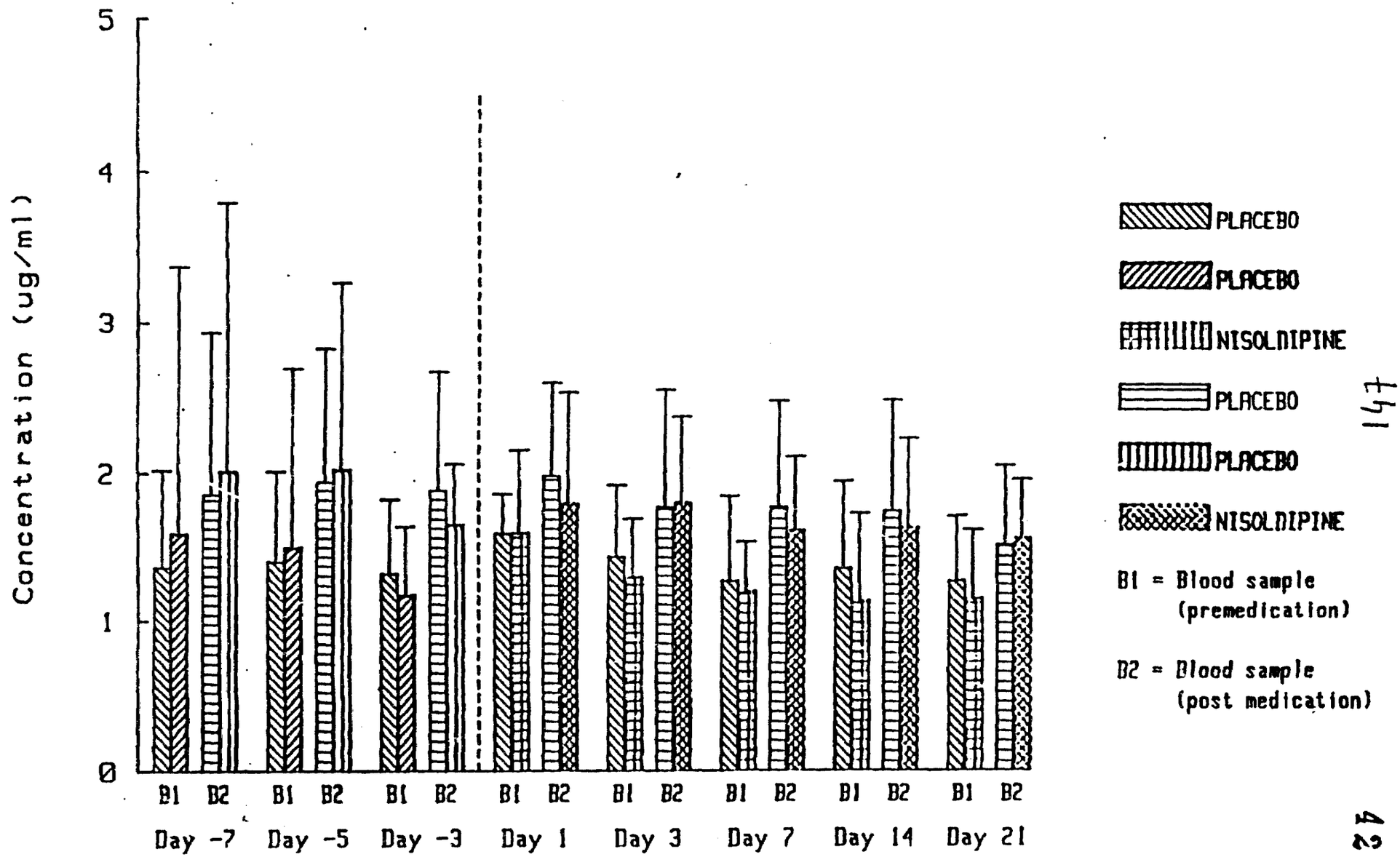
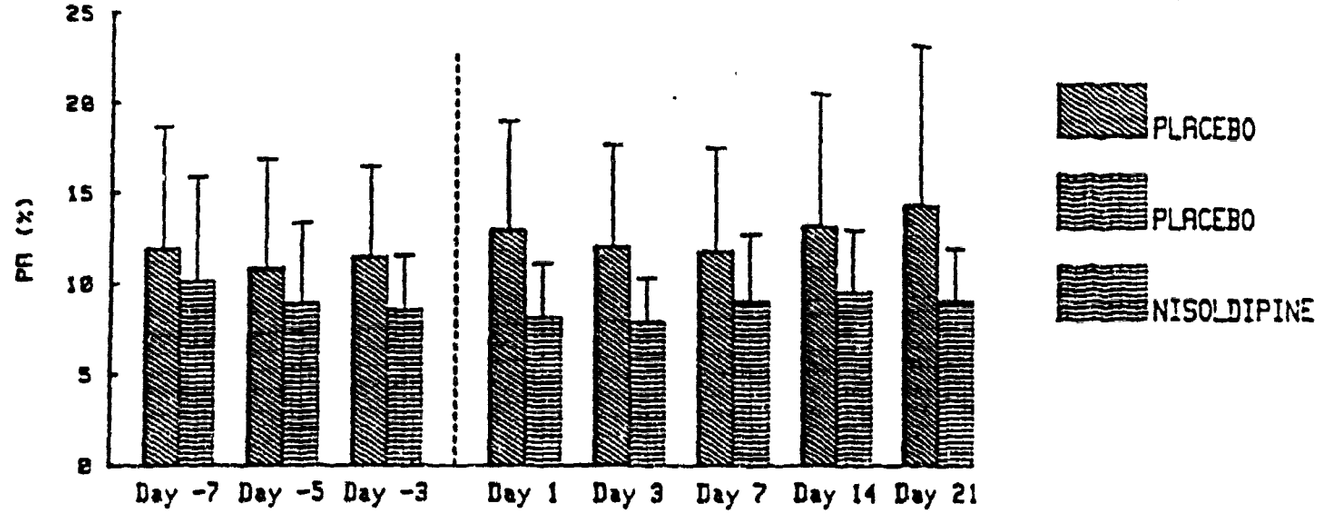
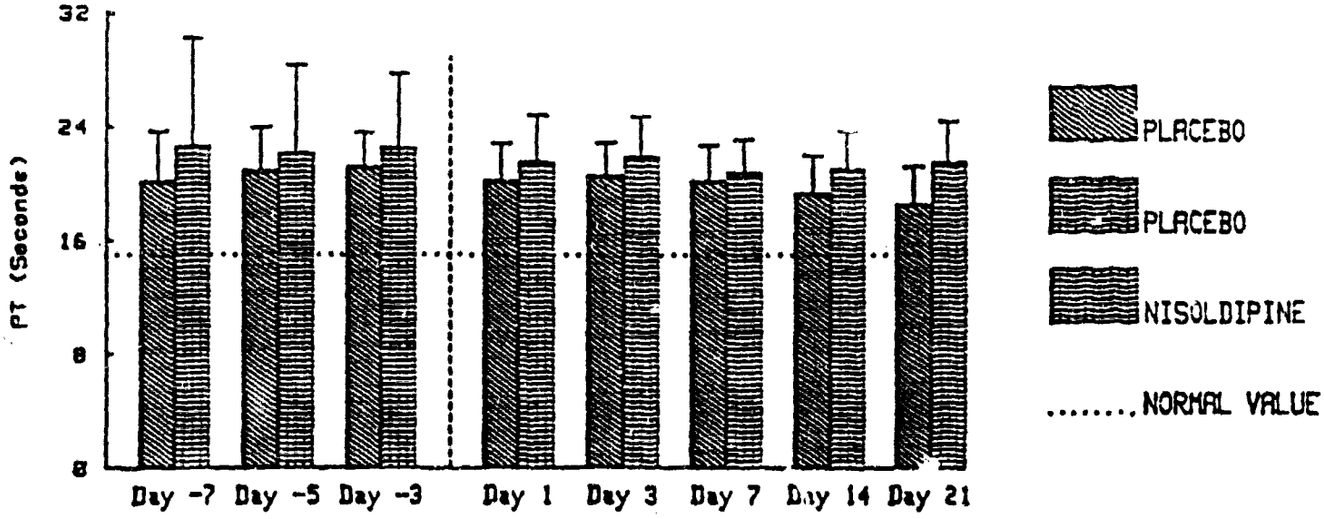


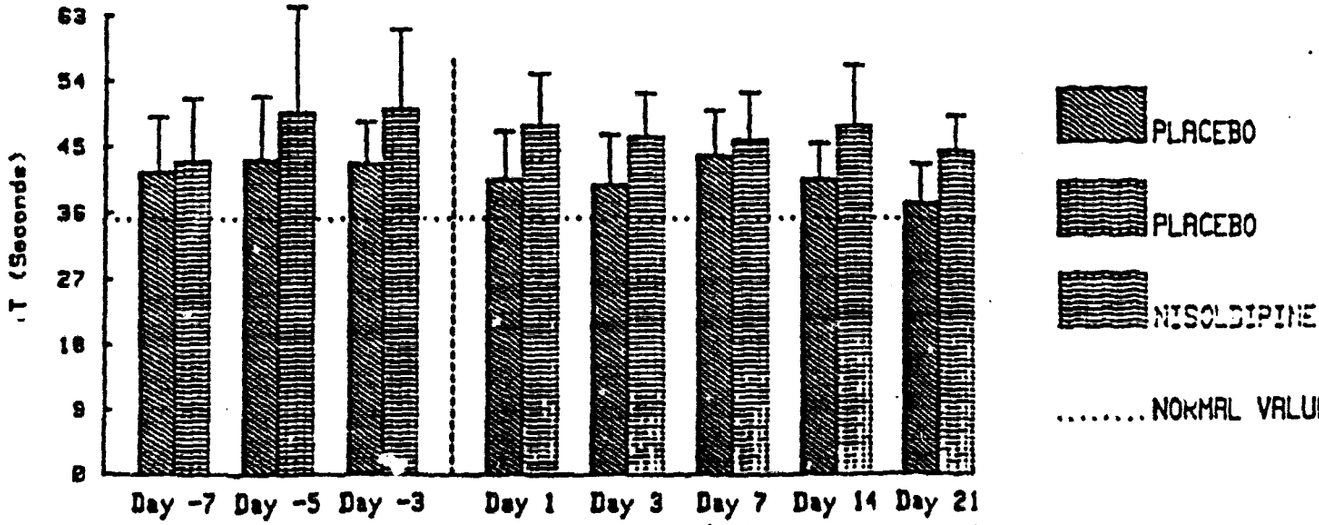
FIGURE 2

Annexure 6 (b)

UOFS 5/84
CLOTTING PROFILE







Comparison of pharmacokinetics and tolerability of nisoldipine C.C. given in combination with placebo, cimetidine or ranitidine.

STUDY #: 738. VOLUME: 1-53-54 PAGES: 6-02-2368-2961

INVESTIGATORS:

OBJECTIVES:

To investigate the pharmacokinetic drug/drug interaction and tolerability of nisoldipine C.C. given in combination with placebo, cimetidine, or ranitidine respectively.

FORMULATION:

-Nisoldipine 20 mg C.C. tablets (batch #: 523341/9).
-400 mg Tagamet^{R} tablet batch # 888/90H30.
-150 mg Zantac^{R} tablet batch # ON 448.
-0.5 mg placebo tablet containing lactose, corn starch and microcrystalline cellulose, batch # 523 196/20.

STUDY DESIGN:

12 healthy male volunteers between the ages of 24 to 35 years participated in this randomized complete block design. Each subject received one of the following treatments:
-treatment b1: 400 mg Tagamet bid for 6 days (days 1-6).
-treatment b2: 150 mg Zantac bid for 6 days.
-treatment b3: 0.5 gm placebo administered for 6 days.
Single oral doses of 20 mg Nisoldipine C.C. was administered on study day 5 of each period. There was a one week washout period between treatments.
6 ml blood samples were taken starting on day 5 of each study period at the following times post dose administration: 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 14, 16, 20, 24, 28, 32, 36 and 48 h. Further blood samples were drawn on study days 1, 4, 5 predose to determine trough levels of cimetidine or ranitidine and on study days 1 and 42 hours post administration for peak levels.

RESULTS:

Figure 1 shows the geometric mean plasma concentration for nisoldipine alone, with cimetidine and with ranitidine while Table 4 to 6 summarize the main pharmacokinetic parameters for nisoldipine for the 3 different treatments. Table 7 shows the geometric mean peak and trough concentrations for cimetidine and ranitidine.
The results show that cimetidine had a more pronounced effect on the pharmacokinetics of nisoldipine as compared to ranitidine. Cimetidine increased nisoldipine's AUC by 55 % and CMAX by 65 % while it decreased TMAX from 9.11 to 4.25 hours. On the other hand, ranitidine caused a small but statistically significant decrease in The AUC (12 %) and CMAX (15 %) of nisoldipine compared to placebo.

Conclusion:

Cimetidine seem to have a pronounced effect on nisoldipine pharmacokinetic parameters (since there was more than 50 % increase in some parameters of interest). Therefore great caution should be exercised when both of these drugs are administered concomitantly, the patients should be monitored and dose adjustments made as necessary. However, the interaction with ranitidine seem to be small enough and therefore is not expected to have any clinical significance.

TABLE 1

Nominal Concentration [µg/l]	Day 1	Day 2	Day 3	mean [µg/l]	SD	CV [%]	$Q^x \frac{\text{actual}}{\text{nominal}}$
19.60	21.14	20.95	20.98	21.01	0.102	0.48	1.07
7.37	7.96	7.64	7.50	7.70	0.230	3.06	1.04
0.49	0.46	0.45	0.45	0.45	0.005	1.28	0.91

Table 3: Data for inter-assay variation for nisoldipine in plasma.

Nominal Concentration [µg/l]	Sample 1	Sample 2	Sample 3	mean [µg/l]	SD	CV [%]	$Q^x \frac{\text{actual}}{\text{nominal}}$
19.60	20.97	22.28	19.70	20.98	1.29	6.15	1.07
7.37	7.69	7.46	7.36	7.50	0.17	2.26	1.02
0.49	0.44	0.46	0.46	0.45	0.01	2.55	0.93

Table 4: Data for intra-assay variation for nisoldipine in plasma.

TABLE 2

Nominal Concentration [µg/l]	Sample 1	Sample 2	Sample 3	$\bar{x}$ [µg/l]	SD	CV [%]	Q $\frac{\bar{x}_{actual}}{nominal}$
13.74	12.35	13.44	12.49	12.76	0.59	4.65	0.93
176.44	186.08	194.86	184.91	188.62	5.44	2.88	1.07
373.72	366.10	355.19	351.31	357.53	7.67	2.14	0.96

Table 3a: Data for intraassay variation for cimetidine in plasma

Nominal Concentration [µg/l]	Means of Triplicates Day 1	Day 2	Day 3	$\bar{x}$ [µg/l]	SD	CV [%]	Q $\frac{\bar{x}_{actual}}{nominal}$
13.74	16.15	12.76	15.49	14.83	1.84	12.38	1.08
176.44	185.37	188.62	206.46	193.48	11.36	5.87	1.10
373.72	410.89	357.53	395.65	388.02	27.49	7.08	1.04

Table 3b: Data for interassay variation of cimetidine in plasma

TABLE 3

Nominal Concentration [µg/l]	Sample 1	Sample 2	Sample 3	$\bar{x}$ [µg/l]	SD	CV [%]	Q $\frac{\bar{x}_{actual}}{nominal}$
1104.52	1114.58	1036.45	962.44	1037.82	76.08	7.3	0.94
393.95	400.32	391.58	373.66	388.52	13.59	3.5	0.99
112.82	115.98	113.69	130.16	119.94	8.92	7.4	1.06

Table 8: Data for intra-assay variation for ranitidine in plasma (Study No. 36/90-02.03.NE)

Nominal Concentration [µg/l]	Means of Triplicate- Day 1	Day 2	Day 3	$\bar{x}$ [µg/l]	SD	CV [%]	Q $\frac{\bar{x}_{actual}}{nominal}$
1104.52	1042.40	1037.82	1061.37	1047.20	12.49	1.2	0.95
393.95	369.92	388.52	349.08	369.17	19.73	5.3	0.94
112.82	112.92	119.34	137.10	123.32	12.44	10.1	1.09

Table 9: Data for inter-assay variation of ranitidine in plasma (Study No. 36/90-02.03.NE)

NONCOMPARTMENTAL PHARMACOKINETIC PARAMETERS OF NISOLDIPINE

Parameter: Unit :	Cmax [ug/l]	tmax [h]	AUC(0-tn) [ug*h/l]	Cmax,norm [g/l]	AUC(0-tn,norm) [g*h/l]
MEAN	1.05	9.11	14.97	3.82	54.17
SDEV	0.29	4.81	4.48	0.79	12.74
GEO.MEAN	1.01	7.51	14.37	3.73	52.87
GEO.SDEV	1.33	2.07	1.35	1.27	1.26
LOW.CON.	0.86	4.98	12.15	3.26	46.48
UPP.CON.	1.19	11.32	17.00	4.27	60.14
MEDIAN	1.00	10.00	13.82	3.94	48.56
MIN	0.54	2.00	9.73	2.01	39.33
MAX	1.64	16.00	22.08	5.22	79.04
COUNT	12.00	12.00	12.00	12.00	12.00

Table 13

Noncompartmental pharmacokinetic parameters of Nisoldipine following p.o. administration of respectively one tablet 20 mg Nisoldipine CC (o.a.d. on day 5) and Placebo coated tablet (b.i.d. on days 1 to 6) (treatment B3).

Arithmetic Means, Standard Deviations, Geometric Means, Geometric Standard Deviations, 95% Confidence Limits of Geometric Mean, Medians, Minimum, Maximum and Number of Cases.

NONCOMPARTMENTAL PHARMACOKINETIC PARAMETERS OF NISOLDIPINE

Parameter: Unit :	Cmax [ug/l]	tmax [h]	AUC(0-tn) [ug*h/l]	Cmax,norm [g/l]	AUC(0-tn,norm) [g*h/l]
MEAN	1.74	4.25	23.20	6.43	86.04
SDEV	1.08	3.28	12.70	3.98	47.75
GEO.MEAN	1.47	3.42	19.02	5.41	70.03
GEO.SDEV	1.87	1.91	2.18	1.91	2.24
LOW.CON.	1.03	2.37	12.23	3.76	44.39
UPP.CON.	2.09	4.94	29.60	7.79	110.48
MEDIAN	1.48	2.75	20.56	5.65	73.22
MIN	0.37	1.50	2.25	1.23	7.47
MAX	4.49	12.00	48.88	16.07	175.00
COUNT	12.00	12.00	12.00	12.00	12.00

Table 11

Noncompartmental pharmacokinetic parameters of Nisoldipine
following p.o. administration of respectively one tablet 20 mg Nisoldipine CC (o.a.d. on day 5) and Tagamet (R) (400 mg Cimetidine b.i.d. on days 1 to 6) (treatment B1).

Arithmetic Means, Standard Deviations, Geometric Means, Geometric Standard Deviations, 95% Confidence Limits of Geometric Mean, Medians, Minimum, Maximum and Number of Cases.

NONCOMPARTMENTAL PHARMACOKINETIC PARAMETERS OF NISOLDIPINE

Parameter: Unit :	Cmax [ug/l]	tmax [h]	AUC(0-tn) [ug*h/l]	Cmax,norm [g/l]	AUC(0-tn,norm) [g*h/l]
MEAN	0.90	8.37	13.30	3.26	48.81
SDEV	0.41	3.90	5.97	1.30	22.43
GEO.MEAN	0.84	7.25	12.20	3.07	44.59
GEO.SDEV	1.47	1.84	1.54	1.42	1.55
LOW.CON.	0.68	5.13	9.57	2.51	34.75
UPP.CON.	1.04	10.26	15.55	3.74	57.21
MEDIAN	0.81	8.99	11.76	3.18	46.35
MIN	0.50	2.50	6.53	1.91	24.97
MAX	1.98	12.02	24.98	6.56	93.34
COUNT	12.00	12.00	12.00	12.00	12.00

Table 12

Noncompartmental pharmacokinetic parameters of Nisoldipine
following p.o. administration of respectively one tablet 20 mg Nisoldipine CC (o.a.d. on day 5) and Zantic (R) (150 mg Ranitidine b.i.d. on days 1 to 6) (treatment B2).

Arithmetic Means, Standard Deviations, Geometric Means, Geometric Standard Deviations, 95% Confidence Limits of Geometric Mean, Medians, Minimum, Maximum and Number of Cases.

Cimetidine and Ranitidine

The following Table 4 shows the geometric mean peak and trough concentrations for cimetidine and ranitidine.

Analyt	Day 1 0 h	Day 1 2 h	Day 4 0 h	Day 4 2 h	Day 5 0 h
Cimetidine	n.d.	747.75	66.06	768.65	60.75
Ranitidine	n.d.	468.70	100.42	542.02	93.72

Table 4: Geometric mean cimetidine and ranitidine concentrations [µg/l] (peak/trough) on study days 1 and 4

FIGURE 1

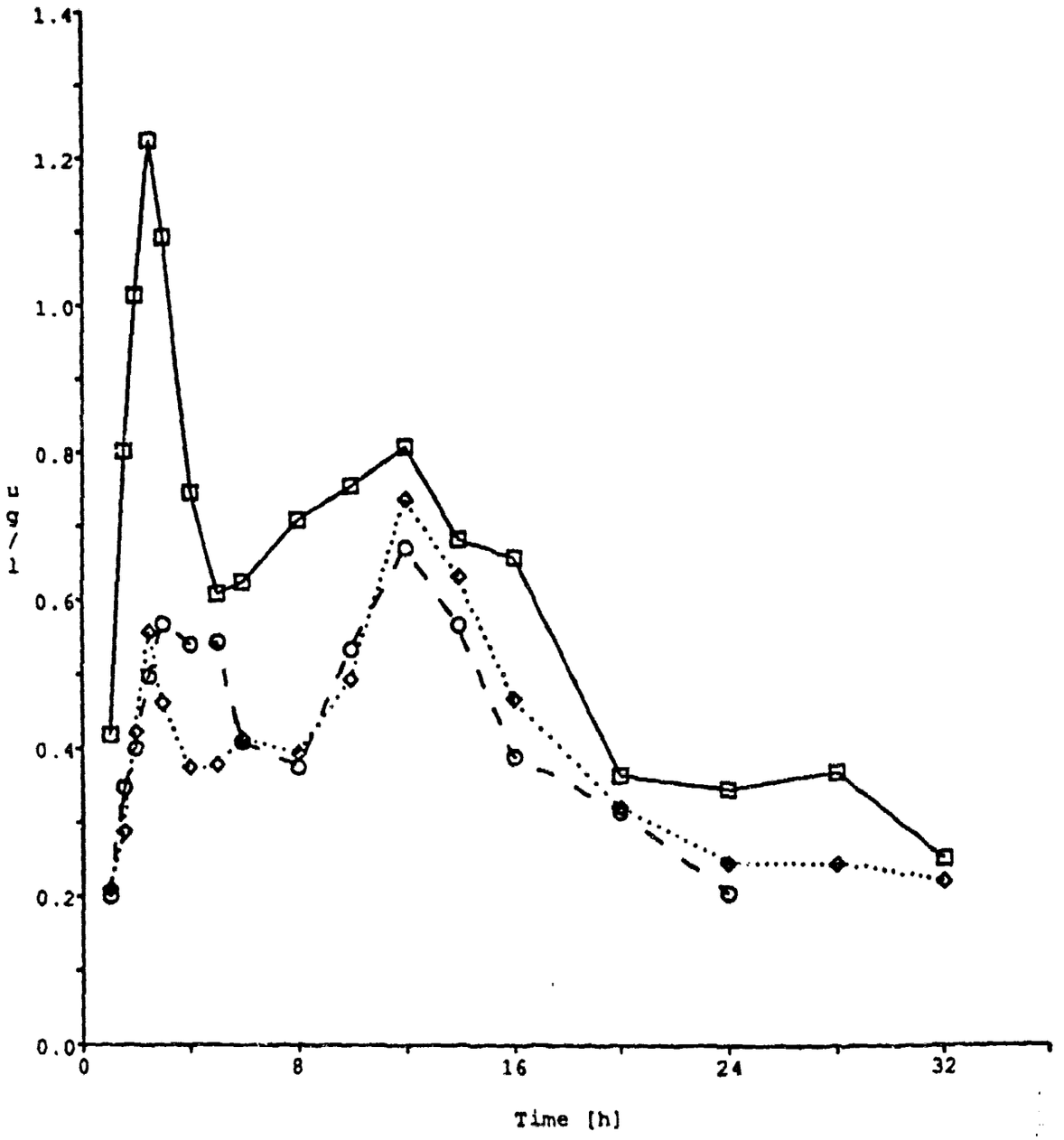


Figure 1

Synoptic plot of geometric mean concentrations of Nisoldipine [ug/l] vs time [h].

Geometric mean not calculated if more than 1/3 single concentrations are <0.18 or no sample. Concentrations <0.18 calculated as 0.09.

To investigate the existence of a possible interaction between nisoldipine and ranitidine.

STUDY: 385. VOLUME: 1-55 PAGES: 06-02-2962-3520.

INVESTIGATOR:

OBJECTIVES:

1-To determine if ranitidine alters nisoldipine kinetics.

2-to determine if a pharmacodynamic interaction exists either as a result of a pharmacokinetic interaction or independent from such an interaction.

FORMULATIONS:

-nisoldipine 20 mg tablets, batch # 929490, expiration date 10-03 1986.
-ranitidine tablets 150 mg.

STUDY DESIGN:

16 healthy male volunteers between the ages of 18 and 45 years participated in this placebo controlled, double blind crossover study.
300 mg ranitidine (single dose) or placebo were given for 3 days preceeding the test days (when 20 mg nisoldipine was given).
Venous blood samples were collected according to the following schedule: 0, 30, 60, 90, 120, 150, 180 minutes and 4, 8, 10 and 24 hours after drug administration.
Supine blood pressure, heart rate and systolic time intervals were measured at 0, 0.5, 1.5, 2, 3, 4, and 24 hours after medication.

RESULTS:

Figure 1 shows the mean nisoldipine plasma concentrations with and without ranitidine while Table 1 gives CMAX and AUC for the 2 treatments.
According to the sponsor,the results show that there was no difference in CMAX between the two treatments but the AUC was significantly greater when ranitidine was given with nisoldipine compared to when nisoldipine was given alone. However, the sponsor claims that when subject 13 was omitted, there was no longer any differences between the two treatments.

COMMENTS:

If one examines Figure 1, it can be seen that the CMAX was higher with the placebo treatment as compared to the ranitidine treatment. However, both CMAX values appear less than the stated values in Table 1. Additionally, ranitidine seems to have delayed and lowered the CMAX value when nisoldipine was given with ranitidine compared to when it was given with placebo.
The sponsor is asked to explain the discrepancy between the results of Figure 1 and the mean values presented in Table 1.

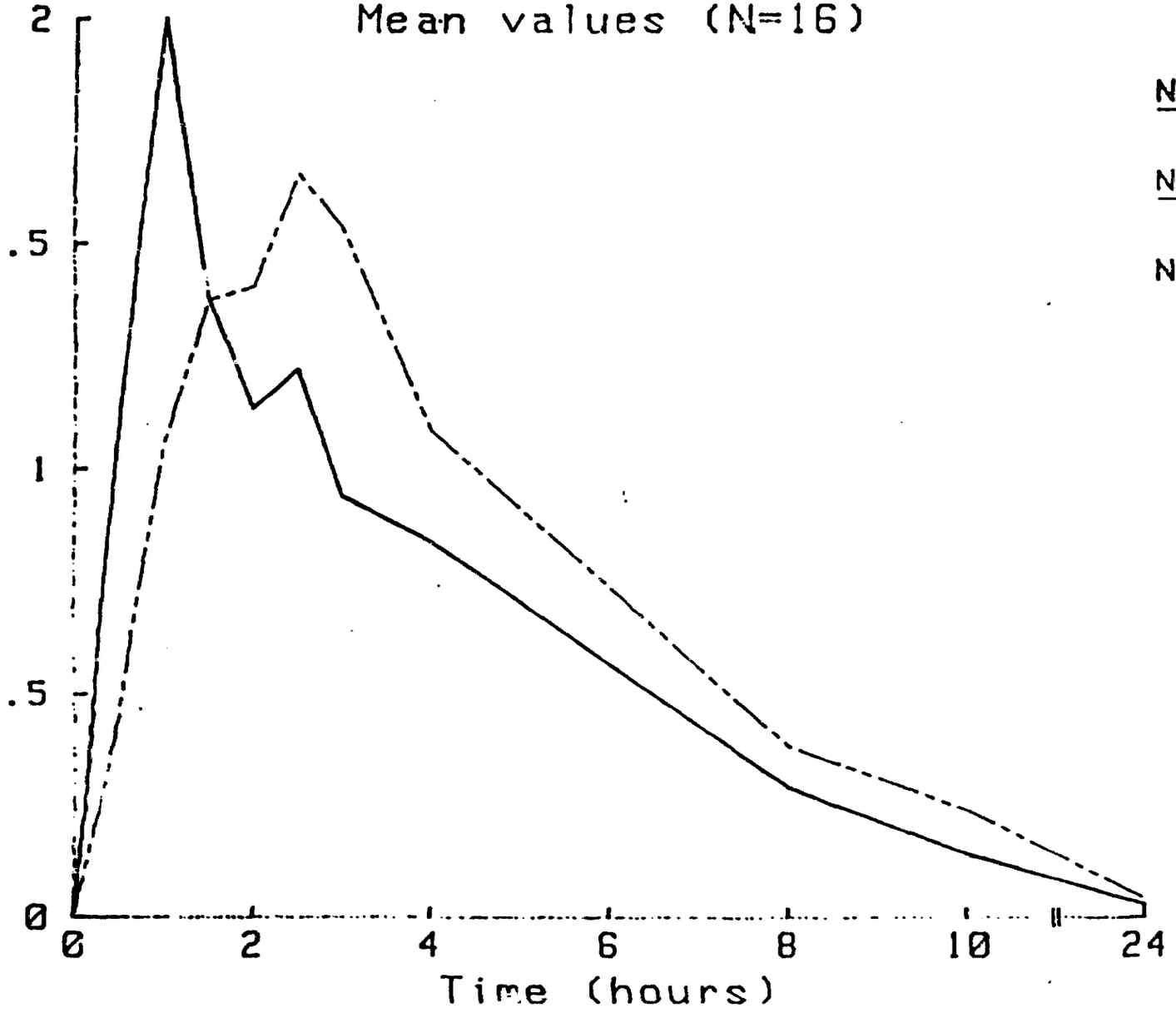
CONCLUSION:

No conclusion can be made from this study whether the pharmacokinetics of nisoldipine are altered by the coadministration of ranitidine.

FIGURE 1

U.O.F.S. 3/85 (STUDY No. 0385)

PLASMA CONCENTRATION
Mean values (N=16)



N + Placebo

N + Ranitidine

N=Nisoldipine

160

54

TABLE 1

12.1 Pharmacokinetic variables

The results are summarised below:

	C_{max} (ng/mℓ)					T_{max} (h)			
	Mean	SD	1	2	B	Mean	SD	1	2
1	2,833	2,245	-	NS	1	2,250	1,966	-	NS
2	2,791	1,858	-	-	2	2,375	1,688		-
	Mean	SD	AUC			Mean	SD	MRT (h)	
1	8,145	4,112	-	*	1	4,749	2,655	-	NS
2	10,100	3,343	-	-	2	5,695	3,041	-	-

1 : Nisoldipine plus placebo

2 : Nisoldipine plus Ranitidine®

Interaction study. Comparative investigation of safety, tolerability, pharmacodynamics and pharmacokinetics of Nisoldipine C.C. and propranolol given alone and in combination at steady state.

Study #: 704. VOLUME: 1-56 PAGES: 6-02-3623-4013.

INVESTIGATOR:

OBJECTIVES:

Comparative investigation of safety, tolerability, pharmacodynamics and pharmacokinetics of nisoldipine C.C. and propranolol given alone and in combination at steady state.

FORMULATIONS:

-Nisoldipine CC 20 mg tablets batch # 523231.
-Dociton^{R}= 40 mg propranolol.

STUDY DESIGN:

This was a randomized, 3 fold crossover multiple dose interaction study. 12 healthy male volunteers between t' :ges of 23 and 40 years participated in this 5 day study. Nisoldipine C.C. 20 mg once a da, or/and Dociton tablets with 40 mg propranolol (tid) were given together with 100 mls of water.
Blood samples in nisoldipine treatments were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 20, 24, 28, 32, 36 and 48 hours after drug administration.
Blood samples in the propranolol treatment were collected at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 8.5, 9, 10, 12, 14, 16, 16.5, 17, 18, 20, 22, 24, 26, 28, 32 and 48 hours post dose.
Trough plasma concentrations were measured in the morning (8 AM) on study days 2 to 4.

RESULTS:

Figure 1 shows the mean geometric mean nisoldipine plasma concentration with and without propranolol administration while Table 1 gives the main pk parameters for both the 2 treatments. Figure 2 shows the mean geometric propranolol concentratrion for both corresponding treatments while Table 2 summarizes the most important pharmacokinetic parameters for propranolol.

Conclusion:

The results show that the nisoldipine plasma concentrations were slightly increased by propranolol coadministration while propranolol plasma concentrations were slightly decreased. This very small interaction between these 2 drugs is not expected to have any consequences from the clinical point of view.

FIGURE 1

' 0704

Plasmaconcentration vs. timeprofiles of Nisoldipine
with and without concomitant Propranolol treatment

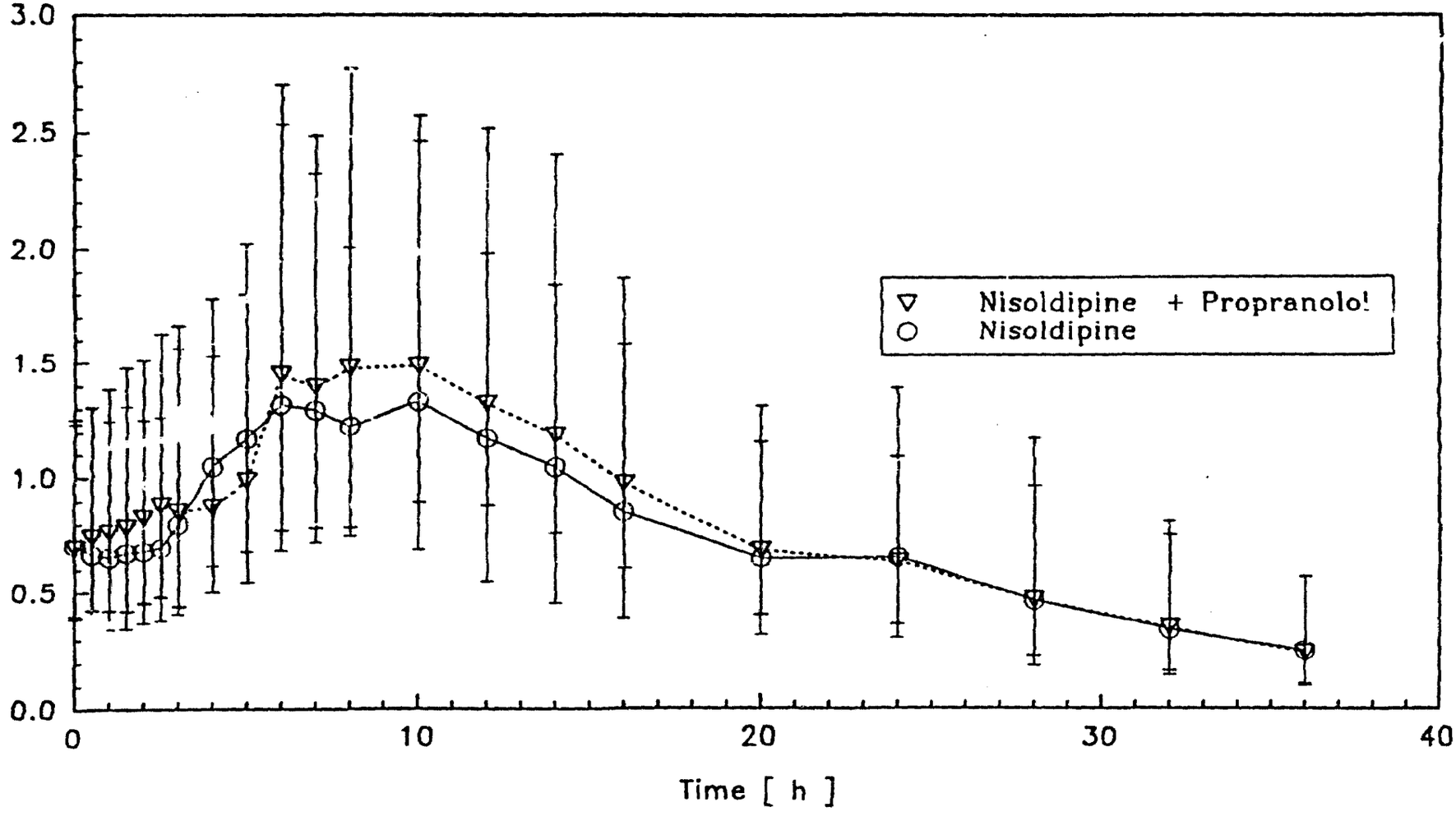


Fig. 6.1.1

FIGURE 2

/ 0704

Plasmaconcentration vs. timeprofiles of Propranolol
with and without concomitant Nisoldipine treatment

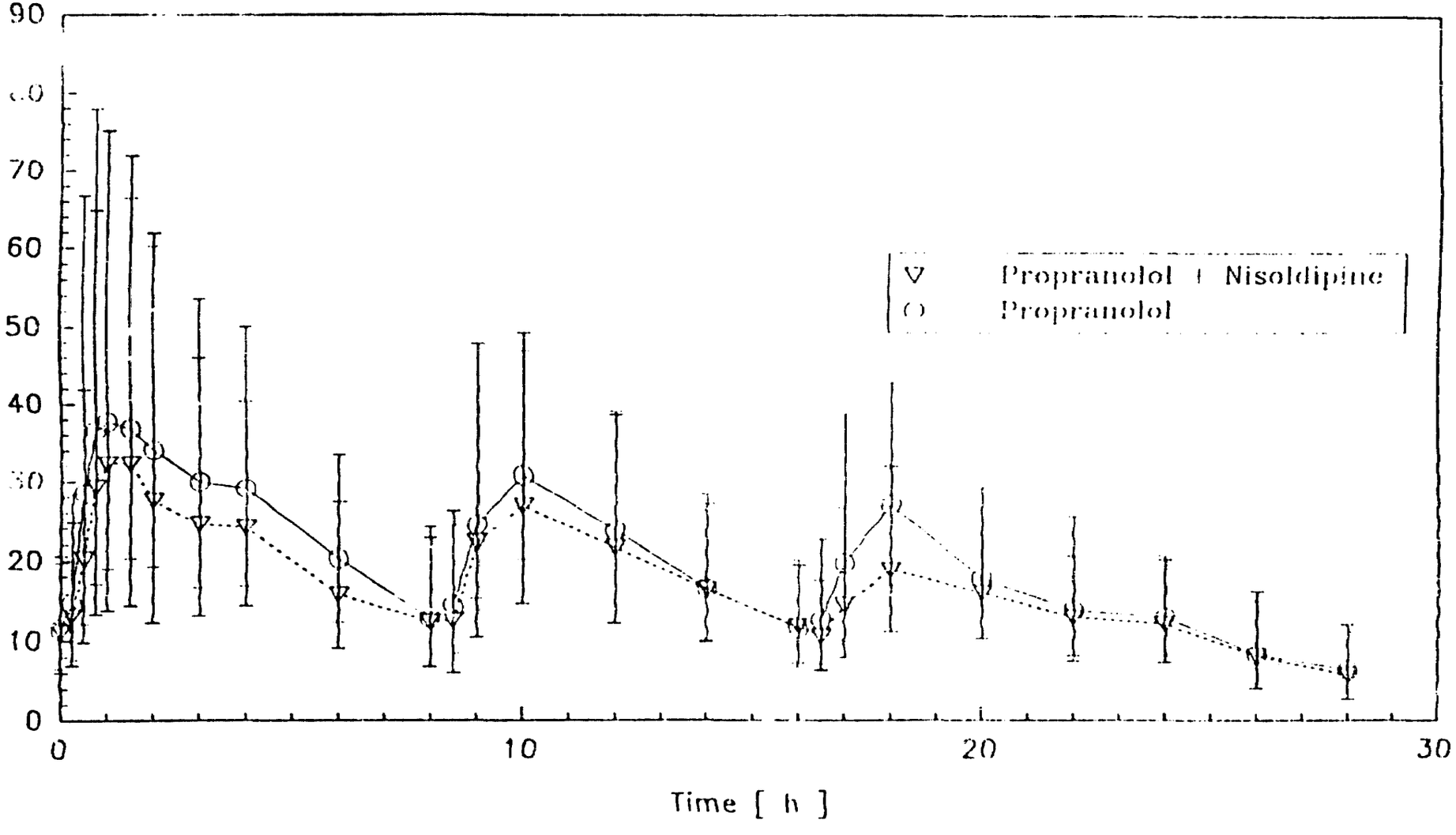


Fig. 6.1.2

255

TABLE 1

/ STUDY NO.704

Estimates of Pharmakokinetic Parameters of Nisoldipine
(Geometric Means and SD) and Quotients to Results of Nisoldipine in %
(Geometric mean and SD,90% Conf.Interv.Referring to Analysis of Variance and Dunnett–Test)

Parameter Nisoldipine		Treatment Nisoldipine	Nisoldipine +Propranolol	Quotient Nisoldipine + Propranolol / Nisoldipine geo.mean	geo S.D.	90% C.I.
$AUC_{norm\ (0-24)}$	geo.mean [g*h/l]	96.26	103.24	107.3	1.38	90.6 – 127
	geo.SD	1.71	1.5			
C_{max}	geo.mean [ng/ml]	1.87	2.01	107.1	1.49	87.0 – 131
	geo.SD	1.81	1.86			
$t_{1/2}$	geo.mean [h]	13.02	10.53	80.9	1.45	66.8 – 97.9
	geo.SD	1.63	1.29			
fluctuation $_{(0-24)}$	geo.mean	1.33	1.40	105.2	1.62	81.9 – 135.
	geo.SD	1.56	1.37			

991

06 02 3868

TABLE 2

/ STUDY NO.704

Estimates of Pharmakokinetic Parameters of Propranolol
(Geometric Means and SD) and Quotients to Results of Propranolol in %
(Geometric mean and SD,90% Conf.Interv.Referring to Analysis of Variance and Dunnett–Test)

Parameter Propranolol		Treatment Propranolol	Propranolol +Nisoldipine	Quotient (Propranolol+Nisoldipine)/Propranolol in % geo.mean	geo.S.D.	90% C.I.
$AUC_{norm\ (0-24)}$	geo.mean [g*h/l]	1072.47	923.02	86.1	1.76	64.3 – 115.
	geo.SD	1.61	1.65			
$AUC_{norm\ (0-8)}$	geo.mean [g*h/l]	421.20	356.49	84.6	2.03	58.6 – 122.
	geo.SD	1.75	1.76			
C_{max}	geo.mean [ng/ml]	46.50	39.94	85.9	2.45	53.9 – 136.
	geo.SD	1.67	1.81			
$t_{1/2}$	geo.mean [h]	3.50	4.37	124.7	1.45	102.7 – 151.
	geo.SD	1.32	1.40			
fluctuation $_{(0-24)}$	geo.mean	1.77	1.70	95.8	1.57	75.9 – 121.
	geo.SD	1.42	1.35			
fluctuation $_{(0-8)}$	geo.mean	1.34	1.33	99.2	1.48	80.9 – 121.
	geo.SD	1.37	1.31			

Studies of the hemodynamic and pharmacokinetic interactions between the beta blockers atenolol and propranolol and the calcium antagonist nisoldipine in normotensive subjects.

Study: 3982. Volume: 112. Pages: 06-04-7235-7341.

Investigators:

Objectives:

1- to examine the pharmacokinetic and hemodynamic interactions of atenolol and propranolol with nisoldipine in normotensive patients.

Formulation:

-20 mg nisoldipine capsules.
-100 mg atenolol (Stuart Pharmaceuticals Ltd).
-160 mg propranolol (ICI Ltd).

Study Design:

This study was a randomized double blind placebo controlled study of 2 groups of 8 normotensive subjects between the ages of 19 and 40 years.
One group of subjects received 100 mg atenolol while the other group received propranolol 160 mg once daily. These active treatments were taken once daily for three weeks and matching placebo tablets were taken once daily during a fourth week. There was a 1 week washout period between weeks 2 and 3. For 2 weeks out of the total period additional treatment was administered: either nisoldipine 20 mg once a day or matching placebo capsules each for 1 week. The following treatment combinations were evaluated:
1 beta blocker + first dose nisoldipine.
2-beta blocker + steady state nisoldipine.
3-placebo + placebo.
4-beta blocker + placebo.

Blood pressure and heart rate erect and supine were measured at baseline and after 0.5, 1, 1.5, 2, 3, 4, 6 and 8 hours.

The extent of beta blockade was assessed by the heart rate response to sub-maximal exercise (75 % of a pre-determined maximum) for 5 minutes on a bicycle ergometer at two times:2.5 (morning) and 5 hours (afternoon).
Plasma for drug assays were collected at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6 and 8 hours.
Hepatic blood flow was assessed 1 hour after drug administration by measurement of the clearance of indocyanine green.
Effective renal plasma flow and glomerular filtration rate were determined 1 hour after drug administration from the clearance of radiopharmaceuticals.

Results:

1-Pharmacokinetics:

Figure 1 shows the mean plasma concentrations for atenolol with and without nisoldipine while Figure 2 shows the plasma profile for propranolol with and without the calcium channel blocker. Table 1 gives a summary of the derived pharmacokinetic for atenolol while Table 2 gives the same pk parameters for propranolol.
The results show that coadministration of nisoldipine with atenolol sharply increased the atenolol CMAX (from 455 to 540 ng/ml) and to a lesser extent the AUC. The same trend of results was observed for propranolol whereby both the CMAX and AUC for acute and chronic propranolol were increased. However, there was no significant difference in propranolol AUC comparing the combination with acute and chronic calcium antagonist.

2-Liver blood flow and renal clearance:

The results shown in Table 3 and 4 respectively show that both atenolol and propranolol when given alone cause a slight decrease in the apparent liver blood flow. However the addition of nisoldipine to either atenolol or propranolol caused a significant increase in the apparent liver blood flow. This increase was slightly attenuated with the chronic administration of nisoldipine.

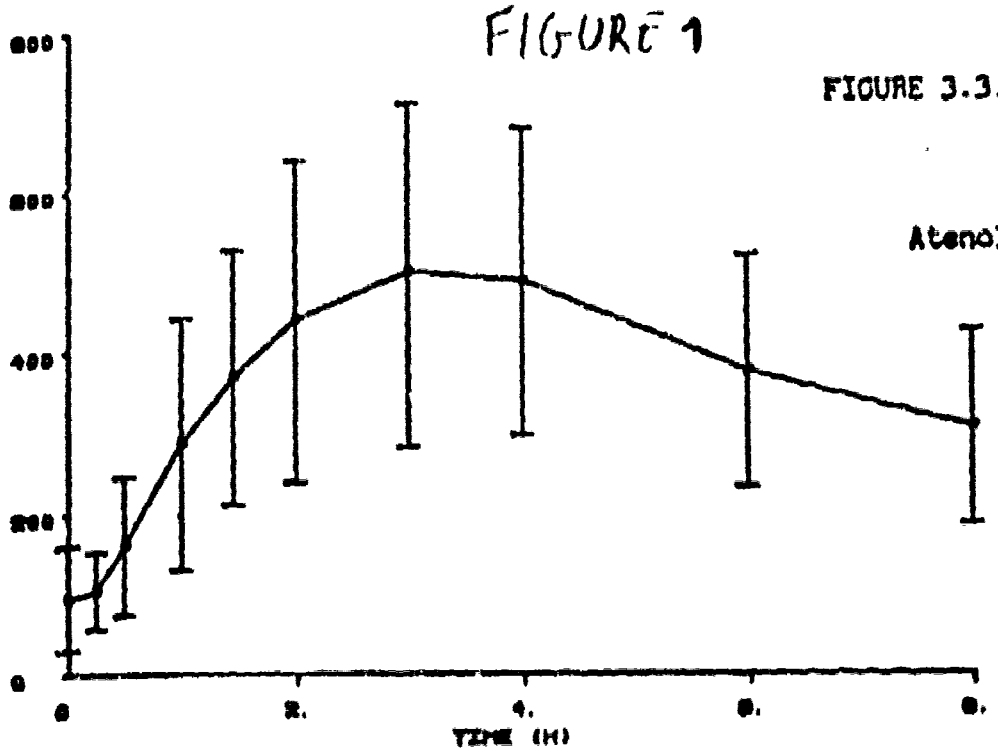
As for the renal function, it was not affected by any of the treatments.

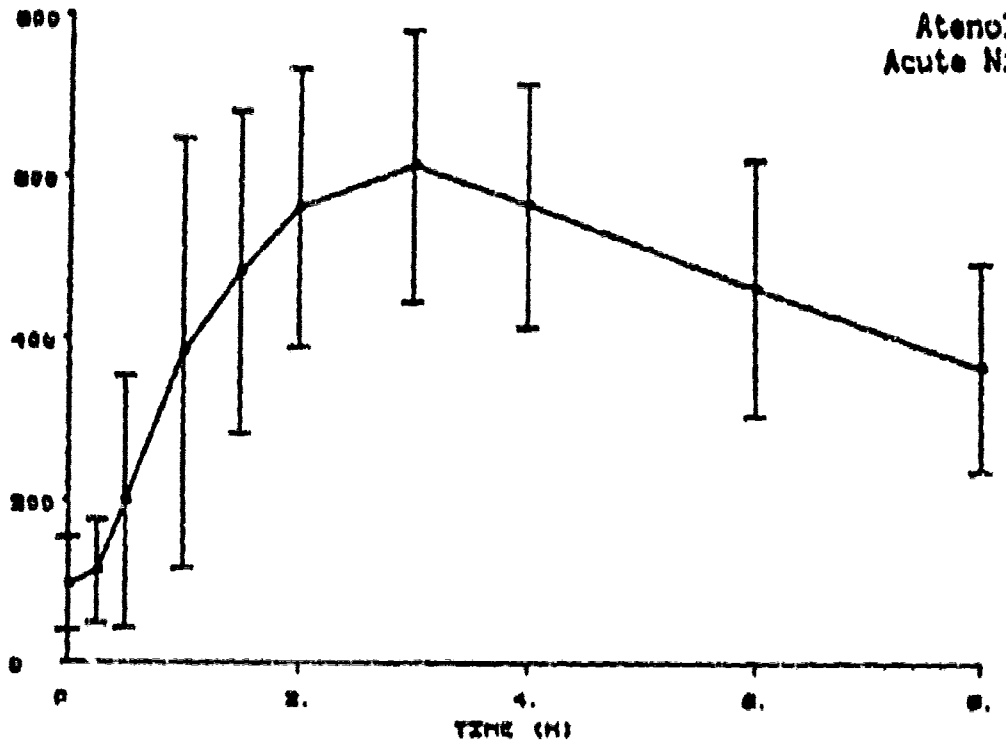
3-Blood pressure and heart rate:

Figure 3, 4 and 5 show the supine systolic blood pressure, supine diastolic blood pressure and supine heart rate for the atenolol treatment while Figure 6, 7 and 8 show the corresponding graphs for the propranolol treatment.
The results show that both atenolol and propranolol caused a significant decrease in both supine and erect systolic and diastolic blood pressure as compared to placebo. The addition of nisoldipine caused a further slight reductions in blood pressures. This further reductions in blood pressures were generally accompanied by a slight increase in heart rate.

FIGURE 1

FIGURE 3.3.1





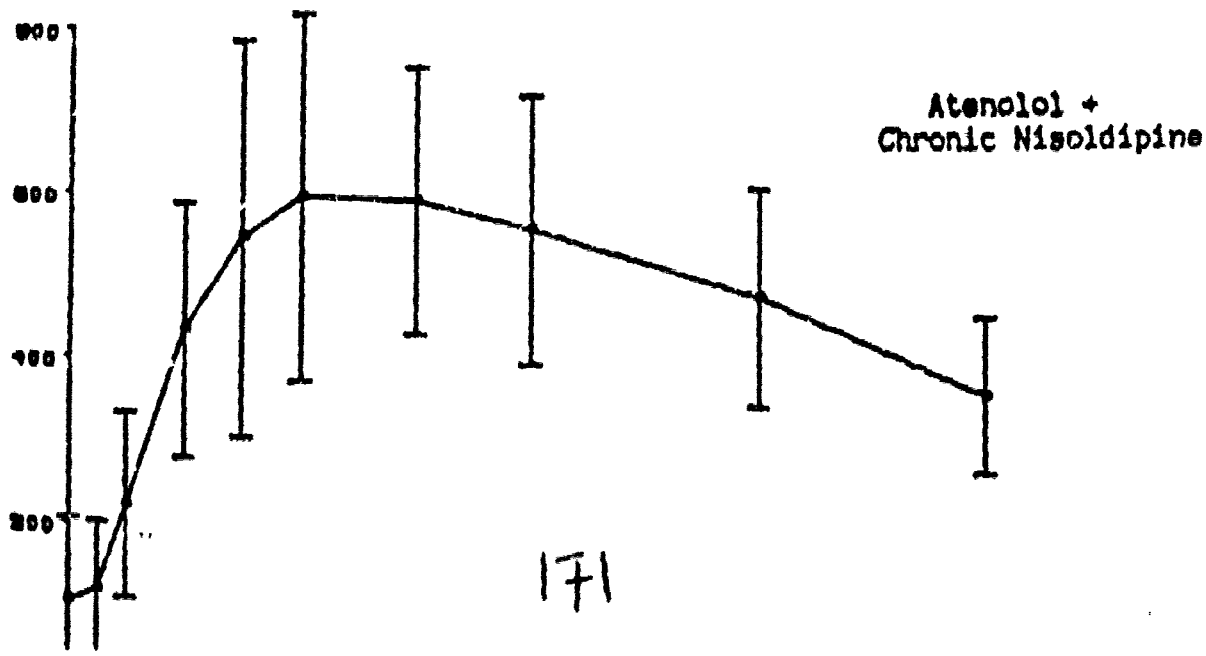
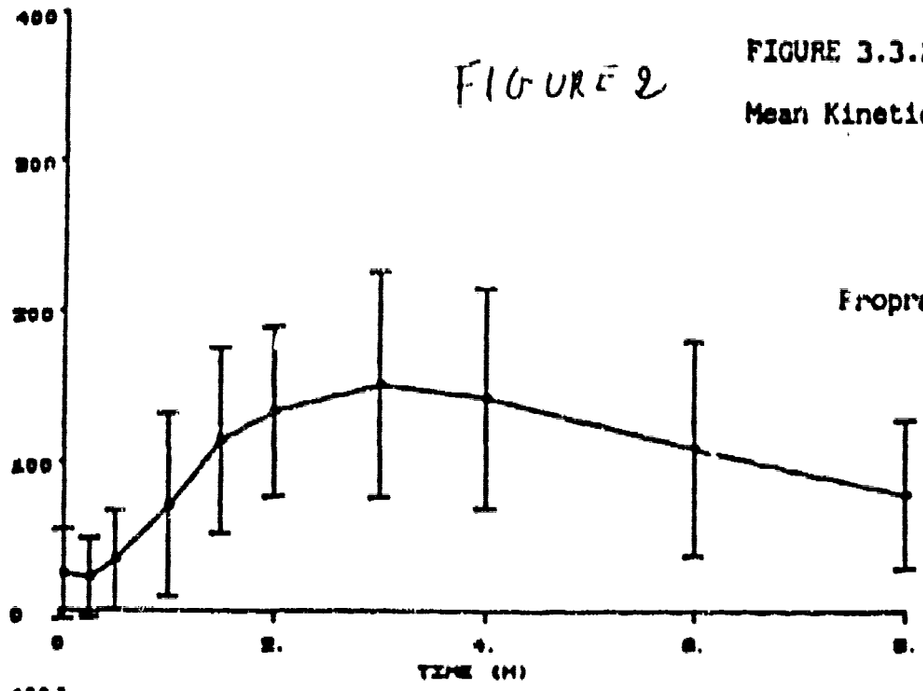


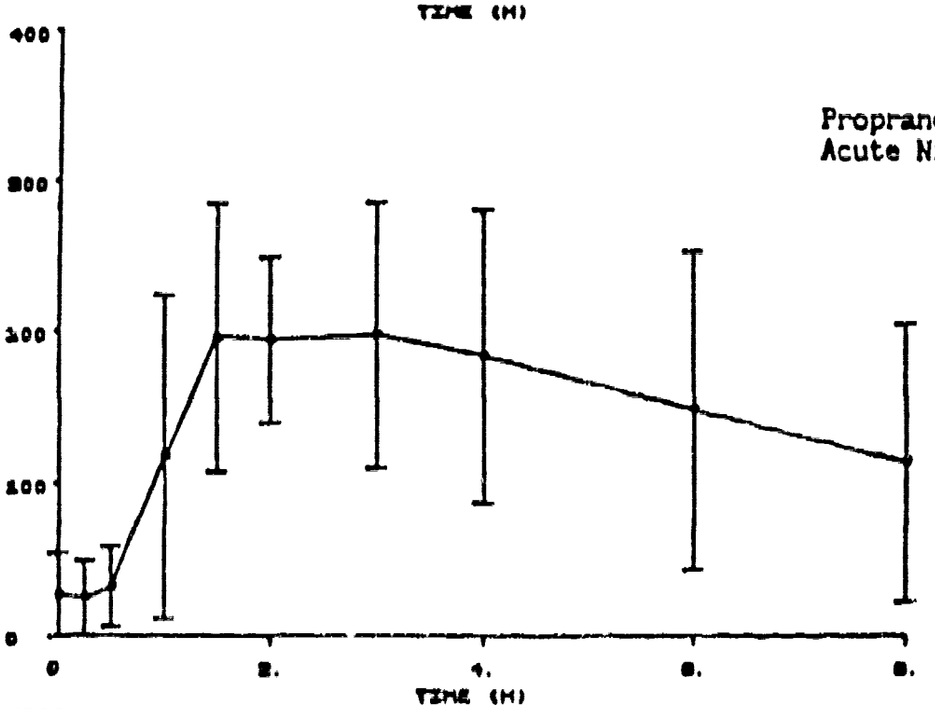
FIGURE 2

FIGURE 3.3.2

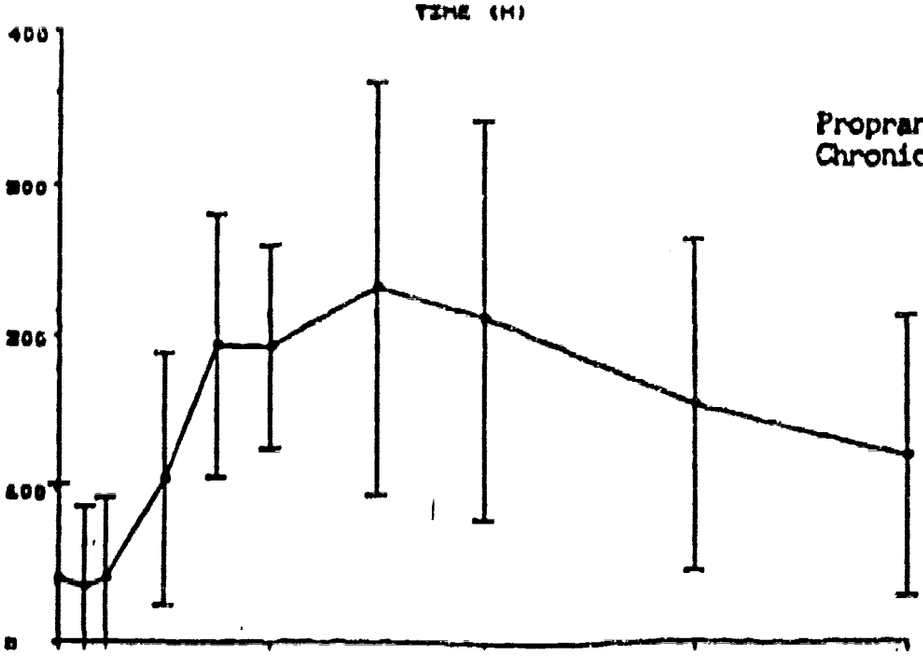
Mean Kinetic Profiles



Propranolol Alone

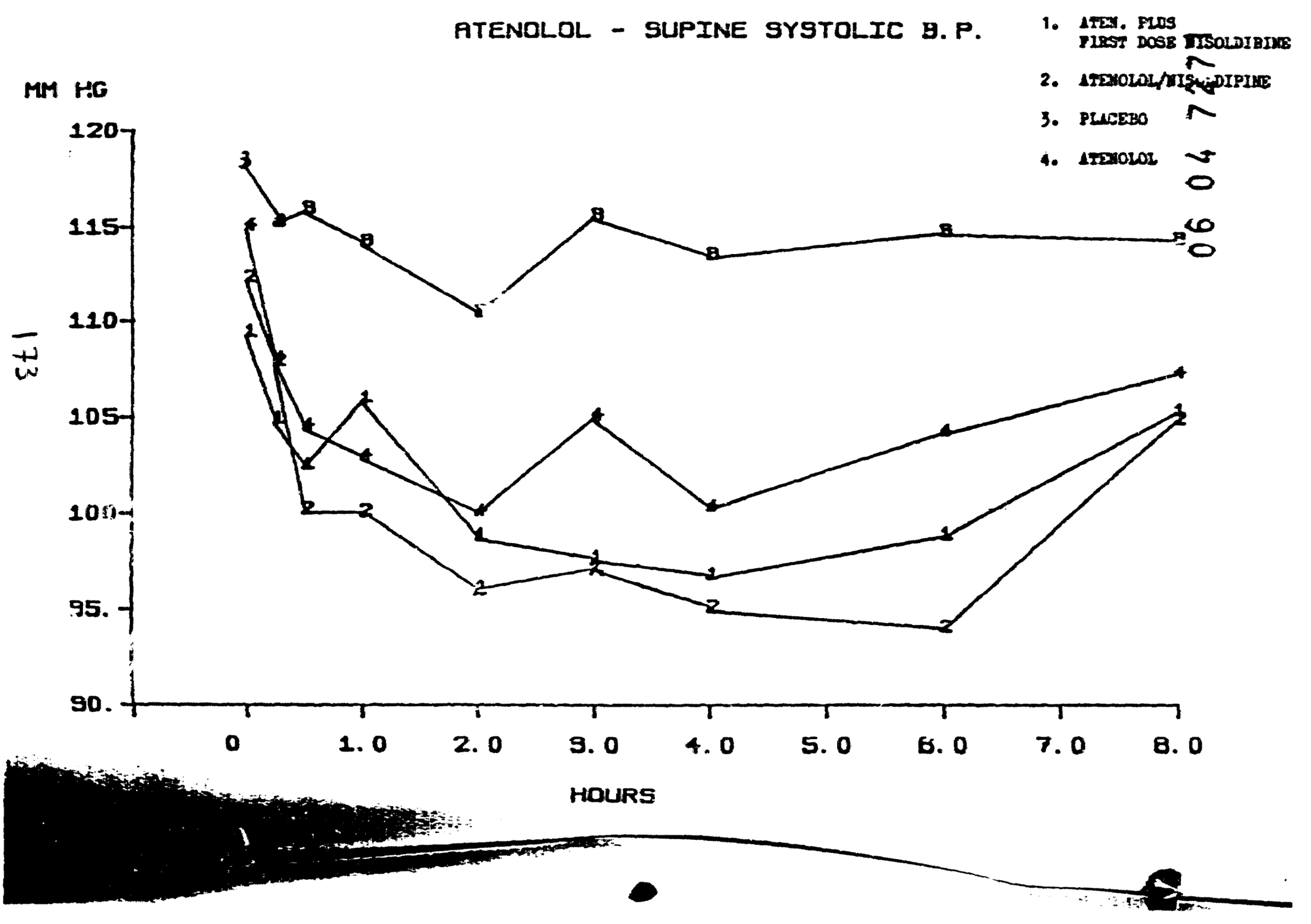


Propranolol +
Acute Nisoldipine



Propranolol +
Chronic Nisoldipine

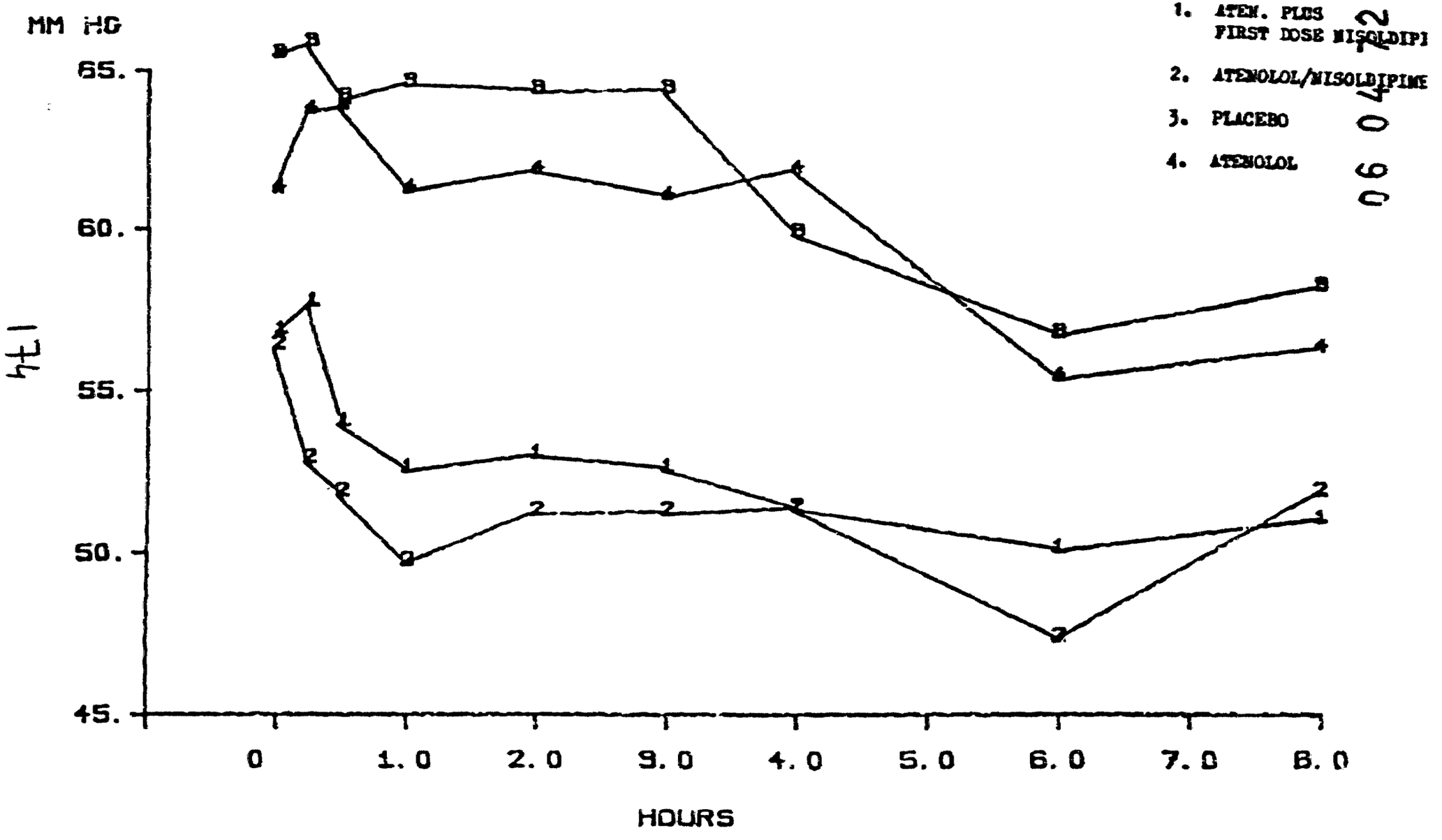
FIGURE 3.


ATENOLOL - SUPINE SYSTOLIC B.P.
1. ATEN. PLUS FIRST DOSE NISOLDIBINE
2. ATENOLOL/NIS...DIPINE
3. PLACEBO
4. ATENOLOL
MM HG
120
115
110
105
100
95.
90.
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
HOURS

35

JUNE 4, 1982

ATENOLOL - SUPINE DIASTOLIC B.P.



htl

06 04 272

Figure 5.168

ATENOLOL - SUPINE HEART RATE

1. ATEN. PLUS FIRST DOSE NI.. .IPINE
2. ATENOLOL/NISOLDIPINE
3. PLACEBO
4. ATENOLOL

BEATS/MIN

65.
60.
55.
50.
45.
40.

0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0

HOURS

40

FIGURE 6.1.7

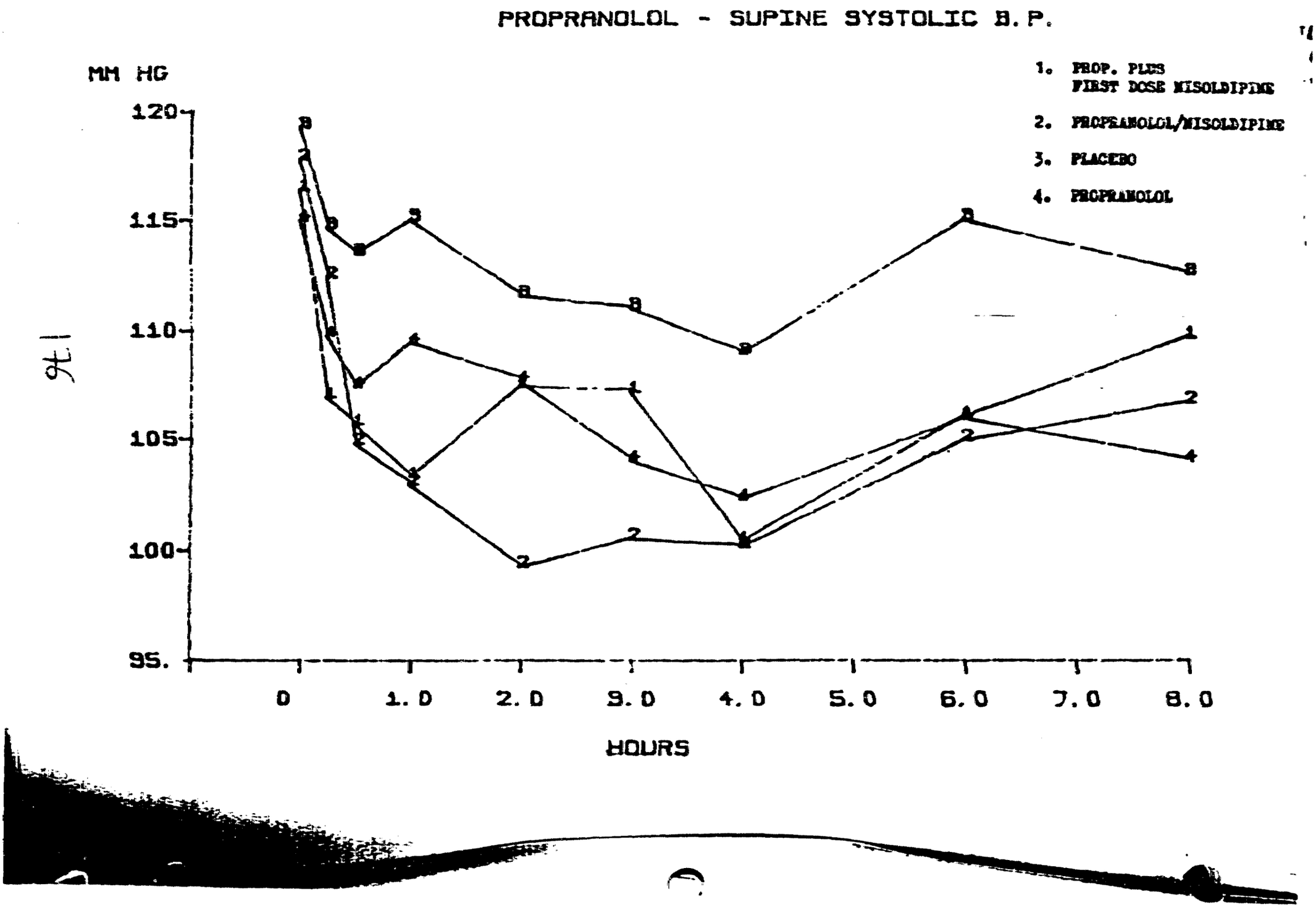


FIGURE 7.1.8

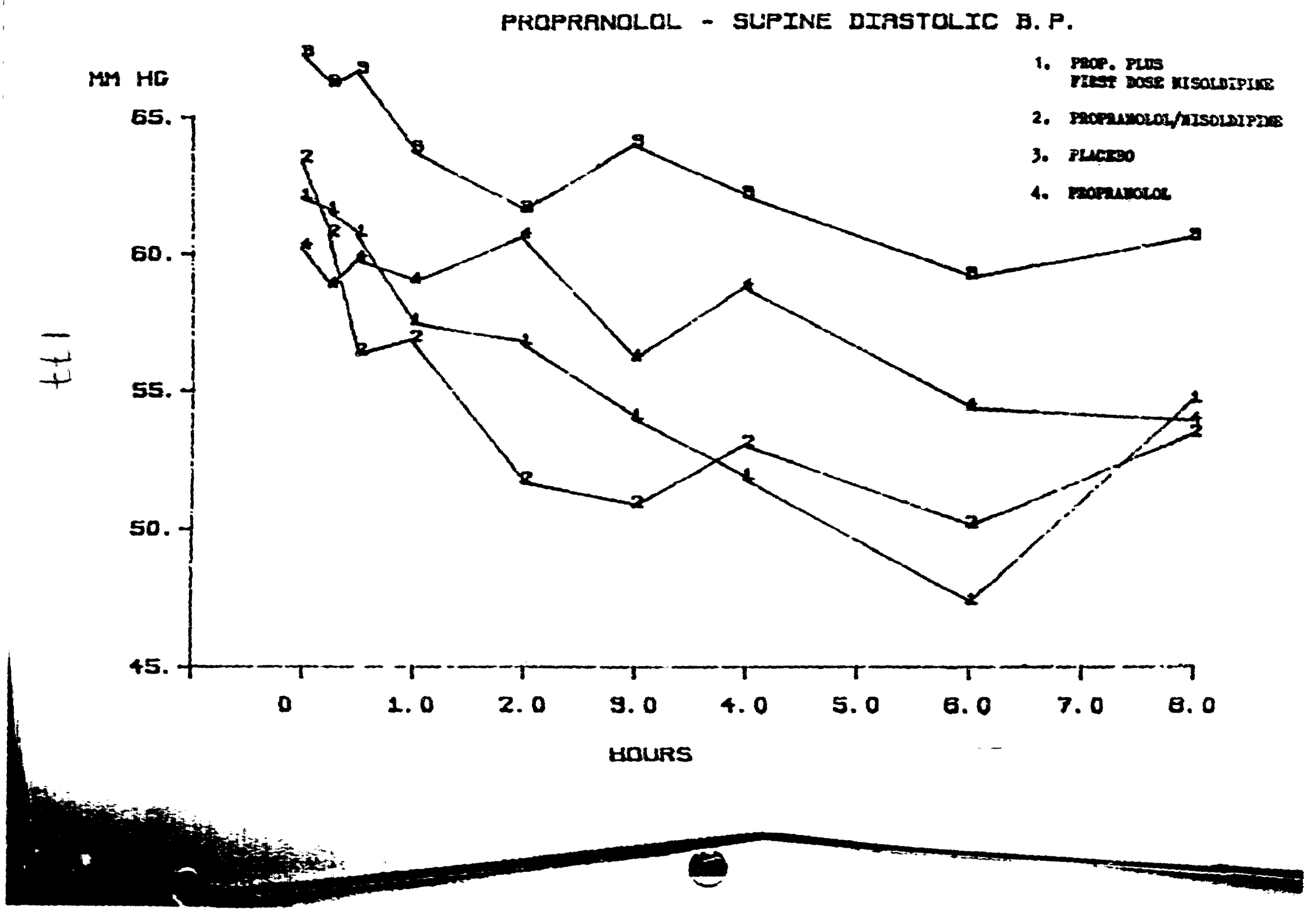

PROPRANOLOL - SUPINE DIASTOLIC B.P.
MM HG
65.
60.
55.
50.
45.
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
HOURS
1. PROP. PLUS FIRST DOSE NISOLDIPINE
2. PROPRANOLOL/NISOLDIPINE
3. PLACEBO
4. PROPRANOLOL

FIGURE 8.

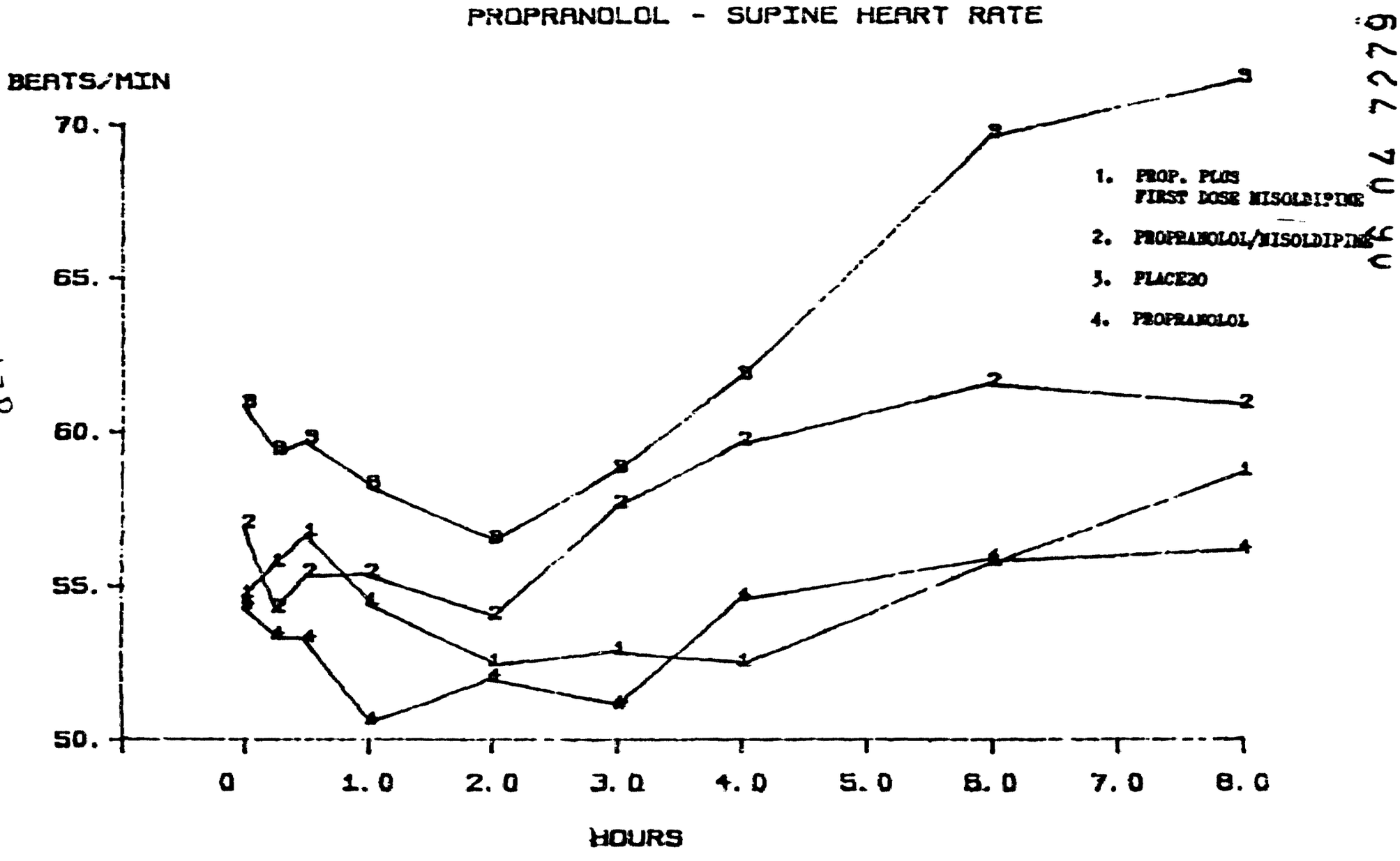


TABLE 1.3.1. DERIVED PHARMACOKINETIC PARAMETERS: ATENOLOL

Subject	AUC (ng.h/ml) CA	CA + AN	CA + CN	$\beta t_{1/2}$ (h) CA	CA + AN	CA + CN	t_{max} (h) CA	CA + AN	CA + CN	ΔC_{max} (ng/ml) CA	CA + AN	CA + CN
1	7584	8373	7832	5.4	6.7	7,3	4.1	3.0	3.0	580	652	569
2	7981	6003	6488	11.6	10.5	6.0	3.5	1.3	3.0	411	382	541
3	3553	4557	3975	9.1	4.8	5.7	2.5	2.2	2.7	463	562	365
4	2181	6079	4745	7.4	11.0	7.0	1.0	2.4	1.9	192	330	404
5	5658	7351	7091	5.9	4.9	3.4	3.2	4.4	3.8	479	583	667
6	5677	4267	5432	8.1	5.5	6.7	2.2	3.6	1.2	422	383	498
7	5190	9076	7992	7.0	7.6	8.9	4.2	3.3	2.9	441	548	514
8	9006	10189	11195	8.2	8.0	8.1	2.3	1.6	2.0	651	780	823
Mean ± S.D.	5854 ± 2291	6987 ± 2130	6844 ± 2269	7.8 ± 1.9	7.4 ± 2.4	6.6 ± 1.7	2.9 ± 1.1	2.7 ± 1.0	2.6 ± 0.8	455 ± 135	540 ± 159	547 ± 146
	ns*	ns		ns	ns		ns	ns		p<0.02	ns	
	ns			ns			ns			p<0.05		

ns* = (0.1 > p > 0.05)

TABLE 3.3.2. DERIVED PHARMACOKINETIC PARAMETERS: PROPRANOLOL

Subject	AUC (ng.h/ml) CA	CA + AN	CA + CN	$\beta t_{1/2}$ (h) CA	CA + AN	CA + CN	t_{max} (h) CA	CA + AN	CA + CN	ΔC_{max} (ng/ml) CA	CA + AN	CA + CN
9	739	806	1378	2.7	2.2	3.4	2.6	1.8	3.1	125	195	177
10	1470	2074	2002	4.1	6.0	5.6	1.8	0.9	1.4	195	224	207
11	4235	5460	7441	10.7	8.1	13.9	3.8	4.5	2.5	225	349	363
12	1336	1272	1901	4.3	5.3	6.6	3.3	3.3	2.0	134	122	179
13	1283	1821	1452	7.2	3.9	4.4	0.7	1.5	1.7	121	266	200
14	570	828	643	3.4	2.5	2.6	1.7	1.2	1.0	101	221	170
15	1297	1829	2469	4.1	5.2	6.0	5.4	3.6	4.2	108	175	196
16	1521	2693	2557	6.2	8.1	6.1	3.3	1.1	1.6	133	224	273
Mean ± S.D.	1556 ± 1135	2098 ± 1501	2482 ± 2099	5.4 ± 2.6	5.2 ± 2.3	6.1 ± 3.6	2.8 ± 1.5	2.2 ± 1.4	2.2 ± 1.0	143 ± 44	222 ± 67	221 ± 66
	p<0.01	ns		ns	ns		ns	ns		p<0.005	ns	
	p<0.03			ns			ns			p<0.002		

TABLE 3.[redacted] APPARENT LIVER BLOOD FLOW: ATENOLOL PLUS NITSOLDIPINE

Subject		Placebo	CA	CA + AN	CA + CN
1	LBF	2.17	1.50	2.55	2.69
	Vd	4.95	4.28	3.78	5.37
2	LBF	2.35	1.95	3.33	2.47
	Vd	5.60	4.60	6.01	4.65
3	LBF	1.61	1.77	1.71	1.98
	Vd	3.37	3.02	2.15	2.78
4	LBF	1.32	1.06	1.74	1.75
	Vd	3.41	2.65	2.33	2.96
5	LBF	1.61	1.88	2.04	1.69
	Vd	2.53	4.77	3.38	2.21
6	LBF	1.65	1.23	3.05	2.76
	Vd	3.33	2.93	4.47	4.97
7	LBF	0.94	0.83	1.56	1.92
	Vd	2.98	2.68	3.92	3.91
8	LBF	1.66	1.13	3.07	2.05
	Vd	3.34	2.22	4.34	3.85
Mean ± S.D.	LBF	1.66 ± 0.44	1.42 ± 0.42	2.38 ± 0.71	2.16 ± 0.42
		ns			
		p<0.01			
		p<0.01			
Mean ± S.D.	Vd	3.69 ± 1.04	3.40 ± 1.00	3.79 ± 1.24	3.83 ± 1.12

TABLE [redacted] APPARENT LIVER BLOOD FLOW: PROPRANOLOL PLUS NISOLDIPINE

Subject		Placebo	CP	CP + AN	CP + CN
9	LBF	2.12	1.73	3.04	3.24
	Vd	3.34	3.88	4.33	4.84
10	LBF	1.74	1.58	2.22	2.79
	Vd	3.16	2.63	4.99	4.83
11	LBF	1.89	1.14	2.36	2.87
	Vd	5.44	3.14	4.50	4.60
12	LBF	1.75	1.54	3.02	2.44
	Vd	3.48	3.34	4.21	3.97
13	LBF	1.40	1.10	2.39	2.64
	Vd	3.39	3.13	3.67	4.79
14	LBF	1.94	1.72	2.53	2.63
	Vd	3.68	3.01	3.23	2.91
15	LBF	0.96	1.15	1.25	1.28
	Vd	2.34	3.82	2.25	2.78
16	LBF	0.83	0.71	1.35	0.96
	Vd	1.18	2.28	2.77	1.53
Mean	LBF	1.58	1.33	2.27	2.36
± S.D.		± 0.47	± 0.36	± 0.67	± 0.80

p<0.05

p<0.01

p<0.01

Mean	Vd	3.25	3.15	3.74	3.78
± S.D.		± 1.21	± 0.54	± 0.94	± 1.24

Clinicopharmacological investigations on interaction of nisoldipine and digoxin.

STUDY #: 413. VOLUME: 1-57 PAGES: 6-02-4014-4166

INVESTIGATORS:

OBJECTIVES:

To investigate the possibility of pharmacodynamic and/or pharmacokinetic interaction taking place between nisoldipine and digoxin.

FORMULATION:

-Nisoldipine 10 mg tablets (batch #: 974386).
0.1 mg Beta acetyldigoxin (Novodigal mite®).

STUDY DESIGN:

8 healthy male volunteers participated in this non controlled observational study which was statistically a fixed sequence study.
Each subject received on days 1 and 2, 0.6 mg/day of Novodigal mite tablets. Starting on day 3 the daily dose was reduced to 0.3 mg of acetyldigoxin. This doses was maintained up to the last study day (day 22). Nisoldipine was additionally taken in the morning and the evening before meals starting with day 9 until the last study day.
The plasma and urine sampling schedule was not given in this study.

RESULTS:

Table 1 shows the main pharmacokinetic parameters for digoxin. Unfortunately, no pharmacokinetic parameters could be calculated for nisoldipine because only peak and trough levels were measured. The sponsor concluded that coadministration of nisoldipine did not have any effect on the pharmacokinetics of digoxin.

Comments:

The results of the study could not be considered definitive as to whether an interaction with nisoldipine C.C. and digoxin occurs, since the immediate release formulation was used and no assay validation was provided. Therefore, one cannot judge whether the results of the study are valid and capable of detecting an interaction if it exists.

table C: pharmacokinetics of Digoxin, mean values of 6th to 8th day (Digoxin), 20th to 22th day (Digoxin + Nisoldipine) and Quotient of 20th to 22th day/6th to 8th day nonparametric 95%-confidence limits of quotient

		Digoxin	Digoxin + Nisoldipine	quotient (%)	95% confidence limit
plasma concentration [mg/ml]	median	0.564	0.625	106.4	106.9
	min,max	0.381, 0.714	0.393, 0.828	101.1, 134.6	102.7, 120.3
renal elimination [%]	median	54.0	62.7	110.7	112.2
	min,max	47.2, 63.5	51.6, 73.4	92.4, 139.4	100.8, 124.6
Cl - total [ml/min/kg]	median	6.04	5.64	94.9	94.2
	min,max	4.84, 7.75	4.43, 6.95	74.0, 98.6	83.7, 97.4
Cl - renal [ml/min/kg]	median	4.06	4.65	103.5	103.7
	min,max	3.55, 4.70	3.16, 4.85	81.4, 137.5	89.2, 119.5

END

BT

J.H.M. Research & Development, Inc., 5776 Second Street, N.E., Washington, D.C. 20011

NDA 20356

Koeden

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 20-356

FEB 2 1995

Miles Inc.
Pharmaceutical Division
Attention: Nancy Motola, Ph.D.
400 Morgan Lane
West Haven, CT 06516-4175

Dear Dr. Motola:

Please refer to your March 31, 1993 new drug application resubmitted on August 3, 1994 under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Nisocor (nisoldipine) Tablets, 10, 20, 30 and 40 mg.

We acknowledge receipt of your amendments and correspondence dated May 31, June 20 and 27, July 18 and 29 (two), September 8 and 16, October 19, November 8, 9, 17, 18 and 21, and December 16, 20 (two), 22 (three) and 28, 1994; and January 20, 23, and 27, 1995.

This new drug application provides for the use of Nisocor in the treatment of hypertension.

We have completed the review of this application including the submitted draft labeling and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the enclosed marked-up draft labeling. Accordingly, the application is approved effective on the date of this letter.

The final printed labeling (FPL) must be identical to the enclosed marked-up draft labeling. Marketing the product with FPL that is not identical to this draft labeling may render the product misbranded and an unapproved new drug.

The approved dissolution specifications are as follows:

3 hours
6 hours
12 hours NLT

Please submit fifteen copies of the FPL as soon as it is available, in no case more than 30 days after it is printed. Please individually mount ten of the copies on heavy weight paper or similar material. For administrative purposes this submission should be designated "FINAL PRINTED LABELING" for approved NDA 20-356. Approval of this labeling by FDA is not required before it is used.

Should additional information relating to the safety and effectiveness of the drug become available, revision of that labeling may be required.

In addition, please submit three copies of the introductory promotional material that you propose to use for this product. All proposed materials should be submitted in draft or mock-up form, not final print. Please send one copy to the Division of Cardio-Renal Drug Products and two copies of both the promotional material and the package insert directly to:

Food and Drug Administration
Division of Drug Marketing, Advertising and Communications, HFD-240
5600 Fishers Lane
Rockville, Maryland 20857

Validation of the regulatory methods is ongoing. At the present time, it is the policy of the Office not to withhold approval because the methods are being validated. Nevertheless, we expect your continued cooperation to resolve any deficiencies that may occur.

Please submit one market package of the drug when it is available.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any questions please contact.

Mr. David Roeder
Consumer Safety Officer
(301) 594-5300

Sincerely yours,

Robert Temple, M.D.
Director
Office of Drug Evaluation I
Center for Drug Evaluation and Research

Enclosure

NISOCOR

(nisoldipine)

Extended Release Tablets

For Oral Use

DESCRIPTION

NISOCOR (nisoldipine) is an extended release tablet dosage form of the dihydropyridine calcium channel blocker nisoldipine. Nisoldipine is 3,5-pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, methyl 2-methylpropyl ester, $C_{20}H_{24}N_2O_6$, and has the structural formula:

supply formula →

Nisoldipine is a yellow crystalline substance, practically insoluble in water but soluble in ethanol. It has a molecular weight of 388.4. NISOCOR tablets consist of an external coat and an internal core. Both coat and core contain nisoldipine, the coat as a slow release formulation and the core as a fast release formulation. NISOCOR tablets contain either 10, 20, 30, or 40 mg of nisoldipine for once-a-day oral administration.

Inert ingredients in the formulation are: hydroxypropylcellulose, lactose, corn starch, crospovidone, microcrystalline cellulose, sodium lauryl sulfate, povidone and magnesium stearate. The inert ingredients in the film coating

are: hydroxypropylmethylcellulose, polyethylene glycol, ferric oxide, and titanium dioxide.

CLINICAL PHARMACOLOGY

Mechanism of Action

Nisoldipine is a member of the dihydropyridine class of calcium channel antagonists (calcium ion antagonists or slow channel blockers) that inhibit the transmembrane influx of calcium into vascular smooth muscle and cardiac muscle. It reversibly competes with other dihydropyridines for binding to the calcium channel. Because the contractile process of vascular smooth muscle is dependent upon the movement of extracellular calcium into the muscle through specific ion channels, inhibition of the calcium channel results in dilation of the arterioles. *In vitro* studies show that the effects of nisoldipine on contractile processes are selective, with greater potency on vascular smooth muscle than on cardiac muscle. Although, like other dihydropyridine calcium channel blockers, nisoldipine has negative inotropic effects *in vitro*, studies conducted in intact anesthetized animals have shown that the vasodilating effect occurs at doses lower than those that affect cardiac contractility.

The effect of nisoldipine on blood pressure is principally a consequence of a dose-related decrease of peripheral vascular resistance. While nisoldipine, like other dihydropyridines, exhibits a mild diuretic effect, most of the antihypertensive activity is attributed to its effect on peripheral vascular resistance.

Nisoldipine pharmacokinetics are independent of the dose in the range of 20 to 60 mg, with plasma concentrations proportional to dose. Nisoldipine accumulation, during multiple dosing, is predictable from a single dose.

Nisoldipine is relatively well absorbed into the systemic circulation with 87% of the radiolabeled drug recovered in urine and feces. The absolute bioavailability of nisoldipine is about 5%. Nisoldipine's low bioavailability is due, in part, to pre-systemic metabolism in the gut wall and this metabolism decreases from the proximal to the distal parts of the intestine. Food with a high fat content has a pronounced effect on the release of nisoldipine from the coat-core formulation and results in a significant increase in peak concentration (C_{max}) by up to 300%. Total exposure, however, is decreased about 25%, presumably because more of the drug is released proximally. This effect appears to be specific for nisoldipine in the controlled release formulation, as a less pronounced food effect was seen with the immediate release tablet. Concomitant intake of a high fat meal with NISOCOR should be avoided.

Maximal plasma concentrations of nisoldipine are reached 6 to 12 hours after dosing. The terminal elimination half-life (reflecting post absorption clearance of nisoldipine) ranges from 7 to 12 hours. C_{max} and AUC increase by factors of approximately 1.3 and 1.5 respectively from first dose to steady state. After oral administration, the concentration of (+) nisoldipine, the active enantiomer, is about 6 times higher than the (-) inactive enantiomer. The

plasma protein binding of nisoldipine is very high, with less than 1% unbound over the plasma concentration range of 100 ng/mL to 10 mcg/mL. Nisoldipine is highly metabolized; 5 major urinary metabolites have been identified. Although 60 - 80% of an oral dose undergoes urinary excretion, only traces of unchanged nisoldipine are found in urine. The major biotransformation pathway appears to be the hydroxylation of the isobutyl ester. A hydroxylated derivative of the side chain, present in plasma at concentrations approximately equal to the parent compound, appears to be the only active metabolite, and has about 10% of the activity of the parent compound. Cytochrome P_{450} enzymes are believed to play a major role in the metabolism of nisoldipine. The particular isoenzyme system responsible for its metabolism has not been identified, but other dihydropyridines are metabolized by cytochrome P_{450} IIIA4. Nisoldipine should not be administered with grapefruit juice as this has been shown, in a study of 12 subjects, to interfere with nisoldipine metabolism, resulting in a mean increase in C_{max} of about 3-fold (ranging up to about 7-fold) and AUC of almost 2-fold (ranging up to about 5-fold). A similar phenomenon has been seen with several other dihydropyridine calcium channel blockers.

Special Populations:

Renal dysfunction: Because renal elimination is not an important pathway, bioavailability and pharmacokinetics of NISOCOR were not significantly different in patients with various degrees of renal impairment. Dosing adjustments in patients with mild to moderate renal impairment are not necessary.

Geriatric: Elderly patients have been found to have 2 to 3 fold higher plasma

concentrations (C_{max} and AUC) than young subjects. This should be reflected in more cautious dosing (See Dosage and Administration).

Hepatic Insufficiency: In patients with liver cirrhosis given 10 mg NISOCOR, plasma concentrations of the parent compound were 4 to 5 times higher than those in healthy young subjects. Lower starting and maintenance doses should be used in cirrhotic patients (See Dosage and Administration).

Gender and Race: The effect of gender or race on the pharmacokinetics of nisoldipine has not been investigated.

Disease States: Hypertension does not significantly alter the pharmacokinetics of nisoldipine.

Pharmacodynamics

Hemodynamic Effects

Administration of a single dose of nisoldipine leads to decreased systemic vascular resistance and blood pressure with a transient increase in heart rate. The change in heart rate is greater with immediate release nisoldipine preparations. The effect on blood pressure is directly related to the initial degree of elevation above normal. Chronic administration of nisoldipine results in a sustained decrease in vascular resistance and small increases in stroke index and left ventricular ejection fraction. A study of the immediate release formulation showed no effect of nisoldipine on the renin-angiotensin-aldosterone system or on plasma norepinephrine concentration in normals. Changes in blood pressure in hypertensive patients given NISOCOR were dose related over the range of 10 - 60 mg/day.

Nisoldipine does not appear to have significant negative inotropic activity in intact animals or humans, and did not lead to worsening of clinical heart

failure in three small studies of patients with asymptomatic and symptomatic left ventricular dysfunction. There is little information, however, in patients with severe congestive heart failure and all calcium channel blockers should be used with caution in any patient with heart failure.

Electropyhysiologic Effects

Nisoldipine has no clinically important chronotropic effects. Except for mild shortening of sinus cycle, SA conduction time and AH intervals, single oral doses up to 20 mg of immediate release nisoldipine did not significantly change other conduction parameters. Similar electrophysiologic effects were seen with single iv doses, which could be blunted in patients pre-treated with beta-blockers. Dose and plasma level related flattening or inversion of T-waves have been observed in a few small studies. Such reports were concentrated in patients receiving rapidly increased high doses in one study; the phenomenon has not been a cause of safety concern in large clinical trials.

Clinical Studies in Hypertension

The antihypertensive efficacy of NISOCOR was studied in 5 double-blind, placebo-controlled, randomized studies, in which over 600 patients were treated with NISOCOR as monotherapy and about 300 with placebo; 4 of the five studies compared 2 or 3 fixed doses while the fifth allowed titration from 10 - 40 mg. Once daily administration of NISOCOR produced sustained reductions in systolic and diastolic blood pressures over the 24 hour dosing interval in both supine and standing positions. The mean placebo-subtracted reductions in supine systolic and diastolic blood pressure at trough, 24 hours post-dose, in these studies, are shown below. Changes in standing blood

pressure were similar:

MEAN SUPINE TROUGH SYSTOLIC AND DIASTOLIC BLOOD PRESSURE CHANGES (mm Hg)

NISOCOR Dose (mg/day)	10 mg	20 mg	30 mg	40 mg	60 mg	10-40mg titrated
Systolic:	8	11	11	14	15	15
Diastolic	3	5	7	7	10	8

In patients receiving atenolol, supine blood pressure reductions with NISOCOR at 20, 40 and 60 mg once daily were 12/6, 19/8 and 22/10 mm Hg, respectively. The sustained antihypertensive effect of NISOCOR was demonstrated by 24 hour blood pressure monitoring and examination of peak and trough effects. The trough/peak ratios ranged from 70 to 100% for diastolic and systolic blood pressure. The mean change in heart rate in these studies was less than one beat per minute. In 4 of the 5 studies, patients received initial doses of 20 - 30 mg NISOCOR without incident (excessive effects on blood pressure or heart rate). The fifth study started patients on lower doses of NISOCOR

Patient race and gender did not influence the blood pressure lowering effect of NISOCOR. Despite the higher plasma concentration of nisoldipine in the elderly, there was no consistent difference in their blood pressure response except that the 10 mg dose was somewhat more effective than in non-elderly patients. No postural effect on blood pressure was apparent and there was no

evidence of tolerance to the antihypertensive effect of NISOCOR in patients treated for up to one year.

INDICATIONS AND USAGE

NISOCOR is indicated for the treatment of hypertension. It may be used alone or in combination with other antihypertensive agents.

CONTRAINDICATIONS

NISOCOR is contraindicated in patients with known hypersensitivity to dihydropyridine calcium channel blockers.

WARNINGS

Increased angina and/or myocardial infarction in patients with coronary artery disease: Rarely, patients, particularly those with severe obstructive coronary artery disease, have developed increased frequency, duration and/or severity of angina, or acute myocardial infarction on starting calcium channel blocker therapy or at the time of dosage increase. The mechanism of this effect has not been established. In controlled studies of NISOCOR in patients with angina this was seen about 1.5% of the time in patients given nisoldipine, compared with 0.9% in patients given placebo.

PRECAUTIONS

General:

Hypotension: Because nisoldipine, like other vasodilators, decreases

peripheral vascular resistance, careful monitoring of blood pressure during the initial administration and titration of NISOCOR is recommended. Close observation is especially important for patients already taking medications that are known to lower blood pressure. Although in most patients the hypotensive effect of NISOCOR is modest and well tolerated, occasional patients have had excessive and poorly tolerated hypotension. These responses have usually occurred during initial titration or at the time of subsequent upward dosage adjustment.

Congestive Heart Failure: Although acute hemodynamic studies of nisoldipine in patients with NYHA Class II-IV heart failure have not demonstrated negative inotropic effects, safety of NISOCOR in patients with heart failure has not been established. Caution therefore should be exercised when using NISOCOR in patients with heart failure or compromised ventricular function, particularly in combination with a beta-blocker.

Patients with Hepatic Impairment: Because nisoldipine is extensively metabolized by the liver and, in patients with cirrhosis, it reaches blood concentrations about 5 times those in normals, NISOCOR should be administered cautiously in patients with severe hepatic dysfunction (See Dosage and Administration)

Information for Patients: NISOCOR is an extended release tablet and should be swallowed whole. Tablets should not be chewed, divided or crushed. NISOCOR should not be administered with a high fat meal. Grapefruit juice, which has been shown to increase significantly the bioavailability of nisoldipine and other dihydropyridine type calcium channel

blockers, should not be taken with NISOCOR

Laboratory Tests: NISOCOR is not known to interfere with the interpretation of laboratory tests.

Drug Interactions: A 30 to 45% increase in AUC and C_{max} of nisoldipine was observed with concomitant administration of cimetidine 400 mg twice daily. Ranitidine 150 mg twice daily did not interact significantly with nisoldipine (AUC was decreased by 15 - 20 %). No pharmacodynamic effects of either H_2 antihistamine were observed.

Pharmacokinetic interactions between nisoldipine and beta-blockers (atenolol, propranolol) were variable and not significant. Propranolol attenuated the heart rate increase following administration of immediate release nisoldipine. The blood pressure effect of NISOCOR tended to be greater in patients on atenolol than in patients on no other antihypertensive therapy.

Quinidine at 648 mg bid ~~increased~~ decreased the bioavailability (AUC) of nisoldipine by 26%, but not the peak concentration. The immediate release, but not the coat-core, formulation of nisoldipine increased plasma quinidine concentrations by about 20 %. This interaction was not accompanied by ECG changes and its clinical significance is not known.

No significant interactions were found between nisoldipine and warfarin or digoxin.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Dietary administration of nisoldipine to male and female rats for up to 24 months

(mean doses up to 82 and 111 mg/kg/day, 16 and 19 times the maximum recommended human dose {MRHD} on a mg/m^2 basis, respectively) and female mice for up to 21 months (mean doses of up to 217 mg/kg/day, 20 times the MRHD on a mg/m^2 basis) revealed no evidence of tumorigenic effect of nisoldipine. In male mice receiving a mean dose of 163 mg nisoldipine/kg/day (16 times the MRHD of 60 mg/day on a mg/m^2 basis), an increased frequency of stomach papilloma, but still within the historical range, was observed. No evidence of stomach neoplasia was observed at lower doses (up to 58 mg/kg/day). Nisoldipine ~~tested~~ was when tested negative in a battery of genotoxically assays, including the Ames test and the CHO/HGRPT assay for mutagenicity ~~mutagenicity and clastogenicity tests~~. and there in vivo mouse micronucleus test and in vitro CHO cell test for clastogenicity. When administered to male and female rats at doses of up to 30 mg/kg/day (about 5 ~~and 6~~ times the MRHD on a mg/m^2 basis ~~respectively~~) nisoldipine had no effect on fertility.

Pregnancy Category C: Nisoldipine was neither teratogenic nor fetotoxic at doses that were not maternally toxic. Nisoldipine was fetotoxic but not teratogenic in rats and rabbits at doses resulting in maternal toxicity (reduced maternal body weight gain). In pregnant rats, increased fetal resorption (post-implantation loss) was observed at 100 mg/kg/day and decreased fetal weight was observed at both 30 and 100 mg/kg/day. These doses are, respectively, about 5 and 16 times the MRHD when compared on a ~~body~~ mg/m^2 basis ~~surface area basis~~. In pregnant rabbits, decreased fetal and placental weights were observed at a dose of 30 mg/kg/day, about 10 times the MRHD when compared on a ~~body surface area~~ mg/m^2 basis. In a study in which pregnant monkeys (both treated and control) had high rates of abortion and mortality, the only surviving fetus from a group exposed to a maternal dose of 100 mg nisoldipine/kg/day (about 30 times the MRHD when compared on a ~~body~~ mg/m^2 basis)

~~surface area basis~~) presented with forelimb and vertebral abnormalities not previously seen in control monkeys of the same strain. There are no adequate and well controlled studies in pregnant women. NISOCOR should be used in pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers: It is not known whether nisoldipine is excreted in human milk. Because many drugs are excreted in human milk, a decision should be made to discontinue nursing, or to discontinue NISOCOR, taking into account the importance of the drug to the mother.

ADVERSE EXPERIENCES

More than 6000 patients world-wide have received nisoldipine in clinical trials for the treatment of hypertension, either as the immediate release or the NISOCOR extended release formulation. Of about 1,500 patients who received NISOCOR in hypertension studies, about 55% were exposed for at least 2 months and about one third were exposed for over 6 months, the great majority at doses of 20 to 60 mg daily.

NISOCOR is generally well-tolerated. In the U.S. clinical trials of NISOCOR in hypertension, 10.9% of the 921 NISOCOR patients discontinued treatment due to adverse events compared with 2.9% of 280 placebo patients. The frequency of discontinuations due to adverse experiences was related to dose, with a 5.4% discontinuation rate at 10 mg daily and a 10.9% discontinuation rate at 60 mg daily.

The most frequently occurring adverse experiences with NISOCOR are those related to its vasodilator properties; these are generally mild and only

occasionally lead to patient withdrawal from treatment. The table below, from U.S. placebo-controlled parallel dose response trials of NISOCOR using doses from 10 - 60 mg once daily in patients with hypertension, lists all of the adverse events, regardless of the causal relationship to NISOCOR, for which the overall incidence on NISOCOR was both >1% and greater with NISOCOR than with placebo.

Adverse Event	Nisoldipine (%) (n=663)	Placebo (%) (n=280)
Peripheral Edema	22	10
Headache	22	15
Dizziness	5	4
Pharyngitis	5	4
~~Asthenia~~	4	4
Vasodilation	4	2
Sinusitis	3	2
Palpitation	3	1
Chest Pain	2	1
Nausea	2	1
Rash	2	1

not greater on Nisocor

Only peripheral edema and possibly dizziness appear to be dose related.

Adverse Event	Placebo	NISOCOR 10 mg	NISOCOR 20 mg	NISOCOR 30 mg	NISOCOR 40 mg	NISOCOR 60 mg
(Rates in %)	N=280	N=30	N=170	N=105	N=139	N=137

Peripheral Edema	10	7	15	20	27	29
Dizziness	4	7	3	3	4	10

The common adverse events occurred at about the same rate in men as in women, and at a similar rate in patients over age 65 as in those under that age, except that headache was much less common in older patients. Except for peripheral edema and vasodilation, which were more common in whites, adverse event rates were similar in blacks and whites.

The following adverse events occurred in ≤1 % of all patients treated for hypertension in U.S. and foreign clinical trials, or with unspecified incidence in other studies. Although a causal relationship of NISOCOR to these events cannot be established, they are listed to alert the physician to a possible relationship with NISOCOR treatment.

Body As A Whole: cellulitis, chills, facial edema, fever, flu syndrome, malaise,

Cardiovascular: atrial fibrillation, cerebrovascular accident, congestive heart failure, first degree AV block, hypertension, hypotension, jugular venous distension, migraine, myocardial infarction, postural hypotension, ventricular extrasystoles, supraventricular tachycardia, syncope, systolic ejection murmur, T wave abnormalities on ECG (flattening, inversion, nonspecific changes), venous insufficiency,

Digestive: abnormal liver function tests, anorexia, colitis, diarrhea, dry mouth, dyspepsia, dysphagia, flatulence, gastritis, gastrointestinal

hemorrhage, gingival hyperplasia, glossitis, hepatomegaly, increased appetite, melena, mouth ulceration,

Endocrine: diabetes mellitus, thyroiditis,

Hemic and Lymphatic: anemia, ecchymoses, leukopenia, petechiae,

Metabolic and Nutritional: gout, hypokalemia, increased serum creatine kinase, increased nonprotein nitrogen, weight gain, weight loss,

Musculoskeletal: arthralgia, arthritis, leg cramps, myalgia, myasthenia, myositis, tenosynovitis,

Nervous: abnormal dreams, abnormal thinking and confusion, amnesia, anxiety, ataxia, cerebral ischemia, decreased libido, depression, hypesthesia, hypertonia, insomnia, nervousness, paresthesia, somnolence, tremor, vertigo,

Respiratory: asthma, dyspnea, end inspiratory wheeze and fine rales, epistaxis, increased cough, laryngitis, pharyngitis, pleural effusion, rhinitis, sinusitis,

Skin and Appendages: acne, alopecia, dry skin, exfoliative dermatitis, fungal dermatitis, herpes simplex, herpes zoster, maculopapular rash, pruritus, pustular rash, skin discoloration, skin ulcer, sweating, urticaria,

Special senses: abnormal vision, amblyopia, blepharitis, conjunctivitis, ear pain, glaucoma, itchy eyes, keratoconjunctivitis, otitis media, retinal detachment, tinnitus, watery eyes, taste disturbance, temporary unilateral loss of vision, vitreous floater, watery eyes,

Urogenital: dysuria, hematuria, impotence, nocturia, urinary frequency, increased BUN and serum creatinine, vaginal hemorrhage, vaginitis.

experience with

In addition to NISOCOR, there is extensive experience with the immediate release formulation of nisoldipine. Adverse events were generally similar to

those seen with NISOCOR. Unusual events observed with immediate release nisoldipine but not observed with NISOCOR, were one case each of angioedema and photosensitivity. Spontaneous reports from postmarketing experience with the immediate release formulation of nisoldipine have not revealed any additional adverse events not identified in the above listings.

OVERDOSAGE

There is no experience with nisoldipine overdosage. Generally, overdosage with other dihydropyridines leading to pronounced hypotension calls for active cardiovascular support including monitoring of cardiovascular and respiratory function, elevation of extremities, judicious use of calcium infusion, pressor agents and fluids. Clearance of nisoldipine would be expected to be slowed in patients with impaired liver function. Since nisoldipine is highly protein bound, dialysis is not likely to be of any benefit; however, plasmapheresis may be beneficial.

DOSAGE AND ADMINISTRATION

The dosage of NISOCOR must be adjusted to each patient's needs. Therapy usually should be initiated with 20 mg orally once daily, then increased by 10 mg per week or longer intervals, to attain adequate control of blood pressure. Usual maintenance dosage is 20 to 40 mg once daily. Blood pressure response increases over the 10 - 60 mg daily dose range but adverse event rates also increase. Doses beyond 60 mg once daily are not recommended. NISOCOR has been used safely with diuretics, ACE inhibitors, and beta-

blocking agents.

Patients over age 65, or patients with impaired liver function are expected to develop higher plasma concentrations of nisoldipine. Their blood pressure should be monitored closely during any dosage adjustment. A starting dose not exceeding 10 mg daily is recommended in these patient groups.

NISOCOR tablets should be administered orally once daily. Administration with a high fat meal can lead to excessive peak drug concentration and should be avoided. Grapefruit products should be avoided before and after dosing. NISOCOR is an extended release dosage form and tablets should be swallowed whole, not bitten or divided.

HOW SUPPLIED

NISOCOR extended release tablets are supplied as 10 mg, 20 mg, 30 mg, and 40 mg round film coated tablets. The different strengths can be identified as follows:

Strength	Color	Markings
10 mg	Oyster	**891** on one side and **MILES 10** on the other side.
20 mg	Yellow Cream	**892** on one side and **MILES 20** on the other side.
30 mg	Mustard	**893** on one side and **MILES 30** on the other side.
40 mg	Burnt Orange	**894** on one side and **MILES 40** on the other side.

NISOCOR Tablets are supplied in:

	Strength	NDC Code
Bottles of 30	10 mg	0026-8911-30
	20 mg	0026-8921-30
	30 mg	0026-8931-30
	40 mg	0026-8941-30
Bottles of 100	10 mg	0026-8911-51
	20 mg	0026-8921-51
	30 mg	0026-8931-51
	40 mg	0026-8941-51
Unit Dose Packages of 100	10 mg	0026-8911-48
	20 mg	0026-8921-48
	30 mg	0026-8931-48
	40 mg	0026-8941-48

The tablets should be protected from light and moisture and stored below 86°F (30°C). Dispense in tight, light-resistant containers.

Distributed by:

Miles Inc.

Pharmaceutical Division

400 Morgan Lane

West Haven, CT 06516 USA

Made in Germany

(Revise throughout labeling)

Nisocor

891130 NDC 0026-8911-48

[redacted]

(nisoldipine)
Extended Release Tablets
10 mg
100 Tablets Unit Dose

891130 NDC 0026-8911-48

NIS® CC

(nisoldipine) Extended Release Tablet

10 mg
100 Tablets
Unit Dose

For institutional use only.
RECOMMENDED STORAGE:
STORE BELOW 86°F (30°C).
Each tablet contains 10 mg nisoldipine.
Tablets should be swallowed whole, not bitten or divide
Dosage: See accompanying prescribing information.

MILES

Distributed By:
Miles Inc.
Pharmaceutical Division
400 Morgan Lane
West Haven, CT 06516
Made in Germany

891130 NDC 0026-8911-48

NIS® CC

nisoldipine)
Extended Release Tablets
10 mg
100 Tablets Unit Dose
Each tablet contains 10 mg nisoldipine
For institutional use only
Caution: Federal (USA) law prohibits dispensing without prescription.

MILES

Distributed By:
Miles Inc.
Pharmaceutical Division
400 Morgan Lane
West Haven, CT 06516
Made in Germany

PN101072 3/93 ©1993 Miles Inc. 2836 Printed in USA

W 74 mm x H 106 mm x D 72.5 mm
Panel opposite the glue flap is 1/8" shorter

Filename: NIS CC 10 mg Carton/PN101072
Job #: 18356
Control #: 2836
Date: 3/4/93

04 03 0000019

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

CSO

Food and Drug Administration
Rockville MD 20857

MAR 25 1994

NDA 20-356

Miles Inc.
Pharmaceutical Division
Attention: Nancy C. Motola, Ph.D.
400 Morgan Lane
West Haven, CT 06516-4175

Dear Dr. Motola:

Please refer to your March 31, 1993 new drug application submitted under section 505(b)(1) of the Federal Food, Drug and Cosmetic Act for nisoldipine coat core tablets.

We also acknowledge receipt of your amendments and correspondence dated May 24 (two), June 3 and 8, July 15, 16, and 26 (two), August 2 and 17, September 2, 13 (two), 16, 24, 27, and 28, October 7 (two) and 14, November 4 (two), 10, 16, and 23 (two), and December 6, 9, and 29, 1993; and January 28, February 7, 8, and 24 (two), and March 15, 1994.

We have completed our review and find the information presented is inadequate and the application is not approvable. Under section 505(d) of the Act and 21 CFR 314.125(b) the data and information submitted do not establish safety and efficacy of nisoldipine (BAY k 5552) coat core tablets. The deficiencies may be summarized as follows:

1. Your proposed expiry date cannot be properly evaluated with the data submitted. Therefore, please submit additional stability data, especially in support of the shelf life specification limits proposed for the nitropyridine compound.

2. Before the application can be approved, must include a stereochemical identity test to demonstrate that the new drug substance is a racemic mixture as described in the enclosed FDA policy statement for new stereoisomeric drugs.

3. You have not responded to our July 1, 1993 request (item #6) for the maximum time that will be allowed between manufacture and packaging of the tablets.

4. Please submit the updated version of the procedure that will include the system suitability information

5. Please submit labeling for the unit dose blisters.

6. We remind you that any reprocessing procedures must be approved, and you must submit a proprietary name as soon as possible sc that we will have sufficient time to review it.

Although the clinical data appear to provide adequate support for the hypertension indication, . Although nisoldipine significantly improved exercise tolerance at trough in two of the four major clinical trials, this was accompanied by an effect on ischemia in only one and on time to angina in only one. Two other studies of good size showed no significant effect on any measure. Although the peak effects were slightly more prominent, especially at 40-60 mg, an effective angina drug should work throughout the dosing interval. In the largest and longest trial (D89-042), the only controlled trial lasting more than two weeks, nisoldipine CC was less effective than in the other major studies in its effect even on peak measurements.

As noted, nisoldipine was not shown to be

As noted in the February 28 and March 2, 1994 telephone discussions between Dr. Charles Resnick of this Agency and Drs. Fred Sundermann and Michael Porter of Miles, we are concerned about the occurrence of brain tumors in nisoldipine-treated male rats and stomach tumors in nisoldipine-treated male mice (specifically, granular cell tumors of the rat brain and papillomas of the mouse stomach).

The tumor findings could possibly have an impact on the approvability of your application. We are awaiting your submission of historical control data on the these tumors as well as other information requested by Dr. Resnick. The nisoldipine carcinogenicity studies will likely be considered by our Carcinogenicity Assessment Committee (CAC) and, should this occur, Miles will be invited to make a presentation.

Within 10 days after the date of this letter, you are required to amend the application, notify us of your intent to file an amendment, or follow one of your other options under 21 CFR 314.120. In the absence of any such action FDA may proceed to withdraw the application. Any amendment should respond to all the deficiencies listed. We will not process a partial reply as a major amendment nor will the review clock be reactivated until all deficiencies have been addressed.

Should you have any questions, please contact:

Mr. David Roeder
Consumer Safety Officer
Telephone: (301) 443-4730

Sincerely yours,

Robert Temple, M.D.
Director
Office of Drug Evaluation I
Center for Drug Evaluation and Research

Enclosure

FDA'S POLICY STATEMENT FOR THE DEVELOPMENT OF NEW STEREOISOMERIC DRUGS

I. INTRODUCTION AND BACKGROUND

Stereoisomers are molecules that are identical in atomic constitution and bonding, but differ in the three-dimensional arrangement of the atoms. For the purpose of this document, the stereoisomeric pairs of greatest interest are those with one or more asymmetric (chiral) centers whose enantiomers (individual stereoisomers) are mirror images. They have essentially identical physical (except for optical rotatory) and chemical (except in a chiral environment) properties.

This document focuses on issues relating to the study and pharmaceutical development of individual enantiomers and racemates. Such stereoisomers usually require specialized chiral techniques for their correct identification, characterization, separation and measurement. They are often readily distinguished by biological systems, however, and may have different pharmacokinetic properties (absorption, distribution, biotransformation, and excretion) and quantitatively or qualitatively different pharmacologic or toxicologic effects.

When stereoisomers are biologically distinguishable, they might seem to be different drugs, yet it has been past practice to develop racemates (i.e., compound with 50:50 proportion of enantiomers). The properties of the individual enantiomers have not generally been well studied or characterized. Whether separated enantiomers should be developed was largely an academic question because commercial separation of racemates was difficult. Now that technological advances (large scale chiral separation procedures or asymmetric syntheses) permit production of many single enantiomers on a commercial scale, it is appropriate to consider what FDA's policy with respect to stereoisomeric mixtures should be. Development of racemates raises issues of acceptable manufacturing control of synthesis and impurities, adequate pharmacologic and toxicologic assessment, proper characterization of metabolism and distribution, and appropriate clinical evaluation.

It should be noted that the term "stereoisomers" is a general one for all isomers that differ only in the orientation of the atoms in space. Stereoisomers include not only the mirror image enantiomers, but also geometric (cis/trans) isomers and diastereoisomers (isomers of drugs with more than one chiral center that are not mirror images of one another). Diastereoisomers and geometric isomers are both chemically distinct and pharmacologically different (unless they are interconverted *in vivo*) and are generally readily separated without chiral techniques. Geometric isomers and diastereoisomers therefore should, with the rare exception of cases where *in vivo* interconversion occurs, be treated as separate drugs and developed accordingly. There is no reason to consider developing mixtures of geometric isomers or diastereoisomers unless they

fortuitously represent a reasonable fixed dose combination (see 21 CFR 300.50). Even in that case, whether the optimal ratio of the two isomers is the ratio produced by an undirected or unmodified synthesis should be critically examined. In general, geometric isomers have been developed as single isomers. Practice with respect to diastereoisomers has been variable. These categories of stereoisomers will not be considered further in this document.

Examination of cases in which the properties of enantiomers have been evaluated reveals 1) instances in which both members had similar desirable activities (both enantiomers of dobutamine are positive inotropes; both ibuprofen enantiomers are anti-inflammatory agents; both enantiomers of warfarin and phenprocoumon are anticoagulants; the enantiomers of bupivicaine both produce local anesthesia, the enantiomers of the quinolones and the β-lactam antibiotics are all antibacterial), 2) instances in which one member of a pair was pharmacologically active and the other inactive (l-propranolol is a β-blocker; d-propranolol is not), and 3) cases in which the enantiomers had completely different activities (d-sotalol is a type 3 antiarrhythmic while l-sotalol is a β-blocker) or h different concentration-response relationships for a given property. While inactivity of one member of a pair might be considered trivial, there are instances in which toxicity has been linked to one member of a pair of stereoisomers, not necessarily the active isomer (granulocytopenia is related to the d-isomer of levodopa; vomiting is caused by the d-isomer of levamisole; and myasthenia gravis symptoms were no longer observed when the d-isomer was removed from d,l-carnitine), and there are examples of an effect on the disposition of one member of a pair by the other. In addition, there are many cases in which enantiomers have been shown to have different pharmacokinetic behavior. Differences in pharmacokinetic behavior may not pose a major therapeutic problem although it can make non-chiral blood level assays difficult to interpret with respect to activity and confuse interpretation of non-clinical data if the pharmacokinetic properties of the isomers in animals differ from those in humans.

While some enantiomeric pairs have had interesting and useful therapeutic properties (e.g., dl-sotalol, dl-dobutamine), there is no reason to expect the optimum ratio of the components to be the 1:1 ratio of a racemate (i.e., the dose-response curves would not usually be expected to be congruent).

Despite the problems identified with some racemates, the common practice of developing racemates has resulted in few recognized adverse consequences. Although it is now technologically feasible to prepare purified enantiomers, development of racemates may continue to be appropriate. However, currently available information suggests that the following should be considered in product development:

1. Appropriate manufacturing and control procedures should be used to assure stereoisomeric composition of a product with respect to identity, strength, quality and purity. Manufacturers should

notify compendia of these specifications and tests.

2. Pharmacokinetic evaluations that do not use a chiral assay will be misleading if the disposition of the enantiomers is different. Therefore, techniques to quantify individual stereosiomers in pharmacokinetic samples should be available early. If the pharmacokinetics of the enantiomers are demonstrated to be the same or to exist as a fixed-ratio in the target population, an achiral assay or an assay that monitors one of the enantiomers may be used, subsequently.

II. POLICY

General

The stereoisomeric composition of a drug with a chiral center should be known and the quantitative isomeric composition of the material used in pharmacologic, toxicologic, and clinical studies known. Specifications for the final product should assure identity, strength, quality, and purity from a stereochemical viewpoint.

To evaluate the pharmacokinetics of a single enantiomer or mixture of enantiomers, manufacturers should develop quantitative assays for individual enantiomers in *in vivo* samples early in drug development. This will allow assessment of the potential for interconversion and the absorption, distribution, biotransformation, and excretion (ADBE) profile of the individual isomers. When the drug product is a racemate and the pharmacokinetic profiles of the isomers are different, manufacturers should monitor the enantiomers individually to determine such properties as dose linearity and the effects of altered metabolic or excretory function and drug-drug interactions. If the pharmacokinetic profile is the same for both isomers or a fixed ratio between the plasma levels of enantiomers is demonstrated in the target population, an achiral assay or an assay that monitors one of the stereoisomers should suffice for later evaluation. *In vivo* measurement of individual enantiomers should be available to help assess toxicologic findings, but if this cannot be achieved, it would be sufficient in some cases to establish the kinetics of the isomers in humans.

Unless it proves particularly difficult, the main pharmacologic activities of the isomers should be compared in *in vitro* systems, in animals and/or in humans.

A relatively benign toxicologic profile using the racemate would ordinarily support further development without separate toxicologic evaluation of the individual enantiomers. If, however, there are toxic findings other than those that are natural extensions of the pharmacologic effects of the drug, and especially if they are unusual or occur near the effective dose in

animals or near the planned human exposure, toxicologic evaluation of the individual isomers in the study where the toxicity was detected should be undertaken.

FDA invites discussion with sponsors concerning whether to pursue development of the racemate or the individual enantiomer. All information developed by the sponsor or available from the literature that is relevant to the chemistry, pharmacology, toxicology, or clinical actions of the stereoisomers should be included in the IND and NDA submissions.

Specific

Chemistry:

The chemistry section of the application should contain the requisite information to assure the identity, quality, purity and strength of the drug substance and drug product. In addition, the following considerations should be taken into account when dealing with chiral drug substances and drug products.

Methods and Specifications

Drug Substance

Applications for enantiomeric and racemic drug substances should include a stereochemically specific identity test and/or a stereochemically selective assay method. The choice of the controls should be based upon the substance's method of manufacture and stability characteristics.

Drug Product

Applications for drug products that contain an enantiomeric or racemic drug substance should include a stereochemically specific identity test and/or a stereochemically selective assay method. The choice of the controls should be based upon the product's composition, method of manufacture and stability characteristics.

Stability

The stability protocol for enantiomeric drug substances and drug products should include a method or methods capable of assessing the stereochemical integrity of the drug substance and drug product. However, once it has been demonstrated that stereochemical conversion does not occur, stereoselective tests might not be needed.

Labeling

The labeling should include a unique established name and a chemical name with the appropriate stereochemical descriptors.

Pharmacology/Toxicology:

Pharmacology

The pharmacologic activity of the individual enantiomers should be characterized for the principal pharmacologic effect and any other important pharmacological effect, with respect to potency, specificity, maximum effect, etc.

Pharmacokinetic Profile

To monitor *in vivo* interconversion and disposition, the pharmacokinetic profile of each isomer should be characterized in animals and later compared to the clinical pharmacokinetic profile obtained in phase 1.

Toxicology

It is ordinarily sufficient to carry out toxicity studies on the racemate. If toxicity other than that predicted from the pharmacologic properties of the drug occurs at relatively low multiples of the exposure planned for clinical trials, the toxicity study where the unexpected toxicity occurred should be repeated with the individual isomers to ascertain whether only one enantiomer was responsible for the toxicity. If toxicity of significant concern can be eliminated by development of a single isomer with the desired pharmacologic effect, it would in general be desirable to do so. The Agency would be pleased to discuss any cases where questions exist regarding the definition of "significant toxicity".

Impurity Limits

It is essential to determine the concentration of each isomer and define limits for all isomeric components, impurities, and contaminants on the compound tested preclinically that is intended for use in clinical trials. The maximum allowable level of impurity in a stereoisomeric product employed in clinical trials should not exceed that present in the material evaluated in nonclinical toxicity studies.

Developing a Single Stereoisomer After the Racemate is Studied

To develop a single stereoisomer from a mixture that has already been studied non-clinically, an abbreviated, appropriate pharmacology/toxicology evaluation could be conducted to allow the existing knowledge of the racemate available to the sponsor to be applied to the pure stereoisomer. Bridging studies would usually include the longest repeat-

dose toxicity study conducted (up to 3 months), and the reproductive toxicity segment II study in the most sensitive species, using the single enantiomer. These studies should include a positive control group consisting of the racemate. If there is no difference between the toxicological profile of the single stereoisomeric product and the racemate, no further studies would be needed. If the single enantiomer is more toxic, the explanation should be sought and the implications for human dosing considered.

Clinical and Biopharmaceutical:

Where little difference is observed in activity and disposition of the enantiomers, racemates may be developed.

In some situations, development of a single enantiomer is particularly desirable (e.g., where one enantiomer has a toxic or undesirable pharmacologic effect and the other does not). A signal that should trigger further investigation of the properties of the individual enantiomers and their active metabolites is the occurrence at clinical doses of toxicity with the racemate that is not clearly expected from the pharmacology of the drug or the occurrence of any other unexpected pharmacologic effect with the racemate. These signals might be explored in animals but human testing may be essential. It should be appreciated that toxicity or unusual pharmacologic properties might reside not in the parent isomer, but in an isomer-specific metabolite.

In general, it is more important to evaluate both enantiomers clinically and consider developing only one when both enantiomers are pharmacologically active but differ significantly in potency, specificity, or maximum effect, than when one isomer is essentially inert. Where both enantiomers are fortuitously found to carry desirable but different properties, development of a mixture of the two, not necessarily the racemate, as a fixed combination might be reasonable.

If a racemate is studied, the pharmacokinetics of the two isomers should be studied in Phase 1. Potential interconversion should also be examined. Based on Phase 1 or 2 pharmacokinetic data in the target population, it should be possible to determine whether an achiral assay or monitoring of just one enantiomer where a fixed ratio is confirmed will be sufficient for pharmacokinetic evaluation.

If a racemate has been marketed and the sponsor wishes to develop the single enantiomer, evaluation should include determination of whether there is significant conversion to the other isomer, and whether the pharmacokinetics of the single isomer are the same as they were for that isomer as part of the racemate.

FEB 2 1995

CSO Application Overview

Application: NDA 20-356
Nisoldipine Coat Core Tablets

Sponsor: Miles Pharmaceuticals

NDA Receipt Date: April 1, 1993

NDA Resubmission Date: August 3, 1994

User Fee Goal Date: February 3, 1995

Date of Overview: November 28, 1994

Background

NDA 20-356 provides for the use of a sustained release (once-daily) formulation of nisoldipine in the treatment of hypertension. No formulation of nisoldipine is currently approved in the U.S.

A non-approval letter was issued on March 25, 1994, that listed deficiencies in the Chemistry, Pharmacology, and Clinical sections. The firm responded fully to this letter on August 3, 1994. In resubmitting the NDA, they

Review

Chemistry

Reviewer: Danute Cunningham

Reviews: 6/7/93 11/29/93 2/4/94 9/16/94

Ms. Cunningham's review of the application has been completed.

The trade name "Nisocor" has been approved by the Nomenclature Committee.

The facility inspection has been completed and was found to be satisfactory. We received a satisfactory response to our FUR on November 22, 1994.

The deficiencies outlined in the environmental assessment review were sent to the firm on October 27, 1994. The response from the firm has not been received yet.

Pharmacology

Reviewers: Xavier Joseph, D.V.M.
Sidney Stolzenberg, Ph.D.

Review: September 2, 1994

The reviewers comments have been incorporated into the draft labeling. The application went before the CAC, and the recommendations from that committee have also been incorporated into the labeling. The minutes of the CAC meeting have not been completed yet.

Biopharmaceutics

Reviewer: Patrick Marroum, Ph.D.

The Biopharm Day was held for this application, revisions have been made, and the draft is now under supervisory review.

Dr. Marroum has made a number of comments including extensive revisions of the labeling (see pp 13-16 of Dr. Marroum's review). The labeling recommendations have been incorporated into the draft package insert. He has also recommended that the dissolution specifications be revised as follows:

from:	to:
3 hours 15 to	3 hours
6 hours	6 hours
12 hours NLT	12 hours NLT

Statistical

Reviewer: Nancy Smith

Review (hypertension): 1/4/94

Dr. Smith has reviewed only the hypertension indication. There were no serious problems identified in the review.

Clinical

Reviewers: Shaw Chen, M.D., Ph.D. (Clinical Pharmacology) 2/16/94
Phil Dern, M.D. (Safety): 9/27/93
Cristobal Duarte, M.D. (hypertension): 8/4/93
Norman Stockbridge, M.D., Ph.D. (angina): 8/4/93

The reviewers recommend approval for the hypertension indication.

The final safety update is under review.

DSI Audits

Four of the seven requested DSI audits are completed. There have been no problems so far.

Labeling

Dr. Chen has provided a marked up copy of the package insert.

David Roeder
Consumer Safety Officer

dr/9-4-94/9-27-94/10-28-94/11-23-94/11-28-94

cc: NDA 20-356
HFD-110
HFD-111/DRoeder

Roeder

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

Date: 10/25/94

From: Shaw T. Chen, M.D., Ph.D., Medical Group Leader, HFD-110
Through: Director, Division of Cardiorenal Drug Products, HFD-110 NOV 21 1994
To: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: NDA 20-356, Nisoldipine Core-Coat for Hypertension, Approvability

OVERVIEW

This memorandum and the attached material constitute the Division's recommendation that NDA 20-356, Nisoldipine Core Coat (referred to as CC formulation) Tablets be approved for treatment of hypertension.

This package is being transmitted with a draft Summary Basis of Approval (SBA) prepared by the sponsor, which has not been edited by the Division but appears to be accurate in its contents to serve as one of the references for secondary/tertiary reviews of the application. In the draft SBA, any description or interpretation of the data different from that of this memo should be disregarded.

As one of the new team approaches, the primary medical review of the NDA were conducted in parallel by the following medical officers:

Clinical Pharmacology:	Dr. Chen
Hypertension -Efficacy:	Dr. Duarte
Hypertension -Safety:	Dr. Dern

Pharmacology sections of the application were also reviewed concurrently by two reviewers (Drs Joseph and Stolzenberg); a synoptic summary of all pharmacologic issues has been prepared by Dr. Joseph. As of the date of this memo, the chemistry, biopharmaceutical, pharmacological and statistical reviews have been completed. There are no major, unresolved preclinical issues which may affect the action recommended. Related labeling have been suitably edited.

Nisoldipine is a new calcium channel blocker of the dihydropyridine type and structurally related to nifedipine. It appears to be a less active inotrope than nifedipine *in vitro* but the two were not distinguishable in intact animals. There are no major efficacy or safety issues that should preclude the approvability of this drug for the hypertension.

The adverse experiences in the NDA have been amended with the First Safety Updates of 08/17/93. Selected major trials should be inspected before final approval of the application.

PRECLINICAL EVALUATIONS

Chemistry

There are no outstanding issues regarding the manufacturing and analytical controls. Final inspection will be scheduled.

Preclinical Pharmacology

Nisoldipine has been adequately characterized with respect to its preclinical pharmacokinetic and pharmacodynamic properties. There are no outstanding issues related to animal toxicity or carcinogenicity which may affect approvability of the drug.

Changes in proposed labeling, as recommended by the pharmacology reviewers, are summarized and commented below. They have been adopted with minor modification.

- Negative findings in carcinogenic studies should be qualified with the dosages studied, comparison with human dose should be based on both body weight and surface area calculations.
- Fetotoxicities in animals are suggestive, not conclusive. However, detailed description of the problematic monkey studies is not necessary. Again, basis of safety margin (toxic animal dose vs maximal human dose) should be specified (body weight and surface area).
- The pharmacology reviewers do not think malformation is increased in rabbits. Other recommendations related to fetotoxicity in rats/rabbits, sections of *Labor and Delivery*, and *Nursing Mothers* are all appropriate (Pharmacology Review, p 145).

CLINICAL PHARMACOLOGY

Pharmacokinetics/Pharmacodynamics

At the proposed dosages of 10-40 mgs, the **pharmacokinetic** profile of nisoldipine CC formulation supports a once-daily regimen. Compared with the immediate release (IR) form, availability of nisoldipine from the CC tablets was prolonged with lower C_{max} and higher AUC over 24 hrs. Bioavailability of nisoldipine CC was low for the unchanged drug but linear and dose-proportional over the range of 10-60 mg; it accumulates moderately after multiple oral dosing (7 days). While nisoldipine is extensively metabolized, the only active metabolite contributes about 10% of the pharmacologic effects.

The states of both hepatic and renal functions are potentially important for pharmacokinetics of the active drug, since nisoldipine is extensively metabolized and excretion of the metabolites is predominantly renal. Bioavailability of the parent drug was indeed increased by 4-5 fold in patients with hepatic failure, but changes in AUC and C_{max} due to various degree of renal impairment were only transient and diminished with multiple dosing. Plasma levels of nisoldipine were also higher in the elderly but dosage adjustment may not be required (see Efficacy -Hypertension). Nisoldipine metabolism probably involves P_{450} cytochrome system (as nifedipine), but no attempt to identify isozyme has been documented. Modest changes in bioavailability of CC nisoldipine were observed with

concomitant use of ranitidine (decreased 15-20%), cimetidine (increased 30-45%), quinidine (reduced by 25%), and propranolol ($t_{1/2}$ shorter by 20%). These interactions are probably of no significant clinical consequences, but labeling has been edited accordingly.

As noted in the Clinical Pharmacology and Biopharmaceutical Reviews, the problem of dose-dumping when nisoldipine CC is administered in a non-fasted state or with grapefruit juice (see biopharm review of Study 770) can not be ignored. Nisoldipine CC should not be administered concomitantly with meal or grapefruit juice, but instead after overnight fast and 1-2 hours before breakfast. Appropriate instructions to avoid dose administration in such settings have been included in the labeling.

Nisoldipine is a vasodilating antihypertensive with pharmacodynamic activities similar to other approved calcium channel blockers. Cardiovascular and hemodynamic effects of nisoldipine have been fairly well-established. Correlation between nisoldipine dose, plasma level and blood pressure reduction was good over the recommended dosage range.

Nisoldipine has no appreciable inotropic effects, but its clinical advantages over nifedipine has not been documented. Except for T-wave changes mostly at high doses (see Safety), nisoldipine had minimal electrophysiology activities. There is some evidence that iv nisoldipine improves coronary blood flow, but its anti-ischemic effect was not established in clinical pharmacology studies.

Nisoldipine did not affect regional blood flow in kidney or liver, and has no significant pharmacologic activities on non-cardiovascular systems.

Biopharmaceutics

Issues raised in the Bio-pharmaceutical Review are commented as follows.

- The ratio of two enantiomers (and other metabolites) in special patient groups were not determined. Since no surprising clinical effects were observed in these patients which may required explanation, such data are not relevant for approval or prescription instruction.
- Nisoldipine is metabolized by the P-450 enzyme system, but the specific iso-enzyme involved has not been identified. While metabolism of nisoldipine is not expected to be significantly different from that of nifedipine, the study should be done post-approval, however.
- Inconsistent C_{max} (by 2-folds) obtained after a 30 mg dose in two small pharmacology studies may be related to the variability in dissolution and need further clarification, as different blood pressure reductions from placebo were also noted in the efficacy trials (see below). Since no efficacy/safety problems were attributed to this variation, approval is not affected.
- Assay validation was described in most of the studies, but missing in a few reports. It is reasonable to assume that same assay was used in all trials.
- Variations in dose-proportionality in two Phase II studies were small and of no clinical significance.
- Pharmacokinetics and metabolism sections of the labeling have been edited to accommodate the recommendations of Biopharmaceutic Review.

CLINICAL: EFFICACY

Major Trials Supporting Approval

Nisoldipine has been evaluated as an antihypertensive treatment in 1,914 patients at dosages up to 80 mg/day. The efficacy data supporting approval were derived from the results of 7 double-blind, parallel placebo controlled studies in 1,360 patients with hypertension, 886 of whom received nisoldipine. Long-term efficacy was supported by five open-label, 6-12 month follow-up of 554 patients (Studies X89-039, X90-019, X90-006, 675, 690).

The primary efficacy endpoint in each of these studies was the change in supine diastolic blood pressure (SDBP) from baseline at the end of dosing interval (trough effect) after 4-9 weeks of therapy. Data from five of the seven studies should be considered for major evidence of efficacy:

Study	Doses, mg/day	Duration		Remarks
D88-054	10, 20, 30	QD	4 wks	fixed dose
D89-026	10-40	QD	9 wks	dose titrated per response
D90-019	30, 60	QD	6 wks	fixed dose (after week 1)
D89-039	20, 40	QD	8 wks	fixed dose (after week 1)
D90-006	10, 20, 30	QD	6 wks	fixed dose (after week 1)

In the last three trials listed above, high doses were phased in after one week of low dose treatment. It should be noted that in Study D89-039, another group of 15 patients were randomized to receive nisoldipine CC 80 mg qd, but the arm was terminated due to safety concerns before collection of efficacy data. Nisoldipine was also compared with verapamil 240 mg (additional group of 78 patients) in this parallel placebo controlled study.

The remaining two controlled studies may provide instructions on how to use nisoldipine in a practical setting, but are not very useful as primary evidence for assessing efficacy of nisoldipine (vs placebo in general population who are not treated with other concurrent antihypertensive agents). Nisoldipine was evaluated in patients all receiving atenolol 50 mg qd as background therapy in one study (D89-029), and in the other (Study D90-029), lisinopril, hydrochlorothiazide (HCTZ) and placebo were compared in the presence of nisoldipine in all groups. Results of these studies will be commented in the Section of "Comparison/Combination with other Antihypertensives".

Overall Treatment Effects vs Placebo

The **primary efficacy** data in Table 1 on Page 6 demonstrate that nisoldipine, at 20-60 mg qd, is a consistently and significantly more effective antihypertensive agent than placebo with adequate duration of activity for once daily treatment. At this dose range, the placebo-subtracted net decreases in SDBP at trough ranged from 3.6 to 9.9 mmHg after 4-9 weeks of therapy. Treatment effects were less consistent for the 10 mg dose, but was superior to placebo in the larger trial (D90-006) with a decent drop in SDBP. Similar results were obtained for supine systolic blood pressures (SSBP) (same Table) and standing blood pressures (excluding the smallest trial, D88-054, results not shown in this memo).

The **percentages of responders** (SDBP reduction of ≥ 10 mmHg at trough or to ≤ 90 mmHg) are also summarized in Table 2 on next page. In the major trials, the response rates for 20-60 mg/day were in the range of 17-45% more than that of placebo. Again, less patients (around 17% over placebo) responded to 10 mg dose.

With respect to blood pressure changes and response rate, there were no significant differences between various statistical analyses, i.e. per-protocol or intent-to-treat (final visit).

Dose Response

The **dose-response** relationship, at trough, has been examined within the range of 10-60 mg once daily (Tables 1 & 2, in the associated Figure 1, % response curve was shifted on the dose axis for clarification). While dose of 10 mg/day was not consistently better than placebo as monotherapy, it is appears that blood pressure reduction may increase further at doses above 60 mg (but not for % responders). Although there are evidence from small pilot studies of hypertensive patients that dose-response for 30-90 mg/day was rather flat (Study D90-022, see Clinical Pharmacology Review), the finding should be accepted with reservation because dosages were forced-escalated rapidly in that study. There was a concern, also in the same early phase study, of asymptomatic T-wave changes at high doses (see Safety below). However, when doses were increased slowly as in efficacy trials, less patients reported the same abnormality. Besides, such ECG changes were common in hypertensive patients and their clinical meaning are not yet clear. Thus, effective doses of nisoldipine CC range from 20 to 60 mg once daily, with a weak support for the high-end limit.

Correlation between blood nisoldipine level and blood pressure reduction has been demonstrated at trough in several clinical trials.

Time-Effect Relationship

While only once-daily regimen was used in clinical trials and no direct comparison with other dosing schedule was performed, appropriate **dosing interval** for nisoldipine was established in the following studies:

	Studies	
Peak/Trough Effect	D88-054, D90-019, D89-039, D90-006	10-60 mg/day, QD
24-hour BPs	D88-054, D90-019, D89-039, D90-006	10-60 mg/day, QD

For the doses studied, the placebo-subtracted **trough-to-peak** ratios appeared to be acceptable, ranging from 70 to 100% for SDBP and SSBP.

NISOLDIPINE Hypertension Efficacy							
Final visit, Placebo subtracted,			these	are significantly different from placebo			

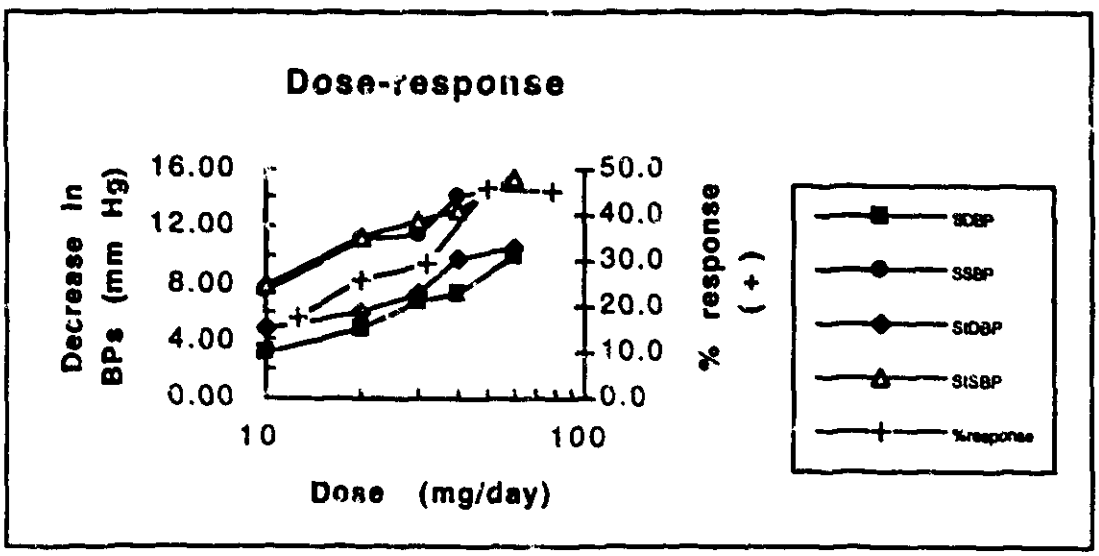
TABLE 1: Change in supine BPs from baseline at trough

		Last		regimen:					
Analy-		Vist	mg/day:	10	20	30	40	60	10-40
sis	protocol	Wks	N/arm						titrated
SDBP	D88-054	1-4	30	2.96	3.61	4.53			
ITT	D89-026	1-9	72						8.35
	D90-019	1-6	74			6.66		9.91	
	D89-039	1-8	76		4.03		7.33		
	D90-006	1-6	52	3.21	6.65	8.00			
	wtd avg		SDBP	3.12	4.81	6.70	7.33	9.91	
SSBP	D88-054	1-4	30	5.31	8.43	7.65			
ITT	D89-026	1-9	72						14.73
	D90-019	1-6	74			9.86		14.90	
	D89-039	1-8	76		7.33		13.96		
	D90-006	1-6	52	8.84	17.78	15.88			
	wtd avg		SSBP	7.55	10.98	11.44	13.96	14.90	

TABLE 2: Response Rate (trough SDBP < 90 or drop by > 10)

		Last		regimen:					
Analy-		Vist	mg/day:	10	20	30	40	60	10-40
sis	protocol	Wks	N/arm						titrated
ITT	D88-054	1-4	30	16.7	16.7	28.8			
	D89-026	1-9	72						40.9
	D90-019	1-6	74			17.7		44.8	
	D89-039	1-8	76		26.5		45.8		
	D90-006	1-6	52	17.5	29.9	45.3			
	wtd avg		%respons	17.2	25.8	29.0	45.8	44.8	

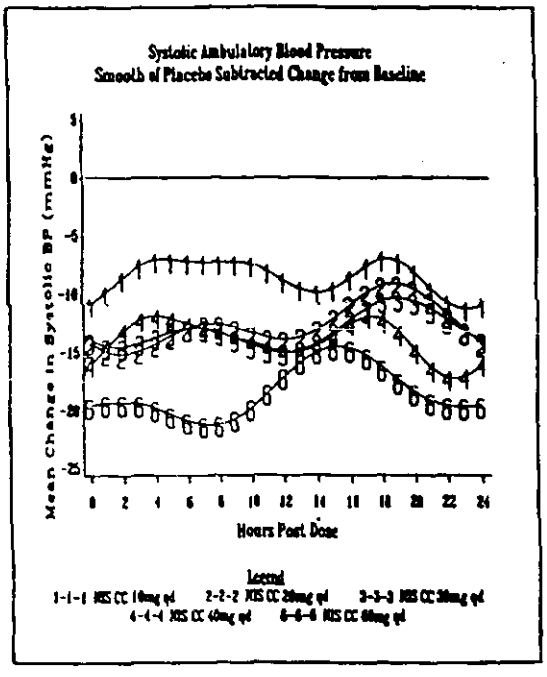
Figure 1

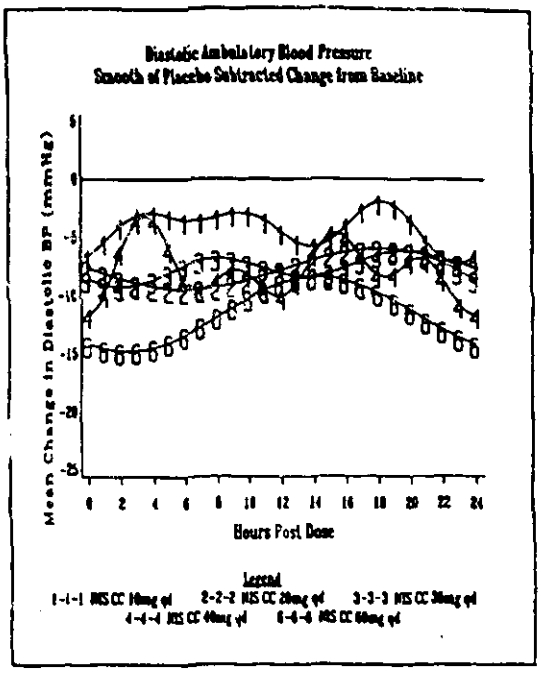


Total of 359 patients from the listed studies were pooled for analysis of **24 hours** ambulatory blood pressure change, the majority were white (65%) and male (60%). As shown by the 24-hour blood pressure curves, treatment effects of at least 5 mmHg reduction in SDBP over placebo were maintained during 24 hours for doses 20 mg and above (see Figure 2 below).

It is concluded that although other dosing schedules have not been evaluated, once-daily treatment with nisoldipine CC 20-60 mg per day appeared to be adequate to cover the dosing period.

Figure 2





Responses in Demographic Groups

In post hoc analyses (see draft SBA), nisoldipine appeared to be equally effective in **male/female**, with a slightly more pronounced dose-response relationship in the male patients. Despite increased bioavailability in the **elderly**, dose-response was less evident in such patients and there were no significant differences in blood pressure reduction between groups of age below and above 65 years. While blood pressure responses to nisoldipine were numerically greater in **black** than in white patients, such retrospective finding should not be described in the labeling or used in promotion. Not surprisingly, response to nisoldipine was greater in patients with higher **baseline blood pressure**, with a more significant dose-response relation.

Comparison/Combination with other Antihypertensives

Nisoldipine was compared or combined with the following antihypertensive agents in 3 controlled trials.

Comparison groups			Studies
nisoldipine+atenolol	vs	placebo+atenolol	D89-029
nisoldipine+lisinopril	vs	nisoldipine+placebo	D90-029
nisoldipine+HCTZ	vs	nisoldipine+placebo	D90-029

While results of the first study listed above indicated that concomitant atenolol did not affect the efficacy of nisoldipine CC, the second study suggested that some patients may have further response when a diuretic or ACE inhibitor is added. Up to one third of all patients received additional antihypertensive therapies in long-term, uncontrolled, follow-up studies. Overall, not much weight can be placed on these active controlled data for the efficacy claim.

Long-Term Efficacy

Long-term effectiveness of nisoldipine was evaluated in five open-label studies up to one year. Without a placebo control, reductions in supine blood pressures from baseline appeared to be sustained in more than 80% of 554 patients treated with nisoldipine for 6 months to one year

CLINICAL: SAFETY

Database

The database appeared to be adequate for analysis of the safety of nisoldipine, which includes cumulative experiences of nearly 4,200 hypertensive patients as of 10/29/93. Of 1,466 patients# (921 in US trials) who were treated with nisoldipine Coat-Care formulation, about 55% were exposed for at least 2 months (approx. 33% over 6 months) and a great majority were on doses of 20 to 60 mg.

The majority of comparative experience was based on the results of 6 randomized, double-blind, parallel group, placebo-controlled trials of 4-9 week duration (all U.S. double-blind controlled trials, see list in Efficacy Section)*, which included 678 patients on nisoldipine and 280 patients on placebo.

Data from the first 120-day Safety Update were not incorporated into the following summary, however, the numbers added were small and did not change the safety profile of the drug (see Reviews of Safety Update by Dr. Dern).

Comparative Experiences

There were no surprising findings in the safety profile of nisoldipine CC used in hypertensive patients. Overall frequency and rates of some specific adverse clinical experiences and abnormal laboratory findings were more common in nisoldipine than placebo treated patients, but none were serious or unexpectedly frequent.

The percentage of nisoldipine-treated patients reporting an **adverse event** in controlled trials (68%, N=678) was higher than that in the placebo group (53%, N=280). Among the adverse experiences, the following were more common for nisoldipine than placebo with incidence of $\geq$ 3%:

ADE	Nisoldipine(%) N=678	Placebo(%) N=280
peripheral edema	22	10
headache	22	15
dizziness	5	4
asthenia	4	4
vasodilatation	4	2
palpitation	3	1

\# From draft SBA. Different numbers of trials and patients exposed were given in Integrated Summary and Draft SBA, the later is probably more updated. Some calculations shown below were based on data from Integrated Summary of Safety.

* Foreign data also included some placebo-controlled safety experiences (D90-006). However, a great majority of the non-U.S. studies were not controlled and thus were not considered in comparative experiences. Nisoldipine was used in all treatment groups in one U.S. study (D90-029), but results of that study were not excluded from the comparative analysis.

As expected, the most commonly reported adverse events were related to nisoldipine's vasodilating effects. Most were mild and infrequently leading to withdraw.

While the overall frequency of adverse experiences was not affected significantly by patient **age, sex, race, or body weight** in the controlled trials, some minor differences in the incidence of a few adverse events may change the tolerability of nisoldipine in demographic subgroups. Headache appeared to be less common in the elderly, which would be surprising if the adverse event is pharmacokinetics-related (see Clinical Pharmacology). Peripheral edema was more frequent in female (as suspected with other dihydropyridine agent) and heavier patients (>185 lbs), but the sex difference was only seen in foreign studies, not in the U.S. controlled trials. Compared to blacks, incidences of headache and edema were slightly higher in whites.

The percentage of nisoldipine-treated patients **withdrawn due to adverse clinical experiences** was higher than that of placebo (7.8 vs 3.2%, U.S. controlled trials only), and dose-related (up from 5.4% at 10 mg to 10.9% at 60mg). The reasons for withdrawal were mostly related to nisoldipine's pharmacologic activities and within the scope of common adverse experiences:

Reasons for withdrawal	Nisoldipine(%) N=678	Placebo(%) N=280
headache	3.8	0.4
peripheral edema	2.9	0.4
vasodilatation	1.5	0.0
nausea	0.9	0.0
palpitation	0.9	0.0
dizziness	0.7	0.4

There were 2 **deaths** (car accident and metastatic prostate cancer) in nisoldipine-treated patients in controlled trials (non-U.S. studies only), compared with two deaths in the placebo groups. None were considered drug-related. Other **serious events** occurred with similar frequencies in nisoldipine (2.0%) and placebo group (1.5%). However, they are dose-related (increased from 0.2% at 20 mg to 4.5% at 60 mg) and half of these serious events led to withdrawal.

Abnormal **laboratory** findings in controlled trials were both rare and no different between nisoldipine and placebo groups. In U.S. controlled trials, incidences of such reports were in the range of 0-4% for hematology, 0-2% for hepatic functions, 0-1% for creatinine/BUN and 0-6% for lipid profile. While there were more reports of increased fasting blood glucose in nisoldipine than in placebo group from non-U.S. controlled trial, the phenomenon was not dose-related, not seen in the U.S., and cases of increases to above 140 mg/dl were not more frequent (than placebo).

Overall Exposures

In general, the overall safety experiences in all patients treated with nisoldipine in all clinical trials were not unexpectedly different from those described above for controlled trials.

Approximately 62% of all patients reported one or more **adverse events**, while the incidence was lower in European trials (43% vs 75% in U.S.). Prominent complaints were similar to those in controlled trials (e.g., 18% headache, 15% edema in U.S. Trial).

In all clinical trials, about 9% were **withdrawn** due to adverse experiences (10% in U.S. studies), not too different from that of comparative experiences. There was no additional death other than those noted above in the comparative experiences. Accumulative experiences of abnormal **laboratory** findings in all U.S. studies were also similar to that in the controlled trials.

Class Specific Safety Issues

As noted above, adverse experiences relatively specific to calcium channel blockers were also reported in nisoldipine-treated patients. They were not more severe or frequent than in other members of the class; however, the database may not be large enough for detecting some of the rare events.

Clinically significant **hypotension** and other related adverse experiences in nisoldipine treated patients were not common and rarely resulted in withdrawal. As described earlier in time-effect relationship, at doses that produced adequate trough blood pressure reduction, average peak response was not excessive. In all U.S. and non-U.S. trials, symptomatic hypotension occurred in about 0.2% and syncope was reported in 0.1% of patients. **Orthostatic** hypotension and related symptoms were slightly more common, reported in approx. 0.4% of patients on nisoldipine monotherapy, but very few were considered serious and required intervention. Overall, hypotensive reactions to nisoldipine treatment did not appear to be more frequent or severe than those with other calcium channel blockers. Appropriate warning related to hypotensive reaction is included in the draft labeling.

Like other dihydropyridines, nisoldipine has no significant effects on electrophysiology or **cardiac rhythms**. Tachycardia was reported in about 1% of all nisoldipine-treated patients, with a small mean changes in heart rate (< 1bpm, placebo-adjusted). It is most likely due to hypotensive reflex, rarely led to withdrawal, and occurred equally frequently in placebo groups. Some minor changes in ECG (QRS) were noted more frequently than that in placebo group, especially in patients receiving concomitant atenolol, but the magnitudes were of no clinical meaning. While dose (plasma level) and magnitude of BP reduction-related **T-wave flattening/inversions** were observed in a small phase II study (D90-022) with rapid dose escalation, such ECG finding was less clearly related to dose and not as frequent (similar to that in placebo groups) in a retrospective but blinded analysis of data from three efficacy trials. It is somewhat re-assuring that no angina or thallium test-documented ischemia were reported in any of the patients with T-wave changes in Study D90-022.

Limited experiences with concomitant use of nisoldipine and atenolol, lisinopril or HCTZ have not identified any unexpected safety or tolerability issue. Combination of nisoldipine with HCTZ or lisinopril may increase slightly the incidences of asymptomatic hypotension, tachycardia, palpitation and dizziness. Rebound hypertension after withdrawal has not been a problem with other dihydropyridines and was not significant in a small pharmacodynamic study for nisoldipine.

PEDIATRIC/GERIATRIC USE

There are no clinical trials assessing the efficacy or safety of nisoldipine in pediatric patients, either completed or in progress. The sponsor claimed that the drug has little potential for use in children and thus did not commit to any study in hypertensive children.

Efficacy and safety of nisoldipine as treatment for hypertension in the elderly (65 year and older) are not significantly different from that of general patient population.

DRAFT LABELING

The draft labeling submitted by the sponsor has been edited.

CONCLUSIONS

Nisoldipine appeared to be an effective and safe treatment for hypertension.

While there is little doubt that nisoldipine at 20-60 mg/day is an antihypertensive more effective than placebo, it is not certain if the entire useful dose range has been fully explored. Nisoldipine should be started at 10 mg once daily and titrated slowly (e.g. every few weeks) to 60 mg according to blood pressure response.

It is recommended that nisoldipine be approved with the edited draft labeling.

Shaw T. Chen, M.D.,Ph.D.

cc:
ORIG: NDA- 20-356
HFD-110
HFD-110/CSO
HFD-110/SChen/10/26/94

Roedev

DEC 19 1994

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

Date: 12/15/94

From: Shaw T. Chen, M.D., Ph.D., Medical Group Leader, HFD-110
Through: Director, Division of Cardiorenal Drug Products, HFD-110 Lipicky
To: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: NDA 20-356, Nisoldipine Core-Coat for Hypertension, Approvability

This is in response to the comments and questions raised in your draft memo of 12/13/94 regarding some labeling issues for the above application.

1. As stated in the Secondary Review (Dose Response), we also think that the effective dosage range is 20-60 mg/day and the recommended doses should include 60 mg. Usual maintenance doses in the labeling were changed to 20-40 mg in the Division's draft, which were concurred in your memo. We agree with your reasoning that dose titration should start at 20 mg.

2. With respect to worsening of angina, we totally agree that nisoldipine is no different from other dihydropyridine type calcium channel blockers and it is neither a safety nor an approvability issue. In the controlled angina trials, there was no significant increase in angina attack rate and worsening of angina was not a frequently reported event in hypertension studies. As you noted, the open label, non-controlled data in angina studies were not much useful for us to draw any conclusion. This was not discussed in details in the secondary review because the sponsor had withdrawn the angina claim after the primary review was completed and it was not clear then to me that we could describe the angina data for review of hypertension indication.

3. We understand that the effects of food and grapefruit on the kinetics of nisoldipine CC are different. They were put together only in "Information for Patients", as they are both "food". The wording in your marked-up draft certainly described the problem much clearer.

4. A cleaned-up draft of package insert with your mark-ups has been prepared.

Shaw T. Chen, M.D.,Ph.D.

cc:
ORIG: NDA- 20-356
HFD-110
HFD-110/CSO
HFD-110/SChen/12/15/94

Koeder

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

Date: 12/22/94

From: Shaw T. Chen, M.D., Ph.D., Medical Group Leader, HFD-110
Through: Director, Division of Cardiorenal Drug Products, HFD-110
To: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: NDA 20-356, Nisoldipine Core-Coat for Hypertension, Labeling

We did not get to see your memo in final prior to responding to your memo. There was one question that you asked that was not answered in our response of 12/17/94.

There were 2 "food studies" conducted by Miles. One of them was not the FDA "high fat" and studied only the 20 mg tablet (Study 666, it was more than average but not high) and in that study there was no evidence of "dose-dumping'. The other study was conducted using the FDA "high fat" meal and in that study there was dose dumping. However, in this study (D92-045-02), 30 and 40 mg tabs were used and the 20 mg dose was not repeated. The food effect on the C_{max} was average 3 fold, and 5 of the 28 subjects had 5-11 fold changes. Thus either the fat content in food is important or dose-dumping by food may be dose-related.

Miles did conduct a pharmacokinetic/pharmacodynamic study, a review of that study was done by Dr. Marroum, in which they found that the pharmacodynamic effects (lowering of blood pressure) was a function of the log of the plasma concentration. So ten-fold changes in plasma concentration make a sizable difference, three-fold changes do not make a big difference. The slope of the concentration-response curve goes over 2 order of magnitude from beginning of effect to definitely over the maximum effect.

Although Dr. Marroum's review criticized the analysis of the study, the qualitative statements above are not materially affected by the quantitative problems that Dr. Marroum found.

So, it seems to us that there is considerable latitude that can be given with respect whether nisoldipine **must** be taken fasting. We do not think it must be taken fasting. To be silent about fasting or fed in the Dosage and Administration section is reasonable but since there are more than 5-fold changes in C_{Max} in 18% of subjects, the Dosage and Administration should probably say "..., preferably in a fasted state (see Clinical Pharmacology)". In Clinical Pharmacology the 11-fold increase in C_{Max} should be stated to be an upper limit when a High fat meal is ingested.

or even other alternatives as were discussed in the 17 M of 12/22/94. RZ

Shaw T. Chen, M.D.,Ph.D.

cc:
ORIG: NDA- 20-356
HFD-110
HFD-110/CSO
HFD-110/SChen/12/22/94

M E M O R A N D U M

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: DEC 16 1994

FROM: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: Nisoldipine Core Coat (NISOCOR, NDA 20-356, Miles)

TO: Dr. Raymond Lipicky, HFD-110

Nisoldipine appears to be a typical dihydropyridine CCB, developed sensibly as a controlled release product. It is possible to argue that doses above 60 mg/day could have larger effects, but the ADE rate seems already to be rising at that level (see, e.g., MOR angina p. 15-16) and I doubt higher doses would be appealing or useful. I do believe the 60 mg dose is well-enough tolerated to be included in the recommended dose range. I have a few questions in addition to comments/questions on labeling.

1. Dose

The proposed D&A was for a starting dose of 10 mg, with titration up to 40. As indicated, I believe 60 mg should be included. Where this dose was studied (90-019, 89-029) it had a numerically larger effect and was adequately tolerated.

The basis for starting at 10 mg is not clear to me. In study 89-026, few patients (5/79) stayed at 10 mg, and not many stayed at 20 (5/79). Starting doses of at least 20 mg were used in studies 88-054, 90-019 (30 mg), 89-039, and 89-029. Only in studies 89-026 (titration) and 90-006 did all patients get 10 mg to start. As 1) the overall response rate at 10 mg is low, 2) there seemed to be no adverse consequence of starting at 20-30, alone or added to a beta-blocker, and 3) extra titration steps are costly and can discourage therapy, I suggest a recommended starting dose of 20 mg, with reductions in the elderly and patients with hepatic dysfunction.

I believe the usual maintenance dose will not be 20-30 mg as proposed but 30-40. In the titration study almost all patients reached 30-40 mg. I was first inclined to change the expected maintenance to 30-40 mg, but the mean effect (especially added to a beta-blocker) of 20 mg was respectable.

Labeling should clearly show D/R of ADE's.

I note that in all studies the relationship of dose to meals was not specified. Is there really need for taking nisoldipine on an empty stomach an hour form meals, as labeling now requires or could this be at least softened somewhat by reference to the trial results? Note also that the extreme effect of food occurred in only one of two studies.

2. Worsened angina

Dr. Stockbridge noted concern about worsened angina. Certainly, all dihydropyridines have been associated with this, presumably resulting from a combination of increased HR and decreased diastolic (coronary perfusion) pressure. Unless I am missing something, however, nisoldipine does not seem unusual. Acute worsening was infrequent, about 1%. I have tabulated the cases of worsening in the controlled phases of the four angina trials, including AMI, increased chest pressure or angina(A) unstable angina(UA) etc. (I called the event UA only if the specific term was used.) I get this:

Study	P1	N10	N20	N30	N40	N60
88-060	4008(A)	1109(A)*	10002(D AMI)	21013(A)		
89-042			5232 (AMI)			
90-010	403(A) 773(UA) 1103(AMI)		416(A)		307(A) 707(A)	
90-015	6004(A)		6013(A) 16014(A)		2008(A) 9016(UA) 36006(A)	28001(A)

* = this patient also had increased AF, which could account for worse angina

I would say there seems no clear evidence here of stimulated worsening by nisoldipine. I note also that the people studied were fairly ill as judged the frequent history of MI and vascular procedures (see following table) and the poor outcome in study 90-015 during the run-in phase (4 deaths).

	88-060	89-042	90-010	90-015
n-single blind lead-in	390	378	408	483
n-randomized	271	302	303	312
prior MI (%)	44-53%	44-53	52-62	40-49
prior CABG (%)	20-36%	14-17	16-20	24-29
prior angioplasty (%)	11-16%	6-15	6-9	19-27
hypertension (%)	37-48%	--	22-32	42-54
diabetes (%)	11-19%	--	12-20	15-19
deaths in run in (2-3 week)	0	0	0	4/483=0.8%

I read Dr. Dern's review after writing the above. He too (p.20-21), I believe, finds no excess (vs placebo) of discontinuations due to worsening angina or AMI on nisoldipine, although he does not necessarily interpret the results as I have. If we take the combined U.S. and non-U.S. angina trials we have:

	Nisoldipine		Placebo	
	n	rate	n	rate
U.S.	438	--	145	--
worse angina	10	2.3	2	1.4
AMI	2	0.5	0	0
NON-U.S.	447	--	189	--
worse angina	3	0.7	1	0.5
AMI	2	0.4	1	0.5
COMBINED	885	--	334	--
worse angina	13	1.5	3	0.9
AMI	4	0.5	1	0.3
Both	17	1.9	4	1.2

All in all, I find it hard to see evidence of increased AMI rate in this (that would be worrisome) and the worsened angina rates also seem very close. In the U.S. trials, the source of the higher rate on nisoldipine, most of the excess is due to the 40 mg group, with lower rates at 20, 30, and 60 mg. I wrote the angina numbers into the labeling (Warning). Despite the small numbers and small differences, Dr. Dern's observation that many of the worsened angina events occur early in the U.S. trials, is worth noting.

It is only in the open label studies that the frequency of worsened angina or AMI/death climbs (to 17%) and it is hard to know the correct comparison for this. There were 2 open-label deaths, surely not too surprising in light of 4 placebo run-in deaths in study 90015. There were 10 open-label AMI's among 305 patients in long-term trials, or 3%. Dr. Stockbridge notes 10 AMIs among 479 nicardipine patients, obviously in the same range as nisoldipine, and 13 AMI deaths in diltiazem long-term trials of 737 people, a 1.8% rate that does not include non-fatal AMIs. All of these numbers would require substantial refinement (patient characteristics, duration) to be used rigorously, but even the crude comparisons make it seem fairly clear that the nisoldipine long-term results are similar to those of nicardipine (another dihydropyridine) and diltiazem with respect to coronary event rate. I am not sure we have useful data for these drugs with respect to the worsened angina/unstable angina question.

I should note that the exacerbated angina/progression question is not related only to If we really do have evidence that nisoldipine causes "hastened progression of underlying CAD" (MOR p.29), we probably should not be approving it for hypertension either (although Dr. Dern makes the case that the history in hypertensive patients in very benign). I do not, however, see any evidence of such hastened progression. I have no doubt that CCB's, or at least dihydropyridine CCB's, can sometimes exacerbate angina, but that is more a matter of labeling, as it is a reversible phenomenon, known to us from other CCB's, and certainly did not seem prominent in the hypertension trials.

3. The effects of food and grapefruit juice are different. Food increases the Cmax but decreases the AUC, i.e., it causes dose-dumping but no apparent showing of systemic metabolism. Grapefruit juice increases both the Cmax and AUC, as would be expected of a substance that blocks metabolism of nisoldipine (as it blocks metabolism of nifedipine, nitrendipine, felodipine and, probably, all dihydropyridine CCB's).

Robert Temple, M.D.

JUL - 7 1994

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
MEDICAL OFFICER'S NDA REVIEW

NDA: 20- 356

DRUG: Nisoldipine

SPONSOR: Miles

DATE SUBMITTED: 17 August, 1993

DATE REVIEWED: 7 July, 1994

REVIEWER: Philip L. Dern M.D.

RESUME:

This is a 120- day safety review and includes both completed and uncompleted trials, foreign and domestic. For uncompleted trials, data is still blinded and treatment is listed as "either drug 1 or drug 2" if these are the possibilities for a particular patient.

I. Deaths
A. Completed US studies

One death is recorded for this update and was also included in the NDA for an on-going trial.

Study # D90- 029-06; Pt 6004.

This 44- yr- old female with a qualification, single- blind BP of 190/111 and a randomization BP of 141/97 died suddenly at home on day 24 of the study. She was on NIS 40 mg qd and HCTZ 25 mg qd. Serum potassium at baseline and last visit were, respectively, 3.9 and 3.6 mEq/l. Autopsy revealed moderate coronary atherosclerosis without thrombi.

B. Completed Non- US studies

Study 752. No deaths.

C. On-going Studies (all non- US)

Study 764; Pt 128.
This 83-yr- old female was being treated with either NIS or lisinopril (LIS). She died at home of acute pulmonary edema and has an associated abdominal infarction.

Study 769; pt 16002.

This 53 yr- old male was being treated with either NIS or atenelol (ATN). The patient had a TIA on 9/91. Baseline BP was 162/105 after three weeks of placebo. He was admitted to hospital on 5/18/92 after 53 days of treatment. He died the following day of a CVA. Bp at last clinic visit was 177/83.

Study 769; pt 17013.

This patient, a male aged 73, was being treated with either NIS or ATN. After 5 days of therapy the patient developed diarrhea, nausea, and vomiting, and, three days later, a fatal MI.

Study 769; pt 54002.

The patient, a male aged 66, was on either NIS or ATN. Baseline BP was 155/93. After 25 days of treatment he developed a CVA and died. BP at last clinic visit was 145/83.

II. Discontinuations due to adverse experiences

A. Completed US studies

REASON WITHDRAWN	DAY	DRUG & DOSE
Edema,erythema	24	NIS40
headache	2	NIS20
headache	1	NIS20
tachycardia,vasodil	1	NIS20
Dizzy, n & v,headach	2	NIS20
Gout	5	NIS20
Faint at phlebotomy	10	NIS40
edema	13	NIS40
edema	19	NIS40
Abn Liver function*	35	NIS40, HCTZ25
Cough	8	NIS40+ LIS20
card. arrest	24	NIS40, HCTZ25
sinus tachycardia	10	NIS40, HCTZ25

* Also abnormal during pre- NIS phase

n.b. There is a notable number of cases withdrawn relatively early in the active dose phase especially for those signs and/or symptoms likely to be due to the vasodilatory action of nisoldipine.

B. Ongoing trials (all non- US):

SELECTED SIGNS/SYMPTOMS	N*
edema	23
migraine	3
headache	28
angina	1
MI	2

* often sign/symptom occurred with others

The above table concentrates on typical findings on nisoldipine therapy (edema, headache) but notes occurrence of migraine. Number of cases of angina and MI is small. Total number of cases withdrawn due to syncope (1), postural hypotension (1), hypotension (1), suggests that excessive BP fall was uncommon. N=1 case was withdrawn for thrombocytopenia.

Among 1370 cases in these foreign studies, 100 were withdrawn due to adverse experiences.

Conclusions:

The withdrawals for adverse effects were often due to the pharmacologic action of nisoldipine and consequent to vasodilation (headache, vasodilation). In this hypertensive population few cases of angina or MI occurred. However, the data base for this report is not cumulative and the number of cases in the completed US trial is too small to provide a good estimate of the risk of angina/MI. The database for the foreign studies is larger and, even though treatment assignment is in doubt, the small number of angina/MI cases is notable (See this Reviewer's review of the hypertension safety segment of the NDA for comments on the association of symptoms/signs of vasodilation and angina/MI).

cc: HFD/110
HFD/110 orig
HFD/110 CSO ✓
HFD/110 pld

OCT 26 1993

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
MEDICAL OFFICER'S REVIEW

NDA: 20-356 (Nisoldipine Coat-Core tablets for exertional angina; Bay K 5552; IND

Sponsor: Miles Inc. Pharmaceuticals Div.

Submission: NDA 120 day Safety Update

Submission date: 17 August 1993.

Receipt date: 19 August 1993.

Review date: 26 October 1993.

Reviewer: N. Stockbridge, M.D., Ph.D.

1. US trials

There is only 1 ongoing US trial, #X90-015, an open-label, long-term follow-on to Study #90-015 (q.v.). There are no new safety data for this trial. However, two subjects who completed this trial and continued to receive nisoldipine coat-core under an individual investigator's IND experienced serious adverse experiences.

Subject was a 69 year old Caucasian female who had generally received 20 mg q.d. The dose was reduced because of dizziness and light-headedness. He experienced chest pain or discomfort for two weeks prior to admission at 734 days for unstable angina. Enzymes did not indicate myocardial infarction and she was discharged after 3 days. She was readmitted with similar history at 770 days, at which time hiatal hernia was diagnosed.

Subject was a 68 year old Caucasian who suffered myocardial infarction while receiving 30 mg q.d. Three months later he was readmitted for severe angina while on 40 mg.

2. Non-US trials

2.1. Study #697

This is a randomized, double-blind, parallel group study being conducted in Germany and Italy. The groups are nisoldipine 40 mg q.d. (n=138) and diltiazem 60 mg t.i.d. (n=136).

Subject #11-009 was a 72 year old female who discontinued after 8 months because of allergic exanthema.

Subject #41-005 was a 55 year old female who discontinued after 3 months because of resting tachycardia.

2.2. Study #701

This is a randomized, double-blind, parallel group study being conducted in Germany. The groups are nisoldipine coat-core 20 mg q.d. (n=70) and nisoldipine immediate release 10 mg b.i.d. (n=72).

Subject 0101 was a 58 year old female with a 2-year history of angina. She discontinued at 5 days with severe burning sensation of the skin, headache, and vasodilation, all of which began with the first dose.

Subject #0115 was a 68 year old female with a 6-month history of angina. She discontinued at 4 months with nausea, inner "trembling", and pressure and heat sensation in hands and feet.

2.3. Study #718

This is an ongoing randomized, double-blind parallel group trial with nisoldipine 20 and 40 mg q.d., and diltiazem 60 and 120 mg b.i.d. and t.i.d.

Subject #303 was a 51 year old male with a 6-year history of angina. He withdrew after

4 days because of chest pressure and palpitations.

Subject #306 was a 54 year old male with an 8-year history of angina. During month 7, he complained of flatulence. After 8 months, he withdrew because of hypotension, malaise, and fatigue.

Study #771

This is an ongoing randomized, double-blind, comparison of nisoldipine 20 to 40 mg q.d. with diltiazem 60 mg t.i.d. or q.i.d.

Subject #3307 was a 55 year old male with a 7 month history of angina and myocardial infarction 12 years previously. He discontinued at 5 months with atrial fibrillation which resulted in prolonged hospitalization; outcome unknown.

Subject #3602 (was a 78 year old male with 2 month history of angina. He suffered cramps from the onset of treatment and discontinued after 4 weeks with the onset of fasciculations.

Subject #3703 was a 63 year old male with a 1-year history of angina. He discontinued after 3 weeks because of vertigo.

2.4. Study #781

This is an ongoing randomized, double-blind trial in Germany comparing 20 and 40 mg q.d. nisoldipine with ISDN 20 and 40 mg b.i.d.

Subject #21. was a 70 year old female with a 3-month history of angina. She discontinued after 3 weeks because of severe headaches and nausea.

Subject #214 was a 62 year old female with a 1-year history of angina. She discontinued after 6 weeks because of leg edema.

Subject #603 was a 64 year old female with a 3-year history of angina. She discontinued after 2 weeks because of severe headaches, diarrhea, restlessness, and general malaise.

Subject #906 , was a 69 year old female with a 3-year history of angina. She discontinued after 2 months because of hair loss.

Subject #1004 was a 68 year old male with a 3-year history of angina and myocardial infarction 1 year previously. He was discontinued after 1 week because of non-compliance.

Subject #1016 was a 58 year old male with a 1-year history of angina and possibly 2 previous myocardial infarctions. He was discontinued after 2 months because of non-compliance.

2.5. Study #761

This is an ongoing open-label trial in Israel with nisoldipine 10 to 60 mg q.d.

Subject #122 was a 59 year old Caucasian male who discontinued after 6 months because of unstable angina.

Subject #404 was a 54 year old Caucasian male who suffered a myocardial infarction at 6 months.

Subject #414 was a 73 year old Caucasian male who discontinued after 4 months because of unstable angina.

Subject #617 was a 95 year old (?) Caucasian male who suffered a myocardial infarction after 7 months.

Subject #100- was a 72 year old Caucasian male who discontinued for constipation after 6 months.

Subject #1017 was a 66 year old Caucasian male who discontinued after 4 months because of intermittent claudication and fissure following prostatectomy.

2.6. Study #762

This is an ongoing randomized, double-blind trial in Italy comparing 20 mg q.d. nisoldipine coat-core with 10 mg b.i.d. nisoldipine immediate release.

Subject #402 was a 21 year old Caucasian male with a 2-month history of angina who had a myocardial infarction at 1 month.

Subject #1504 was a 61 year old Caucasian male with an 11-year history of angina. He

discontinued after 4 weeks because of unstable angina.

Subject #605 was a 69 year old Caucasian male with an 8-month history of angina. He discontinued after 2 weeks because of unstable angina.

Subject #607 was a 64 year old Caucasian male with a 2-year history of angina. He discontinued after 2 weeks because of rash and hypotension.

Subject #615 was a 58 year old Caucasian male with a 2-year history of angina. He discontinued after 2 weeks with rhinitis, edema of the legs, erythema, and pruritus which began on the second or third day of treatment. Symptoms resolved 2 days after withdrawal.

Subject #810 was a 60 year old Caucasian male with a 1-year history of angina. He dropped out after 3 weeks because of unstable angina.

2.7. Study #10011 (X90-010)

This is an ongoing open-label study in Israel with doses 10 to 60 mg q.d.

Subject #101 was a 56 year old Caucasian male who developed thyroiditis and tonsillitis after 4 months.

Subject #103 was a 63 year old Caucasian male who discontinued after 1 month because of unstable angina.

Subject #207 was a 64 year old Caucasian male who was hospitalized after 5 weeks because of unstable angina.

Subject #421 was a 69 year old Caucasian male who discontinued after 3 months because of pedal edema.

Subject #805 was a 63 year old Caucasian male who experienced severe prolonged angina and tachycardia 9 days after beginning treatment. He was off study drug for a short period and later completed 12 months.

Subject #911 was a 70 year old Caucasian female with a history of ophthalmological disease. She had intraocular hypertension for 9 months during study.

Subject #911 was a 65 year old Caucasian male was hospitalized for severe angina after 2 weeks. He subsequently completed 6 months, with complaints of decreased libido. The reason for discontinuation is not explicitly stated.

Subject #913 was a 61 year old Caucasian male with a 3-year history of angina. He complained of flank pain at week 2; the complaint resolved.

Subject #915 was a 52 year old Caucasian male who discontinued after 3 months because of facial flushing.

Subject #1004 was a 71 year old Caucasian male who was hospitalized for unstable angina at 7 months. He completed 12 months of treatment.

Subject #1018 was a 60 year old Caucasian male. He was twice hospitalized for chest pain, the second was after about 12 months of treatment.

Subject #1107 was a 57 year old Caucasian male who developed severe chest pain and underwent CABG 4 days after completing 12 months treatment.

3. Summary

Headache, vasodilation, and peripheral edema remained the common treatment-related adverse events.

Several of the cases described appear to represent acute worsening of angina during treatment. Subject #1107 in Study #10011 may represent a rebound phenomenon.

The information provided in this 120-day safety update do not materially affect conclusions made with the Medical Officer's review (2 August 1993) of safety and efficacy pertaining to the angina indication.

D. Roeder

DIVISION OF CARDIO-RENAL DRUG PRODUCTS

MEDICAL OFFICER'S REVIEW OF NDA AUG 4 1993

NDA Number : 20-356

Name of Drug : Nisoldipine (NIS CC)

Drug Category : Calcium Channel Blocker

Indication : Hypertension

Sponsor : Miles Inc Pharmaceutical Division

Date of Submission : March 31, 1993

Date Received : April 1, 1993

Date Review Completed : July 30, 1993

Reviewer : Cristobal G. Duarte, MD

Background. NIS CC is an extended release tablet dosage form of the dihydropyridine calcium channel blocker Nisoldipine The sponsor has submitted a NDA for approval of Nisoldipine for the treatments of hypertension This review will be concerned only with the efficacy in the treatment of hypertension.

As pivotal protocols in support of the effectiveness of Nisoldipine in the control of hypertension the sponsor is submitting the following studies : D90-006, D90-019, D89-026, D89-029, and D89-039.

Protocol D89-026

Title of Study : " A Pilot Dose-Titration Study of the Safety and Efficacy of Nisoldipine Coat-Core 10 mg, 20 mg, 30 mg and 40 mg versus Placebo in Patients with Mild to Moderate Hypertension ".

Investigators : Ginsberg D, Flamenbaum W, Canzanello V, Townsend R, Winer N,Schnaper H.

Places of Study. Harleysville, Englewood Cliffs, Winston-Salem, Galveston, Kansas City, Birmingham/USA

Objectives. The objectives of this study were :

1. To determine whether Nisoldipine given once daily lowers the blood pressure significantly more than placebo.

2. To determine the efficacy and safety of Nisoldipine when titrated from 10 mg to 40 mg qd.

Inclusion Criteria. Ambulatory patients, male and female, 21 years of age or older, with a history of essential hypertension were eligible for the study. Hypertension was defined as mean supine diastolic blood pressure of 95-115 mmHg.

Exclusion Criteria. Patients with the following conditions were excluded from the study :

1. Labile hypertension.
2. Recent myocardial infarction
3. Patients with cerebrovascular accident or signs suggesting impending MI or CVA, heart failure, angina pectoris, intermittent claudication, major arrhythmia or cardiac conduction disturbances.
4. Insulin-dependent diabetes mellitus, failure of a major organ system, impaired renal function (serum creatinine >2 mg/dl), severe infection, malignancy or psychosis.
5. Patients likely to have impaired drug absorption such as with chronic diarrhea, ulcerative colitis, regional enteritis, diverticulitis, partial or complete gastrectomy or small bowel resection.
6. Women of childbearing potential, alcohol or drug abusers, history of allergy to dihydropyridines.
7. Excluded concomitant medications were : antihypertensive drugs, cimetidine, monoamino oxidase inhibitors, sedatives, tranquilizers, tricyclic antidepressants, neuroleptic drugs, anorectics and decongestants.

Qualification for Randomization. Patients with mild or moderate hypertension discontinued previous antihypertensive treatment and were given a single-blind placebo once daily (regimen A) during a three to four-week qualifying run-in period. There was an optional extension of one week if the blood pressure was not in the qualifying range. Those patients with mean supine diastolic pressure ≥ 95 mmHg to ≤ 114 mmHg after three to four weeks of placebo were randomized and were given regimen B (Nisoldipine or placebo).

Drug-Regimen Protocol. At week 0 qualified subjects were given either Nisoldipine 10 mg qd or placebo qd (regimen B) for 2 weeks. On subsequent visits 5 through 7 (scheduled every two weeks) the once daily dose of Nisoldipine was titrated in a stepwise fashion to 20 mg (regimen C), 30 mg (regimen D), or 40 mg (2X20 mg) (regimen E) if mean trough supine diastolic pressure for that visit was ≥ 85 mmHg. Patients randomized to placebo underwent corresponding dummy titration. Two patients were randomized to Nisoldipine for each patient that was randomized to placebo.

Patients took two tablets before 11 am through the study but did not take the medication on the morning of clinical visits until trough blood pressure has been measured. Patients took the medication fasting or with food.

Patients were seen either weekly or biweekly in the morning throughout the study. At each visit supine and standing blood pressures were measured 24 hours ± 30 minutes after the last dose.

The duration of the double-blind phase was 9 weeks.

Expulsion. A subject was to be dropped from the study if the mean supine diastolic blood pressure was greater than 114 mmHg at any visit or if they had significant physical or laboratory abnormalities or a significant concurrent illness.

They also were to be withdrawn for blatant non-compliance, for missing visits or significant adverse experiences.

Assessment. Patients were seen in the morning at weekly or biweekly intervals. A history was taken at the first visit. Complete physical examination and 12-lead electrocardiogram were done at the first visit, at baseline (after 3 to 4 weeks on placebo) and at the last visit (after 9 weeks of double blind drug). Brief physical examinations were done at all other visits. A chest X-ray was done at the first visit unless a report was available within the previous 6 months.

Blood was drawn for the following laboratory tests at the first visit, after 3 to 4 weeks of single-blind placebo, and after 9 weeks of double-blind drug : CBC, differential, and platelet count, serum glucose, uric acid, calcium, phosphate, sodium, potassium, chloride, bicarbonate, creatinine, BUN, total protein, albumin, cholesterol, triglycerides, CPK, SGOT, LDH, alkaline phosphatase and total bilirubin, Complete urinalysis including microscopic and casts.

The primary endpoint of the study was a change in trough diastolic blood pressure (measured 24 hours after dosing) from baseline (mean of diastolic blood pressure after 3 or 4 weeks of single-blind placebo) to endpoint (the last valid visit on double-blind drug for each valid patient) in the Nisoldipine group compared to the placebo group.

Secondary endpoints were supine systolic blood pressure at trough and standing blood pressure at trough.

Statistical Analysis. The primary efficacy analysis was based on change from baseline in trough supine diastolic blood pressure at endpoint. No analysis based on level of titration achieved was done. Responders were considered those who achieved efficacy results according to the following criteria : blood pressure 90 mmHg or less, at least a 10 mmHg fall in blood pressure from baseline, either of the above and both of the above.

All tests were two-sided and based on the least square means estimated by the model.

Data from previous hypertension studies had suggested that the standard deviation of change from baseline in trough supine diastolic blood pressure at endpoint would be 7.5 mmHg. In order to detect a 5 mmHg difference from placebo in an alpha = 0.05, two tailed tests of

significance, and in order to obtain as much data as possible on the Nisoldipine 40 mg qd, it was decided to randomized 72 patients to Nisoldipine and 36 to placebo. Based on this information, the study, as designed, had 80 % power to detec a significant difference of at least 5 mmHg.

Subjects Studied. Of 166 patients enrolled, 43 were disqualified for randomization. The reasons for which patients did not qualify for randomization id given in the following table :

Mean Diastolic blood pressure at visit 4 did not qualify for randomization (95 mmHg to 114 mmHg)	26
Supine diastolic blood pressure >114 mmHg-	
At any time	4
Non compliance	1
Illness not due to medication	3
Other	9
Total	43

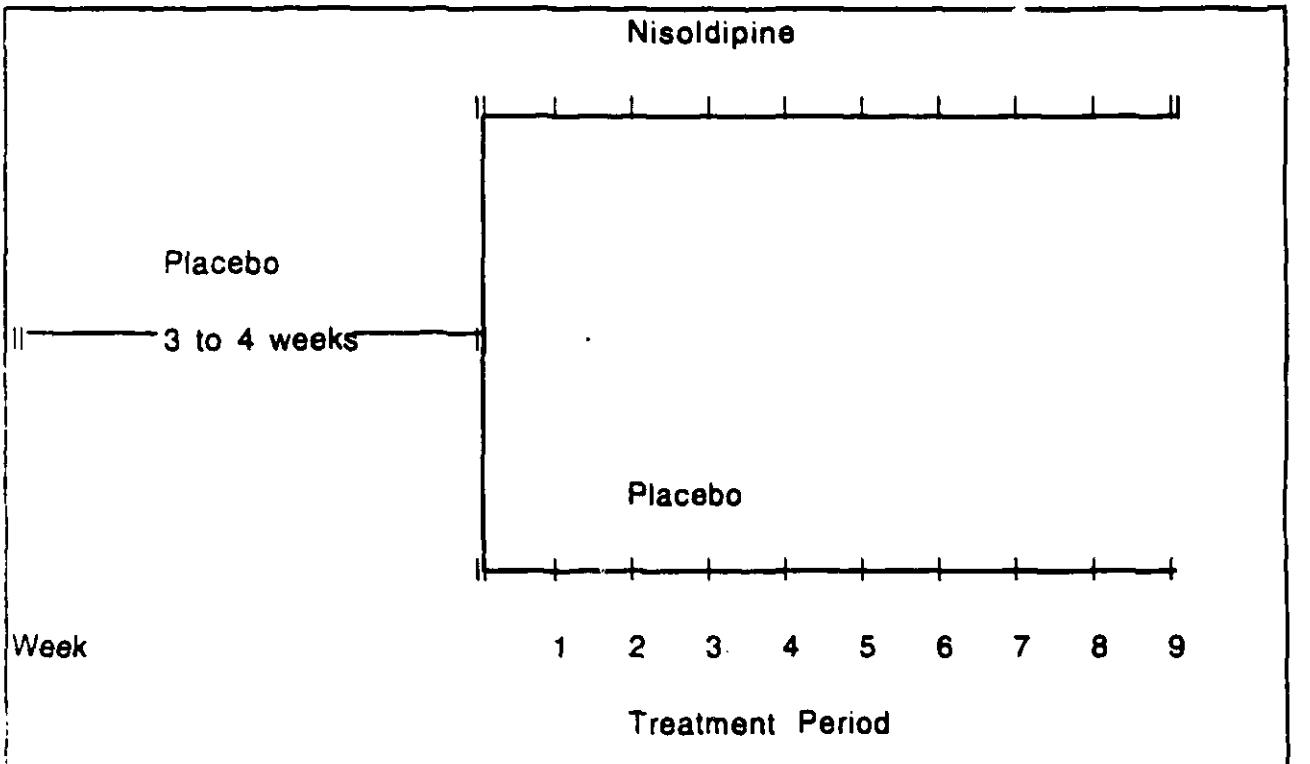
The demography and baseline characteristics of the patients valid for analysis of efficacy is given in the following table :

		Nisoldipine (n=79)	Placebo (n=38)
Sex	Male	46 (58 %)	22 (58 %)
	Female	33	16
Race	Caucasian	53 (67 %)	21 (55 %)
	Black	25	16
	Other	1	1
Age (years)	Mean	53	57
Years of hypertension	Mean	10	14
Baseline blood pressure	Supine	153/100	160/101
	Standing	149/100	156/102

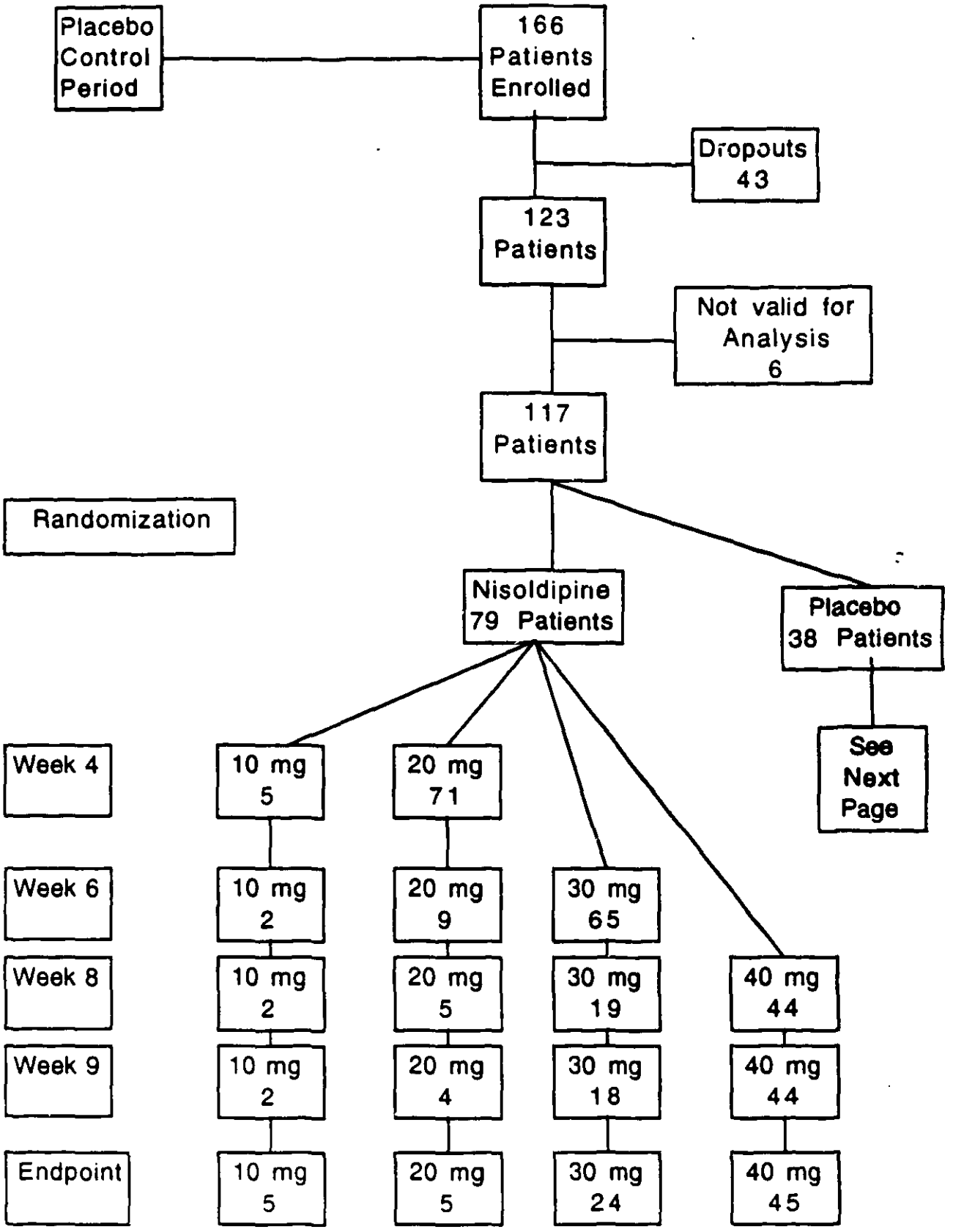
The reasons for discontinuation of double-blind therapy are given in the following table :

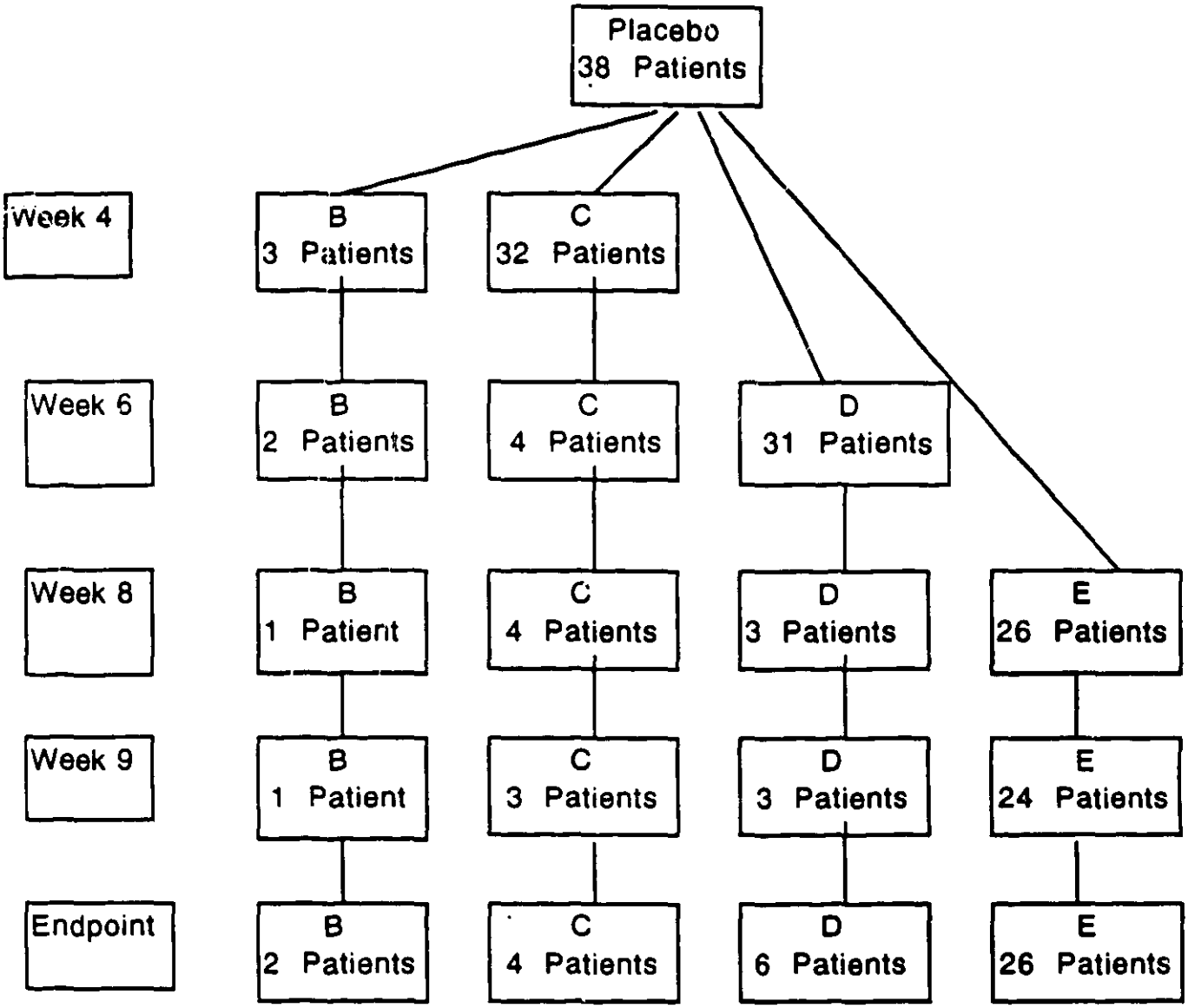
Reason	Nisoldipine n=83	Placebo n=40
Lack of Efficay	0	5
Adverse Event	6	0
Abnormal Laboratory Value	0	1
Lost to Follow-up	3	0
Other	2	9

The protocol that was followed is represented schematically in the following graph



The randomization of patients is given in the following two pages


Placebo Control Period
166 Patients Enrolled
Dropouts 43
123 Patients
Not valid for Analysis 6
117 Patients
Randomization
Nisoldipine 79 Patients
Placebo 38 Patients
See Next Page
Week 4
Week 6
Week 8
Week 9
Endpoint
10 mg 5
20 mg 71
10 mg 2
20 mg 9
30 mg 65
10 mg 2
20 mg 5
30 mg 19
40 mg 44
10 mg 2
20 mg 4
30 mg 18
40 mg 44
10 mg 5
20 mg 5
30 mg 24
40 mg 45


Placebo
38 Patients
Week 4
B
3 Patients
C
32 Patients
Week 6
B
2 Patients
C
4 Patients
D
31 Patients
Week 8
B
1 Patient
C
4 Patients
D
3 Patients
E
26 Patients
Week 9
B
1 Patient
C
3 Patients
D
3 Patients
E
24 Patients
Endpoint
B
2 Patients
C
4 Patients
D
6 Patients
E
26 Patients

Efficacy. Doses of Nisoldipine were titrated from regimen B (10 mg QD) to regimen E (40 mg QD) in 10 mg steps. The following table shows the actual number of patients that were given each dose level and had valid visits at each week for the population of patients valid for efficacy :

			Week of Therapy					
			2	4	6	8	9	End-point
Group	Reg.	Dose	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)
NIS	B	10 mg QD	79 (100)	5(7)	2 (3)	2 (3)	2 (3)	5 (6)
	C	20 mg QD		71 (93)	9 (12)	5 (7)	4 (6)	5 (6)
	D	30 mg QD			65 (86)	19 (27)	18 (27)	24 (30)
	E	40 mg QD				44 (63)	44 (65)	45 (57)
PLA	B		38 (100)	3 (9)	2 (5)	1 (3)	1 (3)	2 (5)
	C			32 (91)	4 (11)	4 (12)	3 (10)	4 (11)
	D				31 (84)	3 (9)	3 (10)	5 (16)
	E					26 (77)	24 (77)	26 (68)

The following table shows trough supine diastolic blood pressure response at different weeks of treatment and at endpoint for both groups for the set of all valid patients.

Trough Supine Diastolic Blood pressure
Mean Change (mmHg) by visit

	Week 2	Week 4	Week 6	Week 8	Week 9	End-point
Nisoldipine						
(n)	(79)	(76)	(76)	(70)	(68)	(79)
Mean Change	-5.7*	-6.5	-10.1*	-10.6*	-10.0*	-9.5*
Placebo						
(n)	38	35	37	34	31	
Mean Change	-3.0	-4.9	-3.4	-4.4	-3.4	-1.2

* Significantly different from placebo

In the following table, changes from baseline at endpoint by treatment regimen at endpoint for all valid patients is demonstrated :

	Nisoldipine			
	Reg B (n=5)	Reg C (n=5)	Reg D (n=24)	Reg E (n=45)
Supine				
Systolic	-8.3	-23.1	-10.9	-16.7
Diastolic	-5.9	-12.4	-9.4	-9.8
Standing				
Systolic	-5.3	-13.5	-10.3	-15.3
Diastolic	-7.2	-9.9	-6.6	-8.6

	Placebo			
	Reg B (n=2)	Reg C (n=4)	Reg D (n=6)	Reg E (n=26)
Supine				
Systolic	-19.7	-8.0	+20.4	-2.2
Diastolic	+ 1.7	-10.0	+6.9	-1.4
Standing				
Systolic	-9.7	-1.3	+13.9	-0.1
Diastolic	-0.7	-7.5	+5.0	-1.6

The responder rates are given in the following table :

Responder Rates
Based on Trough Supine Diastolic Blood Pressure at Endpoint

	Nisoldipine	Placebo
BP<90 mmHg at endpoint	49 %	18 %
At least a 10 mmHg fall at Endpoint	54 %	13 %
Either of the Above	62 %	21 %
Both of the Above	42 %	11 %

The change from baseline in trough blood pressure by treatment for all patients valid for analysis of efficacy is given in the following table :

Supine Diastolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg.B	Reg. B or C	Reg. BC or D	Reg.BCD or E	Reg BCDorE	
Nisoldipine n	79	76	76	70	68	79
Baseline Mean LS	100.36	100.36	100.36	100.14	100.36	100.36
LS Mean Change	-5.65*p	-6.52*	-10.06*p	-10.58*p	-9.98*p	-9.51*p
SE of Change	0.68	0.75	0.88	0.84	0.80	0.89

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg B	Reg B or C	Reg BC or D	Reg BCD or E	Reg BCDorE	
Placebo n	38	35	37	34	31	38
Baseline LSMean	101.45	101.31	101.20	100.52	100.79	101.45
LS Mean Change	-3.04*	-4.89*	-3.39*	-4.42*	-3.36*	-1.16
SE of Change	0.99	1.10	1.26	1.21	1.20	1.29
p Values						
Drug	0.0323	0.2234	0.0001	0.0001	0.0001	0.0001
Drug-Center	0,1582	0.2604	1.381	0.0539	0.0472	0.0332

P Significantly different from placebo
* Significant Change from baseline

Values for supine systolic blood pressure are given in the following tables :

Supine Systolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg B	Reg B or C	Reg BC or D	Reg BDC or E	Reg BCD or E	
Nisoldipine n	79	76	76	70	68	79
Baseline LS Mean	153.11P	153.39	153.41	153,35	153.42	153,11p
LS Mean Change	-8.67*	-10.123*p	-15.04*p	-14.10*p	-15.62*p	-14.73*p
SE of Change	1.33	1.61	1.61	1.61	1.64	1.89
Placebo n	38	35	37	34	31	38
Baseline LS Mean	159.59	158.99	158.23	157.78	159.71	159.59
LS Mean Change	-4.96*	-2.39	-1.90	-4.91*	-5.51*	-0.00
SE of Change	1.92	2.38	2.33	2.31	2.43	2.72

P-Values	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug	0.1140	0.0074	0.0001	0.0015	0.0008	0.0001
Drug Center	0.6059	0.0481	0.0416	0.1372	0.1224	0.0726

p Significantly different from placebo
* Significant change from baseline

Standing Diastolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg.B	Reg.B or C	Reg. BC or D	Reg BDC or E	Reg BCD	or e
Nisoldipine n	79	76	76	70	68	79
Baseline LSMean	100.40	100.31	100.31	100.21	100.27	100.40
LS Mean Change	-5.03*p	-6.24*p	-8.62*p	-10.18*p	-8.29#p	-7.86*p
SE of Change	0.75	0.75	0.85	0.83	0.98	1.00
Placebo n	38	35	37	34	31	38
Baseline LS Mean	102.15	101.85	101.85	101.22	100.89	102.15
LS Mean Change	-2.05	-1.99	-1.87	-4.27*	-3.65*	-1.18
SE of Change	1.08	1.11	1.22	1.20	1.45	1.44
P Values						
Drug	0.0253	0.0021	0.0001	0.0001	0.0095	0.0002
Drug-Center	0.2060	0.0052	0.6446	0.5733	0.4304	0.3160

p Significantly different from placebo
* Significant change from baseline

Standing Systolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg B	Reg B or C	Reg BC or D	Visit BCD	or E Visit BCD	orE
Nisoldipine n	79	76	76	70	68	79
Baseline LS Mean	149.22p	149.54	149.56	149.41	149.57	149.22p
LS Mean Change	-8.37*	-10.78*p	-14.18*p	-14.84*p	-13.76*p	-13.*p
SE of Change	1.34	1.49	1.78	1.73	1.66	1.77
Placebo n	38	35	37	34	31	38
Baseline LS Mean	156.22	155.79	155.30	154.22	155.46	156.22
LS Mean Change	1.94	2.20	2.55	2.49	2.48	2.57
P values						
Drug	0.0832	0.0005	0.0001	0.0002	0.0002	0.0001
Drug-Center	0.1162	0.0979	0.1092	0.2475	0.0099	0.0330

P Significantly different from placebo * Significant change from baseline

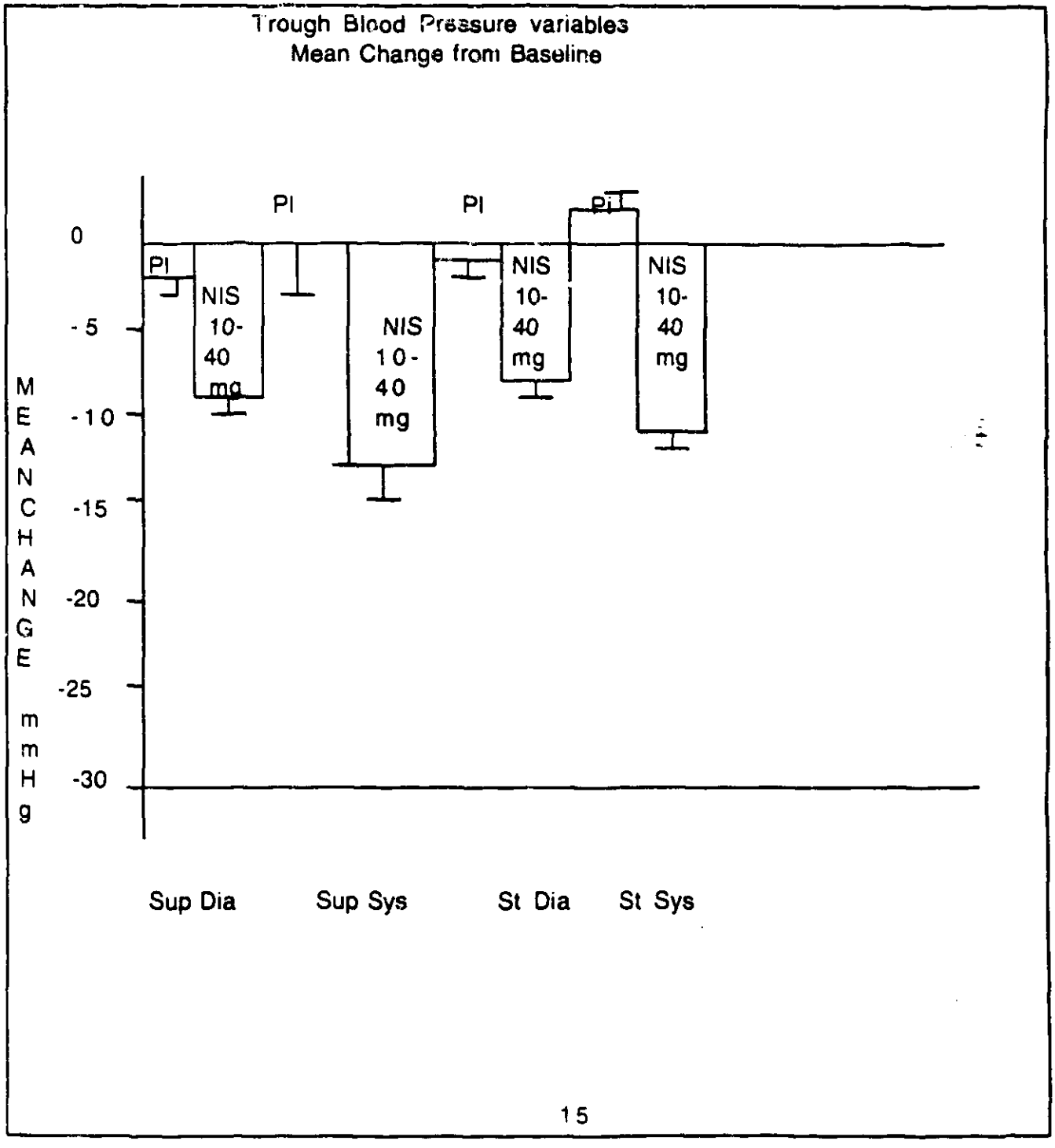
The effect of Nisoldipine on trough supine and standing systolic and diastolic blood pressure at study endpoint in all Nisoldipine treated patients is shown in the following table :

Change from Baseline to Endpoint in Trough Blood Pressures
Mean and SEM in mmHg

	Placebo n=38	Fisoldipine n-79
Supine Diastolic Blood Pressure	-1.16±1.29	-0.51±0.89*
Supine Systolic Blood Pressure	-.000±2.72	-14.73±1.89*
Standing Diastolic Blood Pressure	-1.19±1.44	-7.86±1.00*
Standing Systolic Blood Pressure	+0.89±2.57	-13.00±1.77*

* Significantly different from placebo. $p<0.05$

The change from baseline to endpoint in the primary efficacy blood pressure parameter *supine diastolic blood pressure*, as well as the 3 secondary blood pressure parameters is shown for placebo and all Nisoldipine doses in the figure below :



Conclusion. This was a titration study in which doses of Nisoldipine 10 mg, 20 mg, 30 mg, 40 mg and placebo were evaluated. In the course of the study most patient were moved to the higher doses in order to decrease the blood pressure and very few patients remained in the lower doses (see flow sheets pages 7, 8, and 9). Therefore a dose-range study could not be carried and only a global evaluation was possible. Such assessment demonstrated that Nisoldipine was very effective in lowering the blood pressure (pages 10-15).

Protocol D89-029

Title of Study : " Double-Blind Randomized Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine 20, 40 and 60 mg (2X30 mg) *Core-Coat Tablets vs Placebo in Combination with Atenolol 50 mg* in Hypertensive Patients ".

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Objectives : The objectives of this study were to determine the dose response and safety of 20 mg, 40 mg, and 60 mg Nisoldipine tablets as compared to placebo when administered once daily as additive treatment for hypertensive patients not controlled on once daily Atenolol 50 mg.

Inclusion Criteria. Ambulatory patients, male or female, of age 21 or older, with a history of essential hypertension were eligible for enrollment in the placebo run-in period.

Exclusion Criteria. Criteria foe exclusion were : labile hypertension, renal failure (plasma creatinine > 2.0 mg/dl), significant liver disease, insulin-dependent diabetes mellitus, history or presence of bronchial asthma, obstructive pulmonary disease, significant peripheral vascular disease, recent (3 months) myocardial infraction, cerebrovascular accident, or clinical signs suggesting impending myocardial infarction or cerebrovascular disease. Also excluded were patients with heart failure, major arrhythmias, conduction disturbances greater than first degree block, sinus bradycardia, failure of a major organ system, malignancy, psychosis, impaired absorption (such as chronic diarrhea), pregnancy,

women with childbearing potential, abuse of alcohol or drugs, allergy to dihydroperidines or beta blockers and participation in an investigational drug study within the past 30 days.

Qualifications for Randomization. Patients discontinued all previous antihypertensive medication and were given a single-blind placebo once daily in a 2-week qualifying period (Regimen A). Patients with a mean SUDBP 100-119 mmHg at the end of the placebo run-in period were given 1 capsule containing 50 mg Atenolol and 2 placebo tablets under single-blind conditions for 4 weeks (Regimen B). Patients with mean SUDBP 95-114 mmHg after 4 weeks of single-blind Atenolol were randomly assigned to 1 to 4 treatment groups and given double-blind drug.

Drug-Regimen Protocol (Regimen C). Patients who qualified for randomization received Atenolol 50 mg + Nisoldipine (20 mg, 40 mg, or 60 mg) or Atenolol 50 mg + placebo for 6 weeks.

Drugs for the double-blind period (Regimen C) contained encapsulated Atenolol 50 mg with one of the following :

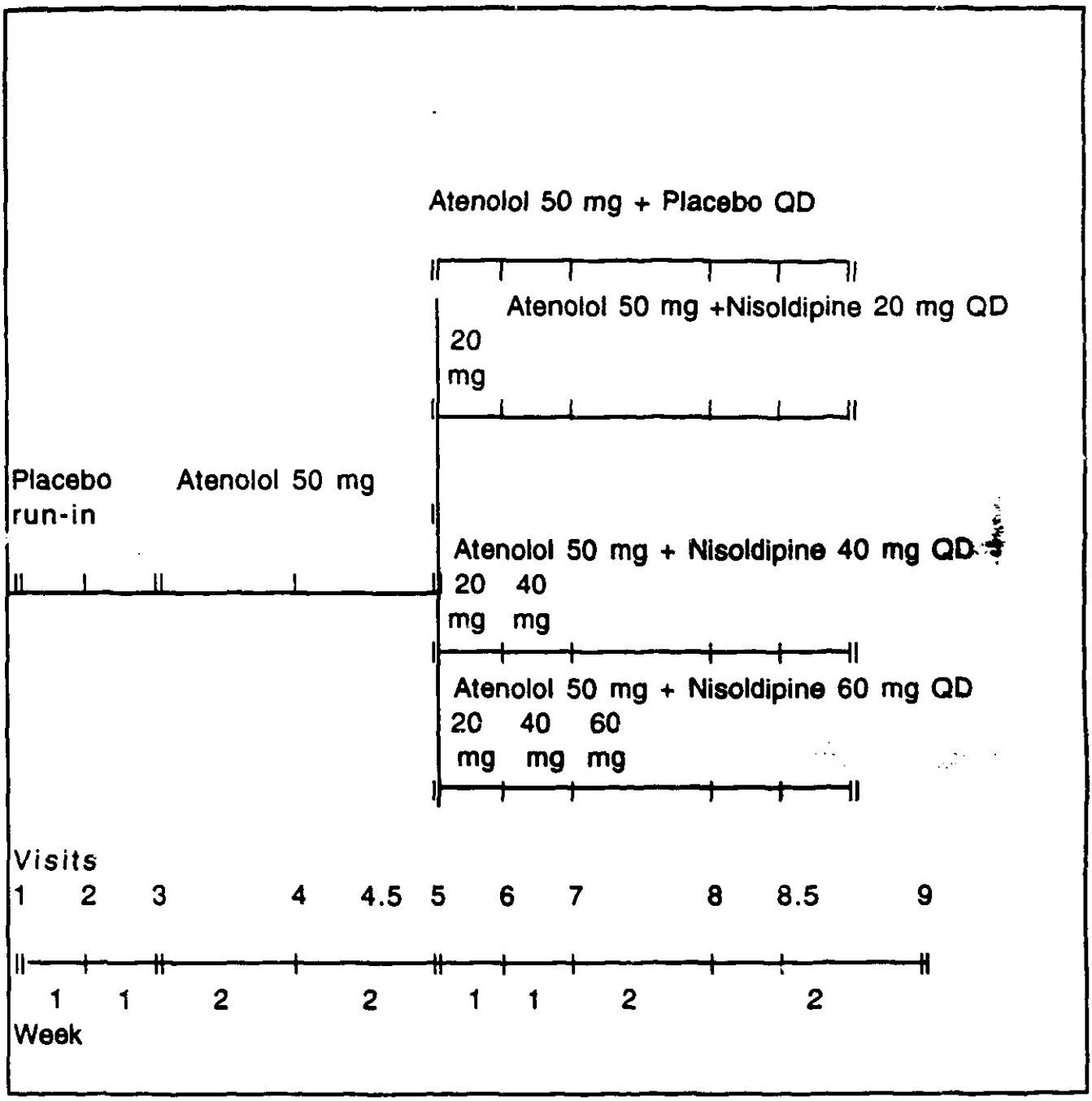
One Nisoldipine 20 mg tablet and
one placebo tablet once daily for 6 weeks

One Nisoldipine 20 mg tablet and
One placebo tablet once daily for 1 week
Forced titrated to
One Nisoldipine 40 mg tablet and
One placebo tablet once daily for 5 weeks

One Nisoldipine 20 mg tablet and
One placebo tablet once daily for 1 week
Forced titrated to
One Nisoldipine 40 mg tablet and
One placebo tablet once daily for 1 week
Forced titrated to
Two Nisoldipine 30 mg tablets once daily for 4 weeks

Two placebo tablets once daily for 6 weeks

The study design is illustrated in the following graph :



Removal of patients from Study or Analysis.. Patients could leave the study at any time if they so wished. Patients could be discontinued if they had significant physical or laboratory abnormalities, or if they had significant concurrent illness or deterioration of their condition. Patients could also be withdrawn if they were blatantly non-compliant. Patients with significant adverse events and those patients with elevations in SUDBP > 114 mmHg were also discontinued from the study.

Statistical Methods. All statistical tests were two-tailed and were conducted at a significant level of 0.05. Pairwise comparisons and within-group changes were tested via the least square means estimated by the model.

Results. . Demographic Characteristics. The demographic characteristics are given in the following table :

	Atenolol+ Nis 20 mg n=61	Atenolol+ Nis 40 mg n=59	Atenolol+ Nis 60 mg n=59	Atenolol+ Placebo n=59
Mean Age (years)	52	54	56	54
Mean wt (lbs)	201	198	198	195
Baseline BP (mmHg)				
Supine	159/101	159/101	162/101	156/110
Standing	154/102	157/103	156/103	152/101
% Male	79	73	70	64
% Caucasian	61	53	58	53
% Diabetic	8	9	14	12
% Mild Hypertensive	60	56	51	59
% Moderate Hypertensives	40	44	49	41

The sponsor states that there were no statistically significant differences between the groups for any of the characteristics examined.

Assessment. Patients were seen in the morning at weekly and biweekly intervals. A history complete physical examination and 12-lead electrocardiogram were taken in the firs visit, at baseline (after 4 weeks of Atenolol) and at the last visit on double-blind drug. Electrocardiograms

were included in visits 7 and 8. Twenty-four hour ambulatory electrocardiograms were taken at some centers. on weeks 4.5 after 3 weeks of single Atenolol and at 8.5 weks of double-blind therapy. Chest X-ray were taken after 2 weeks on placebo.

Laboratory tests performed in the course of the study included blood hematology, serum electrolytes, battery of liver function tests, and urinalysis.

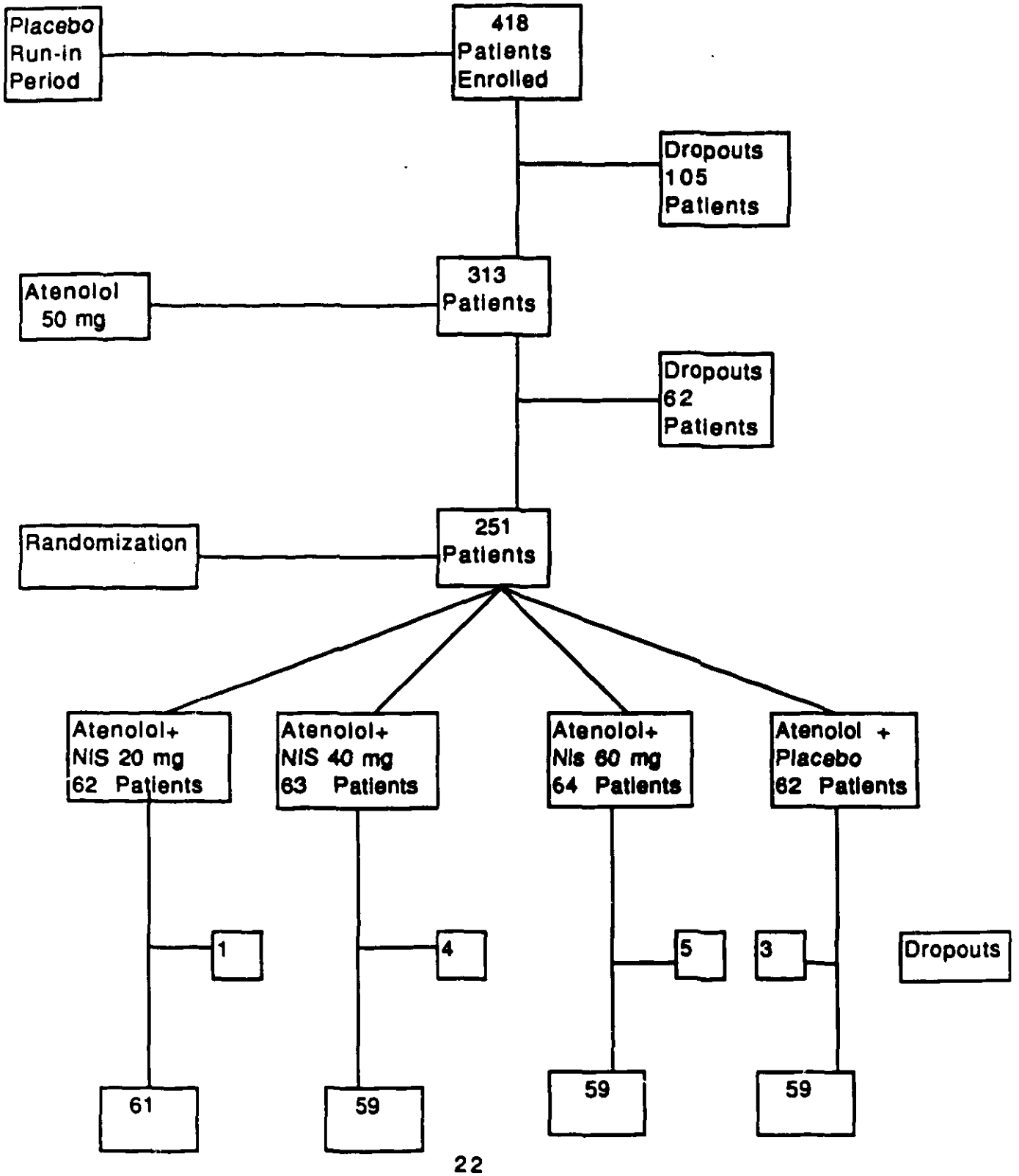
At the end of the single blind Atenolol phase and at the end of the double-blind phase blood was taken at trough for Nisoldipine assay.

At each visit vital signs were taken.

Criteria for Effectiveness. The change from baseline in SUDBP was the primary efficacy variable in this study. The primary time point was the endpoint which was defined as the last double-blind visit for all valid patients. A valid patient was one who had at least 3 weeks of double-blind drug. This criterion was later amended before braking the random code to 19 days. The overall treatment efficacy was determined by the change from baseline in trough SUDBP at endpoint between the average of the three Atenolol-Nisoldipine groups and the Atenolol-Placebo group. Secondary efficacy parameters were supine systolic blood pressure change at trough, standing blood pressure changes at trough, and ambulatory blood pressure trough/peak ratios.

An average decrease in diastolic blood pressure of at least 5 mmHg more than placebo was considered to be clinically meaningful. The actual power for the study was >95 %.

The disposition of the patients is given in the following flow-sheet :


Placebo Run-in Period
418 Patients Enrolled
Dropouts 105 Patients
Atenolol 50 mg
313 Patients
Dropouts 62 Patients
Randomization
251 Patients
Atenolol+ NIS 20 mg 62 Patients
Atenolol+ NIS 40 mg 63 Patients
Atenolol+ Nis 60 mg 64 Patients
Atenolol + Placebo 62 Patients
1
4
5
3
Dropouts
61
59
59
59

The reasons because the patients were withdrawn from the placebo run-in period are given in the following Table :

Mean SUDBP at visit 3 <100 mmHg or > 119 mmHg	=58
Mean SUDBP >119 mmHg during placebo run-in period	= 6
Adverse event during placebo run-in period	=10
Other illness	= 3
Abnormal laboratory value	= 4
Abnormal electrocardiogram	= 2
Noncompliance	= 3
Investigator discretion	= 5
Consent withdrawn	= 9
Lost to follow-up	= 5
Total	105

Patients withdrawn during the single Atenolol period and therefore not randomized :

Mean SUDBP at visit 5 <95 mmHg or >114 mmHg	=37
Mean SUDBP > 114 mmHg on 2 consecutive visits after placebo run-in	= 4
Adverse Event	= 4
Other illness	= 2
Abnormal electrocardiogram	= 1
Noncompliance	= 1
Investigator discretion	= 4
Consent withdrawn	= 4
Lost to follow-up	= 2
Enrolled after enrollment date	= 3
Total	62

The reasons for invalidity for patients that were withdrawn during the treatment period are given in the following table :

Drug Group	Number of Patients	Reasons for Invalidity
Atenolol + NIS 20 mg	1	Less than 19 days on double-blind drug
Atenolol + NIS 40 mg	4	Less than 19 days on double-blind drug
Atenolol + NIS 60 mg	4	Less than 19 days on double-blind drug
	1	Visit 5 diastolic BP not between 95 and 114 mmHg
Atenolol + Placebo	2	Less than 19 days on double-blind drug
	1	Visit 5 diastolic BP not between 95 and 114 mmHg
Total	13	

Effectiveness. The change from baseline in trough blood pressure by treatment for all patients valid for analysisof efficacy are given in the following tables

Supine Diastolic

Drug Group	Visit 6 Week 1	Visit 7 Week 2	Visit 8 Week 4	Visit 9 Week 6	Endpoint
ATN+NIS 20 mg					
N	58	61	61	61	61
Baseline BP	100.56	100.56	100.56	100.57	100.56
Mean Change	-8.53°C	-9.80°C	-9.10°AB	-10.09°	-10.09°
SE	0.85	0.78	C	ABC	ABC
ATN+NIS 40 mg			0.89	0.88	0.88
N	57	58		57	58
Baseline BP	100.75	100.75	58	100.77	100.75
Mean Change	-8.52°C	-11.26°C	100.75	-12.75°C	-12.69°C
SE	0.86	0.81	-12.87°C	0.92	0.91
ATN+NIS 60 mg			0.92		
N	56	59		57	59
Baseline BP	101.12	101.11	58	101.27	101.11
Mean Change	-9.72°C	-12.15°C	101.00	-14.36°C	-14.24°C
SE	0.87	0.80	-12.82°C	0.91	0.90
ATN+PL			0.92		
N	58	59		59	59
Baseline BP	99.93	99.93	59	99.93	99.93
Mean Change	-4.00°	-4.31°	99.93	-4.29°	-4.28°
SE	0.85	0.80	-4.61°	0.90	0.90
			0.91		

Drug Group	Visit 6 Week 1	Visit 7 Week 2	Visit 8 Week 4	Visit 9 Week 6	Endpoint
ATN+NIS 20 mg					
N	61	61	61	61	61
Baseline BP	158.71	158.70	158.70	158.71	158.70
Mean Change	-10.82*C	-12.96* ABC	-13.30* BC	-13.14* ABC	-13.15* ABC
SE					
ATN+NIS 40 mg					
N	58	58	58	57	58
Baseline BP	158.78	158.77	158.77	158.97	158.77
Mean Change	-12.93*C	-19.04*C	-19.03*C	-20.09*C	-19.83*C
SE	2.01	2.11	2.29	2.05	2.05
ATN+NIS 60 mg					
N	58	59	58	57	59
Baseline BP					
Mean	161.46	161.58	161.70	162.01	161.58
Mean Change	-12.29*C	-20.38*C	-21.00*C	-23.43*C	-23.09*C
SE	2.00	2.08	2.28	2.03	2.02
ATN+Pla					
N	59	59	59	59	59
Baseline BP Mean	156.30	156.29	156.29	156.28	156.29
Mean Change	-3.78	-2.51	-0.02	-0.87	-0.85
SE	1.99	2.09	2.26	2.01	2.02

Standing Diastolic

ATN+NIS 20 mg					
N	61	61	61	61	61
Baseline BP	102.15	102.16	102.16	102.16	102.16
Mean Change	-7.50*C	-8.46*C	-7.90* ABC	-8.93* ABC	-8.93* ABC
SE	0.87	1.00	0.89	0.89	0.89
ATN+NIS 40 mg					
N	58	58	58	57	58
Baseline BP	103.42C	103.43C	103.43C	103.45C	103.43C
Mean Change	-8.55*C	-12.23*C	-12.85*C	-15.00*C	-14.93*C
SE	0.89	1.02	0.92	0.93	0.91
ATN+NIS 60 mg					
N	58	59	58	57	59
Baseline BP	102.97	102.94	102.97	103.18	102.94
Mean Change	-8.55*C	-12.23*C	-12.85*C	-15.00*C	-14.93*C
SE	0.89	1.02	0.91	0.91	0.91
ATN+Pla					
N	59	59	59	59	59
Baseline BP	101.13	101.13	101.13	101.12	101.13
Mean Change	-3.29*	-4.24*	-3.19*	-1.96*	-1.95*
SE	0.89	1.02	0.91	0.91	0.91

Standing Systolic

Drug Group	Visit 6 Week 1	Visit 7 Week2	Visit 8 Week4	Visit 9 Week 6	Endpoint
ATN+NIS 20 mg					
N	61	61	61	61	61
Baseline BP	152.33	152.23	152.23	152.20	154.37
Mean Change	-10.69*C	-11.17* ABC	-11.20* ABC	-10.97* ABC	-10.97* ABC
SE	2.00	2.10	2.17	2.04	2.00
ATN+NIS 40 mg					
N	58	58	58	57	58
Baseline BP	156.89	156,87	156.87	156.85	156.87
Mean Change	-13.25*C	-17.66*C	-21.52*C	-22.35*	-22.38*C
SE	2.10	2.18	2.25	2.13	2.11
ATN+NIS 60 mg					
N	58	58	58	57	59
Baseline BP	156.17	156.30	156.33	156.73	156.30
Mean Change	-11.16*C	-19.76*C	-20.50*C	-22.36*C	-22.10*C
SE	2.08	2.15	2.24	2.12	2.08
ATN+Pla					
N	59	59	59	59	59
Baseline BP	152.23	152.23	152.23	152.20	152.23
Mean Change	-3.17	-1.42	-0.90	1.88	1.89
SE	2.07	2.15	2.22	2.08	2.09

P-values

	Visit 6 Week1	Visit 7 Week2	Visit 8 Week 4	Visit 9 Week 6	Endpoint
Drug* Center	0.0203	0.6905	0.1363	0.1813	0.1478
NIS vs PLA	0.0001	0.0001	0.0001	0.0001	0.0001
20 mg vs PLA	0.0002	0.0001	0.0004	0.0001	0.0001
40 mg vs Pla	0.0003	0.0001	0.0001	0.0001	0.0001
60 mg vs Pla	0.0001	0.0001	0.0001	0.0001	0.0001

A: Significantly different from ATN+NIS 40 mg QD
B. Significantly different from ATN+NIS 60 mg
C. Significantly different fron ATN+Pla
* Significant change from baseline

The effect of Nisoldipine on trough SUDBP during the course of the double-blind treatment is shown in the following table :

Placebo Substracted Change in SUDBP
Mean in mmHg

NIS Dose	Week 1	Week 2	Week 4	Week 6
20 mg	-4.53*	-5.49*	-4.49*	-5.80*
40 mg	-4.52*	-6.95*	-8.26*	-8.46*
60 mg	-5.72*	-7.84	-8.21*	-10.07*

* Denotes values when Nisoldipine blood pressure responses are significantly different from placebo, <0.05

The changes from baseline to endpoint in trough blood pressure, men and SEM in mmHg are given in the following table :

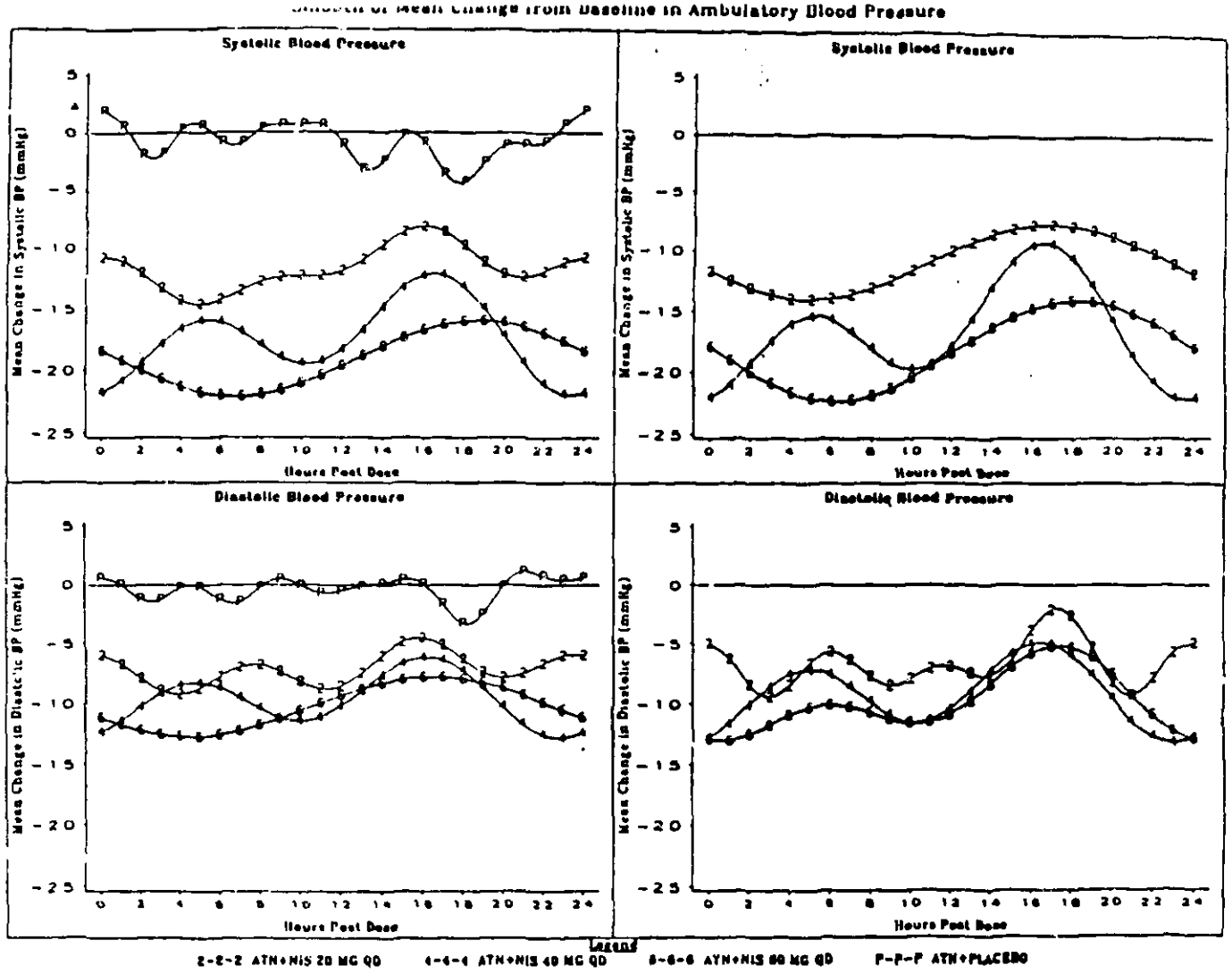
	ATN+PLA n=59	ATN + NIS 20 mg n=61	ATN + NIS 40 mg n=58	ATN + NIS 60 mg n=59
SUDBP	-4.28±0.90	-10.08±0.9[B]	-12.69±0.9*	-14.24±0.9*
SUSBP	-0.85±2	-13.15±2[AB]	-19.83±2*	-23.1±2*
STDBP	-1.95±0.91	-8.93±0.9[AB]	-13±0.92*	15±0.91*
STSBP	+1.89±2.09	-11±2.03*	-22.38±2.1*	-22.10±2.1*

A denotes values NIS 20 mg significantly different from placebo,p<0.05
B denotes values NIS 20 mg significantly different from Nisoldipine 60 mg<0.05.

	ATN+PL:A	ATN+NIS 20 mg	ATN+NIS 40 mg	ATN+NIS 60 mg
Trough response mmHg	% Patients	% Patients	% Patients	% patients
SUDBP ≤90	32.2	55.7*	67.8*	66.1*
Fall in SUDBP ≥10	23.7	50.8*	67.8*	74.6*
SUDBP≤90 or Fall in SUDBP ≥10	39	63.9*	74.6*	78
SUDBP ≤90 and fall in SUDBP ≥ 10	16.9	42.6*	61*	62.7*

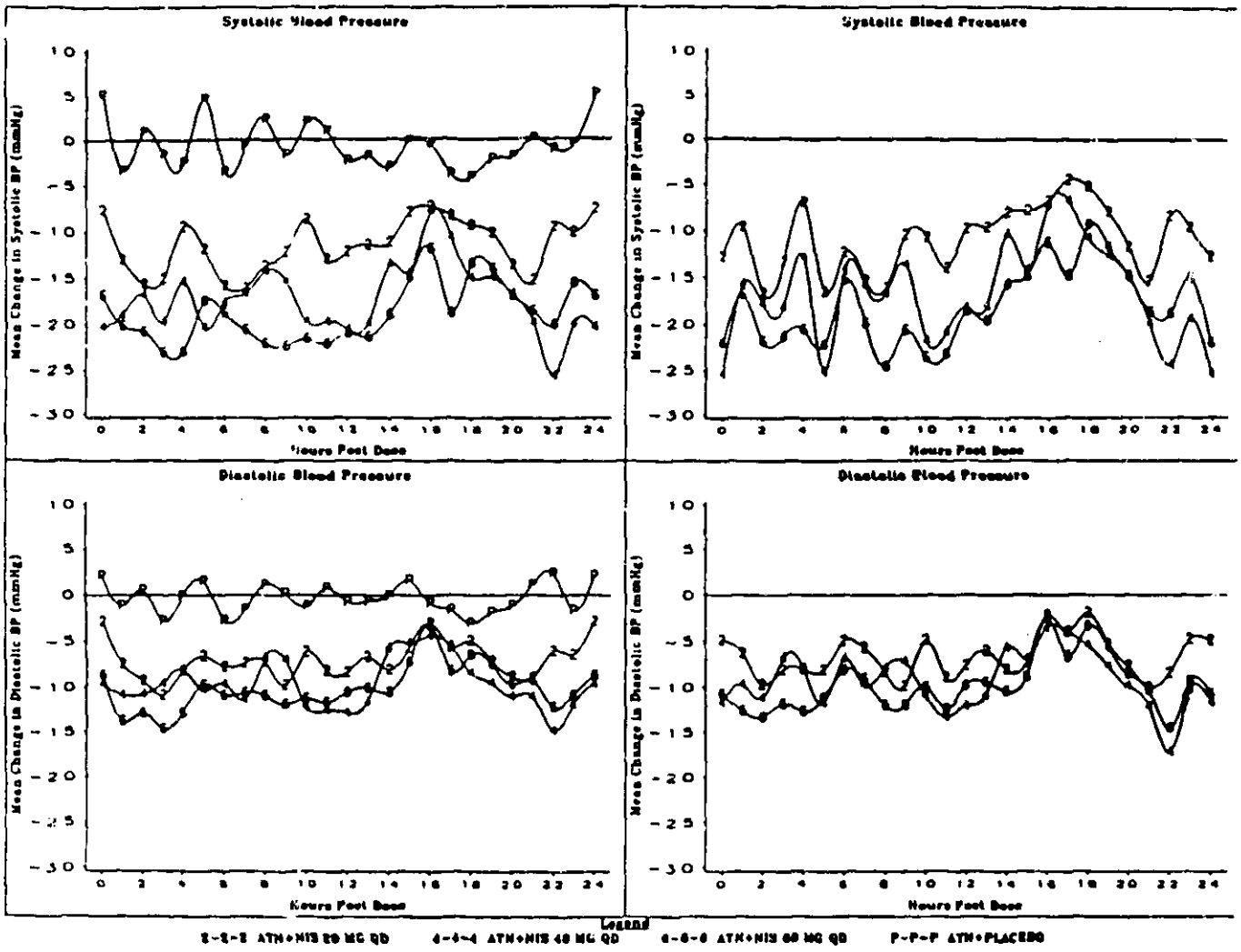
* p <0.01 vs placebo

At 8 centers ambulatory blood pressure monitoring (ABPM) was done after 3 weeks on single blind Atenolol and after 5 weeks of double-blind therapy. Smoothed and unsmoothed means for the ambulatory data are shown in the graphs in the following two pages :



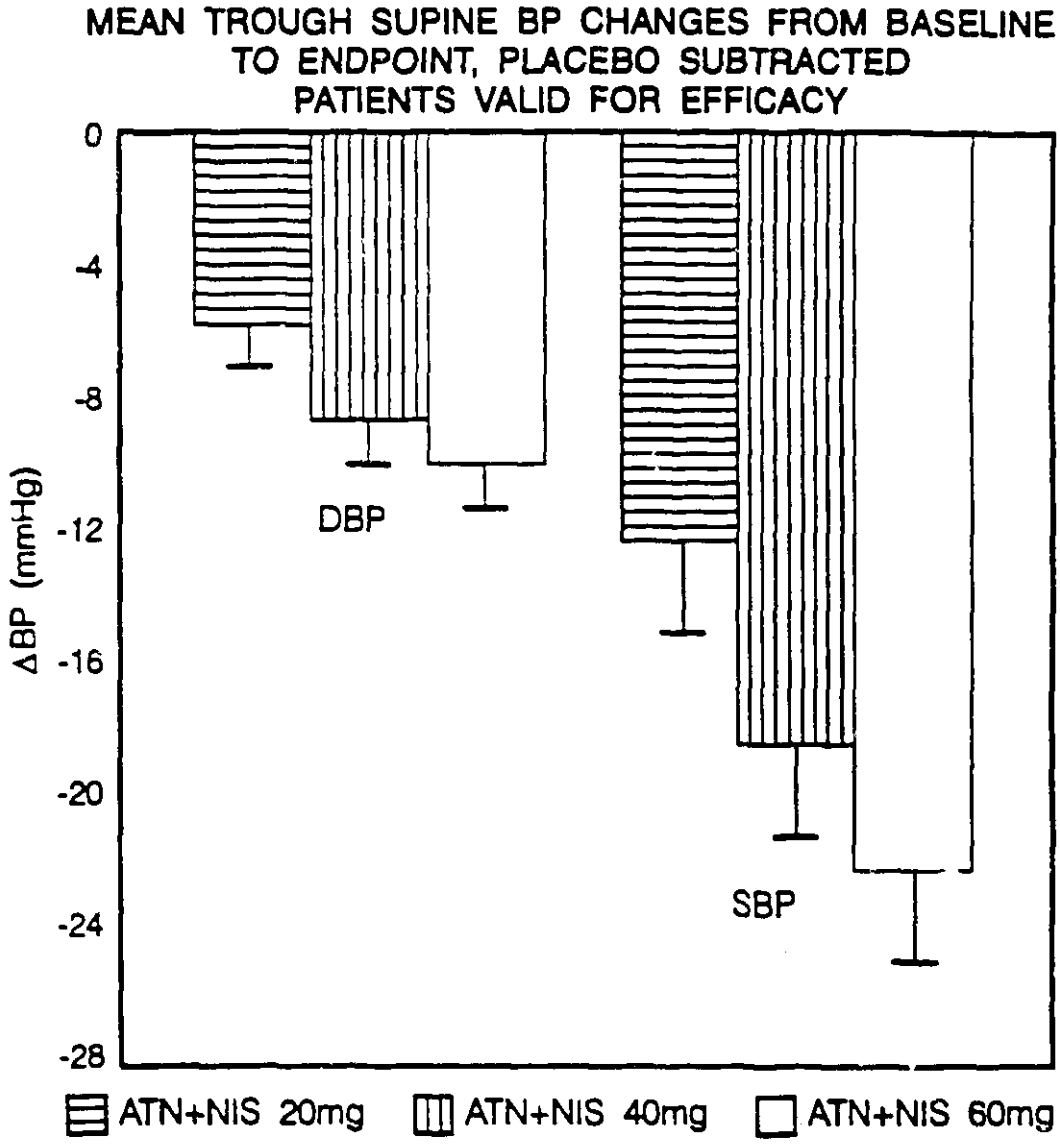
2-2-2 ATN+NIS 20 mg 4-4-4 ATN+NIS40 mg 6-6-6 ATN+NIS 60 mg
P-P-P ATN+Placebo

Figure 10
Mean Change from Baseline in Ambulatory Blood Pressure



Symbols as in previous graph

The placebo-substracted trough SUDBPs are showed in the following graph:



The trough and peak ambulatory blood pressure changes and trough to peak ratios for patients valid for efficacy analysis are given in the following table :

Smoothed Data - Difference from Placebo Group

		Trough mmHg	Peak mmHg	Hours to Peak	Trough to Peak Ratio
Variable	Drug				
Diastolic	ATN+NIS 20 mg QD	-5.0	-9.4	3	53%
	ATN+NIS 40 mg QD	-12.8	-13.1	23	97%
	ATN+NIS 60 mg QD	-12.9	-13	1	99%
Systolic (at corresponding diastolic peak)	ATN+NIS 20 mg QD	-11.7	-13.6	3	86%
	ATN+NIS 40 mg QD	-22.0	-22.0	23	100%
	ATN+NIS 60 mg	-17.9	-19.0	1	94%
Systolic (actual)	ATN+NIS 20 mg QD	-11.7	-14.0	5	83%
	ATN+NIS 40 mg QD	-22.0	-22.0	24	100%
	ATN +NIS 60 mg QD	-17.9	-22.3	6	80%

Pharmacodynamic Results. Trough plasma samples were drawn at visits 5 and 9. Visit 9 samples for all patients were analyzed for Nisoldipine. The results for patients whose treatment regimens did include Nisoldipine are shown in the following table :

	n	Mean Trough Concentration (ng/ml)	Range of Trough Concentrations (ng/ml)
AT+NIS 20 mg	57	1.6	0-7.41
AT+NIS 40 mg	55	2.5	0-12.0
AT+NIS 60 mg	59	3.3	0-10.40

Conclusions. In this protocol Atenolol was used as a positive control and studies were performed with Atenolol in combination with placebo and with NIS in the concentrations of 20 mg, 40 mg and 60 mg. Atenolo with placebo had not significant effect in blood pressure but in combination with NIS demonstrated significant hypotensive effects in relation with the concentration of NIS. Threfore the possibility of drug interaction should be considered. Measurements of NIS in plasma were performed and they increased as would be expected with increasing concentrations of NIS and in relation to their effects on blood blood pressure but unfortunately concentrations of Atenolol in plasma were not measured. In other studies the sponsor did not find clinically relevant drug interaction between Nisoldipine, and the beta blocker Propanol (study 704, PB#21521, Volume 142, pp. 08-17-0010374). However some studies in the literature do not agree with this conclusion.

Elliott et al studying the interactions between Nisoldipine and Atenoloi and Propanolol found that Nisoldipine, in single and multiple doses, significantly increased the peak plasma concentration of Propanolol and Atenolol. There was no evidence that either beta blocker influenced the pharmacokinetics of Nisoldipine. (The interactions between Nisoldipine and two beta-adrenoceptor antagonists : atenolol and propanolol. H.L.Elliott et al. Brit J Clin Pharmacol 1991;32(6):379-85).

Levine et al demonstrated pharacodynamic and pharmacokinetic interactions between Nisoldipine and Propanolol (MAH Levine et al. Pharmacokinetic and pharmacodynamic interactions between Nisoldipine and Propanolol. Clin Pharmacol Ther 1988; 43:39-48).

These contradictions could have been clarified had the sponsor included in this protocol a true placebo group and groups of Nisoldipine without Atenolol. Plasma levels of Atenolol should have been also measured.

Protocol D90-019

Title of Study : " A Double-Blind Randomized Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine Coat-Core Tablets 30 mg, 60 mg (2X30) and 90 mg (3X30) vs Placebo in Hypertensive Patients ".

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Objectives. The objectives of this study were :

1. To determine the antihypertensive efficacy and safety of NIS tablets in doses of 30 mg. 60 mg and 90 mg daily in patients with mild to moderate hypertension.

2. To assess the time and magnitude of peak blood pressure response and the ratio of trough to peak antihypertensive effect by 24-hour ambulatory blood pressure monitoring.

Inclusion Criteria. Ambulatory patients, male and female, 21 to 75 years of age, with a history of mild to moderate essential hypertension were eligible for the study.

Exclusion Criteria. Patients with the following conditions were excluded from the study : labile hypertension, renovascular or other secondary forms of hypertension, patients whose SUDBP after 3 and 4 weeks of placebo run-in were not ≥100 or ≤ 114 mmHg, previous myocardial infarction or cerebrovascular accident, heart failure, frequent arrhythmias, conduction disturbances, angina pectoris, use of other antihypertensive drugs, and many other drugs.

Also excluded were patients with failure of a major organ system, liver, kidney disease, malignancy or psychosis, patients with previous history of gastrointestinal disease which could result in incomplete absorption of the drug, women with childbearing potential, patients with alcohol or drug abuse, or allergy to dihydropyridines

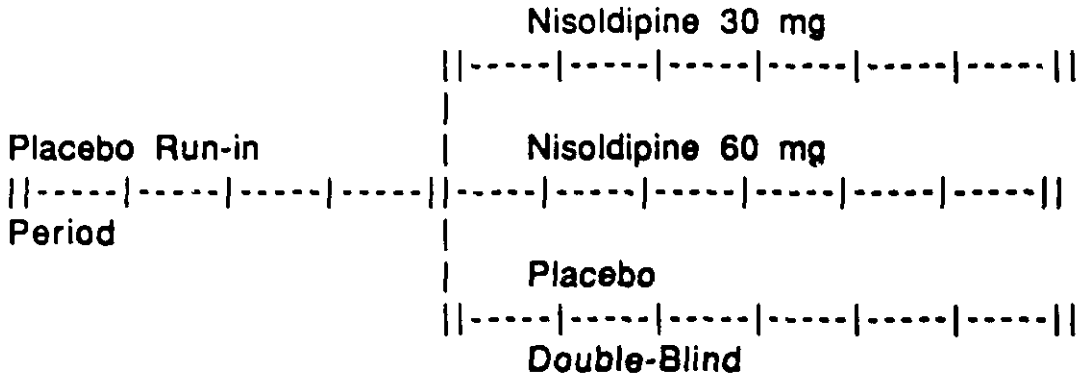
Study Design. . The study consisted of a single-blind placebo run-in period and a treatment period.

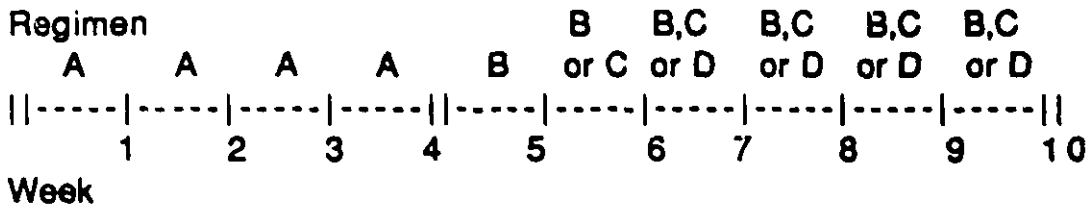
Placebo Run-in Period. During this period of 4 weeks duration pacients discontinued all previous antihypertensive medication and were given a single-blind placebo which consisted of 3 tablets once a day in the morning for a 4-week qualifying run-in period. Then patients with confirmed hypertension, with a trough SUDBP ≥100 to ≤ 114 mmHg after 3 and again after 4 weeks of placebo and whose SUDBP at these 2 visits were within 7 mmHg of each other were admitted into the treatment period.

Treatment Period. After four weeks of single-blind placebo patients with confirmed hypertension were randomized to one of three treatment groups: Placebo, Nisoldipine 30 mg or Nisoldipine 60 mg. Patients randomized to placebo received placebo for the remainder of the study. Placebo randomized to Nisoldipine 30 mg received the same dose for the remainder

of the study. Patients randomized to Nisoldipine 60 mag were given Nisoldipine 30 mg for one or two weeks and the dose was titrated to Nisoldipine 60 mg (2X30) for the final 4 to 5 weeks of the double-blind treatment. A group of patients was to be titrated to 90 mg (3X30) but this arm was discontinued before any patients were randomized because a concurrent high-dose forced-titration clinical pharmacological study showed evidence of symptomatic T wave flattening/or inversion predominantly at doses above Nisoldipine 60 mg.

The study design is shown schematically in the following graph :



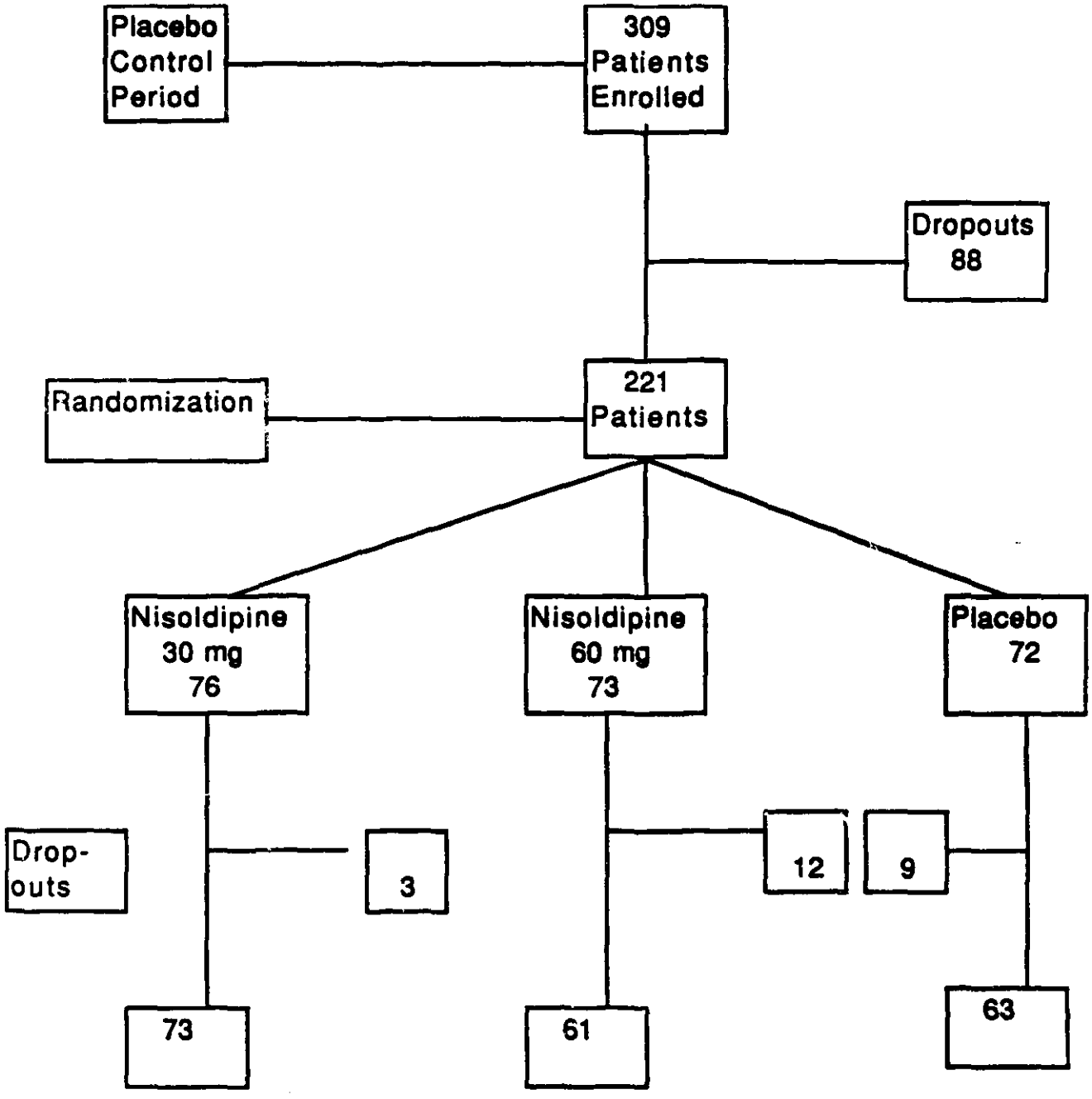


Group	Regimen B	Regimen C	Regimen D
NIS 30 mg	30 mg	30 mg	30 mg
NIS 60 mg	30 mg	60 mg	60 mg
Placebo	Placebo	Placebo	Placebo

Demography. The demography and baseline characteristics in patients valid for analysis of efficacy is given in the following table :

	NIS 30 mg N=76	NIS 60 mg N=66	Placebo N=71
Mean Age (years)	52	52	52
Mean wt (Lbs)	197	197	202
Baseline BP			
Supine	157/104	158/105	155/104
Standing	153/104	154/104	151/103
History of Hypertension (years)	11	9	10
% Male	61	56	52
% Caucasian	58	58	72
% History of Diabetes	9	11	13
% History of Hyperlipidemia	13	12	8
% History of MI	0	2	1
% Mild Hypertensives	62	58	70
% Moderate Hypertensives	38	42	30

The distribution and randomization of patients is seen in the following grapl



The listing of patients who did not qualify for randomization is given in the following table :

Mean supine diastolic blood pressure at visit 4 and at visit 5 did not qualify	45
Adverse events	7
Low diastolic blood pressures	6
Patient request	6
Blood pressure too high	5
Elevated serum transaminase levels	5
Childbearing potential	2
Elevated serum lipids	2
Family considerations	2
Illness not due to study medication	2
Intercurrent medical considerations	2
Administrative problems	1
Change in supine diastolic blood pressure > 7 mmHg from visit 4 to visit 5	1
Low hemoglobin/hematocrit	1
Protocol violation	1
Total	88

The reasons for discontinuation of double-blind therapy population in all randomized patients are given in the following table :

Reason	Nisoldipine		Placebo
	30 mg N=76	60 mg N=73	N=72
Adverse Event	1	11	3
Lack of efficacy	1	0	2
Physician dissatisfied with treatment	0	1	2
Patient dissatisfied with treatment	0	0	2
Lost to follow-up	1	0	0

The listing of dropouts due to adverse events for patients valid for safety analysis is given in the following table :

Drug Group	Adverse Experience Causing Patient to Drop	Day of Onset	Dose/ Duration (Days)	Intensity/ Relation-ship to Drug
Placebo	EKG abnormality	-1	Pla/1	Mild/ Remote
	Cardiac arrest	24	Pla/1	Severe/ Remote
	Frontal headaches	28	Pla/>3	Severe/ Possible
Nisoldipine	Edema lower extremity	24	30 mg/ >7	Moderate/ Probable
	Severe headache	3	30 mg/ >11	Severe/ Possible
	Headache	-1	Pla/5	Severe/ Possible
	Severe headache	0	30 mg/7	Severe/Pos
	2+ ankle edema, bilateral	24	60 mg/ 10	Moderate/ Probable
	3+ pretibial edema, bilateral	24	60 mg/ 10	Severe/ Probable
	Trace edema, bilateral	24	60 mg/ 10	Mild/ Probable
	Atypical chest pain	9	60 mg/5	Mod/Poss
	Headache	12	60 mg/ >1	Severe/ Possible
	3 + ankle edema	33	60 mg/ >3	Severe/ Possible
	Headache,vomiting	7	60 mg/ 1	Severe/ Possible
	Headache	12	60 mg/>1	Sev/Rem
	Confusion,	0	30 mg/>1	Sev/Rem
	Nausea,	0	30 mg/3	Mild/Prob
	Headache	6	30 mg/2	Mod/ Probable

Efficacy

Actual Dosage and Duration of Treatment. Patients were to receive Nisoldipine 30 mg, 60 mg or Placebo over a 6 week double-blind treatment period. Upward titration from Nisoldipine 30 mg to Nisoldipine 60 mg was required in the Nisoldipine 60 mg group after one or two weeks of double-blind medication if trough SUDBP was greater than or equal to 80 mmHg. Placebo and Nisoldipine 30 mg underwent sham titration. The following table shows the number of patients that were given each dose level and had valid visits at each week for the population of patients valid for efficacy :

Double-Blind Visit			6	7	8	9	10	
Week of Double-Blind Therapy			1	2	3	4	6	Endpoint
Group	Regimen	Dose	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Nis 30 mg	B (30 mg qd)		75 (100)	10 (13)	4 (5)	3 (4)	3 (4)	3 (4)
	C (30 mg QD)			66 (87)	71 (95)	71 (96)	70 (96)	73 (96)
NIS 60 mg	B (30 mg QD)		65 (100)	7 (11)	5 (8)	5 (8)	4 (6)	5 (8)
	C (60 mg QD)			59 (89)	59 (92)	59 (92)	58 (94)	61 (92)
Placebo	B (Placebo)		71 (100)	6 (8)	2 (3)	2 (3)	2 (3)	2 (3)
	C (Placebo)			65 (92)	65 (97)	64 (92)	62 (97)	69 (97)

Analysis of Effectiveness. Two hundred thirteen of the 221 enrolled patients had at least one valid blood pressure measurement after randomization and were included in the primary efficacy analysis (endpoint) :76 were randomized to the Nisoldipine 30 mg group, 66 were randomized to the Nisoldipine 60 mg group, and 71 were randomized to the placebo group.

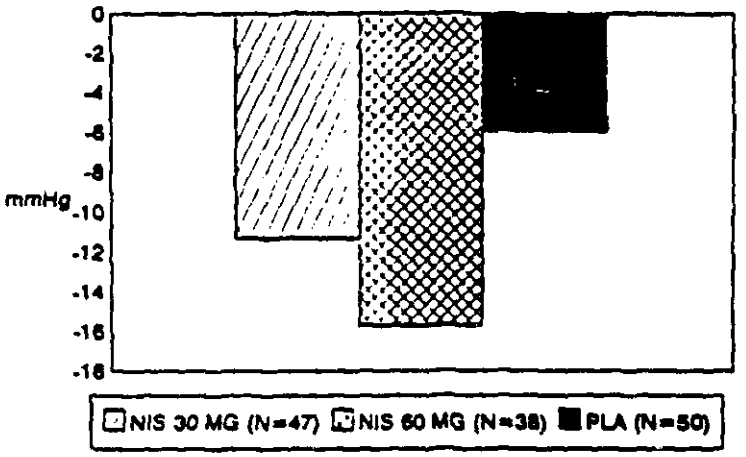
Trough raw means of blood pressure at each visit as well as at endpoint are given in the following table :

	Supine			Standing		
	NIS 30 mg	NIS 60 mg	Placebo	NIS 30 mg	NIS 60 mg	Placebo
Base-line (N)	158/ 104 (76)	158/ 105 (66)	155/ 104 (71)	154/ 104 (76)	155/ 104 (66)	152/ 103 (71)
Week 1 (N)	148/95 (75)	146/94 (65)	152/98 (71)	144/96 (75)	142/94 (65)	149/ 100 (71)
Week 2	147/93 (76)	141/90 (66)	152/98 (71)	143/95 (76)	139/91 (66)	149/99 (71)
Week 3 (N)	144/92 (75)	139/89 (64)	149/97 (67)	140/93 (75)	136/90 (64)	148/98 (67)
Week 4 (N)	142/92 (75)	139/87 (64)	152/98 (66)	140./93 (75)	135/87 (64)	150/99 (66)
Week 6 (N)	145/92 (73)	140/89 (62)	152/98 (64)	140/94 (73)	135/90 (62)	149/ 100 (64)
End-point (N)	146/93 (76)	141/90 (66)	154/99 (71)	141/94 (76)	137/91 (66)	150/ 101 (71)

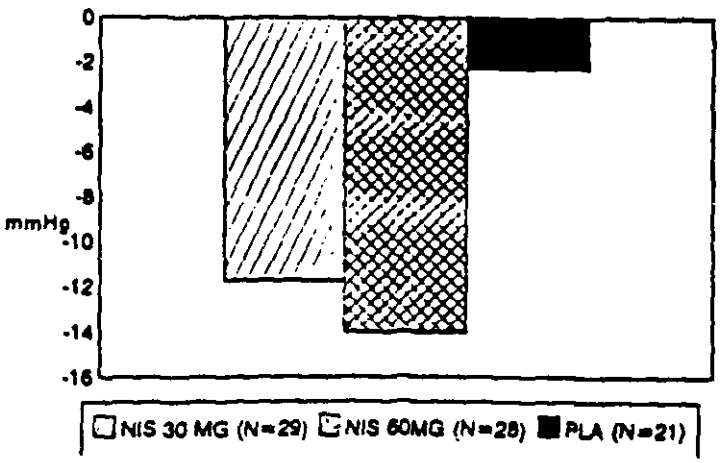
Mean changes (mmHg) in SUDBP at endpoint for patients wit mild (baseline SUDBP ≥ 100 to ≤ 104 mmHg)and moderate (baseline SUDBP ≥ 105 to ≤ 114 mmHg) hypertension are shown in the followings table and figure:

	Nisoldipine 30 mg		Nisoldipine 60 mg		Placebo	
	(N)	Change	(N)	Change	(N)	Change
Mild	47	-11.3	38	-15.7	50	-6.0
Moderate	29	-11.7	28	-14.0	21	-2.3

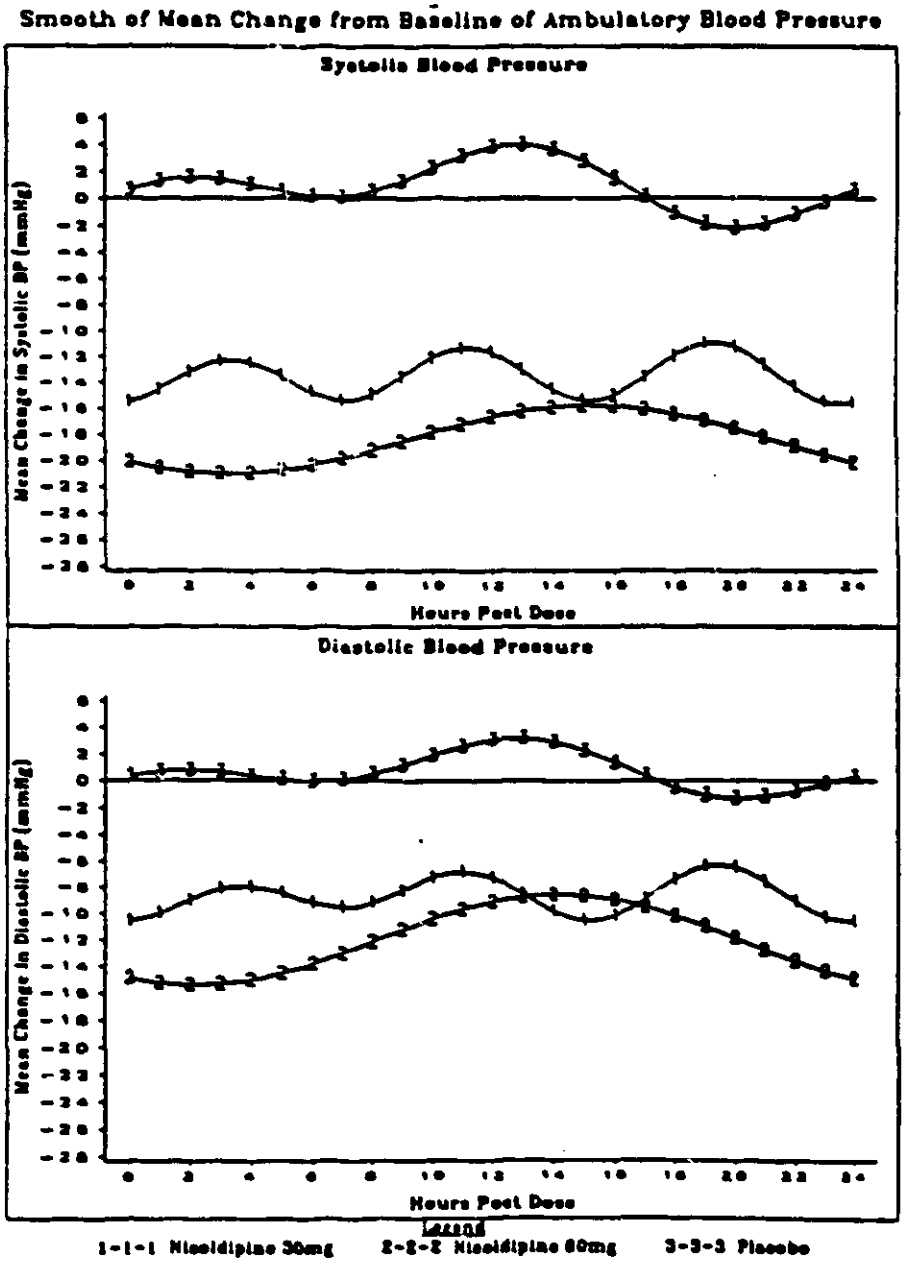
MEAN CHANGE FROM BASELINE (mmHg)
FOR SUPINE DIASTOLIC BP AT ENDPOINT
PATIENTS WITH MILD HYPERTENSION



MEAN CHANGE FROM BASELINE (mmHg)
FOR SUPINE DIASTOLIC BP AT ENDPOINT
PATIENTS WITH MODERATE HYPERTENSION



The 24-hour ambulatory blood pressure profile of mean systolic and diastolic blood pressure responses for the three treatment groups are shown in the following graph :



The ambulatory blood pressure falls and trough to peak ratios change from placebo for patients valid for efficacy analysis are given in the following table :

	N	Peak Hour	Peak value * (mmHg)	Trough* (mmHg)	Trough to Peak ratio
Diastolic BP					
NIS 30 mg	39	13	12.13	9.52	78 %
NIS 60 mg	29	4	15.24	14,22	93 %
Systolic BP (at corresponding time of diastolic peak)					
NIS 30 mg	39	13	17.46	14.52	83 %
NIS 60 mg	29	4	23.13	17.66	76 %
Systolic Blood Pressure (actual)					
NIS 30 mg	39	13	17.46	14.52	83 %
NIS 60 mg	29	5	23.71	17.66	75 %

*Change from placebo, baseline corrected

The mean changes from baseline in diastolic blood pressure over the 24-hour period of ambulatory blood pressure were -8.6 mmHg for Nisoldipine 30 mg, -12.0 mmHg for Nisoldipine 60 mg and +0.7 mmHg for placebo.

Pharmacokinetics Results. Trough blood samples were drawn at all 16 centers at visits 5 an 10. Seven centers also drew blood samples at visit 10.1 at 2 and 12 hours post-dosing. Samples were assayed for Nisoldipine blood levels. Results are presented in the following table :

	N	Mean Concentration (SD) ng/ml	Change in SUBP (Systolic/Diastolic) in mmHg
NIS 30 mg :			
Trough	68	1.5 (1.3)	-12/-11
2 hours post dosing	27	2.3 (1.9)	-17/-15
12 hours post dosing	27	2.1 (1.2)	-19/-17
NIS 60 mg:			
Trough	55	3.2 (2.8)	-17/-16
2 hours post dosing	27	6.0 (5.2)	-20/-19
12 hours post dosing	26	4.9 (2.8)	-21/-22

There was a statistically significant correlation between plasma concentration and change from baseline to endpoint in supine diastolic blood pressure at trough. The greater the correlation, the greater was the decrease in supine diastolic blood pressure. Twenty percent of the variability in the observed change in supine diastolic blood pressure was explained by plasma concentration.

Assessment. The results of this study indicate that Nisoldipine, at the dose of 30 mg and 60 mg daily once daily, is effective in reducing systolic and diastolic blood pressure at trough in patients with mild to moderate hypertension. The reductions in systolic and diastolic blood pressure were greater than 50 percent of peak effect at trough. Furthermore, ambulatory measurements of blood pressure for 24-hours demonstrated that reductions in blood pressure in Nisoldipine-treated patient was maintained through the hours of observation. The effect was more effective with the 60 mg of Nisoldipine than with the 30 mg dose and in the latter more effective than placebo. Pharmacokinetic studies demonstrated that effect on diastolic blood pressure was proportional to the concentration of Nisoldipine in blood.

Side effects were significantly increased by drug administration as compared to control and were greater with the 60 mg Nisoldipine dose than with the 30 mg. Adverse events are to be discussed by another reviewer.

Protocol D89-039

Title of Study : " Comparative Double-Blind Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine 20 mg. 40 mg, 80 mg Coat Core (CC) Tablets vs a Twice Daily Dose of Verapamil SR 240 mg caplets vs Placebo in Hypertensive Patients ".

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Objectives. The objective of this study was to determine the efficacy and safety of once daily doses of Nisoldipine 20 mh, 40 mg and 80 mg to a twice daily dose of Verapamil 240 mg and to Placebo in patients with mild to moderate hypertension.

Inclusion and Exclusion Criteria. Ambulatory male and female patients, 21 years of age or older, with history of mild to moderate hypertension, were eligible for enrollment in this study.

Patients with the following conditions were excluded from this study : recent myocardial infarction or cerebral vascular accident ; herat failure, major arrhythmias, conduction distrurbances, angina pectoris, sinus bradycardia or severe left ventricular dysfunction ; patients with impaired absorption of the drug ; females pregnant or with childbearing potential ; patients with failure of a major organ system such as liver renal disease, malignancy or psychosis ; alcohol abuse or drug intake ; allergy to dihydropyridines, verapamil or other antagonists ; also excluded were patients who participated in another investigational drug study within the previous 30 days.

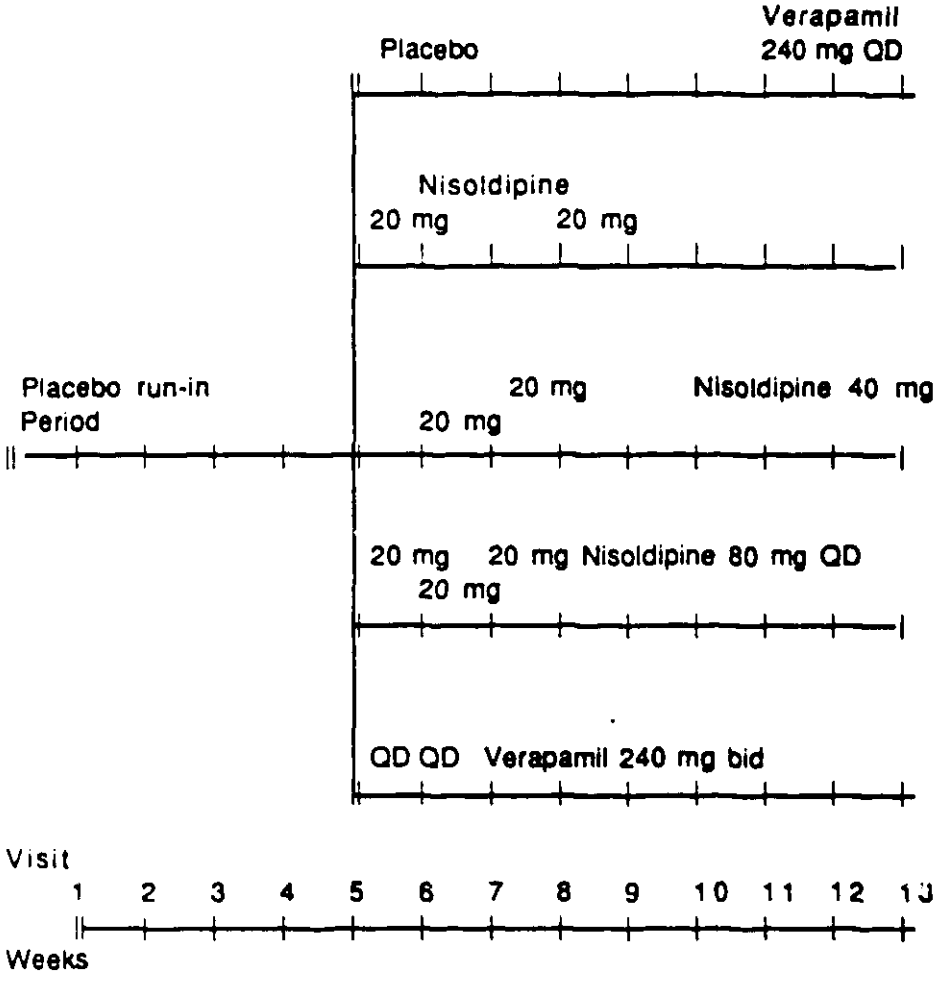
Study Design. The study consisted of a single-blind run-in period and a treatment period.

Single-Blind Run-In Period. Patients were given two placebo tablets and one placebo capsule in the morning and anothe placebo-capsule in the evening each day during a 4-week single-blind-run-in period. Drug for the single-blind placebo run-in period was labeled as Regimen A.

Qualification for Randomization. Patients whose mean SUDBP (the average of 3 readings over a five minute period in the supine position) were 95-114 mmHg after 3 and after 4 weeks on placebo and whose SUDBP after 3 and 4 weeks on placebo were within 7 mmHg of each other were eligible for randomization.

Double-Blind Treatment Period. After the placebo run-in period, a forced titration was designed as follows : Regimen B : Nisoldipine 20 mg, Verapamil 240 mg qd or Placebo which patients took for one week; Regimen C : Nisoldipine 20 mg, Nisoldipine 40 mg, Verapamil 240 mg twice daily or Placebo which patients took for one week ; Regimen D : Nisoldipine 20 mg, Nisoldipine 40 mg, Nisoldipine 80 mg (2 X 40), Verapamil 240 mg twice daily or Placebo which patients took for 6 weeks. After 8 weeks of double-blind drug, patients given Nisoldipine or Verapamil continued on the same drug regimen while patients given Placebo were switched to Verapamil 240 mg qd for the remaining of the 4 weeks of study.

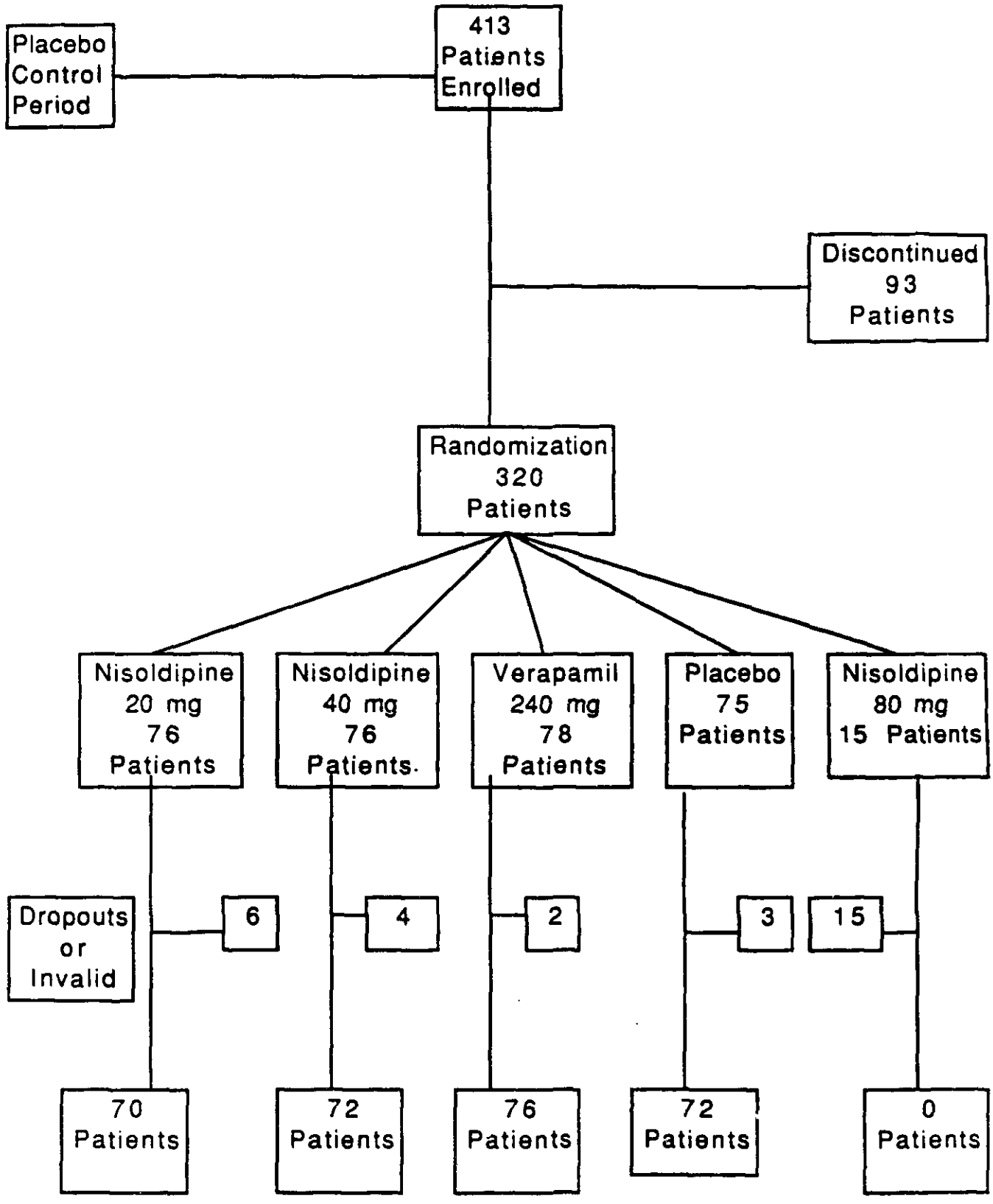
The study design is demonstrated schematically in the following graph :



Demographics. The demographic characteristics are shown in the following table :

	Nisoldipine 20 mg n=70	Nisoldipine 40 mg n=72	Verapamil n=76	Placebo n=72
Mean age (years)	53	54	52	55
Mean wt (lbs)	202	197	198	196
Baseline BP (mmHg)				
Supine	153/100	155/100	151/100	154/100
Standing	151/101	151/101	148/101	151/100
Male	57 %	61 %	55 %	67 %
Black	31 %	24 %	28 %	21 %
History of Diabetes	9 %	1 %	11 %	8 %
History of Hyperlipidemia	3 %	1 %	3 %	10 %
History of MI	3 %	1 %	0 %	1 %
Hypertensives				
Mild	84 %	86 %	83 %	89 %
Moderate	16 %	14 %	17 %	11 %

The distribution of patients and randomization are given in the following graph:



The reasons that disqualified enrolled patients for randomization are given in the following table :

Mean Supine Diastolic Blood Pressure at visit 4 or visit 5 did not qualify for randomization (95 mmHg to 11 mmHg)	47
Adverse events	13
Patient chose to withdraw	9
Other illness/Surgery/Screening abnormality	5
Blood pressure too high off medications for patient's safety	5
Lost to follow-up	4
Elevated transaminases at screening	3
Noncompliance	3
Called to military service	2
Blood pressure too low after in-clinic	1
Inadequate quality control during ambulatory blood pressure	1
Total	93

The number of dropouts during the treatment period and the reasons for elimination from the study are given in the following table :

Nisoldipine 20 mg. N=76

Event	Days on Drug
Palpitations, depression, headache, emesis	2
Headache, shortness of breath, fatigue	3
Headache, flashing, head congestion	4
Headache, flushing, palpitations	6
Peripheral edema	12
Headache, nausea	12
Peripheral edema	24
Peripheral edema	73
Pleural effusion	77
Myocardial infarction	89
Noncompliance	7
Chose to withdraw	54

Nisoldipine 40 mg. N=76

Event	Days on Drug
Headache, rash	1
Headache, nausea	2
Headache, nausea	2
Peripheral edema	10
Peripheral edema	12
Myocardial infarction	13
Headache, tremor, flushing, palpitations hypesthesia, asthenia	14
Peripheral edema	16
Peripheral edema	22
Peripheral edema	40
CVA	41
Chose to withdraw	16
Chose to withdraw	32

Nisoldipine 80 mg. N=15

Headache, flushing, palpitations, chest pain	1
Flushing, palpitation	14
Deep T wave inversion	15
Discontinued	3
Discontinued	4
Discontinued	6
Discontinued	12
Discontinued	13
Discontinued	14
Discontinued	17
Discontinued	20
Discontinued	20
Discontinued	24
Discontinued	28
Discontinued	31

Verapamil 240 mg. N=78

Event	Days on Drug
Headache, dizziness, tachycardia, leg pain, tinnitus	0
Peripheral edema	17
Hypotension	22
Headache, chills, peripheral edema	36
Cholecystitis	7

Placebo. N=75

CVA	6
Fatigue, edema	14
Peripheral edema	32
Lack of efficacy	5
Lack of efficacy	48
Lost to follow-up	21
Lost to follow-up	69
Chose to withdraw	47
Chose to withdraw	62
DBP> 114 mmHg	27
Retinal disorder	55

Efficacy

Criteria for Effectiveness. The change from baseline to endpoint in trough SUDBP (blood pressure measured 24 hours after the previous day's morning dose and 12 hours after the previous day's evening dose) in the Nisoldipine 40 mg group compared to the placebo group was the primary criterion used to determine the effectiveness of the drug.The comparison of Nisoldipine 20 mg to Placebo was of secondary importance.

Secondary efficacy parameters included standing diastolic blood pressure and both standing and supine systolic blood pressure. In addition in eight centers ambulatory blood pressure changes (the difference between measurements made over the 24 hours after 3 weeks of placebo run-in and the 24 hours after 7 weeks of double-blind therapy) were compared among

groups. The 12-hour in-clinic monitoring data were also compared among groups. The peak effect and the time to peak effect were calculated for both the ambulatory and 12-hour in-clinic monitoring. In addition the trough to peak ratio was calculated for ambulatory blood pressure. Plasma samples were drawn at baseline (visit 5) and at visit 11 for analysis of Nisoldipine plasma concentrations.

Statistical Methods. All statistical methods were two-tailed and were conducted at a significance level of 0.05. Pairwise comparisons and within group changes were tested via the least squares means estimated by the model.

Analysis of Effectiveness. The mean blood pressure changes at endpoint (mmHg) for patients valid for efficacy analysis are given in the following table :

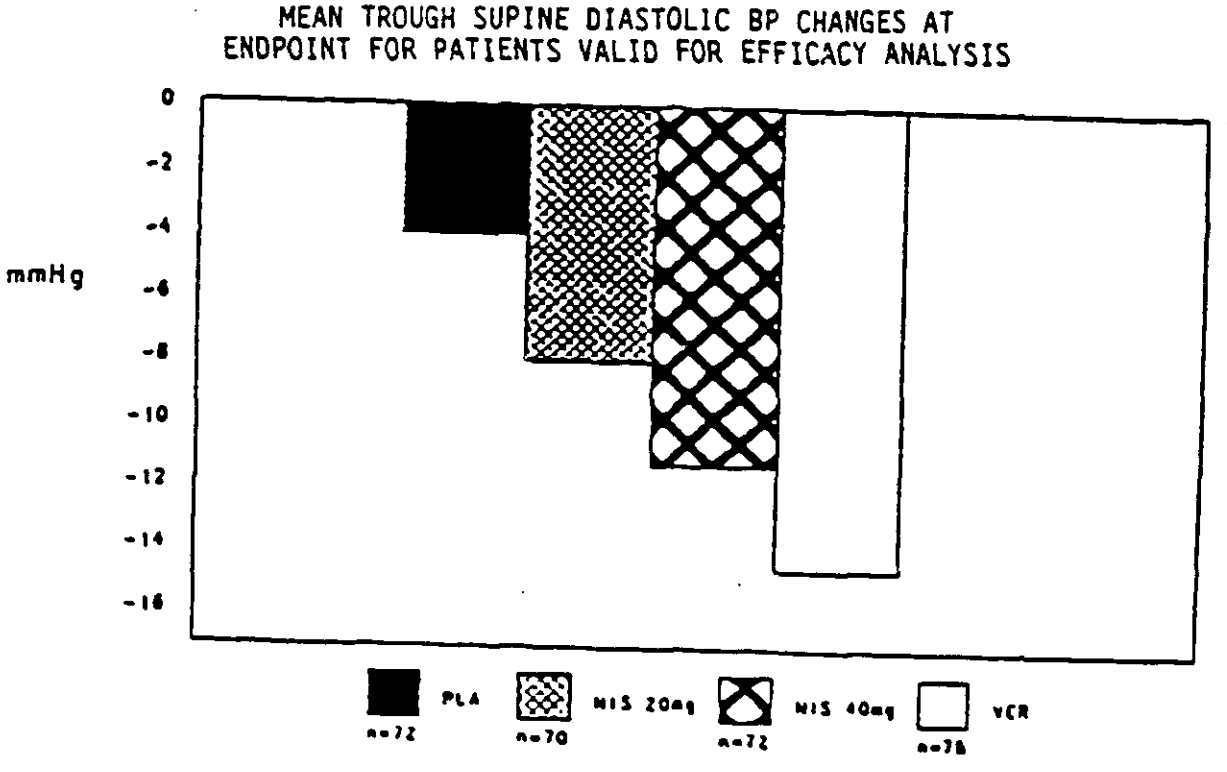
	Nisoldipine 20 mg N=70	Nisoldipine 40 mg N=72	Verapamil N=76	Placebo N=72
Supine				
Diastolic	-8.1 ABP	-11.4 BP	-14.7 P	-4.0
Systolic	-9.6 ABP	-16.2 P	-16.0 P	-2.2
Standing				
Diastolic	-7.1 ABP	-11.8 BP	-13.9 P	-2.0
Systolic	-11.6 BP	-15.4 P	-16.4 P	-2.4

A Significantly different from Nisoldipine 40 mg
B Significantly different from Verapamil
P Significantly different from Placebo

Mean changes (mmHg) in SUDBP at endpoint for patients with mild (baseline SUDBP 95-104 mmHg) and moderate (Baseline SUDBP 105-114 mmHg) are shown in the following table :

	Nisoldipine 20 mg		Nisoldipine 40 mg		Verapamil		Placebo	
	n	Change	n	Change	n	Change	n	Change
Mild	59	-8.4	62	-11.3	63	-14.1	64	-4.1
Moderate	11	-6.5	10	-13.0	13	-18.0	8	-3.5

The effect on SUDBP at endpoint in the Nisoldipine (24 hours after dose), Verapamil group (12 hours after dosa) and Placebo group is shown in the figure below :



The results by visit for SUDBP are shown in the following graph :

	Week 1	Week 2	Week 3	Week 4	Week 6	Week 8
NIS 20 mg						
n	69	70	66	65	68	68
Mean Change	-7.8	-7.2	-8.6	-7.5	-7.9	-8.0
NIS 40 mg						
n	72	72	66	65	65	63
Mean Change	-7.4	-9.8	-9.9	-11.2	-11.0	-11.8
Ver						
n	76	75	73	69	73	71
Mean Change	-5.9	-11.0	-12.8	-13.1	-12.6	-14.6
Placebo						
n	72	72	68	67	69	67
Mean Change	-4.0	-4.7	-5.1	-4.4	-5.7	-4.1

During the secon phase of the double-blind period, the differences between the active drugs decreased, while the Placebo group experienced the expected further decrease in blood pressure after switching to Verapamil. The changes from baseline in trough SUDBP at the two visits in thus phase are presented below :

	Week 10	Week 12
NIS 20 mg	-8.8	-10.1
NIS 40 mg	-12.2	-10.3
Verapamil	-13.1	-11.5
Placebo	-7.3	-7.0

Various demographic variables were examined including sex, weight, age, smoking status, race and baseline blood pressure. Of these only age exhibited a marked difference in blood pressure response. Mean changes from baseline in supine blood pressures for each drug group for patients at least 60 years old vs patients younger than 60 years old are provided in the table below :

	NIS 20 mg		NIS 40 mg		Verapamil		Placebo	
	n	Mean	n	Mean	n	Mean	n	Mean
Diastolic								
Age ≥ 60	28	-10.4	26	-13.6	24	-16.2	23	-4.0
Age < 60	42	- 6.6	46	-10.4	52	-14.1	49	-4.0
Systolic								
Age ≥ 60	28	-14.0	26	-21.2	24	-19.3	23	-2.3
Age < 60	42	-6.8	46	-13.6	52	-14.5	49	-2.2

Responders rate based on trough SUDBP are presented in the following table :

	NIS 20 mg N=70	NIS 40 mg N=69	Verapamil N=76	Placebo N=72
DBP ≤ 90 mmHg	35 (50 %)	50 (69%)	62 (82 %)	19 (26 %)
DBP decrease ≥ 10 mmHg	28 (40 %)	47 (65 %)	59 (78 %)	10 (14 %)

In clinic monitoring was done for 12 hours and 24-hour ambulatory blood pressure monitoring for 24 hours.

The in clinic monitoring, that covered only half of the dosing interval yielded the following results :

Dose	Mean Change Range mmHg/	Hour
Nisoldipine 20 mg	-9.8	12
	-13.5	8
Nisoldipine 40 mg	-10.8	12
	-15.5	4
Verapamil	-11.8	
	-15.1	
Placebo	-2.5	
	-5.5	

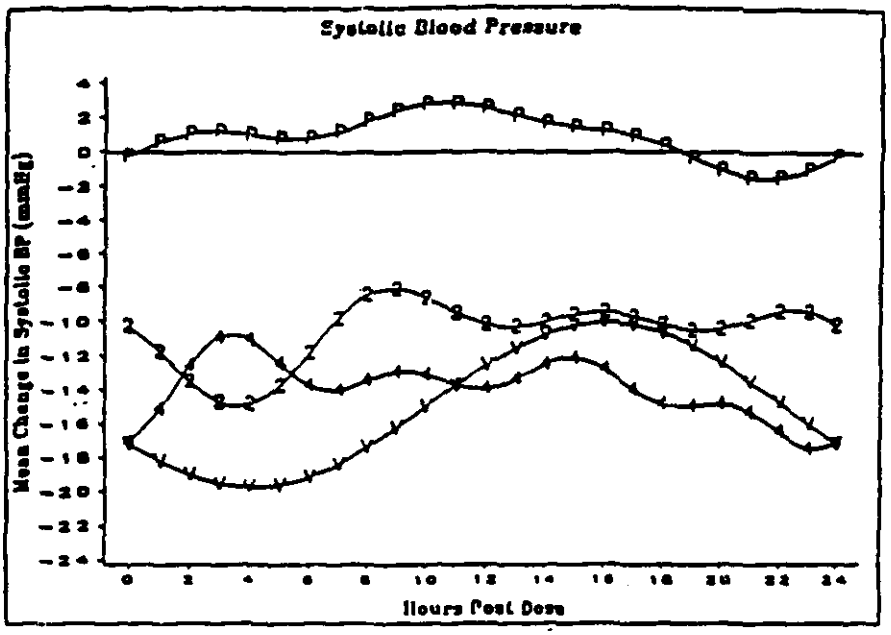
On ambulatory blood pressure monitoring response after 7 weeks of therapy was observed for 24 hours after Nisoldipine 40 mg therapy, 4 hours after Nisoldipine 20 mg therapy, and 4 hours after the morning dose of Verapamil. with blood pressure changes (systolic/diastolic)of -17.5/ -10.7 mmHg, -15.1/10.2 mmHg, and -19.7/-14.3 mmHg respectively. The mean 24-hour systolic and diastolic blood pressure changes during ambulatory blood pressure monitoring were :

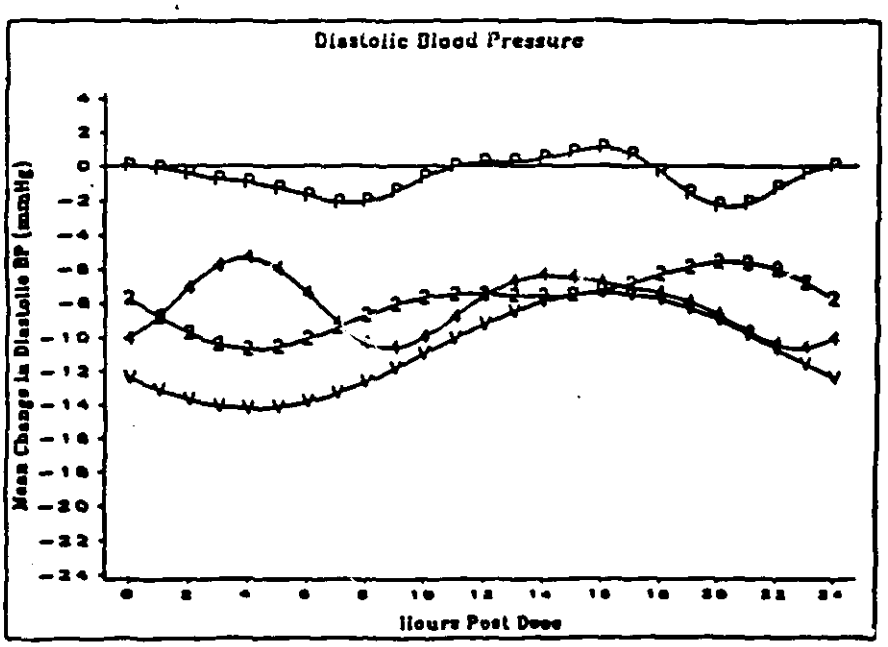
Nisoldipine 40 mg	-13.6/-8.0
Nisoldipine 20 mg	-11.1/-7.9
Verapamil	-14.8/10.8

Based on smoothed ambulatory blood pressure data, the trough/peal ratios for the treatment groups are summarized in the following table :

	Trough mmHg	Peak mmHg	Trough to peak ratio
Diastolic BP			
NIS 20 mg	-6.7	-9.7	69 %
NIS 40 mg	-11.7	-11.7	100 %
Verapamil	-11.1	-12.9	86 %
Systolic BP			
NIS 20 mg	-9.9	-15	66 %
NIS 40 mg	-14.3	-14.3	100 %
Verapamil	-15.9	-20.9	78 %

The unsmoothed change from baseline ambulatory data in systolic and diastolic blood pressure are shown below

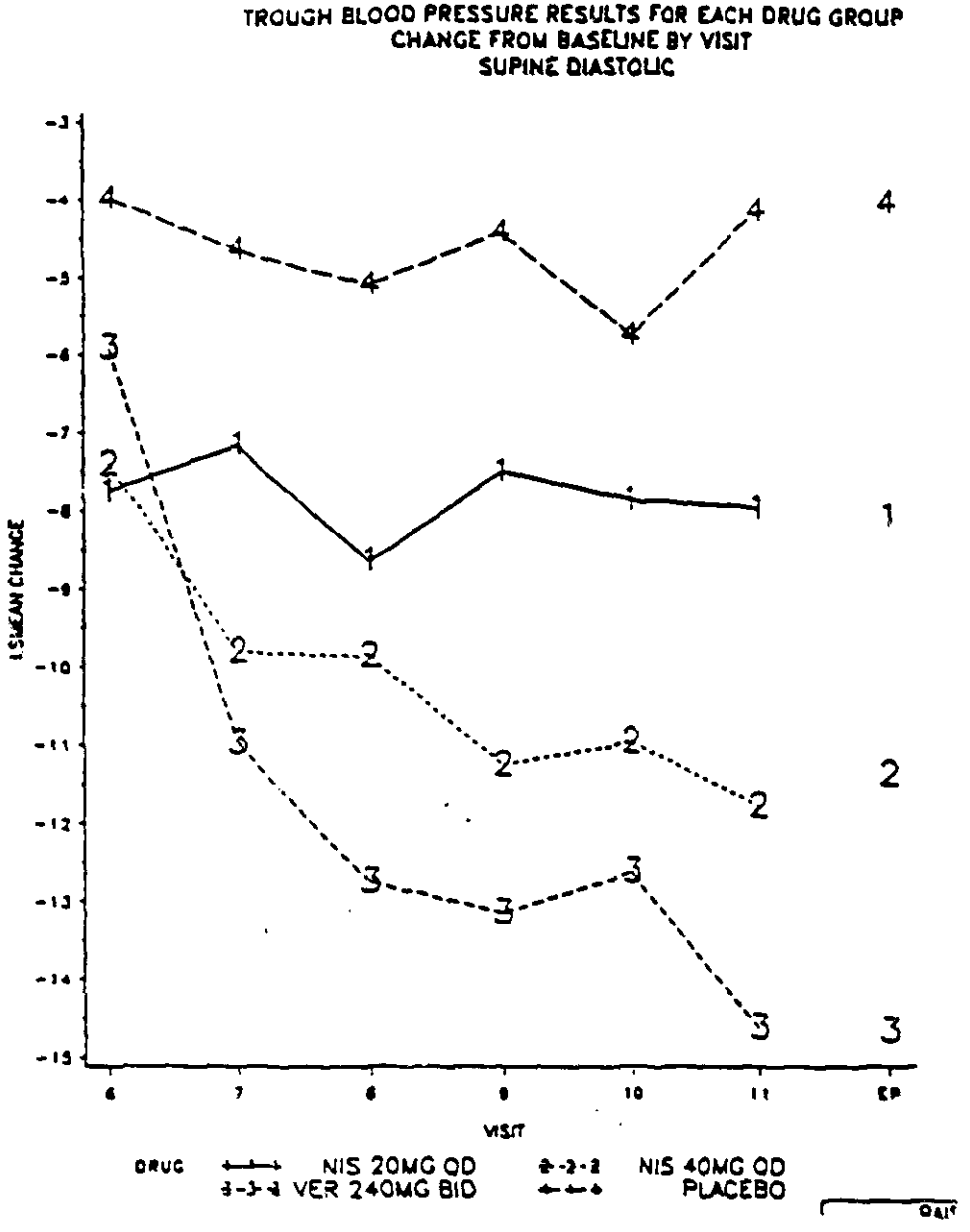




Legend

2-2-2 Nis 20mg 4-4-4 Nis 40mg V-V-V Verapamil P-P-P Placebo

The trough blood pressure results for each drug group change from baseline by visist supine diastolic is given in the following graph :



Pharmacokinetic Results. Trough blood samples were drawn at visits 5 and 11. Visit 11 samples were analyzed for Nisoldipine and results are summarized below :

	n	Range of Concentrations (ng/ml)	Mean Concentrations (ng/ml)
NIS 20 mg	66	0-3.19	1.0
NIS 40 mg	61	0-6.83	2.2
NIS 80 mg	3	0-5.24	2.3

Assessment. The study was initially designed to determine the effectiveness of Nisoldipine at doses of 20, 40, 80 mg, Verapamil and placebo. The 80 mg dose of Nisoldipine was dropped when in another study of a high-dose forced-titration study of Nisoldipine 120 mg daily showed asymptomatic T waves flattening and/or inversion on electrocardiogram predominantly at doses above 60 mg daily.

The 20 and 40 concentrations of Nisoldipine demonstrated to be more effective in lowering the blood pressure than placebo, and the 40 mg more effective than the 20 mg. Also the effectiveness was greater in subjects older than 60 years especially in lowering the systolic blood pressure. Verapamil bid was more effective in lowering blood pressure than any of the concentrations of Nisoldipine.

Peak and trough values were determined by ambulatory blood pressure monitoring and the antihypertensive effect was well sustained at 24 hours after dose administration in all concentrations of Nisoldipine evaluated in this study.

By pharmacokinetic studies the concentration of Nisoldipine in blood was determined and was found to be more elevated after the 40 mg administrations of Nisoldipine than after the 20 mg concentration. There was no major difference between the 40 mg and 80 mg dose of Nisoldipine.

Protocol D90-006

Title of Study : " South-African Multicentre Study to Investigate the Anti-Hypertensive Effect of Three Single Oral Daily Doses of Nisoldipine Administered as a Long Acting "Coat-Core" Tablet Formulation."

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Objectives. The objectives of this study were :

1. To compare the anti-hypertensive efficacy and safety of three daily doses of Nisoldipine coat-core formulation, namely 10 mg, 20 mg and 30 mg with placebo.

2. To study a dose-response relationship for Nisoldipine coat-core.

3. To assess the consistency of anti-hypertensive response over 6 weeks.

Additional objectives were :

1. To describe the blood pressure profile of the last day of therapy by continuous automated ambulatory blood pressure monitoring in a group of patients, and hence :

2. To quantify the trough/peak blood pressure relationship for this therapy.

Inclusion Criteria. Patients with newly diagnosed mild to moderate hypertension were eligible to enter the study. In addition patients with mild to moderate hypertension being treated who, in the opinion of the investigator, were no significantly placed at risk by withdrawal of previous anti-hypertensive medication during the 4-week placebo run-in period could also be enrolled in the study.

Exclusion Criteria. Patients were not eligible if they had labile hypertension, clinical evidence of major arrhythmias, angina pectoris, conductio disturbances or heart failure, or recent or impending myocardial infarction, or a cerebral vascular accident in the previous 3 months, history of allergy to dihydropyridines, type 1 diabetes mellitus, impaired renal function, liver disease, elevated transaminases, treatment with antihypertensives or any other drug that may affect the blood pressure or may interact with the effects of calcium antagonists.

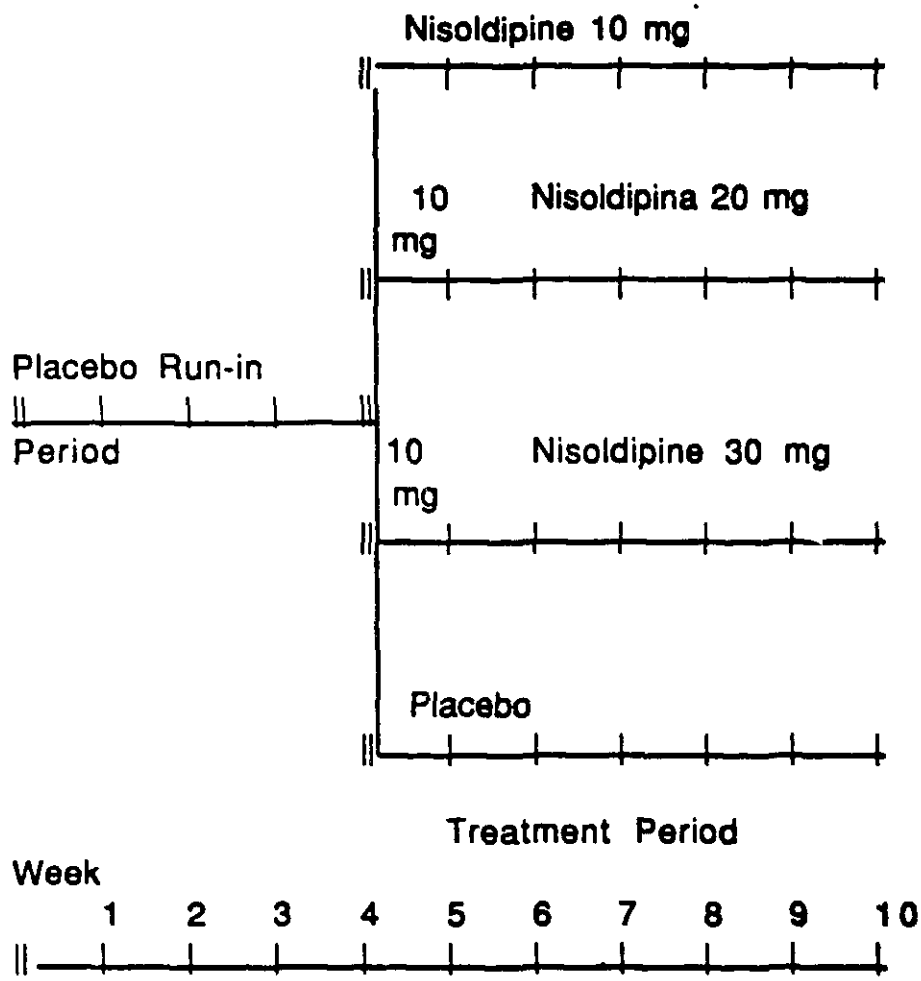
Study Design. This was a 10 week, multi-centre, randomized, placebo controlled, parallel group comparison of Nisoldipine coat-core 10 mg, 20 mg, 30 mg versus placebo. The study consisted of two periods : a single-blind placebo run-in period and a double-blind, randomized, placebo-controlled, group comparison (treatment period.

Placebo run-in Period. During this period of 4 weeks duration all antihypertensive medication was discontinued and one placebo tablet was given to be taken in the morning before breakfast. Patients whose SUDBP was ≥ 95 mmHg and ≤ 114 mmHg at visits 2 and 3 were eligible for enrollment in the active treatment phase.

Treatment Period. Eligible patients were randomized to one of four arms : placebo, 10 mg Nisoldipine, 20 mg Nisoldipine and 30 mg Nisoldipine.

Patients randomized to placebo or 10 mg Nisoldpine were to receive their treatment for 6 weeks. Patients in the two higher dose groups (Nisoldipine 20 mg or Nisoldipine 30 mg) were to receive 10 mg for the first week following by 5 weeks of their randomized treatment in order to avoid rapid exposure to the higher doses.

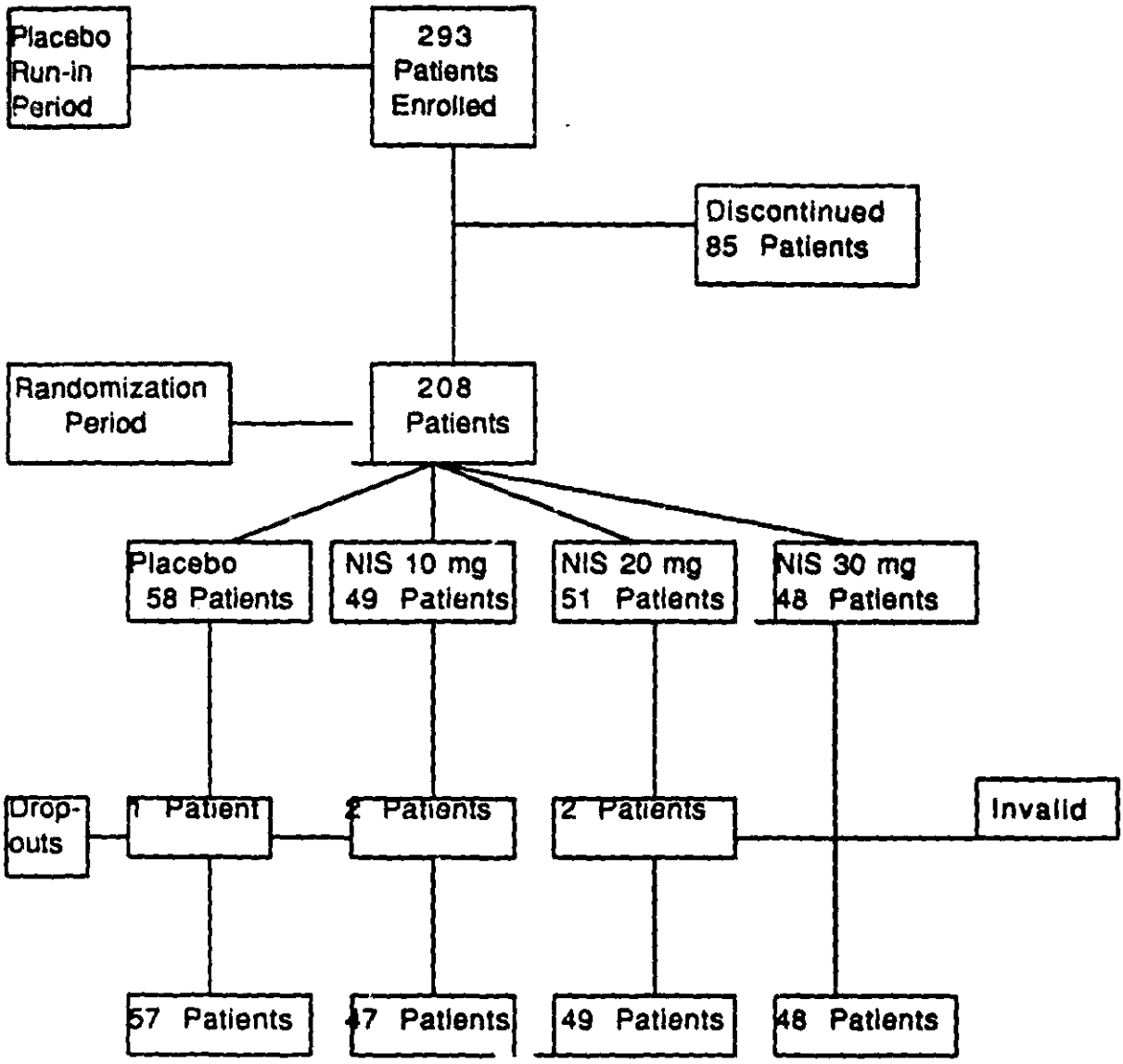
The study design is demonstrated schematically in the following graph :



The demographic information is given in the following table :

		Placebo n=58	NIS 10 mg n=49	NIS 20 mg n=51	NIS 30 mg n=48
Sex (p=0.78)	Male Female	27 (47 %) 31 (53 %)	24 (49 %) 25 (51 %)	20 (39 %) 31 (61 %0	21 (44 %) 27 (56 %)
Race (p=0.98)	Caucasian Black Asian Other	30 (52 %) 27 (29 %) 6 (20 %) 5 (9 %)	24 (53 %) 16 (33 %) 2 (4 %) 5 (10 %)	25 (49 %) 18 (35 %) 6 (12 %0 2 (4 %)	26 (54 %) 14 (29 %) 5 (11 %) 3 (6 %)
Age (years) Mean	Mean Mean=0.2	53	50	55	50
Weight (kg)	Mean (p=0.69)	80.8	77.3	79.7	80.3
Baseline Means BP Supine Systolic Diastolic Standing Systolic Diastolic	 (p=0.17) (p=0.65) (p=0.69) (p=0.09	 163.8 103.5 160.5 105.1	 161.2 104.7 159.7 107.9	 167.2 104.8 163.8 107.4	 164.3 104.4 161.7 107.4
Mild Hypert. n Baseline SDBP Moder. Hypert. N Baseline SDBP		 33 99.7 25 108.5	 25 99.3 24 110.2	 25 99.2 26 110.1	 27 100.1 21 110.1

The distribution and randomization of patients is illustrated in the following graph :



The reasons for patients who did not enter the double-blind treatment period is given in the following table :

Reason	Number of Patients
Supine diastolic blood pressure < 95 mmHg	58
Supine diastolic blood pressure > 114 mmHg	13
Unwilling to continue	5
Patient had raised serum calcium levels	1
Uncontrolled non-insulin dependent diabetes mellitus	1
Raised liver enzymes	3
Left ventricular failure	1
Right ventricular failure when taken off diuretic	1
Major arrhythmias	2
Total	85

Invalid Results and Drop-outs During the Treatment Period. Three patients dropped-out during the treatment period. One patient in the placebo group died after experiencing cerebral hemorrhage 33 days after entering the double-blind treatment period. One patients in the Nisoldipine 10 mg experienced severe tinnitus 30 days after entering the double-blind treatment period. Another patient in the 10 mg Nisoldipine group had a severe headache and dropped 17 days after entering the double-blind treatment period.

Efficacy.

Criteria for Efficacy. The primary variable for assessing efficacy was the trough 24-hour supine diastolic blood pressure (SUDBP), and especifically the change in suDBP from baseline to endpoint (visit 6, week 6 or the last valid visit). The change from baseline in each of the three Nisoldipine treatment groups was compared to the Placebo group. Secondary efficacy variables were supine systolic BP and standing diastolic and systolic blood pressure.

Statistical Analysis. Two types of analysis were followed. The first and primary analysis was the standard endpoint analysis, also referred as the main efficacy analysis. The second was the intent-to treat analysis (ITT).

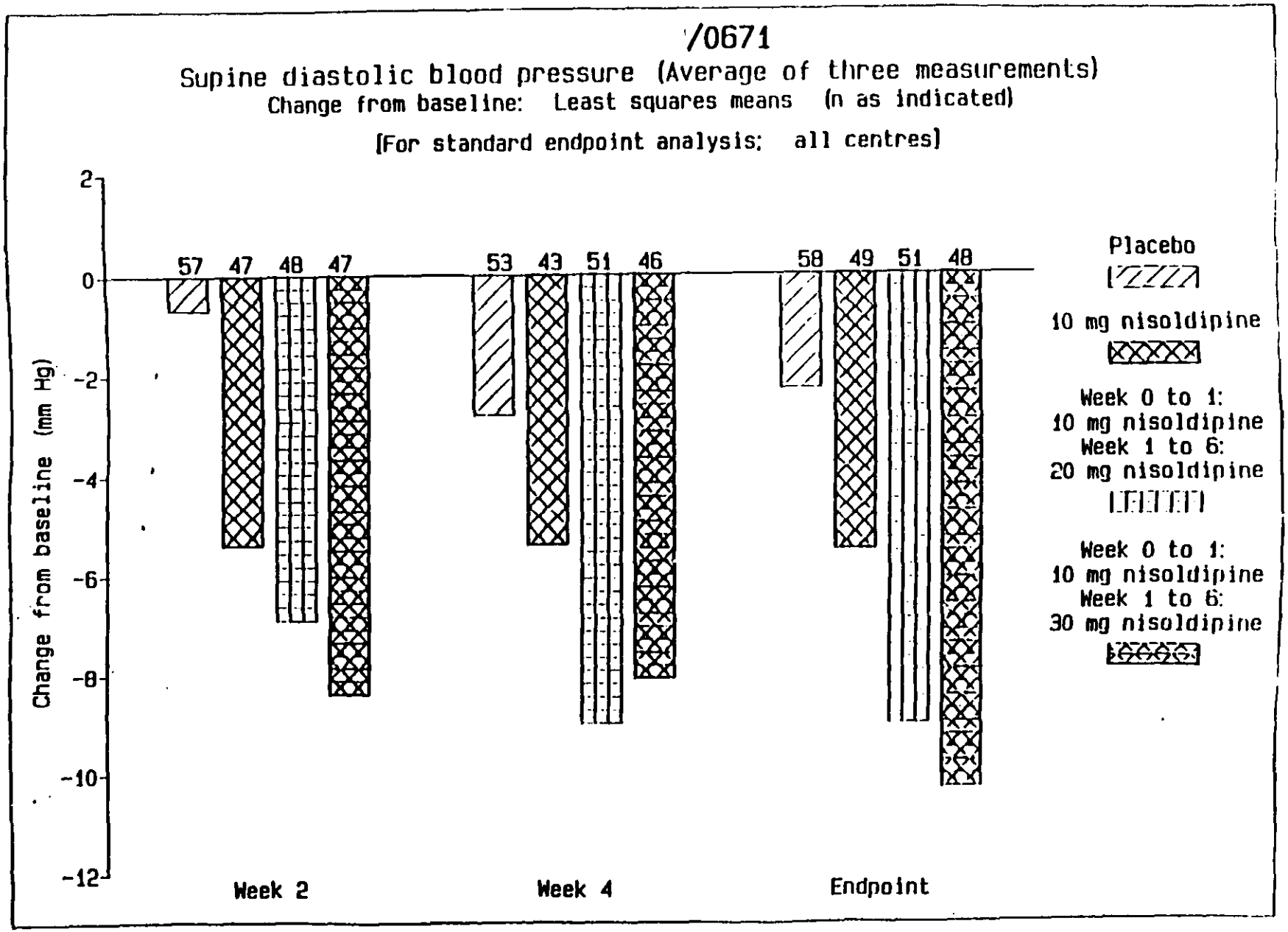
All patients adherent to the protocol with a valid treatment duration of at least 2 weeks on double-blind treatment were included in the main efficacy analysis. These patients completed at least a two-week double-blind treatment period during which they were compliant. and after which the blood pressure was taken between 22.5 h and 25.5 h after the last tablet intake. Patients who discontinued treatment because of lack of efficacy or adverse events were also included. Only 2 patients who received double-blind treatment were considered invalid for the main efficacy

analysis. They were included in the intent to treat analysis. In one patient the baseline measurements were lost
and in another patient only 10 tablets instead of 20 tablets were dispensed.

The results of change from baseline at endpoint in trough blood pressure in all patients valid for the main efficacy analysis (n=206) are given in the following table :

	Placebo n=58	NIS 10 mg n=49	NIS 20 mg n=51	NIS 30 mg n=48
Supine DBP				
Baseline	103.5	104.7	104.8	104.4
Endpoint	101.1	99.3	95.7	94.3
Difference (NIS-Placebo)		-3.2	-6.7	-8.0
Supine SBP				
Baseline	163.8	167.2	167.2	164.3
Endpoint	163.3	149.8	149.8	148.8
Difference (NIS-Placebo)		-8.9	-17.8	-15.9
Standing DBP				
Baseline	105.1	107.9	107.4	107.4
Endpoint	104.6	101.1	98.1	96.9
Difference (NIS-Placebo)		-6.9	-9.2	-10.6
Standing SBP				
Baseline	160.5	159.7	163.8	161.7
Endpoint	160.5	150.6	147.5	145.7
Difference (NIS-Placebo)		-9.5	-15.9	-16.2

The results on SDBP are demonstrated in the following graph :


/0671
Supine diastolic blood pressure (Average of three measurements)
Change from baseline: Least squares means (n as indicated)
[For standard endpoint analysis; all centres]
Change from baseline (mm Hg)
2
0
-2
-4
-6
-8
-10
-12
57 47 48 47
53 43 51 46
58 49 51 48
Week 2
Week 4
Endpoint
Placebo
10 mg nisoldipine
Week 0 to 1:
10 mg nisoldipine
Week 1 to 6:
20 mg nisoldipine
Week 0 to 1:
10 mg nisoldipine
Week 1 to 6:
30 mg nisoldipine

The mean change from baseline in supine diastolic pressure (mmHg) for each treatment group after stratification for age is shown in the following table : (Main efficacy analysis = 206).

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg	Overall Least Square Means
Age < 45 years	-6.5 (12)*	-4.1 (14)	-10.6 (11)	-8.9 (15)	-7.5
Age≥ 45 and < 65 years	-1.4 (38)	-6.9 (28)	-8.3 (31)	-10.7 (27)	-6.8
Age ≥ 65 years	-1.3 (8)	-2.0 (7)	-10.1 (9)	-10.7 (6)	-6.0

* The number of patients used for calculating the mean values are given in brackets.

Results from ANOVA

Age effect : p=0.62
Treatment Effect : p=0.0001
Treatment by age interaction effect : p=0.12.

These results indicate that there is no association between age and the diastolic blood pressure response.

The mean change from baseline in supine diastolic blood pressure (mmHg) for each treatment group after statification for race is given in the following table : (Main efficacy analysis = 206).

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg	Overall Least Square Means
Caucasian	-1.2 (30)*	-4.6 (26)	-7.5 (25)	-9.2 (26)	-5.6
Black	-2.3 (17)	-5.9 (16)	-10.3 (18)	-10.1 (14)	-7.2
Other	-6.3 (11)	-7.0 (7)	-11.5 (8)	-13.1 (8)	-9.5

Analysis of Response and Normalization Rates. Responders were defined as patients who had SDBP of less than or equal to 90 mmHg or patients who had a drop in SDBP of at least 10 mmHg at endpoint. A patient's blood pressure was said to be normalized when satisfied these two conditions, namely, a drop in supine DBP to 90 mmHg or below, and a drop of at least 10 mmHg.

The following table shows the response rates for each treatment group, odds ratio and relative efficacy of each Nisoldipine treatment relative to Placebo from the main efficacy analysis of the last visit:

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg
Total number of Patients	58	49	51	48
Responders	10	17	24	30
Response Rate	17 %	35 %	47 %	63 %
Odds Ratio (OR) NIS relative to Placebo		2.4	4.6	8.8
95 % CI for OR		1.0 ; 5..5	1.8 ; 12	3.6 ; 22

Relative Efficacy (RE) NIS relative to Placebo 95 % CI for RE		2.0 1.0 ; 3.9	2.6 1.5 ; 4.8	3.7 2.1 ; 6.2

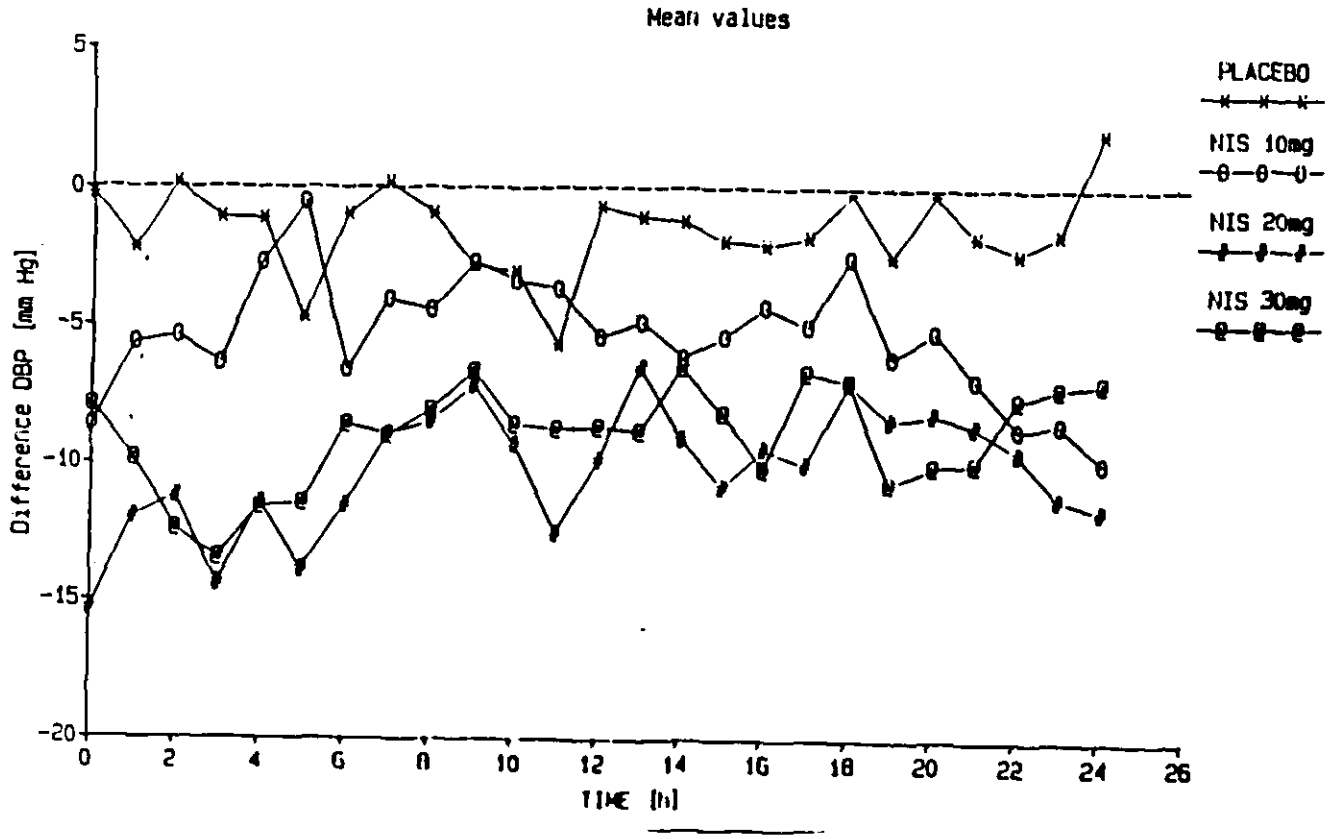
These results can be interpreted as indicating that the response rate for placebo was 17 %, Nisoldipine 10 mg 35 %, Nisoldipine 20 mg 47 % and Nisoldipine 30 mg 63 %. A relative efficacy of 2.6 of Nisoldipine 20 mg vs Placebo means that a positive treatment response is 2.6 times more likely to occur under Nisoldipine 20 mg than placebo. The confidence interval of 1.5 to 4.8 indicates that the true relative efficacy is likely (95% confidence limits) to be at least 1.5 and at most 4.8.

The following table shows the normalization rates for each treatment group, odds ratio, and relative efficacy of each Nisoldipine treatment relative to Placebo from the main efficacy analysis of the last visit :

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg
Total Number of patients	58	49	51	48
Number of Patients Normalization	5	5	13	13
Rate	8.6 %	10 %	25 %	27 %
Odds Ratio (OR) NIS relative to Placebo 95% CI for OR		1.2 0.31 ; 4.8	4.3 1.4 ; 1.3	4.3 1.4 ; 13
Relative Efficacy (RE) NIS relative to Placebo 95 % CI for RE		1.2 0.37 ; 3.9	3.1 1.3 ; 7.3	3.3 1.3 ; 8.1

These results can be interpreted in the same manner as described for the response rates.

Analysis of Ambulatory Blood Pressure Monitoring. Of the 165 patients who entered the ambulatory blood pressure monitoring phase of the study 137 patients were evaluable. The means across patients (change from baseline in diastolic blood pressure) are graphically presented in the following figure



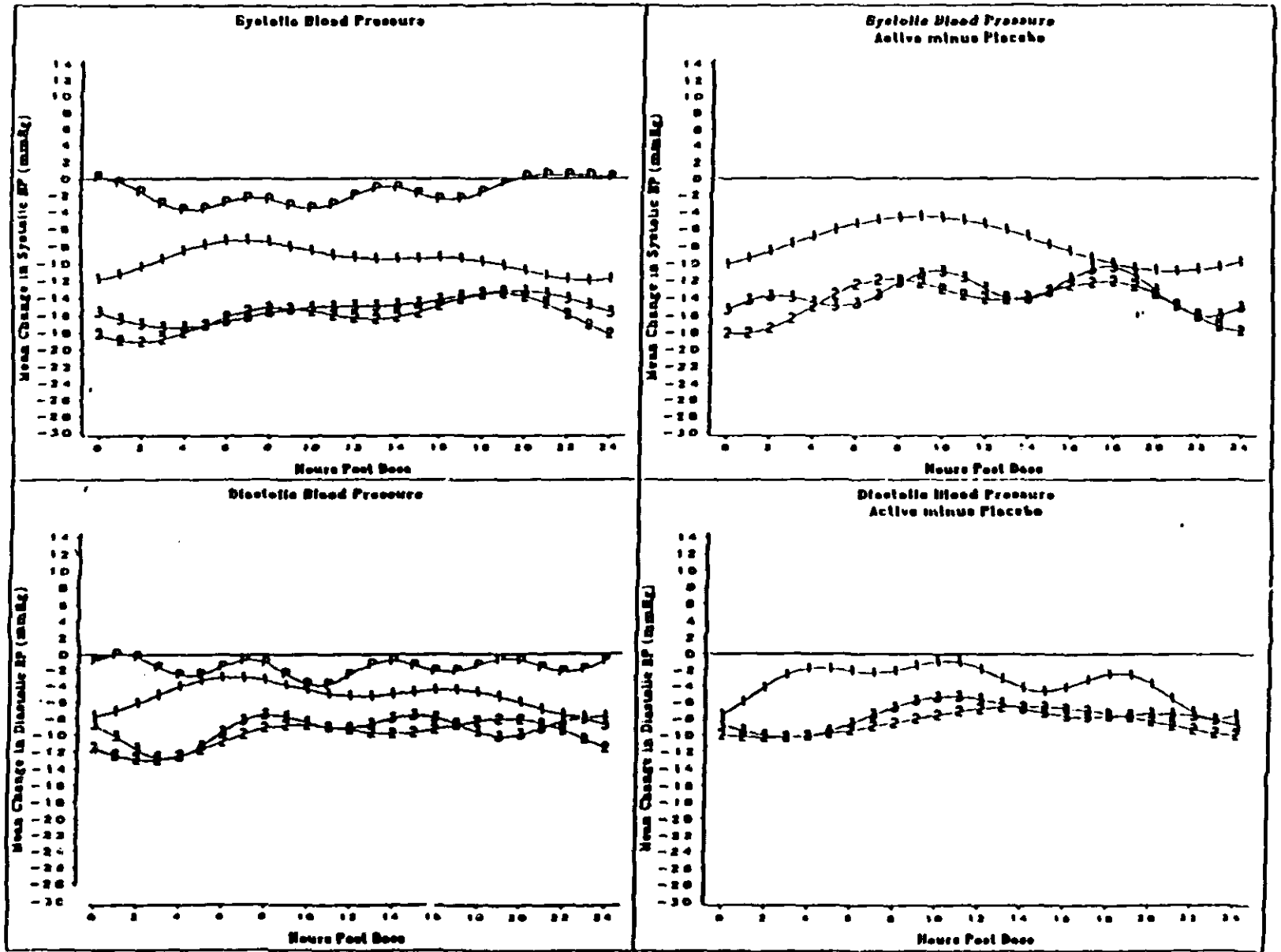
Various clinically meaningful variables could be calculated from the hourly mean diastolic blood pressure profiles. The following table shows results of trough/peak ratios calculated from hourly means of ambulatory monitoring data :

	Trough (mmHg)	Peak (mmHg)	Hour of Peak	Trough to peak Ratio
Diastolic BP				
NIS 10 mg.	-11.95	-11.95	24	100 %
NIS 20 mg	-13.70	-13.70	24	100 %
NIS 30 mg	-9.03	-12.57	2	72 %
Systolic BP *				
NIS 10 mg	-15.70	-15.70	24	100 %
NIS 20 mg	-20.72	-20.72	24	100 %
NIS 30 mg	-18.31	-18.31	24	100%
Systolic BP#				
NIS 10 mg	-15.70	-15.70	24	100 %
NIS 20 mg	-20.72	-20.72	24	100%
NIS 30 mg	-18.31	-10.64	2	172%

* Using timepoint of systolic peak.
# Using timepoint of diastolic peak

The results indicate that there was a good dose-response pattern in both systolic and diastolic blood pressure falls from baseline for placebo Nisoldipine 10 and 20 mg while the fall of Nisoldipine 30 mg was very similar to that in the 20 mg group. The effect of the 3 Nisoldipine group was maintained over the entire dosing period. This is also in evidence by observing the following graph of hourly means in a smoothed curve :

Smooth of Mean ange from Baseline of Ambulatory Blood P sure



Assessment. This study demonstrated that Nisoldipine, at concentrations of 10 mg, 20 mg and 30 mg, was more effective than placebo in lowering the blood pressure, but this effect was not potentiated when the dose was increased from 20 to 30 mg. Ambulatory blood pressure measurements were done which demonstrated that the effectiveness of Nisoldipine extended throughout the 24 hours after administration , trough values frequently being equal to peak values.

It is interesting that in this study this calcium channel blocker demonstrated to have a greater effectiveness in blacks, a patients population usually more refractory to antihypertensive treatment, than in caucasians.

In reference to age, this study concluded that Nisoldipine was more effective in individuals 65 years of age or older. (table page 73). This finding is consistent with those of protocol D89-039 in which Nisoldipine was more effective in this age range especially in lowering systolic blood pressure. (table page 60).

Protocol D88-054

Title of Study : " Comparative Double-Blind Pilot Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine 10, 20, 30 mg Core-Coat Tablets vs Placebo in Hypertensive Patients ".

Principal Investigators and Sites of Investigation :

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Carrollton, TX

Objectives. The objectives of this study were :

1. To test whether Nisoldipine core-coat given 10 mg, 20 mg, 30 mg once daily lowers the blood pressure significantly more than placebo at the end of 24-hour dosing interval (trough).

2. To record blood pressure and pulse rates for four hours after the first dose of double-blind drug to monitor patient response to acute administration of the drug.

3. To determine peak response and calculate ratios of trough to peak effect by 24-hour ambulatory blood pressure monitoring.

Inclusion and Exclusion Criteria. Male or female patients, 21 to 70 years of age, with a history of mild to moderate essential hypertension and a mean supine diastolic blood pressure of 95 to 114 mmHg after three and four weeks of placebo were eligible for the study.

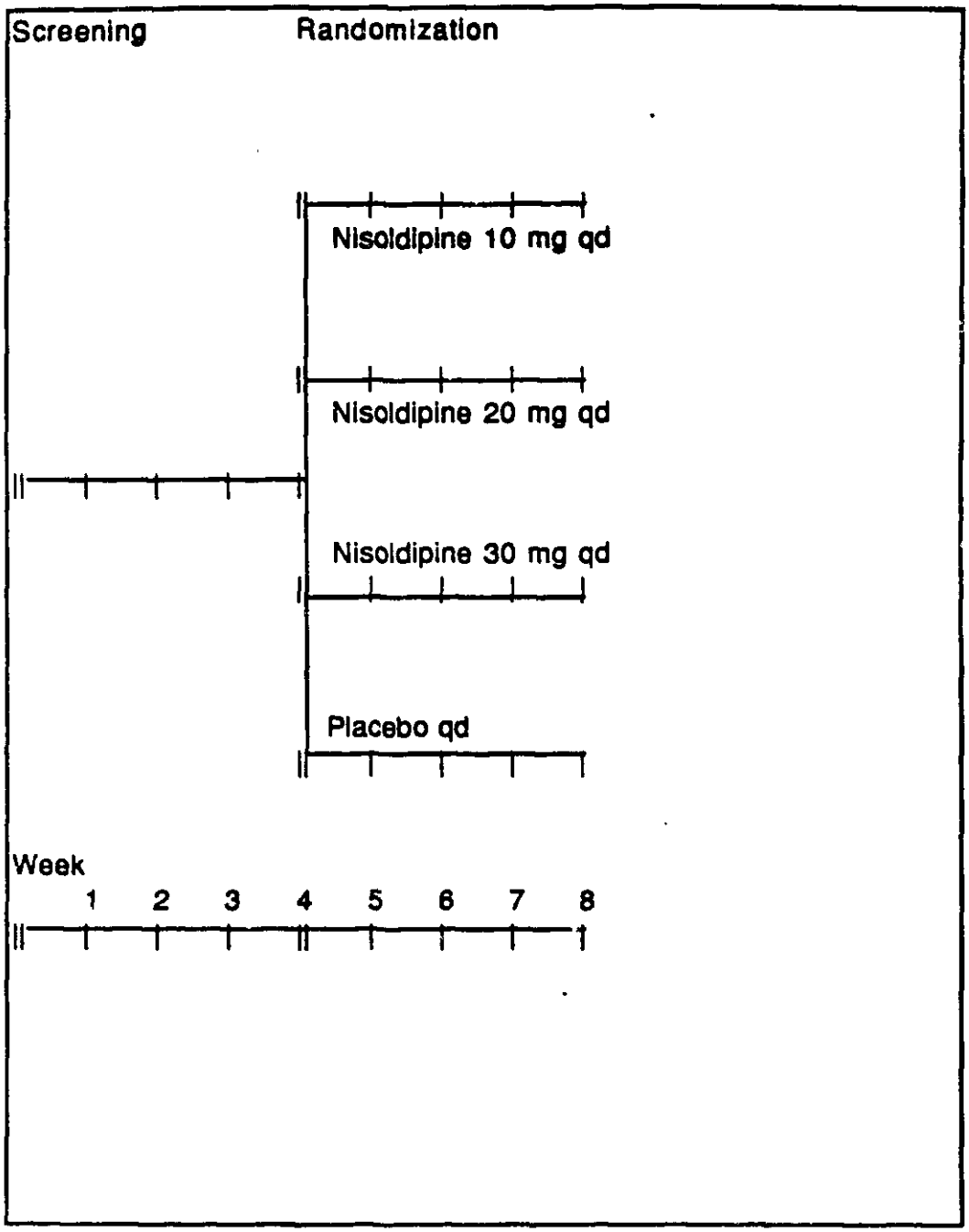
Excluded from the study were patients with labile hypertension, a change in supine diastolic blood pressure greater than 7 mmHg between the last 2 placebo run-in visits, impaired renal or liver function, recent or impending myocardial infarction, or cerebral vascular accident, angina pectoris or intermittent claudication, heart failure, major arrhythmias, conduction disturbance, failure of a major organ system, severe infection, malignancy, psychosis, chronic diarrhea, ulcerative colitis, regional enteritis, diverticulitis, partial or complete gastrectomy or small bowel resection, history of allergy to dihydropiridines, pregnant women or those with childbearing potential and patients known to abuse alcohol or drugs.

Study Design. This was a randomized, double-blind, parallel group, placebo controlled study of eight weeks duration consisting of a screening period and a randomization treatment period.

Screening Period. During this period of 4 weeks duration patients discontinued all previous antihypertensive medication and were given a single-blind placebo once daily. Those patients with a mean supine diastolic pressure ≥ 95 mmHg to ≤ 114 mmHg after three to four weeks of placebo and within 7 mmHg at both visits were transferred to the treatment period.

Randomization Period. Patients were randomized to receive either Nisoldipine 10 mg qd, Nisoldipine 20 mg qd, Nisoldipine 30 mg qd or Placebo qd for four weeks.

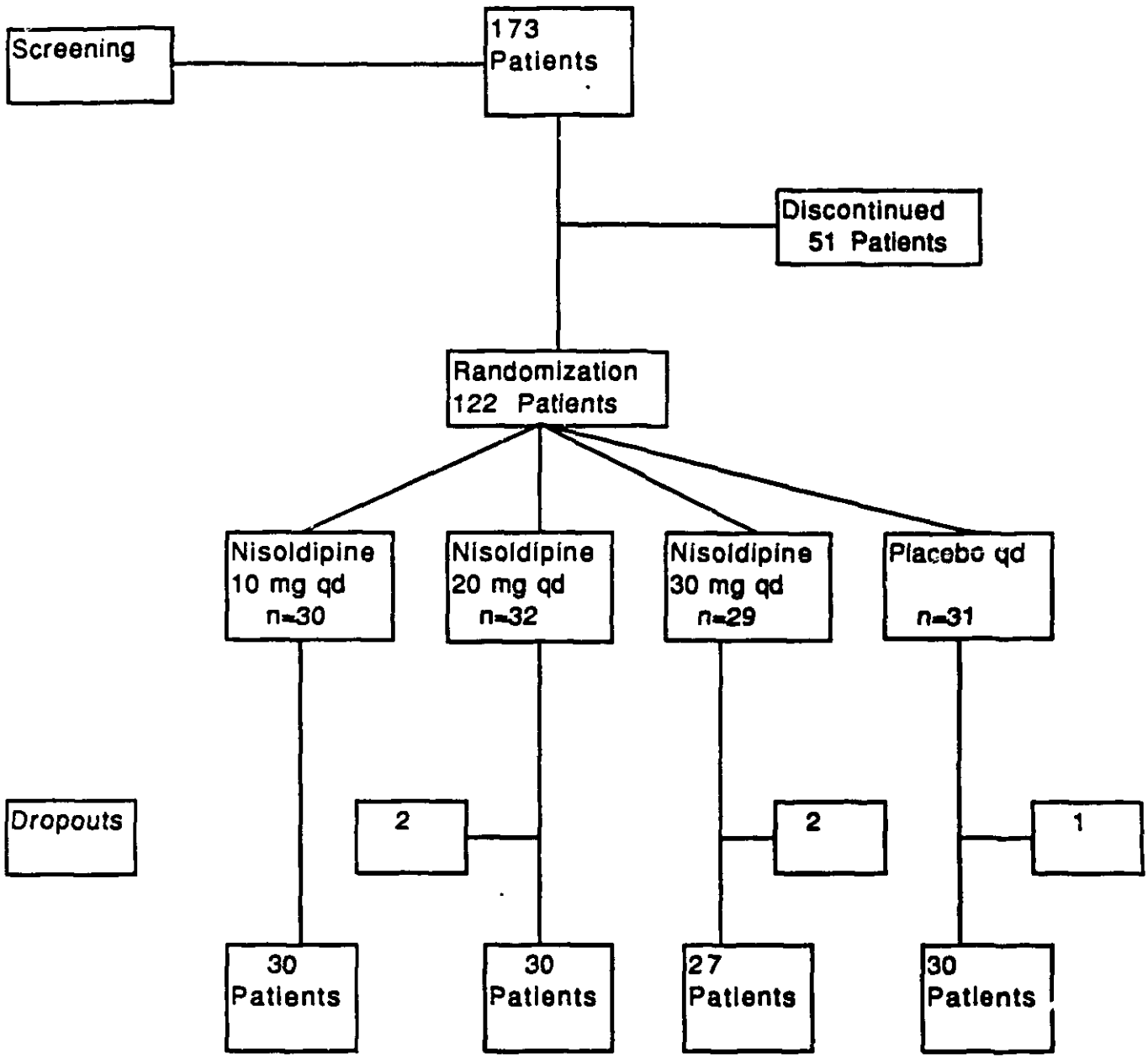
The study design is demonstrated schematically in the following graph :


Screening
Randomization
Nisoldipine 10 mg qd
Nisoldipine 20 mg qd
Nisoldipine 30 mg qd
Placebo qd
Week
1
2
3
4
5
6
7
8

Demography. The demography and baseline characterisitics are given in the following table :

		Nisoldipine 10 mg n=30	Nisoldipine 20 mg n=30	Nisoldipine 30 mg n=29	Placebo n=30
Sex	Male Female	20 (67 %) 10	19 (63 %) 11	19 (66 %) 10	21 (70 %) 9
Race	Caucasian Black Hispanic	23 (77 %) 4 3	23 (77 %) 7 0	20 (69 %) 9 0	23 (77 %) 7 0
Age (years)		56	53	52	51
Weight (lbs)		186	200	207	190
Baseline Blood Pressure mmHg	Supine Standing	146/99 144/100	147/99 144/100	145/99 144/100	148/100 145/101

The distribution of patients and randomization are given in the following graph :


Screening
173 Patients
Discontinued 51 Patients
Randomization 122 Patients
Nisoldipine 10 mg qd n=30
Nisoldipine 20 mg qd n=32
Nisoldipine 30 mg qd n=29
Placebo qd n=31
Dropouts
2
2
1
30 Patients
30 Patients
27 Patients
30 Patients

The reasons that disqualified enrolled patients for randomization are given in the following table :

Reasons for disqualification	Patients
Supine diastolic blood pressure < 95 mmHg	21
+ 7 mmHg difference in supine diastolic blood pressure (visits 4 and 5)	3
Supine diastolic blood pressure >114 mmHg	4
Unable to make scheduled visits	3
Illness not due to study medication	4
Lost to follow-up	2
Abnormal laboratory values	3
Non-compliance	2
Systolic blood pressure above acceptable limit	1
High blood pressure readings during ambulatory monitoring	1
Chest pain at visit 1	1
Chose to withdraw	1
Total	46

The reasons for dropping out during the double-blind randomization period are given in the following table :

Drug Group	Final visit	Days on Drug	Reasons for dropping-out- Severity Drug Relationship
Placebo	7.0	11	Dizziness-Moderate Probable
Nisoldipine 20 mg	6.0	5	Intolerance to all-night visits
	5.5	5	Shortness of breath-Cough Mild-Probable
Nisoldipine 30 mg	8.0	23?	Noncompliance
	6.0	7	Flushing-Severe-Probable

Efficacy

Criteria for Effectiveness. The change in trough supine diastolic blood pressure from baseline to endpoint compared among the groups was the primary efficacy criterion. Endpoint was defined as the last valid visit on the double-blind drug for each valid patient. Patients were to be included in this efficacy analysis if trough blood pressure data was available after at least 7 days on double-blind drug.

Statistical Methods. All tests of significance were performed as two-tailed tests with alpha + 0.05 unless stated otherwise.

Analysis of Efficacy. The mean blood pressures by visit for all patients valid for analysis of efficacy are given in the following table :

Week Post Randomization — Supine Blood Pressure

	Nisoldipine 10 mg Sys/Dia (n)	Nisoldipine 20 mg Sys/Dia (n)	Nisoldipine 30 mg Sys/Dia (n)	Placebo Sys/Dia (n)
Week-1	143/99 (30)	145/99 (30)	144/99 (29)	145/99 (30)
Week 0	145/99 (30)	146/98 (30)	144/98 (29)	147/100 (30)
Week 1	136/91 (30)	136/90 (30)	136/91 (29)	141/95 (30)
Week 2	140/91 (30)	139/92 (29)	133/90 (28)	140/94 (30)
Week 3	138/91 (30)	136/91 (30)	134/89 (28)	142/95 (29)

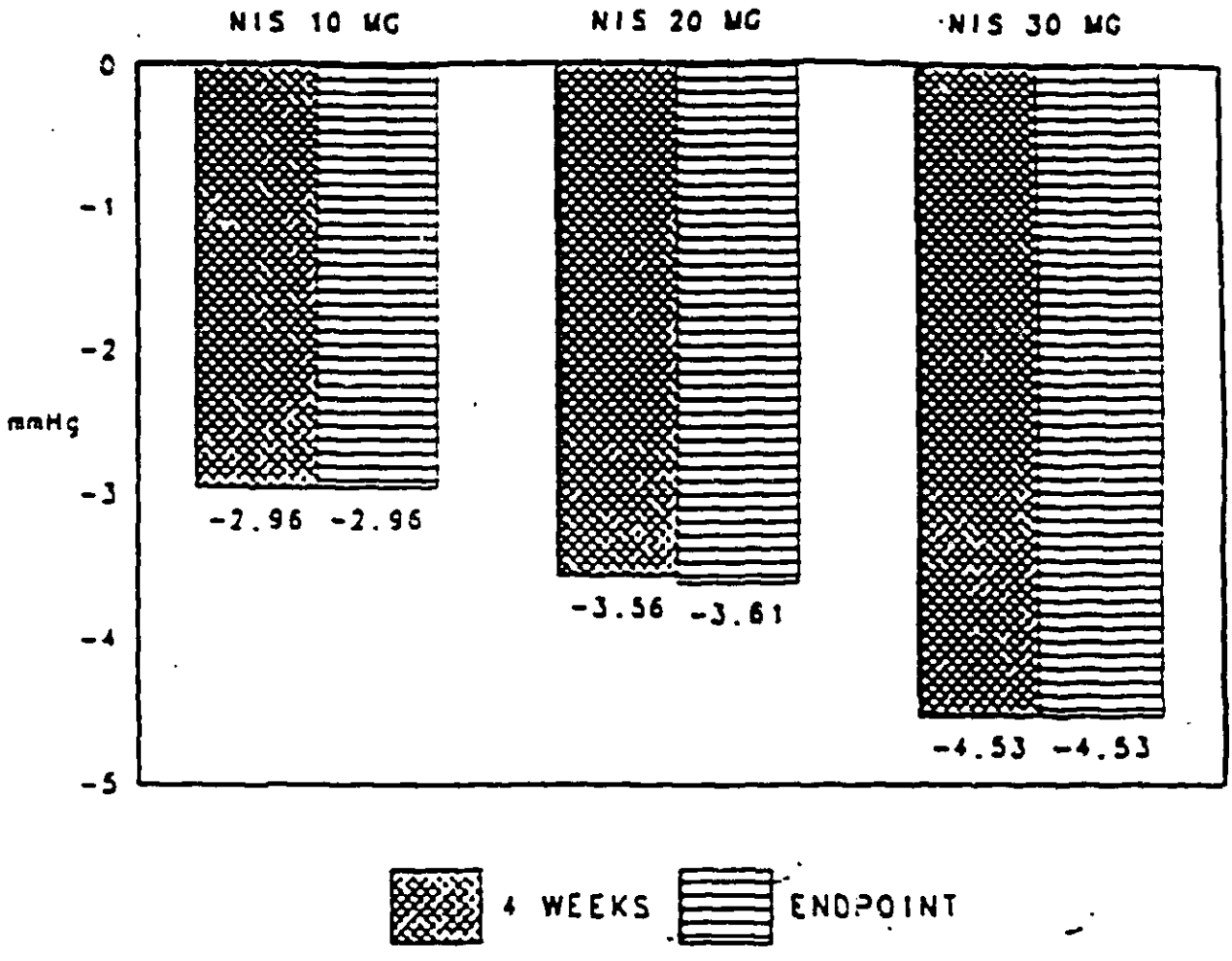
Week 4	137/90 (30)	134/90 (30)	133/89 (27)	144/94 (30)
Endpoint	137/90 (30)	134/90 (30)	133/89 (30)	144/94 (30)

In the following table, the results of the analysis at endpoint are summarized :

	Nisoldipine 10 mg *n*=30	Nisoldipine 20 mg *n*=30	Nisoldipine 30 mg *n*=29	Placebo n=30
Supine				
Systolic	8.4	11.5*	10.7*	3.0
Diastolic	8.3	8.9*	9.9*	5.3
Standing				
Systolic	8.3	11.8*	10.7*	3.4
Diastolic	6.2	7.3	7.0	5.1

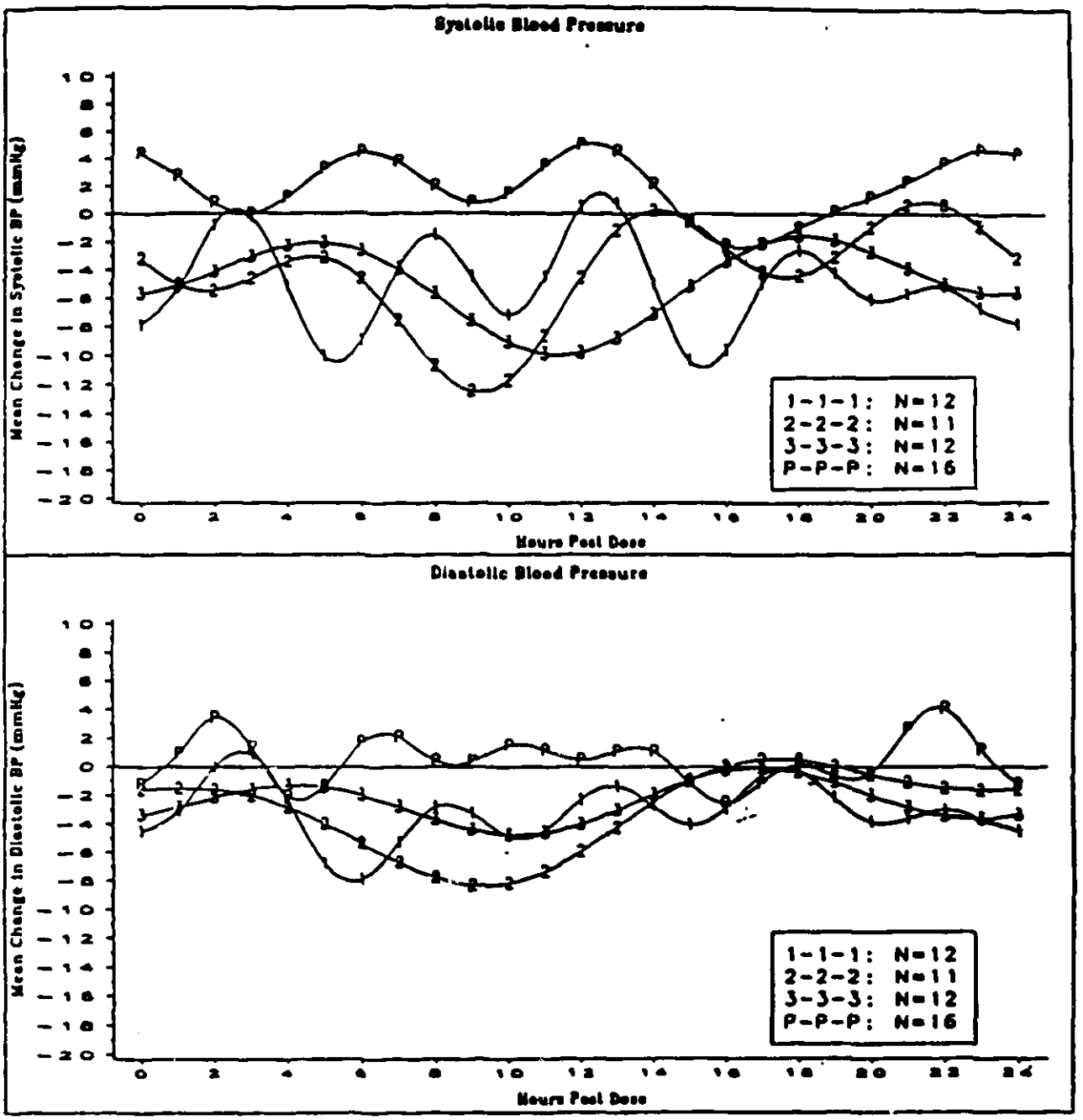
*Significant difference from the placebo group $p<0.05$

The change in trough supine diastolic blocd pressure at 4 weeks and endpoint, placebo subtracted, are shown in the following graph :



Ambulatory monitoring and supine in-clinic blood pressures were smoothed and results are demonstrated in the following graph :

SMOOTH OF MEAN CHANGE FROM BASELINE OF AMBULATORY BLOOD PRESSURE



Legend

1-1-1 NIS 10mg
2-2-2 NIS 20mg
3-3-3 NIS 30mg
P-P-P Placebo

The Trough/Peak ratios from smoothed ambulatory monitoring data for valid patients are given in the following table :

	Nisoldipine		
	10 mg (n=12)	20 mg (n=11)	30 mg (n=12)
Diastolic	7 %	35 %	68 %
Systolic	92 %	43 %	108 %
Peak hour Post-dose	6	9	8
Nisoldipine levels at trough ng/ml	0.82	1.04	1.49

Assessment. This is a small pilot studied carried in a relatively small number of subjects consisting mostly of middle-age caucasian male obese patients. Although the results on blood pressure with the 10 mg dose of Nisoldipine was not significantly different from placebo the 20 and 30 mg doses were but the effect of both did not seem to be very different from each other.

Other Studies. Other studies were performed in which Nisoldipine was administered to patients with renal disease, to cirrhotic, elderly and young people. The effect of food on drug absorption was also investigated. The effects of combination with other antihypertensive agents was studied in long term extension studies.

Study in Cirrhosis. Protocol M.M.R.R. # 1118

Title of Study. "The Effect of Cirrhosis on the Steady-State Pharmacokinetics of Nisoldipine Coat-Core Sustained-Release Tablets ".

This was a single center, non-randomized, non-blinded, comparison of single dose and steady-state pharmacokinetics of Nisoldipine coat-core tablets in cirrhotic and healthy subjects.

Sixteen subjects participated in the study : 8 cirrhotic and 8 healthy subjects. There were 4 males and 4 females in each group. In stage 1 a

single 10 mg dose of Nisoldipine was administered and in stage 11 10 mg of Nisoldipine was administered qd for 7 days.

Results. Administration of Nisoldipine to patients with cirrhosis resulted in a 3 to 4-fold increase in peak plasma concentration and $AUC_{(024)}$. Nisoldipine had little effect on blood pressure in either group.

Assessment. These results are indicative of possibility that the dose of Nisoldipine may need to be adjusted in patients with cirrhosis.

Study in Renal Disease. Report 5837 (R).

Title of Study. "Influence of Renal Function on the Pharmacokinetics of Nisoldipine CC Tablets After Single and Multiple Dosing ".

This was a multicenter, non-blinded, non-randomized, comparative study among 4 groups to compare the effects of renal function on the pharmacokinetics of Nisoldipine CC after a single dose as well as after achievement of a steady state.

A total of 40 patients were enrolled in 3 centers.. The following groups of patients were enrolled :

1. Control. Nine subjects with creatinine clearance > 90 ml/min/1.73 m^2

2. Mild Renal Failure. Twenty subjects with creatinine clearance 61 ≤ 90 ml/min/1.73 m^2

3. Moderate Renal Failure. Nine subjects with creatinine clearance 30 to ≤ 60 ml/min/1,73 m^2

4. Severe Renal Failure. Seven subjects with creatinine clearance < 30 ml/min/1.73 m^2.

Results. Although there was not a statistically significant difference in the Nisoldipine AUC_{norm} between the groups with impaired renal function and the normal control, in the former an increase in plasma Nisoldipine of approximately 2-fold could not be excluded.

Assessment. An increase in plasma levels of Nisoldipine in patients with impaired renal function may require the adjustment of the dose. There were only modest effects on blood pressure across all groups.

The Factor Age . Report 5857 (P).

Title of Study : "A Study to Determine the Single Dose and Steady-State Pharmacokinetic Profile of Nisoldipine Coat-Core (CC) Tablet 20 mg in Elderly and Young Volunteers and in Elderly Hypertensive ".

This was an open, multiple-dose, non-randomized study. Nisoldipine CC was administered at the dose of 20 mg qd for 7 days. Plasma samples were collected and blood pressure and heart rate were measured.

The following groups of patients were studied :

Young Volunteers. Twenty healthy young volunteers, 18 to 23 years of age, completed the study.

Elderly Volunteers. Twenty healthy elderly volunteers, 65 to 84 years of age, completed the study.

Hypertensive Elderly. Eleven hypertensive patients, 66 to 77 years of age, completed the study.

Results. The plasma concentrations of Nisoldipine were higher in elderly volunteers and hypertensive patients than in young volunteers. After multiple dose administration the supine diastolic blood pressure remained essentially unchanged in normal young healthy volunteers but a moderate decrease in elderly healthy volunteers and a significant decrease in elderly hypertensive patients was observed.

The Effect of Diet. Study Number D92-045-02.

Title of Study : " The Effect of Food on the Pharmacokinetics of 30 mg and 40 mg Nisoldipine CC Tablets in Healthy Male Volunteers ".

This study was an open-label, randomized, two-way cross over evaluation of the effect of food on the pharmacokinetics of 30 and 40 mg

Nisoldipine. Subjects were randomized to receive a single 30 mg or 40 mg dose of Nisoldipine either in a fasted or a fed state. After one week washout period there was a crossover to the opposite state.

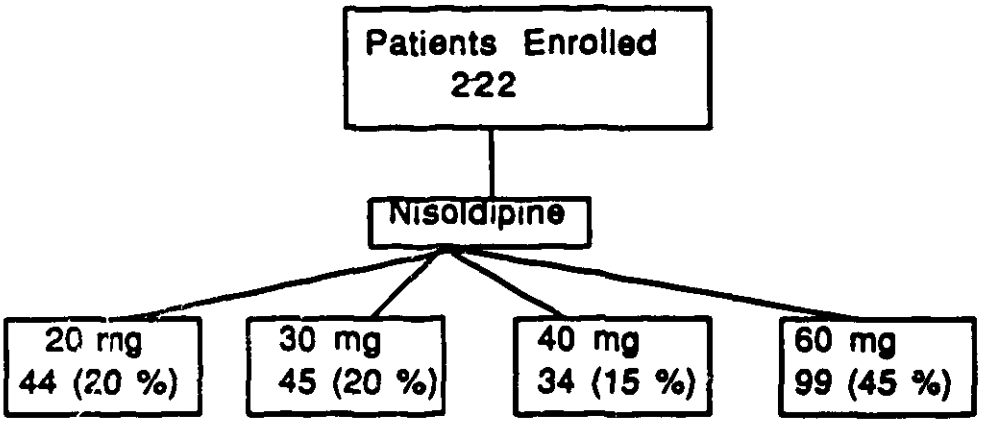
Twenty-eight healthy male subjects between the ages of 18 and 45 years completed the study. There were no significant effects on mean sitting diastolic blood pressures in the fed or fasted states at the 30 or 40 mg. doses.

Long Term Extension Studies. Drug Combination. Protocols X89-039 and X90-019.

These were long term extension studies of the 6-month efficacy studies and safety of Nisoldipine CC in the treatment of mild to moderate hypertension. Patients completing studies D89-039 and D90-019 were given the option of immediately entering an open-label extension protocol.

Patients were initially given Nisoldipine CC 20 mg or 30 mg tablets once a day as initial therapy. Then the dose of Nisoldipine was to be increased sequentially every one or two weeks as tolerated, to 40 mg qd, 60 mg qd and 80 mg qd. or 60 mg qd and 90 mg qd until SUDBP was $\leq$ 90 mmHg. However the maximum dose of Nisoldipine was in fact limited to 60 mg qd before any patient enrolled. Atenolol 50 mg to 100 mg qd and/or Hydrochlorothiazide 24 to 50 mg qd could be added at the investigator's discretion at any time. Thus tablets used were Nisoldipine CC 20, 40, 2X30 mg for monotherapy with the addition of Atenolol 50 and 100 mg and.or Hydrochlorothiazide 25 and 50 mg for combination therapy.

The distribution of patients is shown in the following graph :



With Atenolol
20 Patients (9 %)
1 Patient 25 mg
15 Patients 50 mg
4 Patients 100 mg

With Hydrochlorothiazide
78 Patients (35 %)
44 Patients 25 mg
34 Patients 50 mg

The results are summarized below :

	Supine		Standing	
	SBP mmHg	DBP mmHg	SBP mmHg	DBP mmHg
Baseline	154.0	101.1	149.7	100.3
Endpoint	135.7	86.0	132,4	86.5
Mean Dif	-18.3	-15.2	-17.3	-13.6

Assessment. These were open-label uncontrolled studies in which results were all pooled together and therefore they should not be valid for evaluation of combined therapy.

Total Assessment of Efficacy

Peak Drug Effect on Blood Pressure. The effect of Nisoldipine on blood pressure at the approximate time of peak drug plasma concentration (i.e. the maximal response between 6-10 hours post-dose) in the supine and standing position is shown below for the systolic and diastolic blood pressure.

	Placebo Substracted Change in Peak Blood Pressure Dose Nisoldipine				
	10 mg	20 mg	30 mg	40 mg	60 mg
Study			SUDBP		
D88-054	-11.6	-9.5	-14.1	NA	NA
D89-039	NA	-8.0	NA	-8.3	NA
D90-019	NA	NA	-6.3	NA	-10.6
			SUSBP		
D88-054	-8.6	-7.6	-12.8	NA	NA
D89-039	NA	-15.2	NA	-15.3	NA
D90-019	NA	NA	-13.0	NA	-11.1
			STDBP		
D88-054	-9.3	-7.8	-11.5	NA	NA
D89-039	NA	-7.6	NA	-8.5	NA
D90-019	NA	NA	-6.6	NA	-13.4
			STSBP		
D88-054	-4.7	-11.6	-11.0	NA	NA
D89-039	NA	-14.4	NA	-17.6	NA
D90-019	NA	NA	-15.5	NA	-19.1

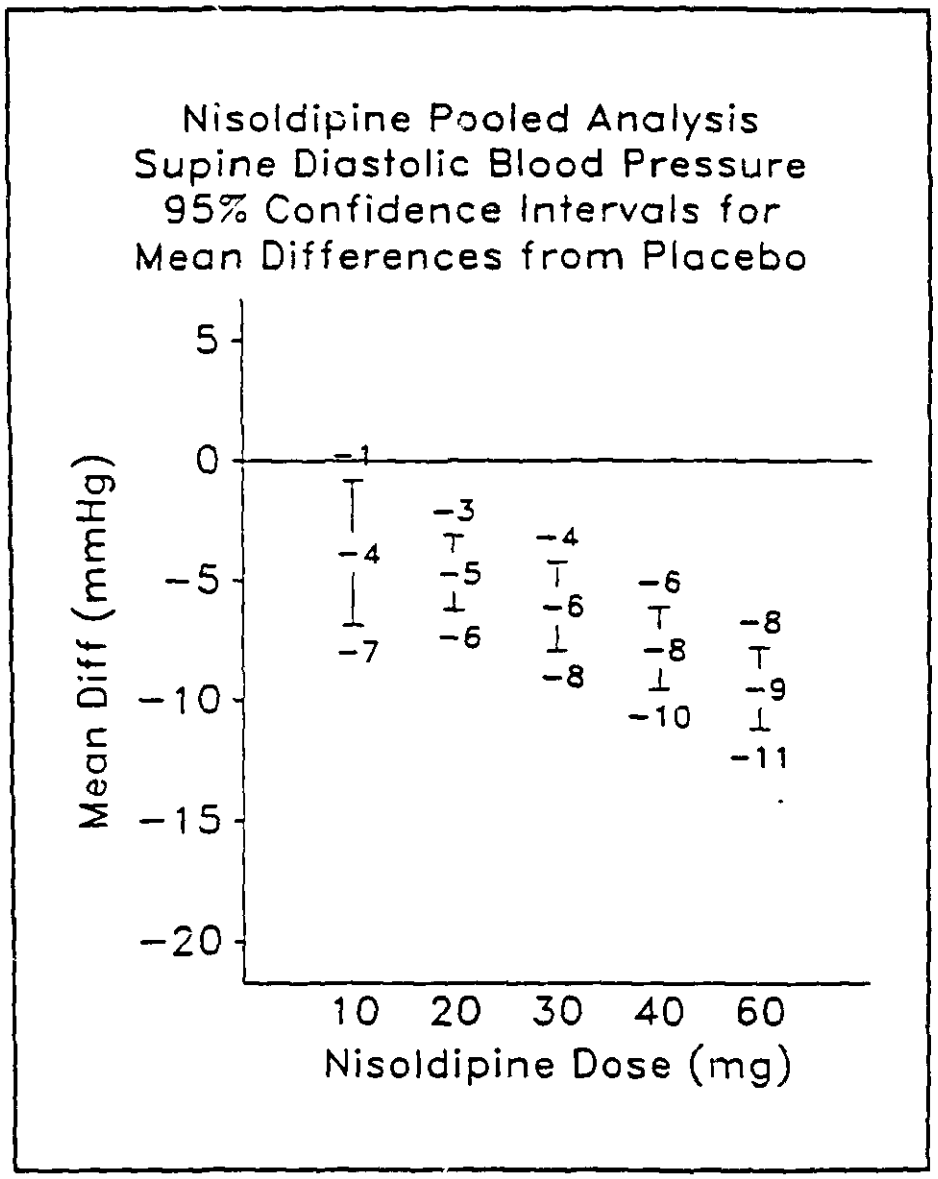
Twenty Four Hour Mean BP Reduction. Ambulatory blood pressure was used in a majority of the clinical trials of Nisoldipine in hypertension. In addition to characterizing the temporal profile of its effect on blood pressure, these data provide an estimate of the time-average reduction in blood pressure for each dosage of the drug. The pooled results of several studies are shown in the following table :

Nisoldipine Dosage (mg)	24 Hour AVG BP Reduction, Mean ± SEM Systolic	Diastolic
Placebo	-0.7±8.7	-0.9±6.3
10	-8.4±11.8	-4.6±7.5
20	-12.7±11.5	-8.4±7.1
30	-12.7±10.8	-7.9±6.9
40	-13.6±12.1	-8.0±6.8
60	-18.4±9.9	-12.0±7.2

The change in trough blood pressure from baseline to endpoint (Mean±SEM in mmHg) is given in the following table :

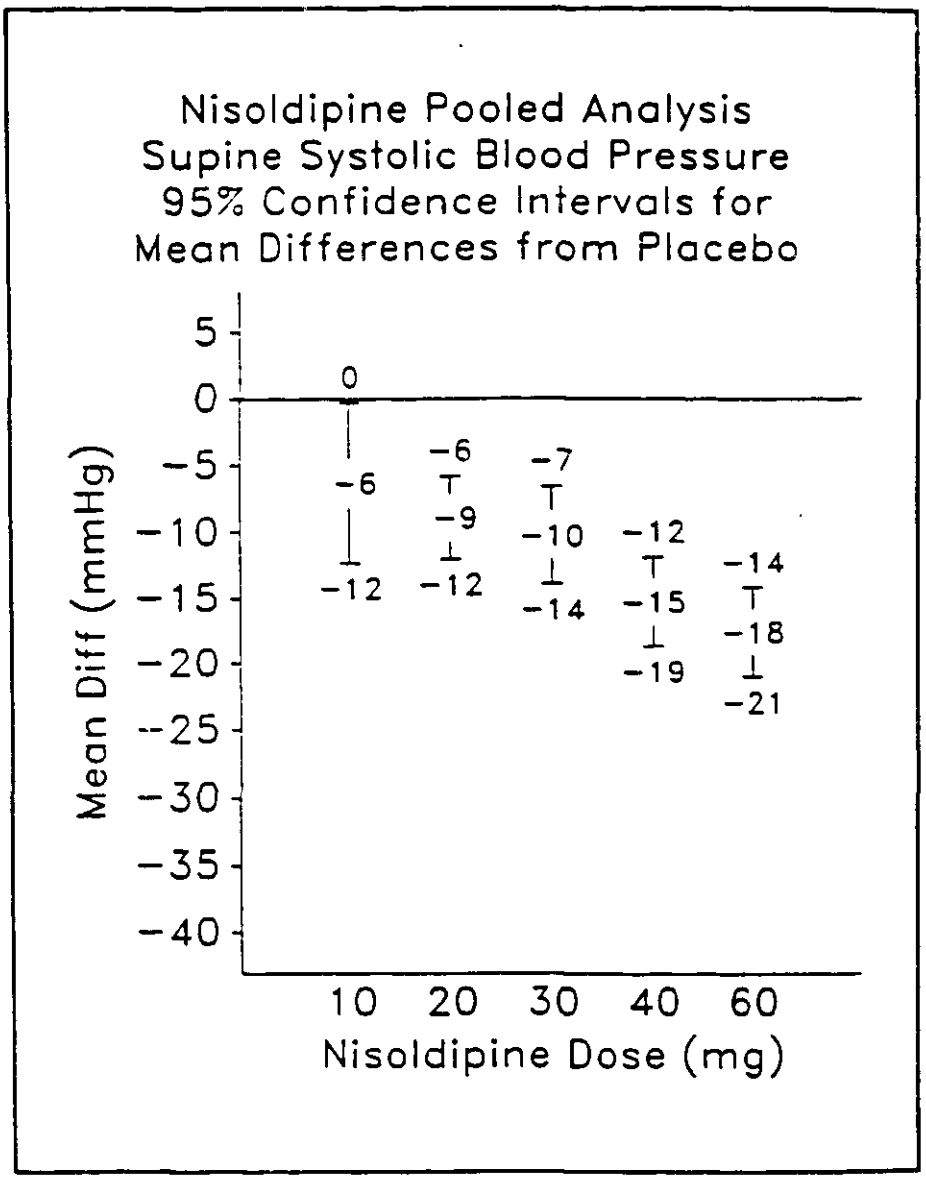
Pooled Dosage	Placebo N=232	Nisoldipine 10 mg N=30	20 mg N=161	30 mg N=105	40 mg N=131	60 mg N=125
SUDBP	-4±0.5	-8.4±1.4	-9.2±0.6	-10.6±0.8	-12.4±0.7	-14.0±0.7
SUSBP	-2.0±0.5	-8.3±2.9	-10.9±1.2	-12.2±1.6	-17.2±1.4	-19.5±1.4
STDBP	-2.7±0.5	-7.0±1.5	-7.9±0.7	-9.0±0.8	-12.6±0.8	-13.6±0.8
STSBP	-1.5±1.0	-8.2±3.0	-11.4±1.3	-12.9±1.6	-18.7±1.5	-19.2±1.5

In the following graph, pooled results of placebo subtracted values for trough SUDBP reduction by dose are demonstrated :



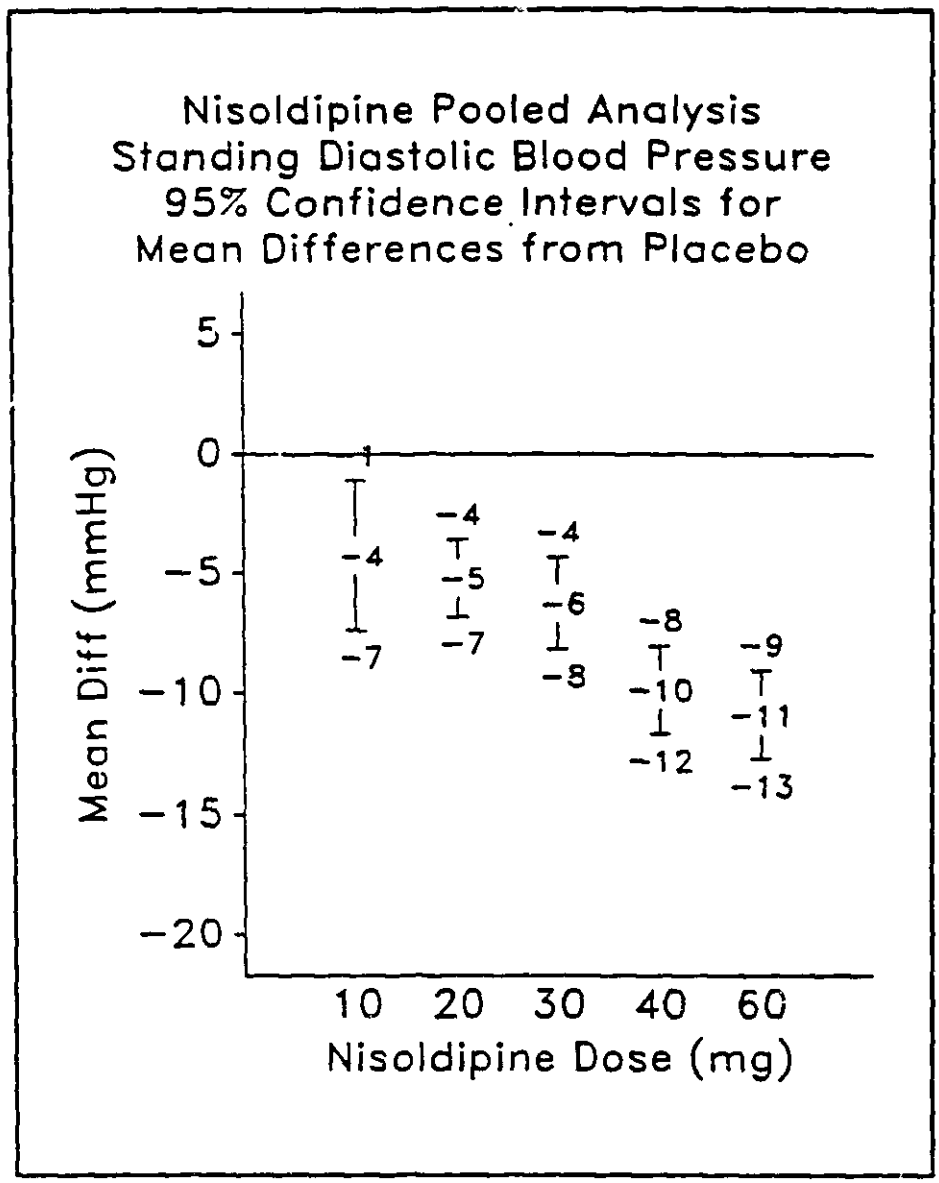
A linear relationship of blood pressure reduction by Nisoldipine in dosages between 10 and 60 mg is apparent without evidence of a plateau.

Similar results for SUSBP are shown in the following figure :



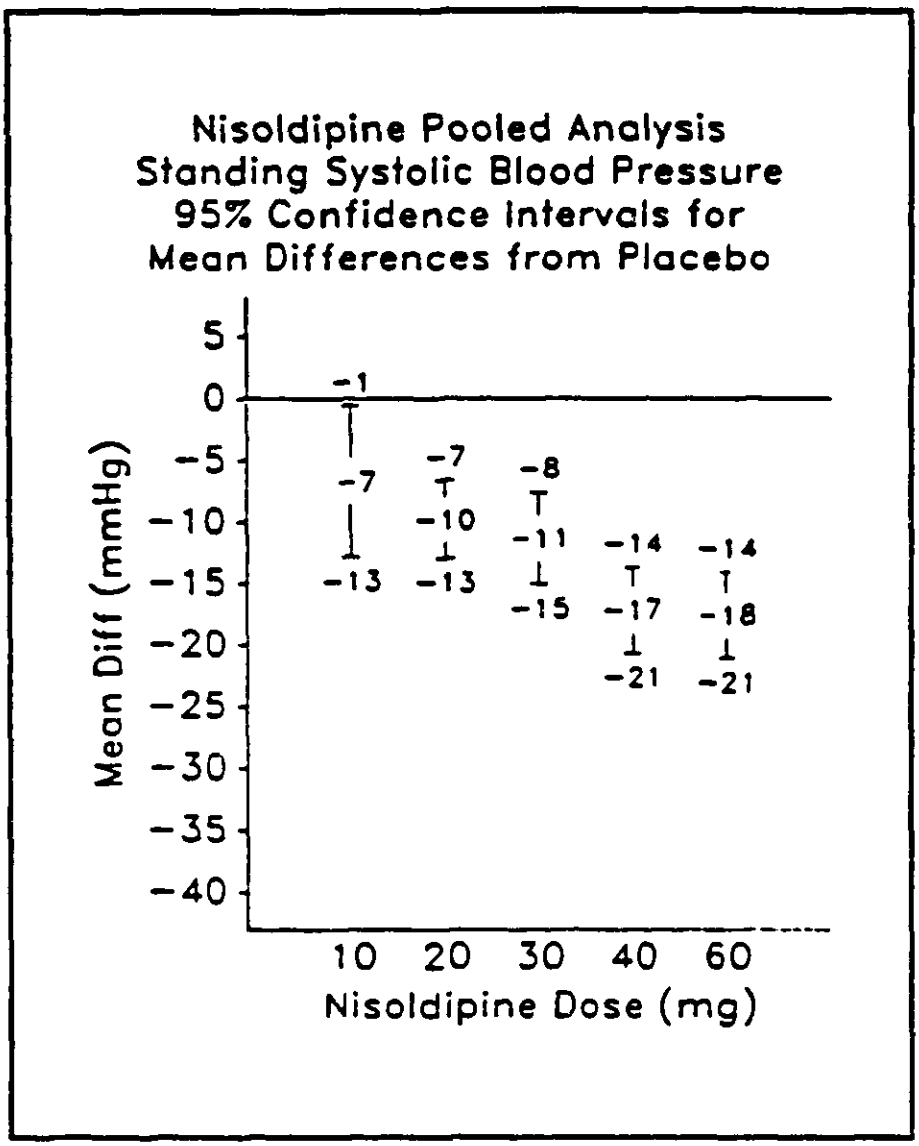
A linear relationship is not as evident as in previous graph but the maximum effect was achieved with 60 mg Nisoldipine dose

Similar results for STDBP are shown in the following figure :



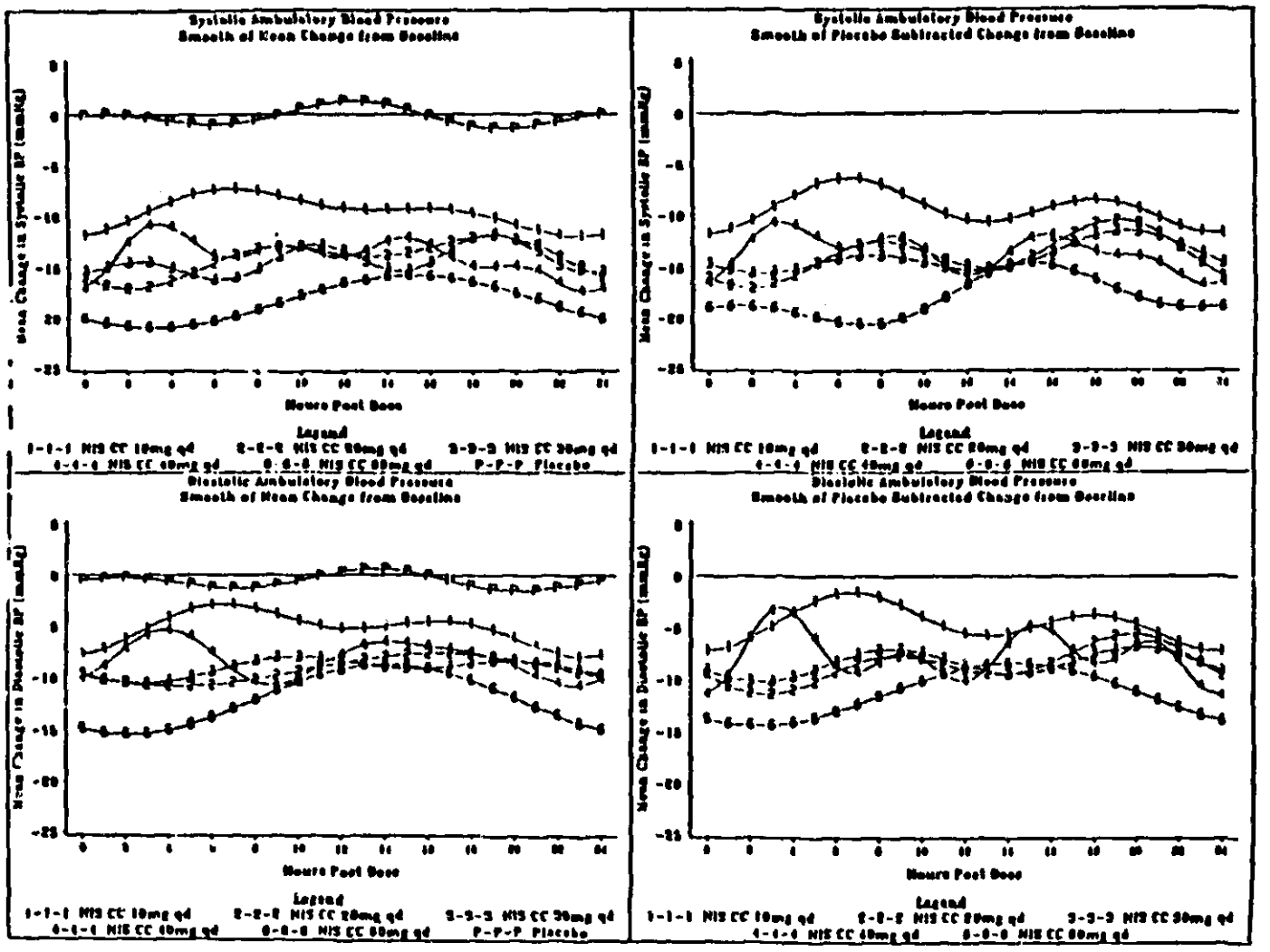
In this case the relationship of blood pressure reduction to dosage is roughly sigmoidal with an apparent plateau at 60 mg.

Similar results for STSBP are shown in the following figure :



The relationship of blood pressure reduction to dosage is sigmoidal with an apparent plateau at 40 mg

A pooled analysis of 24 hour ambulatory blood pressure monitoring is demonstrated in the following 4 graphs :



Through the 24-hour recording there seems to considerable overlapping especially among the higher doses but at trough there is evidence of blood pressure reduction that seems to be dose related.

The effects on diastolic blood pressure at peak and trough and the trough/peak ratios according to dose are given in the following table :

Dosage	Trough/Peak Ratio Diastolic Blood Pressure
10 mg	73 %
20 mg	75 %
30 mg	93 %
40 mg	100 %
60 mg	97 %

Time Course Effect of Nisoldipine. The therapeutic effect of Nisoldipine was achieved early in the course of treatment (approximately 2 weeks) and gradual incremental gain is evident for another 2-4 weeks.

The mean changes in sitting blood pressure from baseline after first dose is given in the following table :

Dose (mg)	N	8 Post-dose Systolic/ Diastolic	24 post-dose Systolic/ Diastolic
Placebo	10	-4.9/-1.9	3.8/-2.2
5	11	-10.4/-4.2	0.3/2.3
10	13	-6.7/-7.1	-0.7/-4.5
20	12	-11.3/-7.8	-5.8/-1.9
30	7	-15.4/-9.6	-13.3/-1.9

Pharmacokinetic and Blood Pressure Results. The mean sitting blood pressure change (mmHg) from baseline at peak and pharmacokinetic parameters (Mean ±SD) at steady state at each dose level is given below :

Dose (mg)	N	8h Post-Dose Sys/Dia	24h Post-Dose Sys/Dia	AUC (0-24h) (ng.h/ ml)	C_{max} (ng/ml)	T_{max} (h)
Placebo	10	-2.5/ -5.9	3.8/ 0.3			
5	11	-9.6/ -4.7	1:9/ -4.3	9.1±5.0	0.7±0.3	9.2±3.0
10	13	-8.9/ -7.1	-5.0/ -4.6	16.2± 3.0	1.1±0.3	6.3±4.8
20	12	-13.2/ -7.6	-5.4/ -4.3	29.4± 11.8	2.3±0.9	4.0±2.4
30	7	-21.7/ -10.5	-11.8/ -5.9	43.2± 23.1	2.9±1.1	5.4±5.0

The mean supine blood pressure change (mmHg) from baseline and pharmacokinetic parameters (mean±SD) at steady state for each dose level is given in the following table :

Dose (mg)	N	8h Post Dose	24 h Post Dose	AUC (0-24h) (ng.h/ ml)	C_{max} (ng/ml)	T_{max} (h)
30	18	-16.4/ -8.4	-14.0/ -10.2	74.28± 7.96	4.79± 0.68	7.22± 0.93
60	18	20.8/ 13.2	16.8/ 15.0	129.76± 12.74	8.48± 0.81	9.08± 1.97
90	9	-22.1± 12.1	-23.0± 13.4	199.31± 16.45	13.02± 1.20	6.78± 2.30
120	3	-30.7/ 25.0	-44.3/ -19.0	226.58± 12.41	14.92± 2.01	4.00± 1.00

To bring up more clearly the relationship between plasma Nisoldipine concentrations and blood pressure decrease, supine diastolic blood pressure changes from baseline at peak (8 h) and trough (24 h) were related to plasma Nisoldipine concentration at this time points using a simple linear regression. Placebo patients were used in this analysis with a plasma Nisoldipine level of Zero. The results for 30 and 60 mg are summarized in the table below :

Timepoint	Nisoldipine Mean Plasma Conc. (ng/ml)	Mean Change in SUDBP (mmHg)	Estimated Slope	Estimated Slope (P-Value)
Day 4, 30 mg (N=18)				
8 hours	3.5	-8.4	-2.55	0.0118
24 hours	2.6	-10.2	-1.42	(0.0689)
Day 8, 60 mg (N=17-18)				
8 hours	6.2	-13.2	-1.14	0.0507
24 hours	5.2	-15.0	-1.39	(0.0027)

Blood Pressure Rebound Upon Withdrawal. Blood pressure rebound was determined 24, 48 and 72 hours after cessation of Nisoldipine 60 mg qd in patients who had reached steady state. There was no evidence of for an exaggerated rebound effect on blood pressure after discontinuance of Nisoldipine at this high dose.

Maintenance of Blood Pressure Reduction in Long Term Studies. There was no evidence of tolerance to the antihypertensive effect of Nisoldipine over 6 months to 1 year of therapy.

Demographic Subgroups. Gender. Trough SUDBP changes from baseline to endpoint for male and female patients are given in the following table :

	Female	Male
Dosage	Nisoldipine-Placebo	Nisoldipine-Placebo
10 mg	-8.85	-2.27
20 mg	-3.21	-5.87
30 mg	-8.47	-6.1
40 mg	-7.82	-8.43
60 mg	-10.31	-10.79

Although dose-response profiles are somewhat erratic the overall effects are similar for men and women.

Race. A comparable analysis of efficacy for race related to dose is demonstrated in the following table :

	White	Black
Dosage	Nisoldipine-Placebo	Nisoldipine-Placebo
10 mg	-3.59	-4.37
20 mg	-4.54	-6.39
30 mg	-7.51	-8.82
40 mg	-6.69	-11.61
60 mg	-11.51	-11.1

Black patients responded with a greater decline in trough SUDBP than did white patients.

Age. In the following table the dose response for patients divided by age less than 65 years and equal or greater than 65 years is demonstrated.

	<65	≥65
Dosage	Nisoldipine - Placebo	Nisoldipine - Placebo
10 mg	-3.69	-6.2
20 mg	-4.98	-5.15
30 mg	-7.31	-5.48
40 mg	-8.21	-8.09
60 mg	-11.08	-8.14

The elderly demonstrated a greater low-dose response and a lesser high-dose response.

Quartile of Baseline Blood Pressure. For Nisoldipine as well as for many other antihypertensive drugs, a higher baseline blood pressure is associated with larger decline on medication. In the table below a dose response according to baseline SUDBP by quartile is demonstrated :

	Q1	Q2
Dosage	Nisoldipine-Placebo	Nisoldipine-Placebo
10 mg	-4.38	-5.97
20 mg	-4.23	-8.18
30 mg	-2.27	-11.49
40 mg	-6.36	-13.81
60 mg	-9.66	-14.16

The relationship of Nisoldipine dosage and decline in blood pressure is least evident in the first quartile and strongest in fourth quartile.

Combination Antihypertensive Therapy. Addition to a background of a beta blocker. One the pivotal studies (D89-029) evaluated the combination of Nisoldipine CC and a beta blocker.To patients who were already receiving Atenolol Nisoldipine was added. The sponsor claims the efficacy of Nisoldipine under these conditions. However there seems to be a drug interaction between these drugs that the sponsor has not recognized (see p. 35 this review).

Long Term Extension Trials. Based on open-label controlled trials and uncontrolled studies of one year duration the sponsor claims that meaningful responses were elicited by the combination of Nisoldipine with diuretics and or/ a beta blocker.

Recommendations. Nisoldipine should be approved as monotherapy for hypertension. The recommended dosage should be 10 mg to 40 mg.

Although the sponsor states that there is no drug interaction between Nisoldipine and beta blockers there are publications stating that such interaction exists (1, 2). This should be stated in the package insert.

Consideration should be given to advising that the dosage may need to be adjusted in patients with renal failure.

There were not well controlled studies of the combination of Nisoldipine with diuretics or other antihypertenive agents. Therefore the claim of efficacy with other drug combination is not well substantiated.

Cristobal G. Duarte, MD - HFD-110

CC.
ORIG. NDA
HFD-110
/HFD-110/ CSO/Roeder
√HFD-110/CGD/30Jul93

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75 pages
purged

SEP 27 1993

NDA20356 P1

DIVISION OF CARDIO- RENAL DRUG PRODUCTS
MEDICAL OFFICER'S NDA REVIEW

NDA: 20 356

DRUG: Nisoldipine Core- coat

SPONSOR: Miles Inc (Pharmaceutical Division)

DATE SUBMISSION: 3 March, 1993

DATE REVIEW: 20 August 1993

REVIEWER: Philip L. Dern M.D. P.L. Dern

RESUME:

This review deals entirely with safety aspects of the above submission; not efficacy. The primary approach is via examination of individual pools of data based on similar studies and provided by the Sponsor in this submission.

I. Hypertension

A. *Exposure*

Although the number of cases treated world-wide with nisoldipine exceeds 6000, according to the Sponsor, relatively few of these, N= 1292, were given nisoldipine core- coat (NIS cc) as shown below in completed studies:

PATIENTS EXPOSED TO NISOLDIPINE IN EACH CATEGORY

FORMULATION	NDA STATUS	INDICATION: ANGINA NON-US	ANGINA US	HYPERTENSION NON-US	HYPERTENSION US	HEART FAILURE NON-US	HEART FAILURE US	CLIN. PHARM. NON-US	CLIN. PHARM. US	OTHER NON-US	OTHER US	TOTAL
COAT-CORE	INCLUDED	624	474	518	778	142		210	183			2329
	EXCLUDED											0
	TOTAL	624	474	518	778	142	0	210	183	0	0	2329
IMMEDIATE RELEASE	INCLUDED	3472	521	2371	10	414	14	755	83	131		7771
	EXCLUDED	411		334		185		421		136		1487
	TOTAL	3883	521	2705	10	599	14	1176	83	267	0	9258
OTHER	INCLUDED	81				103		575				759
	EXCLUDED					31		126				157
	TOTAL	81	0	0	0	134	0	701	0	0	0	916
TOTAL	INCLUDED	4104	995	2887	788	659	14	1540	266	131	0	11382
	EXCLUDED	411	0	334	0	216	0	547	0	136	0	1644
	TOTAL	4515	995	3221	788	875	14	2087	266	267	0	13026

STUDIES IN EACH CATEGORY

FORMULATION	NDA STATUS	INDICATION: ANGINA NON-US	ANGINA US	HYPERTENSION NON-US	HYPERTENSION US	HEART FAILURE NON-US	HEART FAILURE US	CLIN. PHARM. NON-US	CLIN. PHARM. US	OTHER NON-US	OTHER US	TOTAL
COAT-CORE	INCLUDED	4	3	4	7	2		11	6			37
	EXCLUDED											0
	TOTAL	4	3	4	7	2	0	11	6	0	0	37
IMMEDIATE RELEASE	INCLUDED	111	16	87	1	33	2	67	3	8		328
	EXCLUDED	36		21		14		38		7		116
	TOTAL	147	16	108	1	47	2	105	3	15	0	444
OTHER	INCLUDED	11				9		55				85
	EXCLUDED					3		13				16
	TOTAL	1	0	0	0	12	0	68	0	0	0	87
TOTAL	INCLUDED	116	19	91	8	44	2	133	9	8	0	430
	EXCLUDED	36	0	21	0	17	0	51	0	7	0	132
	TOTAL	152	19	112	8	61	2	184	9	15	0	562

The following table provides the total duration of treatment by total daily dose of longest duration for the US NIS CC (total controlled and uncontrolled) cases. The second table provides duration of treatment in the non- US studies.

TABLE 2
TOTAL DURATION OF TREATMENT WITH NISOLDIPINE
BY TOTAL DAILY DOSE OF LONGEST DURATION

POOL OF US CC HYPERTENSION
TOTAL CONTROLLED + TOTAL UNCONTROLLED

	ALL	DURATION									
		2-7 DAYS		8-30 DAYS		31 DAYS-60 DAYS		61 DAYS-180 DAYS		181 DAYS-360 DAYS	
	N	N	%	N	%	N	%	N	%	N	%
DRUG											
NIS CC 10MG QD	37			33	89.2	2	5.4	2	5.4		
NIS CC 20MG QD	203	8	4.4	37	18.2	67	33.0	64	31.5	26	12.8
NIS CC 30MG QD	138	1	0.7	32	23.2	46	34.8	39	28.3	20	14.5
NIS CC 40MG QD	164	3	1.8	13	7.9	56	34.1	81	49.4	11	6.7
NIS CC 60MG QD	189	6	3.2	11	5.8	88	46.6	33	17.5	51	27.0
NIS CC 80MG QD	11	4	36.4	6	54.5	1	9.1				
ALL	776	22	2.8	133	17.1	261	33.6	269	34.7	108	13.9

2. DURATION BY DOSE TABLE NISOLDIPINE (BAY K 5552) - DATA POOL / NON US-STUDIES 10:22 TUESDAY, SEPTEMBER 22, 1992
TOTAL DURATION OF TREATMENT WITH NISOLDIPINE BY TOTAL DAILY DOSE OF LONGEST DURATION

POOL 80: CC - HYPERTENSION - TOTAL STUDIES

INSTITUTE OF BIOMETRY

DRUG	ALL	DURATION															
		NOT RECORDED		1 DAY		2-7 DAYS		8-30 DAYS		31-60 DAYS		61-180 DAYS		181-360 DAYS		> 360 DAYS	
	N	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
>5- <=10 MG	114	1	0.9	2	1.8	1	0.9	6	5.3	49	43.0	5	4.4	17	14.9	33	28.9
>10- <=20 MG	192	1	0.5					5	2.6	56	29.2	6	3.1	39	20.3	85	44.3
30 MG	186	1	0.5							67	44.3	1	0.5	22	20.8	35	33.8
40 MG	184									2	1.1	7	4.3	84	66.5	13	10.6
ALL IN POOL	516	3	0.6	2	0.4	1	0.2	11	2.1	154	29.8	19	3.7	162	31.4	166	31.8

In the non- US studies N= 326 received NIS CC for more than 6 months; N= 164 for more than 1 year. In the US studies the figure for greater than 6 months was N= 131 but, apparently, none were treated longer than 1 year.

Demographic characteristics in this safety evaluation will be related primarily to adverse effects and other safety- related features.

B.Safety

1. Deaths

No deaths occurred in the US NIS CC hypertension studies other than for a single subject receiving placebo. He was aged 68 years, collapsed, and failed to respond to resuscitation efforts. In the non-US NIS CC hypertension studies a placebo case died of cerebral hemorrhage; N= 2 NIS CC cases died, one due to an accident, another due to cerebral metastases from prostatic cancer.

2. Serious ADE

The next table shows the number and percentage of subject in the US NIS CC studies by dose for both cases with serious ADE, which display dose response, and for those withdrawing due to serious ADE, in whom dose response is probably present. The dose response for % of patients with ADE versus dose varys from 0.7% (10mg) to 9.1%(80mg).

Number (%) of Patients with Serious Adverse Events and Withdrawals from Study Participation because of Those Events by Dose of NIS CC							
DOSE OF NIS CC (n)	10mg (151)	20mg (395)	30mg (244)	40mg (292)	60mg (199)	80mg (11)	Total (1292)
NO. OF PATIENTS WITH SERIOUS AEs (% OF PTS ON DOSE)	1 (0.7)	8 (0.2)	2 (0.8)	5 (1.7)	9 (4.5)	1 (9.1)	26 (2.0)
NO. OF PATIENTS WHO WITHDREW BECAUSE OF SERIOUS AE (% OF PTS WITH SAE)	0	3 (38)	0	4 (80)	5 (55)	1 (100)	13 (50)

3. Discontinuations due to ADE

Of the N= 1292 patients (combined US+ non- US NIS CC), N= 25 (2.0%) ADE reports were received. Of these cases N= 13 were withdrawn because of these events. N= 17 of the 25 reports occurred during the double- blind phase of trials.

These N= 13 cases, among others, have narrative comments in Table 15a Pool 6 Vol 521. Each of the narratives on these cases was examined by the Reviewer. The final diagnoses were angina, MI, cellulitis of legs, possible MI, CVA, possible MI, infection, flu, pleural effusion, cholelithiasis, CVA, chest pain, pituitary tumor and berry aneurysm, elevated liver enzymes, edema and erythema plus petechiae, pain in legs with elevated CPK, chest pain.

Of the non- US NIS CC completed studies N= 35 cases on NIS withdrew due to ADEs. A listing, Vol 523 Table 15, provides reasons for discontinuation in N= 11 cases: These include tinnitus, non-response, pheochromocytoma, headache plus edema, atrial fibrillation, impotence, edema(2), vertigo, lack of efficacy, non- compliance. In the remaining cases a cause for discontinuation was not given although co-start terms for side effects were. There were a variable number of such terms for different patients. Most were manifestations of vasodilatation. No withdrawals for laboratory abnormalities were listed.

In the N= 6 placebo- controlled trials (US and non- US) with NIS CC 55/828 (6.6%) discontinued. Another N= 68 cases (11.5%) discontinued from among N= 590 patients on long- term uncontrolled studies. Since all but one of the 6 trial was a US study, the Sponsor focused on them. The following table shows that a dose response exists, except at 30 mg, for withdrawal due to ADE in the US placebo- controlled trials. The Sponsor does not believe dose response is evident, but this reviewer's logistic regression (below) shows a slope coeff t of 3.5 std errors.

Number and Percent of Patients in US Placebo-Controlled Studies Withdrawing Due to Adverse Experiences, by Dose of NIS CC							
	PLA (n=280)	10mg (n=37)	20mg (n=180)	30mg (n=125)	40mg (n=184)	60mg (n=137)	80mg (n=15)
N (%)	9 (3.21)	2 (5.4)	13 (7.2)	5 (4.0)	15 (8.2)	15 (10.9)	3 (20.0)

Table 15a Vol 521, not attached, provides a complete listing of reasons for withdrawal in this group. What is striking is that many subjects have multiple reasons for withdrawal. When more than one reason is listed, no single one is given most weight in this table. There was a suggestion, based on inspection of the table, that peripheral edema and rash might be associated but the number of cases is not more than a few.

The following table shows the most frequently reported ADE in subjects withdrawing due to ADE in the controlled US studies.

Incidence (%) of Most Frequently Reported Adverse Events in Patients Withdrawn Due to Adverse Events in U.S. Placebo-Controlled Studies		
ADVERSE EVENTS	PLA (n=280)	ALL NIS CC (n=678)
Any Body System	3.2	7.8
Headache	0.4	3.8
Peripheral Edema	0.4	2.9
Vasodilatation	0	1.5
Nausea	0	0.9
Palpitation	0	0.9
Dizziness	0.4	0.7

The Sponsor reports that the ratio of the number of ADE to the number of patients discontinuing was greater at lower doses than at higher ones.

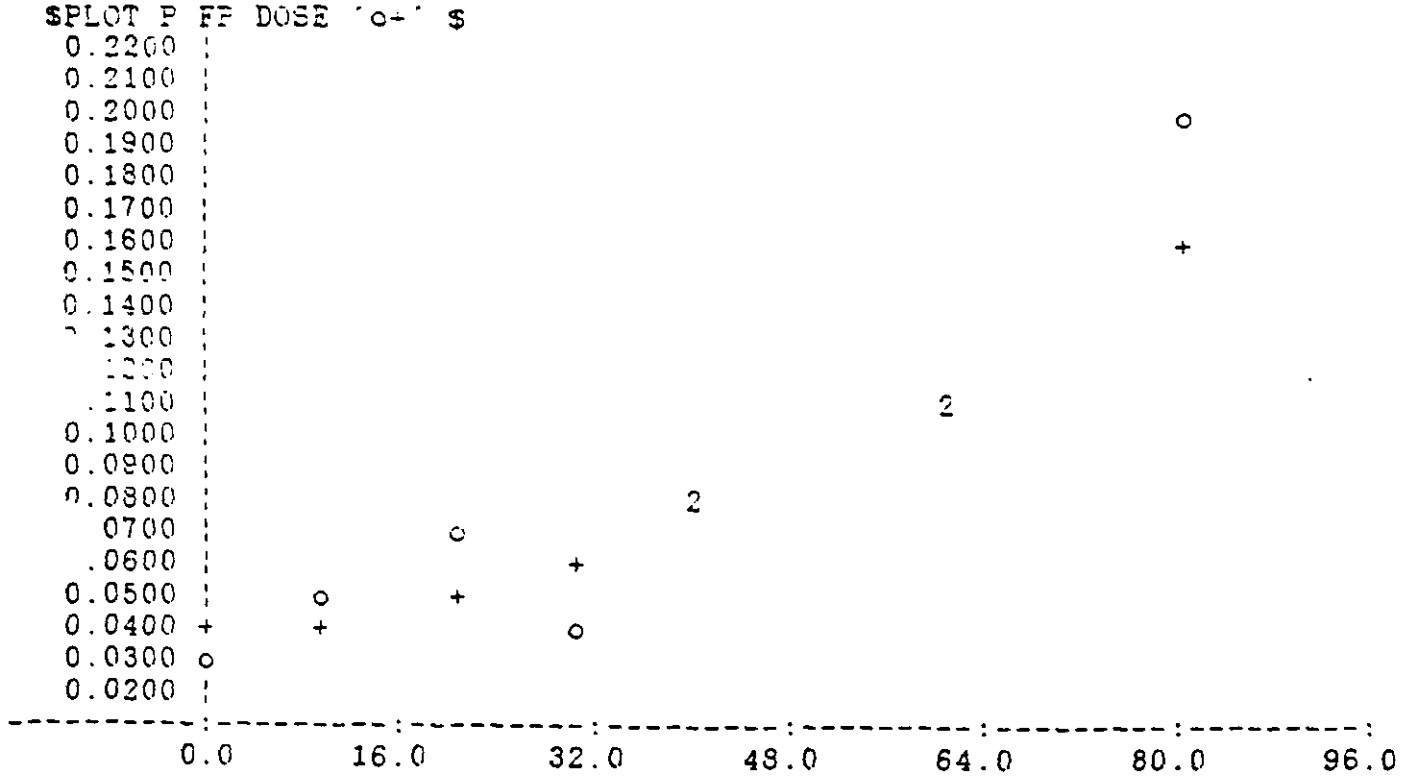
LOGISTIC REGRESSION OF WITHDRAWAL PROPORTION ON DOSE
Y AXIS: PROPORTION WITHDRAWN DUE TO ADE
X AXIS: DOSE MIS MG/DAY

Method: Iteratively- reweighted least squares (GLIM)
(analysis by reviewer)

o = OBSERVED

+ = PREDICTED

US PLACEBO- CONTROLLED HYPERTENSION TRIALS



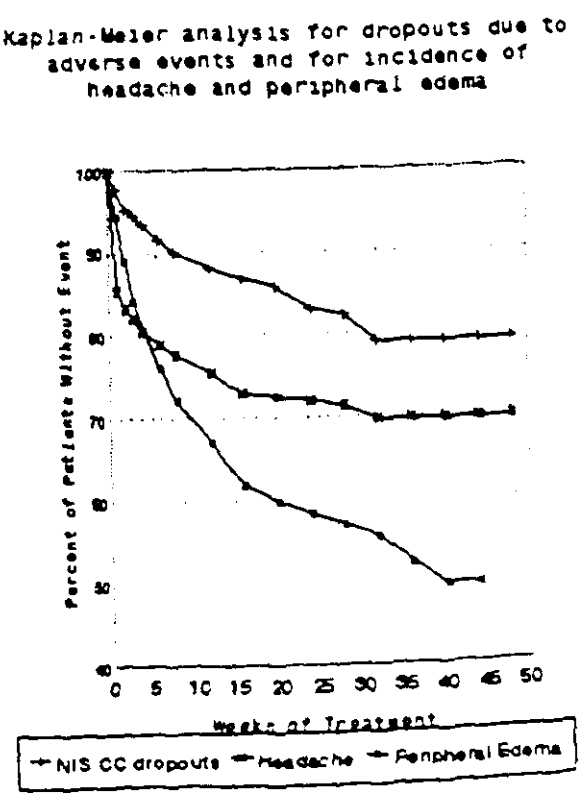
The Sponsor suggests that multiple, but mild, events could be occurring at low doses with more severe ones, though fewer, at high doses. No analysis of this hypothesis is provided.

The following table shows cases withdrawing due to ADE from US uncontrolled studies. Patients are assigned the dose they were on for the longest time. There is a counterintuitive inverse association of withdrawal and dose. One possibility is that subjects on higher doses had the opportunity to have withdrawn on lower ones during the process of upward dose adjustment in these long- term studies.

Number and Percent of Patients in U.S. Uncontrolled Studies Withdrawing Due to Adverse Experiences, by Dose of NIS CC				
	20mg (n=55)	30mg (n=46)	40mg (n=34)	60mg (n=89)
N (%)	12 (21.8)	9 (19.6)	6 (17.6)	8 (9.0)

The most frequent ADE in patients withdrawn during the US uncontrolled studies, shown below, are ordered somewhat differently than in the short- term studies. In particular, peripheral edema is more frequent in the long- term trials probably because of a time-dependence for withdrawal such that headache occurs earlier than edema. This is shown in the following table and in a Kaplan-Meier plot.

Incidence (%) of Most Frequently Reported Adverse Events in Patients Withdrawn Due to Adverse Events in U.S. Uncontrolled Studies	
ADVERSE EVENTS	ALL NIS CC (n=224)
Any Body System	15.6
Peripheral Edema	12.1
Headache	7.6
Rhinitis	4.0
Asthenia	3.6
Dizziness	3.1
Chest Pain	2.7
Vasodilation	2.7



The Sponsor states events causing discontinuation are more severe or higher doses, say 60 mg, than on lower ones. In the absence of a specific analysis taking account of the correct denominators, this may be questioned.

4. Most frequent ADE

The following table lists the most frequent ADE regardless of whether they were associated with withdrawal. Data from US and non- US NIS CC cases are given. The prominance of symptoms related to vasodilation is again seen. The relatively greater incidence of edema in the pooled controlled + uncontrolled studies is also shown for both US and non- US data. In addition, the rates in the non- US studies are overall less than in the US ones.

Incidence Rate (%) of Adverse Experiences (≥3%) in Patients Treated in U.S. and Non-U.S. Studies						
Study Location	Studies conducted in the U.S.			Studies conducted outside the U.S.		
Type of Studies	Placebo-Controlled		Controlled + Uncontrolled	Placebo-Controlled		Controlled + Uncontrolled
Adverse Events	PLA (n=280)	NIS CC (n=678)	NIS CC (n=776)	PLA (n=58)	NIS CC (n=150)	NIS CC (n=516)
Headache	15	22	23	21	23	18
Peripheral Edema	10	22	29	7	12	15
Dizziness	4	5	7	0	4	5
Asthenia	4	4	6	0	1	3
Vasodilatation	2	4	5	0	3	5
Palpitation	1	3	3	3	4	3

5. ADE by Demographic features

ADE by demographic sub- groups is examined in the US placebo- controlled trials. The incidence is given below of ADE selected by the Sponsor for "common observance" with the type of compound used. These are mostly those with higher incidence. There is no aggregation by sex though one might have expected this, say, for edema in females.

Breakdown by Gender of the Incidence (%) of Selected* Adverse Events† in US Placebo-Controlled Studies				
ADVERSE EVENT	NIS CC		PLACEBO	
	Male (n=424)	Female (n=254)	Male (n=172)	Female (n=108)
Any Body System	67	69	50	58
Headache	20	24	14	18
Peripheral Edema	22	21	8	14
Dizziness	5	5	2	8
Asthenia	4	4	4	3
Vasodilatation	4	4	1	4
Palpitation	3	4	1	1

* The above events were selected because of their common observance with dihydropyridine therapy

† The US data includes both adverse events and intercurrent illnesses

In the non- US placebo- controlled trials there was an increase in edema in females treated with NIS CC but data for the placebo group is not provided. The incidence of edema in treated females is about the same as in the US placebo group, above. The more frequent ADEs are shown below by race in all placebo- controlled studies. Rates tend to be higher for headache and edema in Caucasians.

Breakdown by Gender of the Incidence (%) of Selected* Adverse Events in Non-US Placebo-Controlled Studies		
ADVERSE EVENT	NIS CC	
	Male (n=65)	Female (n=85)
Any Body System	33.8	38.8
Headache	23.1	22.4
Peripheral Edema	7.7	15.3
Dizziness	3.1	4.7
Vasodilatation	< 3	4.7
Palpitation	3.1	4.7

* The above events were selected because of their common observance with dihydropyridine therapy

ADE by age are shown below for placebo- controlled studies. Headache is more frequent in younger subjects, interestingly, though edema may be less frequent than in older cases. These trends are present in both US and non- US studies.

Breakdown by Age of the Incidence (%) of Selected Adverse Events† in US and Non-US Placebo-Controlled Studies				
ADVERSE EVENT	US Studies		Non-US Studies	
	≤ 65 Years (n=601)	> 65 Years (n=77)	≤ 65 Years (n=131)	> 65 Years (n=19)
Any Body System	69	57	37	37
Headache	24	5	24	11
Peripheral Edema	21	27	12	16
Dizziness	5	5	4	5
Asthenia	4	0	< 3	5
Vasodilatation	4	4	4	< 3
Palpitation	3	1	5	< 3

† The US data includes both adverse events and intercurrent illnesses

Peripheral edema was more frequent in heavier subjects than in the lighter ones shown below both in US and non- US studies.

The table below shows selected adverse events with incidence rate ≥ 3% broken down by median weight in the US and non-US placebo-controlled studies.

Breakdown by Weight of the Incidence (%) of Selected Adverse Events[1] in US and Non-US Placebo-Controlled Studies				
	US Studies		Non-US Studies	
ADVERSE EVENT	≤ 185 lbs (n = 2801)	> 185 lbs (n = 3981)	≤ median weight (n = 81)	> median weight (n = 681)
Any Body System	64	70	33	39
Headache	20	23	24	22
Peripheral Edema	16	28	10	15
Dizziness	5	5	4	4
Asthenia	4	4	< 3	< 3
Vasodilatation	5	4	< 3	6
Palpitation	3	3	< 3	6

[1] The US data includes both adverse events and intercurrent illnesses

A table, not attached, shows that when ADE are stratified by baseline BP below and above 108 mmHg diastolic, that cases with lower BP tended to have more vasodilatation. The respective rates, 5/537 and 1/141. These are not very impressive.

6. Hemodynamic safety

A. Hypotension

ADE suggestive of hypotension, syncope and "hypotension" were sought. Asymptomatic hypotension was determined by "first dose effect", by trough/peak ratios from in- clinic and 24hr ambulatory BP readings, and by examining supine and standing BP plots. No cases (Sponsor) of syncope in the US NIS CC trials on NIS. N= 6 patients in the US placebo- controlled studies had either "hypotension" or "postural hypotension" on NIS CC. The next table shows data from US placebo- controlled trials.(INSERT tp15 v309)
Note that only a few of the cases show orthostatic hypotension in casual blood pressures. It seems worth while to point out that "dizziness" occurred in about 7% of all the US NIS CC studies and that it is possible that a number of cases had this symptom due to hypotension. Clinical experience shows that "dizziness" is not often distinguished from light- headedness without vertigo due to inadequate questioning of patients.

A first- dose effect was examined in two studies without showing adverse symptomatology but with BP reductions. The following table shows the results of in- clinic BP monitoring. Dose related peak effects are seen.

Mean Pre-dose and Peak Supine and Standing Blood Pressure Changes During In-Clinic Monitoring Periods in US Placebo-Controlled Studies							
		Mean Change in Supine Blood Pressures (mmHg)		Time to Peak (hr)	Mean Change in Standing Blood Pressures (mmHg)		Time to Peak (hr)
Drug Group	n	Pre-dose	Peak		Pre-dose	Peak	
STUDY 088-054 (24-hour period, BPs every hour)							
Placebo	8	-0.9/-3.4	-5.0/-8.8	3	-2.3/-3.6	-6.3/-7.0	22
NIS CC 10mg	7	-11.5/-10.3	-12.6/-11.1	8	-17.4/-8.7	-9.7/-10.6	9
NIS CC 20mg	7	-8.0/-8.3	-7.1/-12.0	14	-9.6/-4.6	-7.1/-10.5	11
NIS CC 30mg	9	-6.8/-12.0	-16.0/-15.9	12	-5.8/-7.4	-14.8/-16.2	11
STUDY 089-039 (12-hour period, BPs every two hours)							
Placebo	35	-4.9/-5.0	-3.4/-5.5	8	-5.7/-3.6	-1.6/-4.9	8
NIS CC 20mg	37	-11.7/-10.8	-16.7/-13.5	8	-14.3/-8.3	-15.2/-12.5	8
NIS CC 40mg	38	-18.0/-12.9	-19.1/-15.5	4	-18.2/-12.1	-22.8/-16.9	4
VER SR	40	-14.6/-14.8	-17.4/-15.1	2	-16.2/-14.9	-21.0/-15.8	8
STUDY 090-019 (12-hour period, BPs every hour)							
Placebo	27	-1.9/-7.2	-6.0/-8.1	7	-7.1/-8.0	-4.8/-7.5	4
NIS CC 30mg	32	-13.3/-13.6	-19.0/-14.4	8	-14.6/-12.8	-16.9/-15.8	5
NIS CC 60mg	28	-19.2/-17.8	-18.8/-18.7	9	-18.1/-16.0	-21.1/-20.3	7

Symptomatic Hypotensive Adverse Events
Occurring in US Placebo-Controlled Studies

Study Number	Patient No. / Drug & Dose	Investigator Term	Days on Drug	Duration (days)	Intensity	Baseline BPs (supine & standing)	BPs (day and blood pressures)
			SYNCOPE				
D89-039	16020 / PLA	Vasovagal Attack	43	1	Moderate	165/107 168/105	day 43: 142/99 143/90
D90-019	1012 / PLA	Feeling Faint at Times	30	>13	Mild	149/107 14[illegible]/10[illegible]	day 28: 155/113 171/113
			HYPOTENSION				
D89-039	13012 / NIS 20mg	Hypotension Post-Dose	62	1	Mild	136/95 135/97	day 62: 129/82 108/70
D89-039	16015 / VER SR 240mg bid	Hypotension	23	>5	Severe	129/100 115/91	day 23: 114/93 98/74
			POSTURAL HYPOTENSION				
D89-039	1005 / NIS 20mg	Dizziness upon standing	30	12	Mild	129/95 130/99	day 41: 136/91 135/95
D90-019	16004 / NIS 30mg	Dizziness (Postural upon standing up)	10	9	Mild	165/105 162/106	day 14: 151/99 146/96
D89-039	1008 / NIS 40mg	Postural Hypotension	16	1	Mild	155/98 141/99	day 16: 129/89 129/85
D89-039	12007 / NIS 40mg	Postural Hypotension	25	1	Mild	157/97 150/97	day 25: 146/89 136/88
D90-019	16007 / NIS 60mg	Postural Dizziness	28	>6	Mild	170/105 166/101	day 33: 145/89 147/90
D89-029	4001 / ATN + PLA	Dizziness upon standup	3	9	Mild	187/105 146/90	day 6: 183/58 150/93 day 14: 153/9[illegible] 9[illegible]/67

N= 5 ambulatory monitoring studies were done. The distribution of doses by study is provided. The second table shows the trough/peak ratios from these studies.

Number of Patients Undergoing 24-Hour ABPM by Dose of NIS CC						
Study & (location)	PLA	10mg	20mg	30mg	40mg	60mg
D88-054 (US)	18	12	11	12		
D89-029* (US)	31		32		32	30
D89-039 (US)	26		24		24	
D90-006 (non-US)	33	33	32	37		
D90-019 (US)	31			39		25

* This study was conducted on the background of atenolol 50mg qd

Trough/peak ratios from the 24-hr ambulatory data are provided below. Note that for systolic BP two methods are used depending on whether peak systolic is a) determined at the time of peak diastolic BP or, b) whether the true peak is used. These ratios are consistant with peaks that are not substantially below the trough values. Note, however, that if in a given subject the trough readings are quite low that a small, further drop at peak might be hypotensive. For that reason trough/peak ratios may not be very good means of exploring for BP reductions for safety purposes.

TABULATION OF TROUGH-TO-PEAK RATIOS ANALYZED DURING 24-HOUR AMBULATORY BLOOD PRESSURE MONITORING (BASELINE- AND PLACEBO-SUBTRACTED RESULTS)						
	DOSE OF NIS CC					VER SR
PARAMETER	10mg	20mg	30mg	40mg	60mg	240mg bid
Diastolic BP Trough/Peak Ratio (%)	73	75	93	100	97	86
Systolic BP* Trough/Peak Ratio (%)	75	83	114	100	101	78
Systolic BP† Trough/Peak Ratio (%)	75	83	93	100	89	78

* Peak values correspond to the time of peak diastolic effect.
† Peak values are the actual maximum systolic effect.

The table below shows the percentage of patients having either a change of 20 mm from baseline or a BP below 100mm Hg. There appears to be a fairly consistant percentage of cases with a fall

in systolic BP regardless of dose except for low numbers in the small sample on 40 mgm NIS. Note that a substantial number of placebo cases also show this degree of fall. Subtraction of the placebo values gives about 4- 8% of cases with reduction below 100 mm systolic. Thus supine reductions of note occurred in some subjects.

TABULATION OF SAFETY PARAMETERS ANALYZED FROM 24-HOUR AMBULATORY BLOOD PRESSURE MONITORING (BASELINE-SUBTRACTED RESULTS)

SAFETY PARAMETER	DOSE OF NIS CC					PLA	VER SR
	10mg (n=45)	20mg (n=67)	30mg (n=88)	40mg (n=24)	60mg (n=29)	(n=106)	240mg bid (n=29)
Diastolic BP Change > 20mmHg from Baseline for at least 1 Hour (% of patients)	82	85	78	79	96	62	96
Systolic BP < 100mmHg for at least 1 Hour (% of patients)	24	26	22	4	20	16	24

The last method of assessing hypotension was to compare supine and standing blood pressures at trough. The correlation was near 1.0 and consistant with little orthostatic hypotension.

B. Reflex tachycardia

One of the effects of a vasodilator is reflex tachycardia. The Sponsor examined this by dose response of pulse rate; by frequency of tachycardia as an ADE; and by ECG HR.

In the US monotherapy studies the mean placebo- subtracted change in HR varied from -1.53/min to +0.48 over the dose range of 10 to 60mgm NIS CC. The change in HR for the combined doses was 0.52. Note that these are not specified as standing readings.

In the US placebo- controlled studies, tachycardia was an ADE in 1% of NIS CC patients. One patient in these studies withdrew for supraventricular tachycardia.Three patients in the US uncontrolled studies had tachycardia contributing to withdrawal. The Sponsor analyzed the transition from normal or low heart rate to high values in the US controlled trials and found this to have occurred in 1.4% of NIS CC patients and in 0.9% of placebo cases. Again, none of these readings are specified as taken standing, a position that might have exaggerated pulse change.

C. Rebound hypertension

A placebo- controlled study D90-022 in hypertensive patients treated for as long as 21 days sought evidence for rebound blood pressure elevations by a 72- hr follow-up after discontinuation of NIS CC. Examination of the Sponsor's table showed that the group means for diastolic BP show no evidence of rebound. However, the systolic BP values are higher at 72 hrs than at baseline except for the highest dose level, 120mg NIS. The systolic BP mean for the placebo group is also elevated at 72 hrs so that it is difficult to ascribe the increase in systolic BP to "rebound". It is more likely that loss of both the placebo and therapeutic effects are involved.

7. Clinical Laboratory Tests

A. US NIS CC placebo- controlled studies

1. Incidence rates of "high" lab abnormalities

The Sponsor provides Table 17a (not attached), in which the rates for "high" abnormalities are given by dose level from 0 mgm (PLAC) to 80mg. Sample sizes are very small for the lowest active dose, 10mgm and the highest, 80mgm. Examination of these rates show no evidence for dose response nor is it likely they would given the exceedingly small rates for the data pooled over doses. In particular, for items with overall higher rates including blood glucose, no dose response trend is seen. BUN has a 3% rate at 40mg NIS, 2% at 60mg, and 0% for placebo. No such trend is seen for creatinine. No trend is seen for increase with dose of serum calcium, alkaline phosphatase, or SGPT is seen.

Rates by dose/body weight are also provided but do not show trends of interest except, possibly, for alkaline phosphatase, which has a rate of 5% at the highest dose/weight level, >.55- <1.2 mgm/kg, versus a placebo rate of 2% and rates in lower active dose/weight levels of 1%.

For "low" values the hematologic values for all US NIS CC studies showed 2% of NIS patients with neutrophils below 1700/microl and 1% in the placebo group. The rate was also 2% in the pooled controlled and uncontrolled studies. No patients with platelets below 100,000/microl are shown. Thus addition of cases with long- term followup did not increase the rate of low values for these two tests.

Hematopoietic Parameter Abnormalities from Studies Conducted in the US

BLOOD CELL LINE	Placebo-Controlled		Controlled + Uncontrolled
RBC	PLA	NIS CC	NIS CC
	n=250	n=588	n=680
% PATIENTS LOW ABNORMAL	3	3	5
WBC	PLA	NIS CC	NIS CC
	n=232	n=553	n=636
% PATIENTS LOW ABNORMAL	5	4	5
% PT WITH NEUTROPHILS < 1700/μL	1	2	2
PLATELETS	PLA	NIS CC	NIS CC
	n=267	n=655	n=751
% PATIENTS LOW ABNORMAL	0	0	0
% PT WITH PLATELET < 100,000/μL	0	0	0

This submission contains a tabulation of mean difference from baseline for both NIS CC (N= 650) and placebo (N= 280) for US placebo- controlled subjects. A tabulation on the following page contains selected laboratory tests from the larger tables. The larger table, Table 21 Vol 522, also has standard deviations. The Reviewer calculated the mean difference +/- 2 SE limits for NIS cc and for placebo for hematocrit, platelets, %neutrophils, glucose, BUN, alkaline phosphatase, and SGPT. All of the 2SE limits for the NIS group overlapped those for the PLAC group for these particular tests so that the treatment groups are not likely to differ by this method.

Examination of 10 lowest or highest values of selected laboratory tests in all US studies(controlled and uncontrolled for individual subjects showed, among the lowest 10, N= 3 instances in which falls in hematocit occurred. None were associated with the lowest 10 values for total wbc or %neutrophils. Respective baseline and lowest values were 34,29; 36,30; and 36,33. The baseline values tended towards being low. The subject with the lowest hematocrit,29, was on many medicines at baseline including Naproxin, insulin, enalapril,labetalol, and glyburide. During the

Renal Function Parameter Abnormalities from Studies Conducted in the US			
RENAL FUNCTION TEST	Placebo-Controlled		Controlled + Uncontrolled
CREATININE	PLA	NIS CC	NIS CC
	n=271	n=646	n=739
% PATIENTS HIGH ABNORMAL	0	0	1
BUN	PLA	NIS CC	NIS CC
	n=269	n=645	n=738
% PATIENTS HIGH ABNORMAL	0	1	1

Hepatic Parameter Abnormalities from Studies Conducted in the US			
LIVER FUNCTION TEST	Placebo-Controlled		Controlled + Uncontrolled
SGOT	PLA	NIS CC	NIS CC
	n=248	n=603	n=692
% PATIENTS HIGH ABNORMAL	3	2	3
% PT >3X NORMAL	0	0	0
SGPT	PLA	NIS CC	NIS CC
	n=224	n=562	n=647
% PATIENTS HIGH ABNORMAL	3	2	4
% PT >3X NORMAL	0	0	0
ALKALINE PHOSPHATASE	PLA	NIS CC	NIS CC
	n=262	n=623	n=713
% PATIENTS HIGH ABNORMAL	2	2	4
% PT >1.25X NORMAL	0	0	0
LDH	PLA	NIS CC	NIS CC
	n=257	n=628	n=717
% PATIENTS HIGH ABNORMAL	3	2	4

All cases of high blood glucose on treatment were high at baseline, usually above 200mg%.

N=7 instances of mildly elevated BUN on treatment were found. Only one of these (value =27) was associated with an increased serum creatinine(1.4,2.0).
Serum calcium increased in association with treatment in N=3 cases baseline, on treatment:9.8,10.5; 10.0,10.5;9.1,10.4) but alkaline phosphatase was not in the highest N=10 for any of these. The last of the above N=3 cases had a low phosphate (2.3,1.9).

Although a number of cases had elevation of alkaline phosphatase during treatment, all were high at baseline. One instance of notable change (112,248), but with a subsequent fall to 150, despite some increase at baseline was more closely examined. The patient was a 64- year-old female with diabetes mellitus, hyperlipidemia, and edema. During a long-term extension trial she was on concomittant medications including atenelol, niacin, and glyburide. A slow rise in alkaline phosphatase over 1 and 1/2 years occurred with 3 high readings.

N=3 instances of elevation of SGPT with concomittant elevation of SGOT were noted (baseline, on treatment: 25,384; 39,209;35,88). The first of these also had elevated total bilirubin (0.6,3.3). This patient was a 63-yr-old male with a history of elevated transaminases on ACE inhibitors.During treatment he received Lovastatin. Despite continued treatment with NIS the final day SGPT and SGOT were well within normal limits though the previous two values, both obtained within a one-week period, were elevated. An additional N=2 instances of elevation of SGOT occurred in the absence of enough rises in the other enzymes to reach the level of the highest 10 values.

increases in serum bilirubin occurred in N=4 cases. One of these had enzyme elevations described just above. Total CPK was elevated during treatment in N=2 patients. In one the MM and BB fractions were normal.

In the N=516 (depending on test) non- US controlled plus uncontrolled studies hematocrits were low in N= 3 cases but in N=2 they tended to increase subsequently. Total leucocytes were low in N=4 cases. In two of these the baseline value was also low. None of these cases were among the 10 lowest %granulocyte values. N=8 low platelet counts occurred in N= 8 cases. In N= 2 of these the baseline values were normal. Except for N=2 cases the on- Rx values were not very low, and though below the assigned normal range, were all above 100,000. In the one of N=2 cases with platelets below 100,000 total wbc was low both at baseline and on treatment. In the other the wbc count was not among the N=10 lowest.

In N= 10 cases elevation of SGOT occurred but baseline values were elevated in these. In two cases use of country- specific normal values might have reduced baseline values to normal. N=7 cases with elevated alkaline phosphatase values occurred. In N= 5 of these the baseline values were also elevated. These elevations ere not associated with values for SGOT in the highest 10. N=6 cases of elevated serum bilirubin were found; in N=4 baselines were high. None were associated with SGOT values in the highest 10.

Serum creatinine was not elevated above normal in any of the values in the highest 10.

8. ECG

A. Background

Because of the finding of t-wave changes in study D90-022 (hypertension), the Sponsor examined that study and three phase III NIS CC trials for such alterations. The Sponsor has provided background information from the medical literature. T wave inversion or flattening occur during rapid reduction of BP with vasodlators. These reductions in BP do not appear to be associated with wall motion abnormalities on 2D echocardiography. Long- term treatment with minoxidil has been carried out with improvement in the initial t-wave changes.

B. The phase II trial D90-022

This was a trial in N= 26 patients with mild to moderate hypertension, mean age about 60 years. This was a forced titration trial 30- 120mg NIS CC with the first two doses given for 4 days each; the next two for 7days each. N=8 subjects were randomized to NIS CC and N=5 developed t- wave flattening on the ECG. The N=120mg dose level was discontinued due to poor tolerance (severe peripheral edema, ECG changes).A second group, N= 10, was randomized to NIS. N=6 of these cases developed t wave flattening and/orinversion with occurrence equal, N=2 cases, at each of the doses, 30,60, and 90 mg. No angina occurred. Thallium scans were reported as negative in N=5 of the first cohort.

The percent with T-wave changes and dose were, respectively, 0%(PLAC); 22%(30mg); 39%(60mg); 64%(90mg); 80%(120mg). The respective sample sizes were 5,18,18,11,5. n.b. The excess over the N=23 that were randomized must represent titration steps.

Stratifying results into cases with normal and abnormal ECG and into 6 and 24 hrs after dosing, showed significant differences between BP falls at 6hrs between the two ECG groups. Differences at 24 hrs (trough) were not significant.

Stratifying normal and abnormal ECGs by AUC and Cmax showed that the abnomal ECG group had larger pharmacokinetic parameters.

The Sponsor relates the ECG changes to the forced- titration design and, by analogy, to the literature reports of T- alterations in rapidly- induced hypotension.

C. Other Phase III trials

ECGs from trials D89-029,039,and D90-019 coded blindly and read by a cardiologist. These ECGs were usually taken at trough. Peak ECGs were obtained in one study. Dosage was up to 80mg NIS CC (N=494 in the pooled studies) or placebo. Mean age was about 55. One study had background atenolol.

In these titration studies the dose assigned to an ECG was, for one analysis, that to which a patient was randomized, not to, say, a lower dose they might have transited from. Another analysis assigned the dose as that on which the event occurred. Two analyses were done; one ignoring baseline ECG events and another eliminating cases with these. It seems likely that more than one event per person could occur since rates were calculated from the "total number of events" divided by the number of cases at risk for the first type of analysis. In the second analysis all subjects were at risk. One analysis was done for peak ECG responses.

The table below shows some evidence of dose response except for the small sample at 80mg.

STUDY D89-039						
ECG abnormality	PLA	ALL NIS CC	NIS CC 20 mg	NIS CC 40 mg	NIS CC 80 mg	VER SR
T flattening	5/70 (7%)	10/155 (6%)	3/71 (4%)	7/69 (10%)	0/15 (0%)	4/71 (6%)
T inversion	3/73 (4%)	11/157 (7%)	4/72 (6%)	6/71 (8%)	1/14 (7%)	3/72 (4%)
either	8/74 (11%)	20/166 (12%)	7/75 (9%)	12/76 (16%)	1/15 (7%)	7/76 (9%)

Similar results were obtained when 'all patients' (regardless of baseline findings) were studied and when the number of ECG tracings was used as the denominator. n.b. there tends to be a dose response in each of the above results except at 80mg.

Results for ECGs taken at peak were unrevealing. The sample sizes were far too small, about N=5 per active dose group.

Study DS029. This is the study with background atenelol in addition to NIS CC.The doses of NIS were 0,20,40, and 60mg.

The respective rates of T flattening or inversion at these doses were 18%,7%,20%13% so there was not much evidence of dose response.

For all cases, regardless of baseline status, there was a significant difference among doses for T-flattening (PLAC 21%; 20 21%; 40mg 41%; 60mg 27%).

For events per number of ECGs results were weaker.

Study D90-019. The rates for T-wave flattening or inversion by dose were PLAC 13%; 30mg 9%; 60mg 5%. Therefore no dose response is seen.

Rates using all patients or number of ECGs in the denominator were not more useful.

ST- segment elevation or depression: Rates for these were very low in each of the three studies (1-2%). Comparing the incidence in the PLAC and pooled NIS CC groups by ST depression and by elevation showed that placebo and active dose rates were each no more than 2%.

There is little evidence from these three studies, taken together, of a dose response for the primary ECG T-wave changes of interest. However, the findings in the Phase II trial with forced titration do show a trend for dose response as well as effects of BP reduction and plasma NIS concentrations on T abnormalities. Thus the findings in the Phase III trials may just be at the opposite end of a spectrum of effects.

Conclusions for Hypertension Safety (NIS CC):

In placebo- controlled trials there is a dose- related incidence of ADE over the range 0 to 80 mgm of NIS CC. The most frequent ADE are those related to the vasodilatory action of the drug - headache and edema. The occurrence of these two ADE is time- dependent with headache occurring relatively early versus edema.

Marked symptomatic hypotension was not prominant with NIS CC although "dizziness" occurred and may have been a manifestation of hypotension. Asymptomatic hypotension in trough readings was not frequent though supine systolic BP values in the region of 100mm Hg were not infrequent in 24- hr ambulatory BP readings. Rebound hypertension was not present overall during monitored withdrawal. ECG T- wave abnormalities, inversion and flattening, occurred during forced titration in a phase II study but in studies in which dosage was increased slowly was not prominant. There was an association of these ECG changes to the degree of BP reduction and to drug blood levels in the forced titration study ECG S-T alteration was infrequent.

Clinical laboratory abnormalities: In the US, placebo- controlled (shorter- term) studies, evaluations by overall rates of abnormality, transition from normal, and overall rates by dose were not very revealing. present. Examination of the N= 10 highest or lowest values, as appropriate, in all US studies, controlled or uncontrolled (long-term), showed a few instances of falls in hematocrit, total wbc count, and %neutrophils. Several instances of increased transaminases, one with increased bilirubin were found. An instance of substantial elevation of alkaline phosphatase occurred. Serum calcium increased in three cases without increases in alkaline phosphatase.

In the non- US controlled and uncontrolled trials (approximately N= 516 NIS cases) several instances of decreases in hematocrit or wbc occurred. Platelet counts fell in some cases but not below 100,000. A number of cases had increases in SGOT but from elevated baseline levels. Serum creatinine was not elevated in the highest ten values.

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Overall Conclusions for Safety of Nisoldipine:

Two major findings in this submission are 1) a substantial incidence of withdrawal associated with signs/symptoms of vasodilation and 2) an increased rate of withdrawal for angina/cad in trials for the angina indication. In the short- term trials the latter withdrawals tended to occur early and to be associated with signs/symptoms of vasodilation but, in the long- term trials they were primarily manifested by an increased rate of withdrawal.

In the US, placebo- controlled, hypertension trials there was a significant dose response for overall withdrawal of about 11% at 60mg NIS and 5.4% at 10 mg. The incidence of headache, not necessarily associated with withdrawal was about 20%. In the long- term, uncontrolled hypertension studies, about 20% of cases had withdrawn by 30 weeks and the cumulative incidence of peripheral edema was more than 40%. Thus whatever blood pressure reduction that is achieved is associated with substantial side effects, most of which are due to the pharmacologic action of the drug in causing vasodilation or to the compensatory mechanisms such as tachycardia.

One might expect some myocardial ischemic phenomena if the vasodilation and tachycardia increased cardiac work out of proportion due the benefits of reduction of afterload through lowering of blood pressure. There was little evidence that the balance was unfavorably affected since, in the hypertension studies the incidence of withdrawal due to angina/cad was about 1%. However, there were instances, especially in the phase 2 rapid, forced- titration trial, of the development of t wave abnormalities.

It is in the angina patients that evidence for ischemic effects of nisoldipine are of greater concern. Even in the short- term, US placebo- controlled trials the rates of withdrawal for angina/chd exceed those on placebo. In addition, it is of particular interest to note that a high proportion of such withdrawals occurred very early at times close to those for withdrawal due to vasodilation. Thus the close similarity of the distribution of withdrawal for vasodilation and that for angina/cad supports a similar mechanism for both. Symptoms of vasodilation were not prominant in the long- term angina trials but in those the number of events was higher, about 10%. Thus for the short- term trials one may use the rates, the timing of withdrawal and/or association of vasodilation; for the long- term trials only the rates are useful. Note that the use of timing of withdrawal and/or any associated vasodilation constitutes an "internal" control.

From a purely safety standpoint, this reviewer is not in favor of approval for the angina pectoris indication in view of the increased rate of chest pain/cad in angina subjects. It is possible that a combination of a beta- blocker and nisoldipine would allow use of the latter drug in angina pectoris. n.b. a single study (0702) carried out in Canada, US, and Israel had very few withdrawals due

to ADE. N= 1 subject withdrew for increasing angina and N= 1 for myocardial infarction out of N= 200 NIS+ atenolol cases. The experience with this combination is as yet insufficient to recommend this combination but it may be a justifiable treatment to explore in future trials.

Note that if the NIS formulation dumps early, one would expect to see early withdrawal or the early occurrence of signs/symptoms of vasodilation.

The hypertension indication, at least for subjects without notable coronary heart disease, is supported by the safety data. . However, if the spectrum of patients selected for treatment includes patients whose hypertension is associated with CHD or as hypertensive subjects develop CHD, some of these may be unable to tolerate NIS therapy. Alternative therapy should be considered in such cases.

The ECG findings in rapid- dose escalation (intervals of less than a week) in hypertensive subjects, while not clearly established as adverse, are in an unfavorable direction and suggest that titration be carried out over the longest intervals consistant with the patient's need for blood pressure control.

Summary of safety recommendations:

1. Nisoldipine, as studied,

2.

3. Subjects with asymptomatic coronary disease constitute a somewhat difficult group with respect to suitability for NIS therapy since many hypertensives responding to this treatment undoubtedly have this condition. Perhaps some clinical judgement needs to be invoked here.

4. In view of the findings of ECG T abnormalities on rapid titration in hypertensive patients, it is suggested that dosage increments be made at the greatest intervals consistant with the need for BP control. The Sponsor's dosing recommendations do not specify the interval between dosage increments. Intervals of only a few days may be too frequent since they were associated with "adverse" ECG changes in hypertensives.

5. Although mono- therapy for hypertension with NIS appears justifiable due to the lack of serious drug- induced effects, there is still a substantial incidence of troublesome symptoms of vasodilation that might be diminished with combination of NIS and beta- blockade. This may also be a suitable and informative area for further trials.

6. Pharmacokinetic studies by the Sponsor show that the mean Cmax was 48% higher when NIS was administered with a meal. It is not clear whether this explains the very early occurrence of vasodilation after dosing and, in the angina patients, withdrawal associated with chest pain. It may not be correct to state that it is known that there are no clinical consequences from dose dumping.

7. Since elderly subjects have a 2- 3 fold higher plasma NIS concentration than younger ones, it may be best to follow these subjects more closely and increase dosage slower.

Philip L. Dern M.D.

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D.K.

NDA REVIEW
Clinical Pharmacology

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

FEB 16 1994

NDA:	20-356
Name of Drug:	Nisoldipine, Coat Core Tabs
Sponsor:	Miles
Indications:	Hypertension
Submitted:	03/31/93
Received:	04/05/93
Assigned:	04/09/93
Reviewed:	08/25/93
Review Completed:	02/14/94
Reviewer:	Shaw T. Chen, M.D.,Ph.D.

Overview of NDA (Clinical Pharmacology)

Nisoldipine is a calcium channel blocker of dihydropyridine derivative type being developed for the treatment of hypertension In this initial application, approvals of a sustained release formulation (coat-core) for indications are requested. As a part of new parallel, team approach, this medical review covers only the areas related to clinical pharmacology.

The sections on clinical pharmacology (Section 8.1 of NDA) contain data of 17 studies, involving 393 patients/subjects, on sustained released formulation (coat-core, referred to as CC in this memo). Of these, 183 participated in 6 U.S. studies. In addition, the submission also includes results of 47 studies on the immediate release preparation (IR), most of which were conducted in foreign countries. Except for three small studies (total 12 normal subjects, copies of publications only), full reports of all studies listed in Section 8.12 were submitted.

Additional pharmacokinetic and bioavailability data were presented in Section 6 of the NDA, which include 129 foreign studies on IR or other non-CC formulations not repeated in the clinical sections (Section 8, as noted above). These studies will be reviewed by the biopharmaceutical group of the Agency and not commented in this report.

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NDH 20356

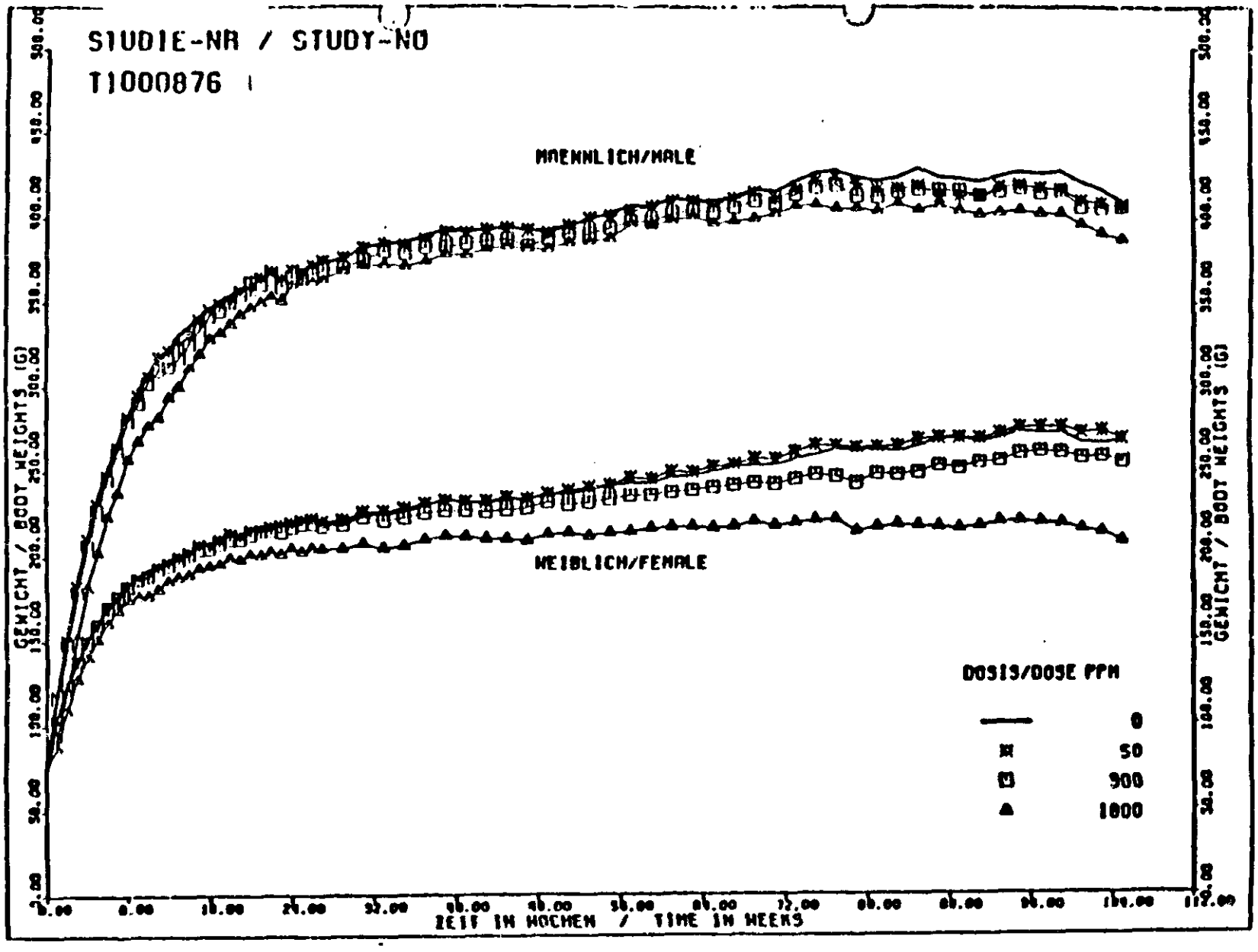


Fig.8 : Body weight curves for male and female rats which received with the feed for 24 months.

Mean Clinical Chemistry Parameters (Male Rats)					
		Dose Group (ppm in diet)			
Parameter	Week	0	50	300	1800
ALP	28	211	201	201	175*
U/L	54	182	174	186	145*
	79	180	156	176	136**
GOT		38.8	39.0	38.9	52.7*
U/L					
Bilirubin	28	3.6	3.1	4.0	4.8*
mcmol/L					
Creatinine	79	53	50	47*	51
mcmol/L	105	57	63	46**	50**
Urea	105	5.80	7.01*	5.69	5.27
mmol/L					
Cholesterol	28	1.98	2.16	2.21*	2.21
mmol/L					
Protein	54	66.5	64.2**	61.0**	61.4**
g/L	105	68.4	67.1*	66.3*	67.8
Sodium	28	142	143	140*	140*
mmol/L	54	141	142	142*	142
	79	140	139	138*	141
Potassium	79	4.8	5.0	5.1*	5.1
mmol/L					
Calcium	28	2.64	2.54*	2.49*	2.55*
mmol/L	79	2.76	2.66*	2.62**	2.63**
	105	2.69	2.66	2.63	2.58*
Aldosterone	55	349.7	360.2	334.9	245.1**
pg/mL					

** Significantly different from control at the 0.05 level*
*** Significantly different from control at the 0.01 level*

Mean Clinical Chemistry Parameters (Female Rats)					
		Dose Group (ppm in diet)			
Parameter	Week	0	50	300	1800
ALP	28	174	140	153	133*
U/L					
CPK	28	98	54*	72	85
U/L	79	43	75	64	77*
GPT	28	54.1	50.5	52.9	66.3*
U/L					
Bilirubin	54	3.0	3.1	3.2	4.3**
mcmol/L	105	4.7	2.9**	3.2*	2.9*
Creatinine	79	56	50	59	57*
mcmol/L	105	71	59	55**	61
Urea	28	7.54	7.27	6.56*	6.30**
mmol/L	79	6.22	5.96	6.51	7.56**
	105	6.09	6.43	6.67*	7.28*
Cholesterol	28	7.54	7.27	6.56*	6.30**
mmol/L	105	2.46	3.01*	2.76	2.96
Glucose	105	4.71	5.25	5.52*	5.22
mmol/L					
Sodium	54	140	138	135**	138
mmol/L					
Potassium	54	4.8	4.8	5.0	5.2*
mmol/L	105	4.5	4.6	4.8*	4.8*
Calcium	28	2.58	2.65	2.59	2.47*
mmol/L	54	2.71	2.65	2.58*	2.52**
Corticosterone	55	36.6	41.7	20.4	19.2*
mcg/DL					

** Significantly different from control at the 0.05 level*
*** Significantly different from control at the 0.01 level*

At the termination of the study, the relative mean weights of adrenals, heart, kidneys and liver of the high dose group (both sexes) were significantly higher than respective control values (page 50A). However, no significant differences were seen in the absolute weights of the above organs except for the increased mean kidney weight of the high dose males. At the interim sacrifice, relative heart and liver weights (high dose males and females) and relative adrenal and kidney weights (high dose females) were significantly increased without any significant changes in absolute weights.

No significant treatment related gross lesions were seen in this study.

At the interim sacrifice, no treatment-related histological findings were observed except for the moderate widening of the zona glomerulosa region of the adrenal cortex of high dose animals. The cells of this zone were large and contained a foamy cytoplasm. Four benign tumors [2 in control females (cystadenoma of thyroid in one and pituitary adenoma in the other) and 2 in high dose males (Leydig cell tumor of testis in one and meningioma of the cerebellum in the other)] were seen at the interim sacrifice.

[Note: The terms "blastoma" and "tumor" are used interchangeably in this NDA.]

The number of rats with benign and/or malignant tumors and the percent of these tumor carriers are given in Table 7. According to sponsor, no treatment-related increased incidence of tumor bearing animals was observed in this study. The incidence of various types of tumors observed at different locations are presented in Tables 8 and 9. Although the incidence of Leydig cell tumor of testes appears to be higher in treated male groups than in control, the differences were statistically not significant.

Analysis of the tumor data by FDA statisticians showed that there was a statistically significant (at 0.05 level) linear trend in brain granular cell tumor (listed also as meningioma in this NDA) in male rats ($p=0.0411$). The incidence of this tumor is as follows: control - 0/50, low dose - 0/50, mid dose - 0/50 and high dose - 3/50 (2 animals at the final necropsy and one at the interim sacrifice). However, pairwise comparison did not reveal any significant difference between control and high dose groups ($p=0.1594$). According to the sponsor, "the incidence rate for granular cell tumors among male rats at terminal kill in the study performed with lay within the spontaneous range for male rats at terminal kill" (Table-page 50e). Spontaneous tumors of meningeal origin (meningioma, meningeal sarcoma or granular cell tumor) were seen in 7 out of 30 studies in male Wistar rats (39-50 rats/study). In 2 studies, 2 rats each were diagnosed with such tumors at terminal kill, whereas in 4 other studies, only one rat each had above tumors.

[Note: Granular cell tumors are believed tc be of meningeal origin and are considered to be a subclassification of meningiomas.(Boorman et. al. 1990. eds. Pathology of the Fischer Rat. Academic Press, Inc.)]

Relevant nonneoplastic findings observed in this study included hypertrophy of the cells of the zona glomerulosa of the adrenal cortex of high dose males and females and increased incidence of progressive nephropathy in high dose females.

d. Two-Week Intravenous Toxicity Study

Testing Facility:

Study Number: Not provided (Pharma Report No.7721)

Study Date: October, 1977

GLP Compliance: Not addressed

Animals: SPF Wistar albino rats, individually housed in Type II Macrolon cages, weighed 125 to 130 g at the initiation of dosing.

Dose Levels: 0, 0.1, 0.3 and 1.0 mg/kg. . dissolved in a 10%:90% mixture of Cremophor EL and physiological saline, was administered as a single iv bolus injection (caudal vein; 1 ml/kg) daily for 14 consecutive days.

Number of Animals: 10/sex/group

Parameters Evaluated: Appearance and behavior (daily), body weight and food and water consumption (weekly), hematology and clinical chemistry (at the termination of the study; 5 rats/sex/group), urinalyses (after 10th treatment), major organ weights and gross and microscopic pathology (more than 30 different tissues/rat; 5 rats/sex from control and high dose groups).

Results: High dose animals showed inertia and dyspnea for about 5 to 15 min following drug administration. Two high dose females died during the study, one after the third dose and the other after the ninth dose. No treatment-related clinical signs or mortalities were seen in low and mid dose group animals.

Intravenous administration of nisoldipine had no significant effect on body weight, food or water consumption and hematologic, blood chemistry and urinalyses parameters. There were no treatment-related gross or histopathological findings, or any evidence of local intolerance at dose levels tested in this study.

MOUSE STUDIES (X. Joseph)

a. 28-day Dietary Dose Rangefinding Study

Testing Facility:

Study Number: T 1003 576

Study Dates: June-July, 1981

GLP Compliance: Study was not conducted according to GLP regulations. The deviations were as follows: a. no phase 1-3 GLP audits, and b. no checking of physico-chemical properties of test substance.

Animals: SPF-bred NMRI mice, individually housed in Type I Macrolon cages, were 4-5 weeks old (average body weights: males-20.0 g, females-19.8 g) at the initiation of the study.

Dose Levels: was mixed with powdered diet by the addition of peanut oil DAB 7 (1%) to obtain dietary drug concentrations of 0, 400, 800, 1200 and 1600 ppm.

Achieved Dose Levels:

Dose (ppm)	Average Drug Intake (mg/kg/day)	
	Male	Female
0	0	0
400	110	124
800	226	271
1200	348	383
1600	429	523

(Note: The drug intake was calculated from the average daily food intake/animal/group for the whole duration of the study.)

Number of Animals: 10/sex/group

Parameters Evaluated: Appearance and behavior (daily), body weight, food and water consumption (weekly), organ weights (heart, lungs, liver, spleen and kidneys) and gross pathology.

Results: No treatment-related clinical signs or mortalities were observed. Significant reductions in body weights (3-7%), compared to concurrent control, were seen in females throughout the study

at dose levels of 800 ppm and above except at 1200 ppm at the end of the study. In males, although body weights were lower than concurrent control (4-9%) at 1200 and 1600 ppm levels, the differences were statistically significant only at 1200 ppm. No significant differences were seen in food and water consumption between treated and control groups. Organ weight findings are given below.

Mean Organ Weights of Male and Female Mice

		Sex	Dose Group (ppm in diet)				
			0	400	800	1200	1600
Body Weight (g)		M	31.8	30.1	31.0	29.5*	30.3
		F	25.5	25.4	24.0*	24.7	24.5*
Heart	(Absolute, mg)	M	0.14	0.15	0.17**	0.15*	0.15
	(Relative, mg/100g)	M	0.44	0.52**	0.53**	0.53**	0.49*
	(Absolute, mg)	F	0.13	0.14	0.14	0.15*	0.14*
	(Relative, mg/100g)	F	0.51	0.54*	0.58**	0.61**	0.57**
Kidneys	(Absolute, mg)	M	0.46	0.46	0.49	0.49	0.48
	(Relative, mg/100g)	M	1.44	1.53	1.59	1.66*	1.58
	(Absolute, mg)	F	0.35	0.34	0.33	0.33	0.34
	(Relative, mg/100g)	F	1.35	1.32	1.38	1.33	1.37
Liver	(Absolute, mg)	M	1.93	1.84	1.82	1.74*	1.72*
	(Relative, mg/100g)	M	6.08	6.09	5.66	5.90	5.66*
	(Absolute, mg)	F	1.47	1.40	1.34	1.39	1.36
	(Relative, mg/100g)	F	5.72	5.53	5.55	5.64	5.56
Lung	(Absolute, mg)	M	0.23	0.26*	0.25	0.23	0.22
	(Relative, mg/100g)	M	0.72	0.88**	0.80**	0.78*	0.74
	(Absolute, mg)	F	0.22	0.21	0.20	0.23	0.22
	(Relative, mg/100g)	F	0.87	0.82	0.82	0.92*	0.89
Spleen	(Absolute, mg)	M	0.09	0.09	0.10	0.09	0.09
	(Relative, mg/100g)	M	0.28	0.31	0.32	0.30	0.29
	(Absolute, mg)	F	0.10	0.09	0.10	0.10	0.09
	(Relative, mg/100g)	F	0.38	0.37	0.41	0.38	0.38

** Significantly different from control at 0.05 level.*
*** Significantly different from control at 0.01 level.*

Relative heart weights in all treatment groups (both sexes) were significantly higher than control; however, absolute heart weights were significantly higher in males only at 800 and 1200

ppm and in females at 1200 and 1600 ppm levels. Both absolute and relative liver weights were lower than control in 1600 ppm males.

No significant gross findings were observed. Histopathological evaluations were not performed in this study.

Based on the results of this study, dietary dose levels of 100, 300 and 900 ppm were selected for the mouse carcinogenicity study.

b. 21 Month Carcinogenicity Study in Mice

Testing Facility:

Study Number: T7010709 (Sponsor's number)

Study Dates: Initiation of dosing - 10/7/81
Autopsy of last animal - 7/7/83

GLP Compliance: Studies were done in accordance with GLP regulations.

Animals:

Strain: Bor:NMRI (SPF HAN)
Sex: Both sexes
Age and Wt: 4 to 6 weeks old/20-22 g
Housing: Individually housed in Makrolon Type I cages.

Mode of Administration of Test Agent: Powdered diet.

Dose Levels: 0, 100, 300 and 900 ppm dosage levels of (Batch No. 662845, purity-98.1%) were used on the basis of results of the 28-day dietary dose rangefinding study. The stability and the concentration of drug in the diet were determined periodically. The concentrations of the drug in diet, at all intervals, were more than 89% of the theoretical values, and the compound was found to be stable in the diet for at least 10 days. However, there is no indication that concentrations of drug in diet were adjusted periodically to maintain a constant mg/kg body weight exposure.

No. of Animals: Equal numbers of males and females (50+20*/sex/dosage level) were used. *Additional 20 mice included in each group were sacrificed 12 months after the initiation of dosing for interim investigations.

Observations/Measurements:

Appearance/Behavior monitored twice daily and a detailed assessment of each individual animal was made on a weekly basis, with particular attention given to posture, general behavior, body surfaces, orifices and breathing and elimination products.

Body weight determinations were done at the beginning of the study, once a week until 27th week and every 2 weeks thereafter and also before the termination of the study.

Food intake was calculated on a weekly basis up to 23rd week and every two weeks thereafter.

Hematological (RBC, WBC, platelet and reticulocyte counts, differential white cell counts, hemoglobin, hematocrit, MCV, MCH and MCHC) and clinical chemistry [alkaline phosphatase, transaminases (ASAT and ALAT), plasma creatinine, urea, blood glucose, cholesterol, bilirubin and total plasma proteins] investigations were done at 12 months (interim sacrifice group) and also at the end of the study (20 animals/sex/treatment group).

Autopsies were done on all mice which died during the course of the study or that were killed in extremis, and also on those that were sacrificed at 12 months and at the termination of the study. Nine major organs were weighed and sections of various organs and tissues (about 38 different tissues/mouse) and gross lesions were preserved for histopathologic evaluation. At 12 months, these evaluations were done only on tissues from 0 and 900 ppm dosage groups and also on any tissue from 100 and 300 ppm groups which looked tumorous macroscopically. At the termination of the study, tissues from 0, 300 and 900 ppm dosage groups were examined histologically (only stomach, pituitary, uterus and liver were examined from 100 ppm group).

Statistical analysis on body weights, clinical laboratory values and organ weights were done using two-tailed U test according to Mann and Whitney, and Wilcoxon. The survival data were analyzed by the statistical software package using the generalized Wilcoxon test. Statistical analysis of tumor findings was done using the death rate method for malignant tumors and the prevalence method for benign tumors (Peto et al.). Because of the high mortality rate in high dose males, the death rate and the prevalence methods were used combined for the analysis of hepatocellular tumor data.

Interim Sacrifice:

Surviving animals from interim sacrifice groups (originally 20 mice/sex/dosage group) were killed at 12 months.

Achieved Dose Levels:

Average daily drug intake* (mg/kg body wt)

Sex	Dose (ppm) 0	100	300	900
Male		19.37	58.06	162.93
Female		24.99	74.36	217.28

(*The drug intake was calculated from the average daily food intake/animal/group for the whole duration of the study.)

Mortality:

Mortality data is summarized below and it is presented graphically in Figures 9 &10.

Mortality of Mice Receiving Nisoldipine in Diet for 21 Months

Daily Dose (ppm in diet)	Number of Mice (M/F)	Number of Dead (M/F)	% Mortality (M/F)
	6 Months		
0	50/50	0/0	0/0
100	50/50	2/0	4/0
300	50/50	0/0	0/0
900	50/50	3/1	6/2
	12 Months		
0	50/50	0/2	0/4
100	50/50	3/5	6/10
300	50/50	0/3	0/6
900	50/50	8/5	16/10
	18 Months		
0	50/50	5/21	10/42
100	50/50	11/19	22/38
300	50/50	7/20	14/40
900	50/50	29/21	58/42
	21 Months		
0	50/50	14/28	28/56
100	50/50	15/34	30/68
300	50/50	19/34	38/68
900	50/50	40/32	80/64

The mortality rates in treated females (all groups) were not significantly different from controls at any given interval. However, the incidence of deaths in females was high in all groups including controls from 12 months onward. The mortality rates in males from 900 ppm group, especially at 21 months, were significantly higher ($p<0.001$) compared to controls or other

Fig.9 : Mortality curves of male mice which received in the diet 21 months

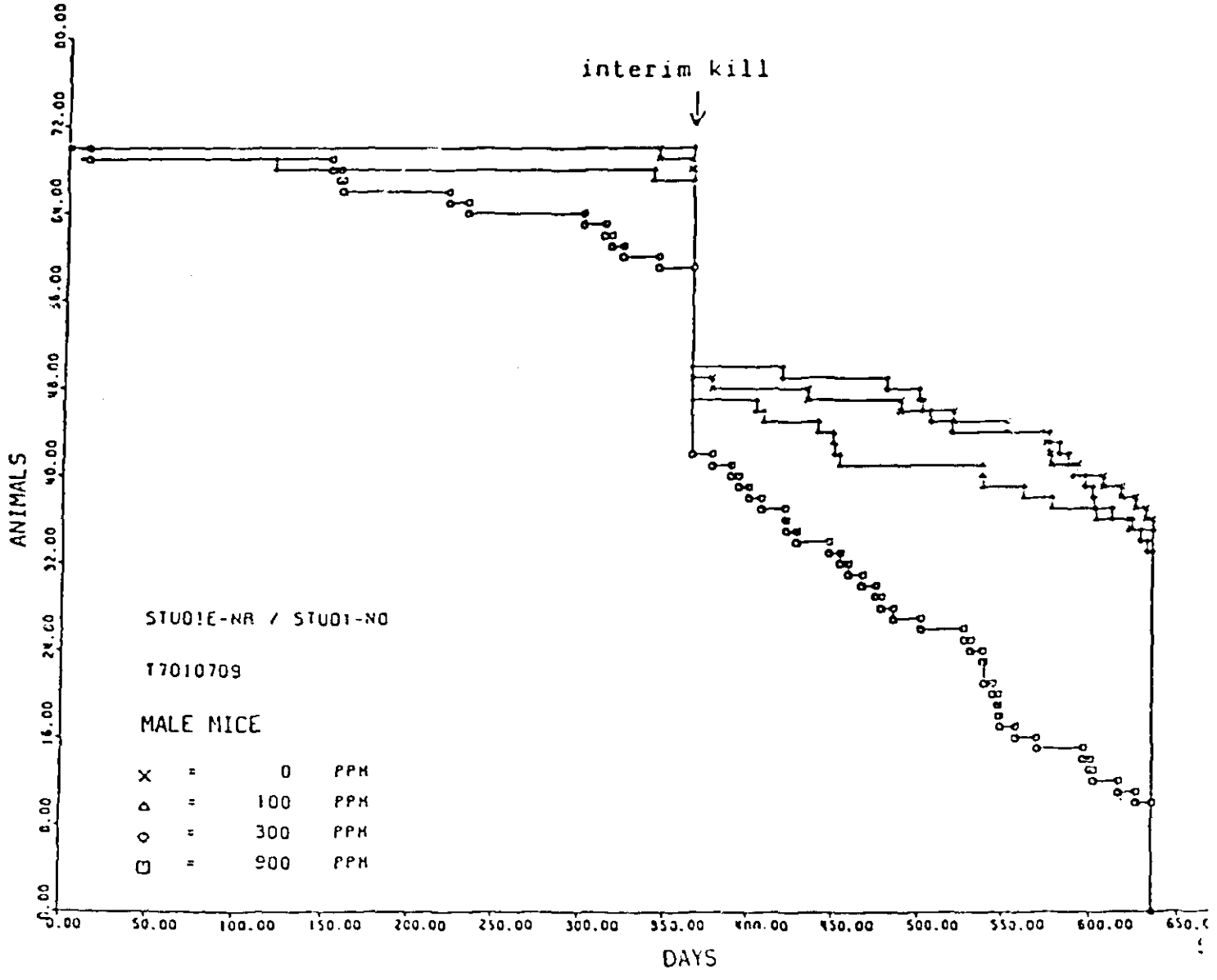
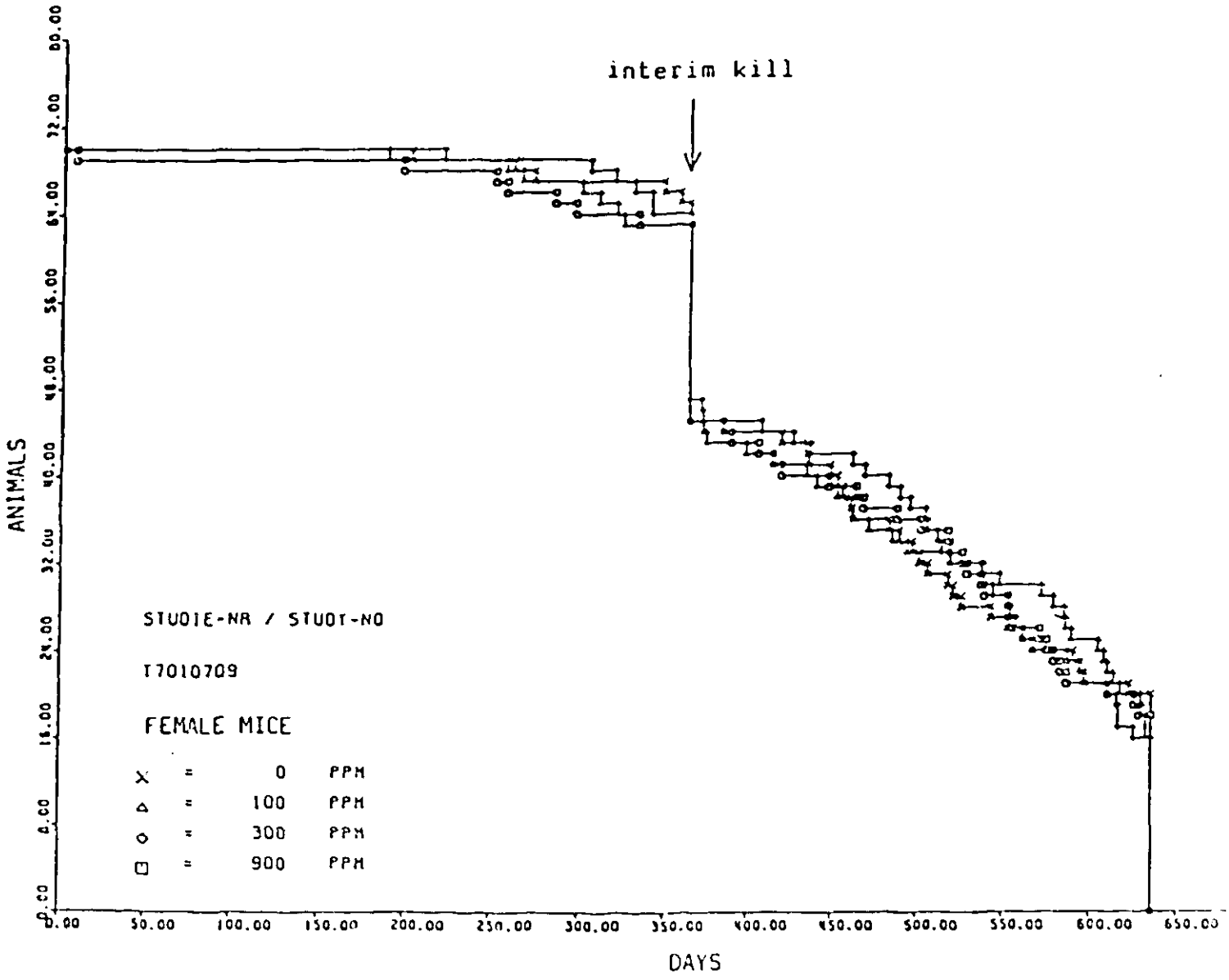


Fig. 10: Mortality curves of female mice which received in the diet
21 months



lower dosage groups. No significant difference noticed in this parameter between males of low or middle dosage groups and controls. The increased mortality of males in the high dose group is partly attributed to pharmacodynamically induced colonic atonies. Autopsy of animals that died or were killed in extremis frequently showed that the large intestine was tightly filled with solid faeces resulting from colonic atony.

Using the Cox and the generalized Wilcoxon methods for testing the heterogeneity in survival distribution, FDA statisticians observed a statistically significant difference (at 0.05 level) in the survival distribution in males, but not in females (for both of the above tests, the p values for males were <0.00001).

Drug Associated Findings:

No treatment related clinical signs were seen in this study. The food intake in males from the 900 ppm group was about 9% less than in control males. Average body weights are presented graphically in Figures 11 & 12. Statistically significant reductions in body weights at certain weeks were seen especially in males of 900 ppm group and to a lesser extent in 100 ppm group. However, at the termination of the study, no significant body weight differences were seen between treatment and control groups (both sexes). Leukocyte counts at 21 months were significantly lower ($p<0.01$) in males (900 ppm) and females (300 and 900 ppm groups) compared to respective controls. However, differential counts did not show any significant variations in the proportion of different cell types between treated and control mice. The hemoglobin and hematocrit values in mid and high dose males were significantly lower at 12 months, but not at 21 months. The blood glucose concentration was significantly higher ($p<0.01$) in males (300 and 900 ppm) at 12 months and also at 21 months (all treatment groups). Females showed a similar increase only at 12 months. All these values were reported to be within the range of historical control values. Significant elevations in blood urea levels were seen only at 12 months in all treated male groups and in high dose females.

Macroscopically, swollen gastric mucous membranes were observed more frequently in treated males than in controls (0 ppm - 5; 100 ppm - 12; 300 ppm - 14; 900 ppm - 14). The incidence of enlarged hearts was more in males of 900 ppm group (0 ppm - 7; 100 ppm - 2; 300 ppm - 11; and 900 ppm - 23). In mice that died or were killed in extremis, the incidence of large intestines impacted with solid feces was higher in both sexes at the highest dosage level (0 ppm - males 5 and female 0; 900 ppm - male 13 and female 9).

The heart and liver weights (absolute and relative) in males (21 months) were significantly increased at 300 and 900 ppm dose levels, but the weights of adrenals (absolute and relative) were significantly decreased in all treated male groups compared to controls. In females, the heart and liver weights were

Figure II : Body weight curves for male mice receiving in their food for 21 months

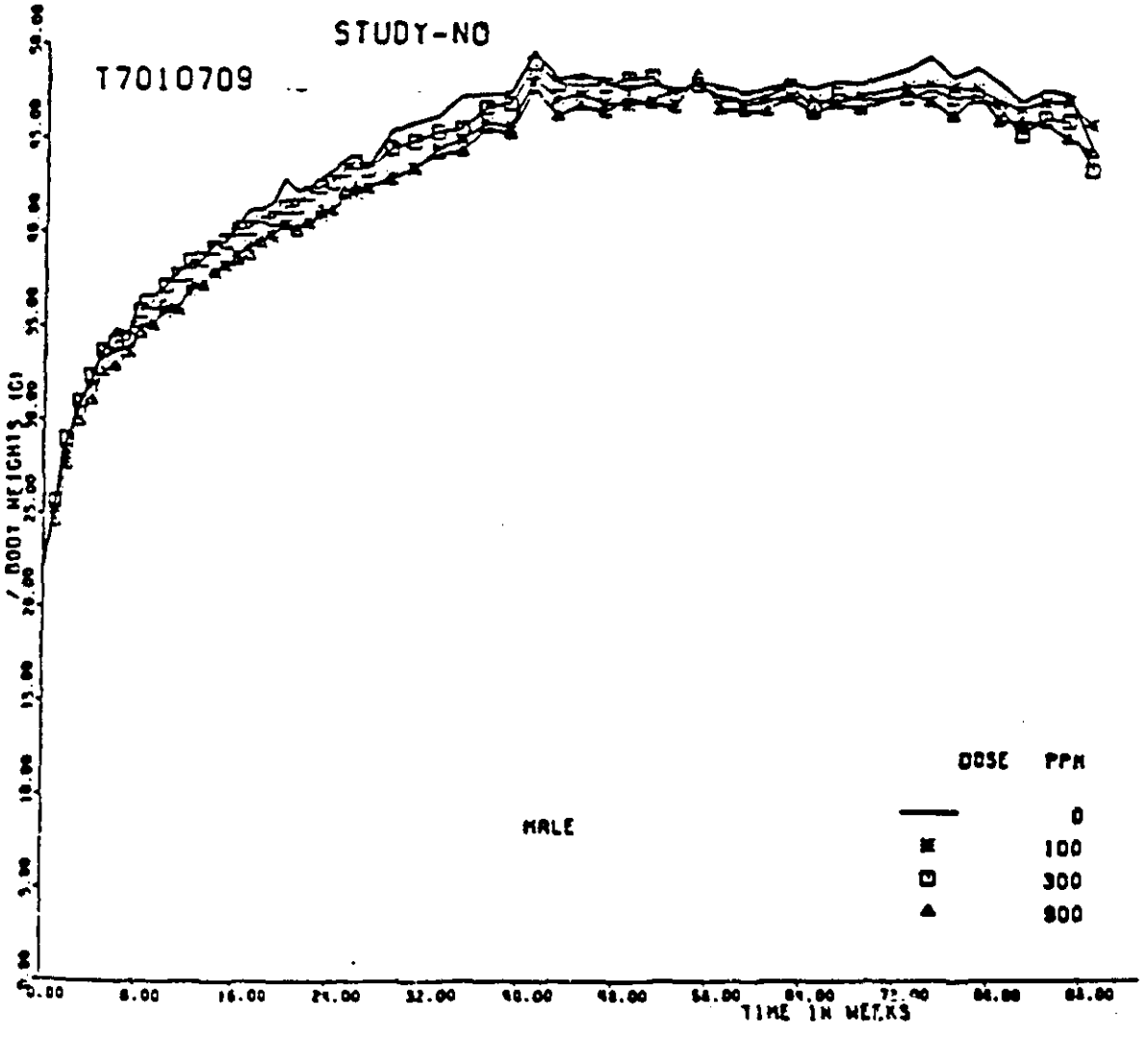
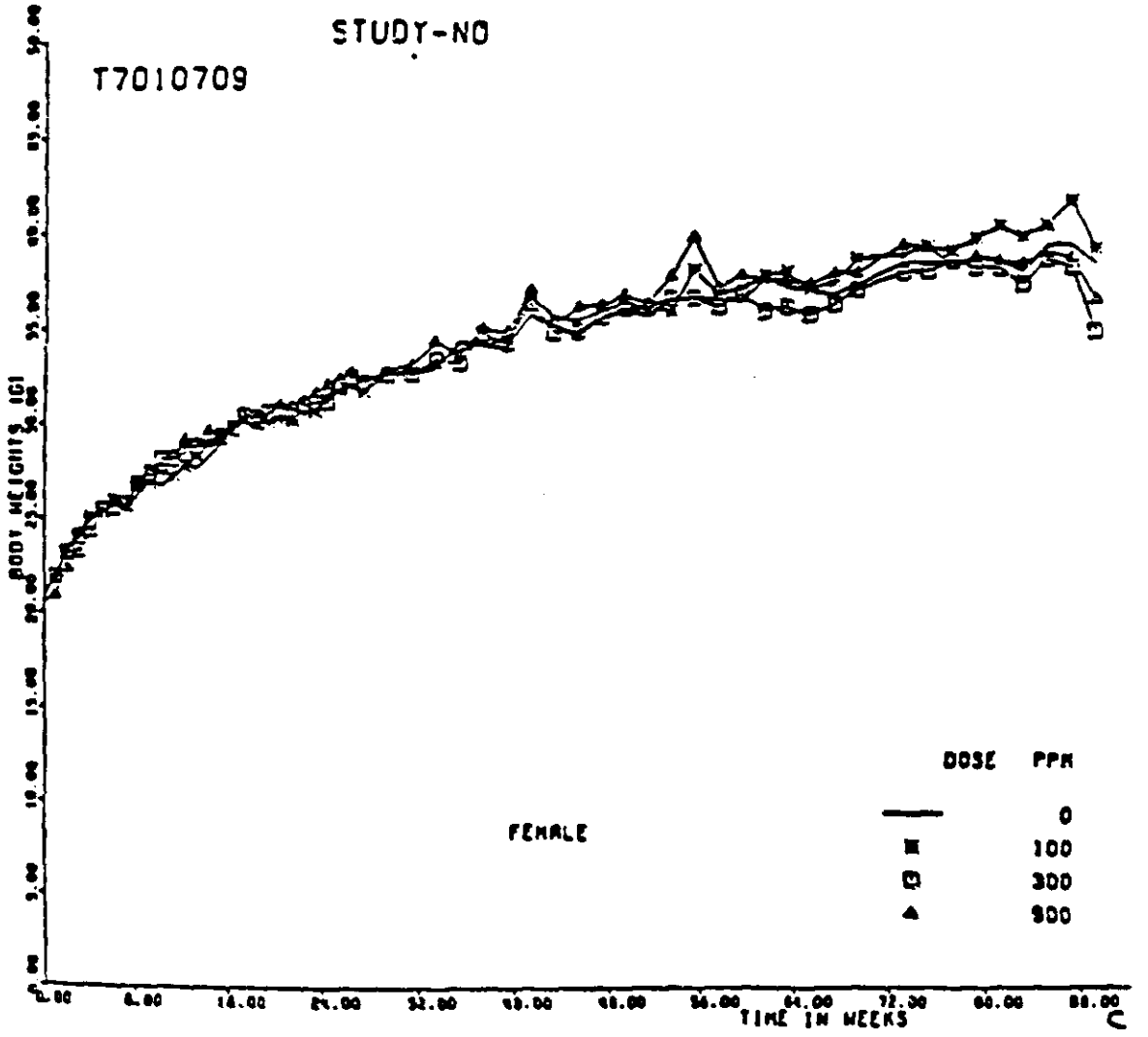


Figure12: Body weight curves for female mice receiving . in their food for 21 months



significantly higher only at the highest dose level. Females that received either 300 or 900 ppm doses had significantly lower kidney weights at 21 months. Significantly increased liver weights (absolute and relative) were seen in mid and high dose females at the interim sacrifice.

Histologically, hyperplastic mucosa of the glandular stomach was found more frequent in treated males than in females. The incidence of this condition is given below.

Incidence of Hyperplastic Mucosa of the Glandular Stomach

Dose (ppm)	Percent affected	
	Male	Female
0	18	6
100	31	16
300	36	10
900	24	16

The above values are reported to be within the range of historical control values (Table 10).

A dose dependent increase in the occurrence of intracytoplasmic vacuoles near the nucleus was seen in the hepatic cells, more in females than in males. Round cell infiltrates in the kidney and senile nephropathy were predominantly found in females. The endometrium was often found to be hyperplastic (0 ppm - 13%; 100 ppm - 34%; 300 ppm - 33%; and 900 ppm - 28%). The above incidences are reported to be within the historical control ranges for this strain of mouse (Table). An increased incidence of pituitary hyperplasia was seen in females (0 ppm - 17%; 100 ppm - 13%; 300 ppm - 22%; 900 ppm - 42%).

The incidences of tumors observed at the interim sacrifice are given below.

Comparative Summary of Tumors at 12 Months According to Location, Type and Malignancy

Sex:	Males		Females	
Dose (ppm in diet):	0	900	0	900
Reticulocytary system: malignant lymphoma	1	1	2	4
Lung: alveologenic carcinoma (malignant)	0	2	1	2
Liver: hepatocellular carcinoma (malignant)	0	1	0	0
Stomach: kerato-acanthoma	0	0	1	0
Number of blastoma carriers	1	4	4	5
Number of malignant tumors	1	4	3	6
Number of benign tumors	0	0	1	0
Number of mice investigated	20	20	20	20

Table 10

Historic control values: NMRI mouse 1980 to 1984

	Number					
Test No.	1	2	3	4	5	6
Adenomatous gastric mucosal hyperplasias in males	9*	20	10	32	5	27
n	50	50	50	44	47	48
%	18	40	20	73	11	56
in females	1*	8	11	14	10	13
n	50	48	49	46	47	48
%	2	17	22	30	21	27
*classified as adenoma						
Liver tumour in males	7	3	9	5	1	6
n	50	50	50	45	46	48
%	14	6	18	11	2	12
Uterine hyperplasias	0	19	21	23	0	33
n	50	46	49	45	45	46
%	0	41	43	51	0	71

Glucose concentration in the plasma: 4.32 - 9.36 mmol/l (male)
4.51 - 7.75 mmol/l (female)
Urea concentration in the plasma: 5.96 - 15.12 mmol/l (male)
4.05 - 14.99 mmol/l (female)

n = Number of organs evaluated

The increased incidence of lymphoma of reticulohistiocytary system (RHS) observed in high dose females is considered to be incidental since the incidence of this tumor at 21 months was higher in the control group than in treated groups.

The overall incidences of benign and/or malignant tumors and the total number of tumor bearing animals (21 months) for both sexes at 0, 300 and 900 ppm dose levels are given in Table 11. There is no significant increase in the neoplasm incidence among treated animals of either sex compared to respective controls (sponsor's analysis). Moreover, there is also no difference in tumor occurrence between 300 and 900 ppm dosage groups. Incidence of tumors according to the location and the type is presented in Table 12. Because of the increased incidence of hepatocellular tumors in males especially in the 900 ppm group (only few females, 900 ppm group, had this type of tumor) at 21 months and also because of the occurrence of hepatocellular carcinoma in a male mouse from interim sacrifice, an additional investigation was carried out by examining more hepatic tissue sections (5 per animal) from male mice of each group for hepatocellular tumor occurrence. This second study showed additional cases of hepatocellular tumors as follows: 1 each from 100 and 900 ppm groups, 4 from 300 ppm and 1 from control groups. Combined incidences of these tumors (males) from the original and additional investigations (49-50 mice/group) and the p values from the trend test (death rate method) are given below. (The results of sponsor's statistical analyses of liver tumor data are summarized in Table 13.)

	Dose(ppm)				p value
	0	100	300	900	
hepatocellular adenoma	2	2	2	3	0.0735
hepatocellular carcinoma	3	4	5	8	0.0015
hepatocellular tumors (all)	5	6	7	11	0.0004

Thus, the sponsor's analysis showed significant positive linear trends (at 0.05 level) for the incidences of hepatocellular carcinoma and hepatocellular tumors (all) in male mice. Analysis of the tumor data by FDA statisticians showed that there were no significant positive linear trends for the incidences of hepatocellular carcinoma (p=0.0762) and hepatocellular tumors (p=0.0514) in male mice. According to FDA statisticians, the above discrepancies in p values observed in sponsor's and FDA analyses are attributed to "1. the sponsor did not apply the survival-adjusted method and 2. the ordinal dose levels 0, 1, 2 and 3 were used in sponsor's analysis."

FDA analysis of tumor data showed a significant positive linear trend for inverted papilloma of pars cutanea of the stomach in male mice (p=0.0072). The incidence of the above tumor is as follows: 0 ppm - 0/50; 100 ppm - 0/49; 300 ppm - 0/50; and 900 ppm - 2/50. Pairwise comparison also showed significant difference between high dose and control groups (p=0.0435). Historical control data from 21 month studies in NMRI mice, conducted during a 7 year period from July 1981 to August 1988, showed that papillomas of the stomach occurred in 2 of the 18 studies evaluated (page 65e), in 1/49 males and 1/47 females examined (amendment to original application dated May 31, 1994). Moreover, incidence rates upto 4% were seen for the above tumor in NMRI control male mice in carcinogenicity studies conducted between 1974 and 1979 (page 65f). Although statistically significant, the incidence rate (4%) observed in the present study for the stomach papilloma is considered to be within the historical control range for NMRI mice.

Significant positive linear trends were also reported by FDA statisticians for the urinary bladder benign stromal tumor in male mice and RHS malignant lymphoma* in females. However, when the incidences of urinary bladder stromal tumors are combined (benign + malignant, benign + polypous, or benign + malignant + polypous tumors), no statistically significant trend was seen. In the case of RHS malignant lymphoma also, if all malignant lymphomas of different locations are combined, then, no significant linear trend was observed.

*Note: The sponsor has listed all malignant lymphomas, irrespective of locations, under RHS system; however, for some lymphomas, the anatomic site (organ) is specified (e.g. lymphoma of adrenal or heart etc.) but for others no site is given (listed only as lymphomas). By using the combined incidences of all lymphomas, no treatment-related increased incidence of this tumor was seen in sponsor's statistical analysis. [According to NTP guidelines (McConnel et al, 1986. Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. JNCI 76: 283-289), lymphomas of all types can be combined for statistical evaluation.]

DOG STUDIES (S.Stolzenberg)

a. 4-Week Oral Administration Study

Testing Facility

Pharma-Report No: 7075
Study No: Not given

Study Dates: 11/8/76 to 12/9/76

GLP compliance: This study predates GLP compliance requirements.

Animals: Purebred beagles, 2 males and 2 females per group were used. At the start of dosing, the animals were 25 to 30 weeks old, with body weights of 7.4 to 11.8 kg.

Dose Levels/Mode of Administration: The test substance (Batch 2/76) was administered at doses of 0, 1, 3 and 10 mg/kg, once daily, 4 to 6 hours before feeding, in a vehicle of polyethylene glycol 400, glycerol and water, in gelatin capsules.

Observations/Measurements: Body weights were obtained before the start of treatment and weekly thereafter. Appearance, behavior, body posture, appetite and feces were checked daily. Neurological investigations (pupillary reflex, patellar reflex and extensor postural reflex) and body temperature measurements (rectal) were conducted pretreatment and after 2 and 4 weeks. Ophthalmoscopy (direct) was performed at pre-treatment and after 4 weeks. ECG measurements (Leads I, II and III) were recorded on the 1st, 11th and 23rd day, immediately before administration and 1 and 24 hours after administration. Femoral artery blood pressure was measured on the 1st, 11th and 23rd day, before administration and 1 and 24 hours after, via a Stratham element, Hellige measuring bridge and Hellige recorder. Blood and 6 hour urine samples were obtained before treatment, then after 1 and 4 weeks, for hematology, blood chemistry and urinalysis. Post-mortem examination included weights of 12 or 13 major organs (including gonads and prostate), gross pathology and complete histopathology (31 or 32 organs).

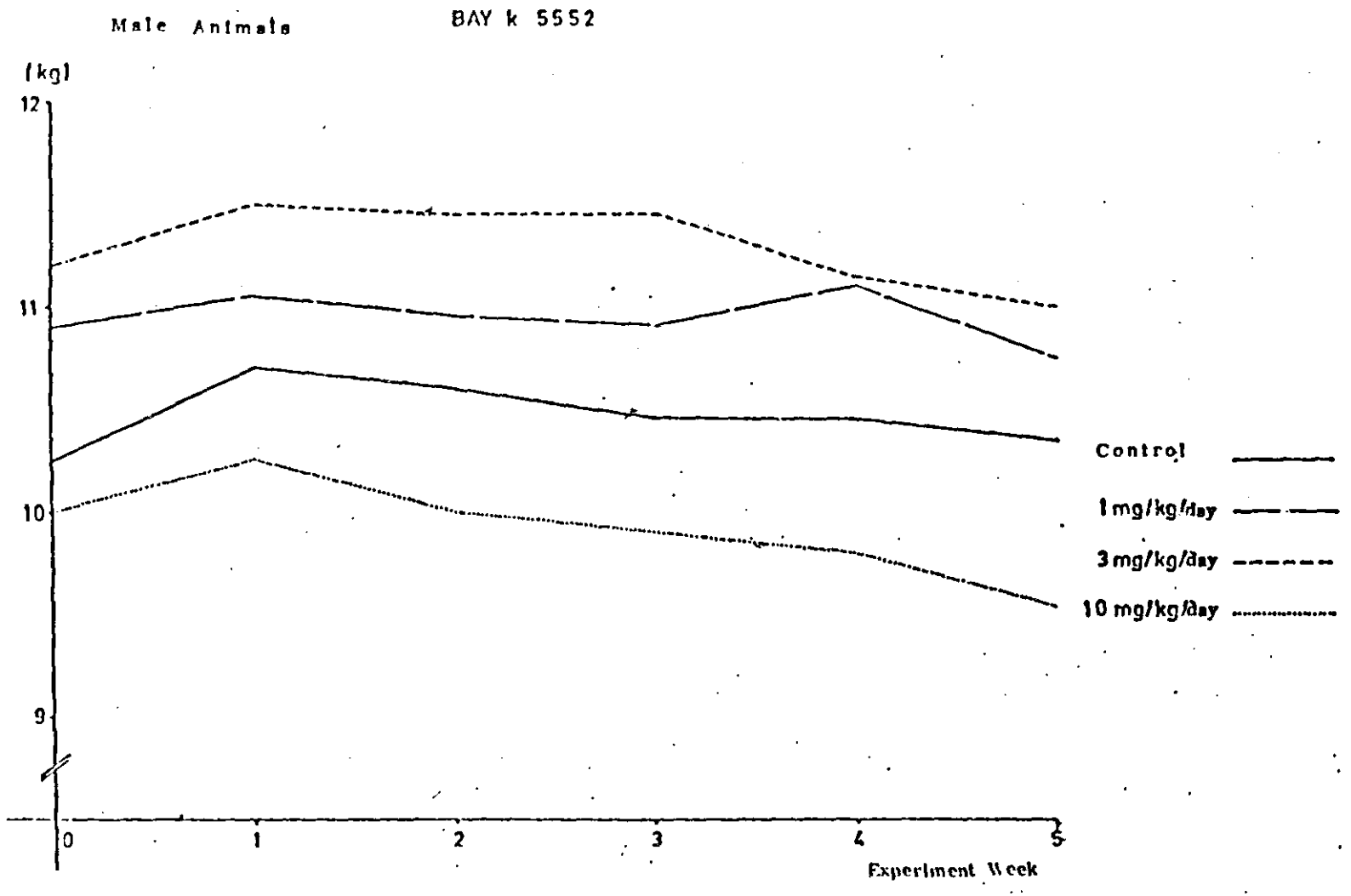
Mortality: There were no deaths.

Drug Associated Findings: Slightly reduced weight gain was observed in the high dose males, with a reduced food consumption in both high dose females and in one high dose male, from the middle of the third week to the end of the study. In the 10 mg/kg treated animals, a distinct ST drop (manifestation of a possible myocardial ischemia) was observed in one male 1 hour after the 1st and 23rd dose, and in one female 1 hour after the 1st dose. No treatment related effects on P or Q waves or QRS complex were observed at any time. Heart rates determined from

ECGs, showed dose dependent increases one hour after dosing on days 1, 11 and 23, and with the high dose, bradycardia was still evident 24 hours after dosing on days 11 and 23. Systolic and diastolic blood pressures at 1 hour post dosing were decreased by a mean of 30 to 50% in all treated groups (dose dependent) on days 1, 11 and 23. As a rule, blood pressures returned to pre-treatment levels by 24 hours after treatment, except after day 1, when they remained lower for the 1 and 10 mg/kg groups.

Although no gross pathology or organ weight changes due to treatment were noted, histopathology revealed that the hearts of both females and 1 of the 2 males on the high dose had myocardial scars in one or both left ventricular papillary muscles. The effect was attributed to hypoxic damage related to vasodilator-induced heart rate increase, "a known damage mechanism in the dog". The ST drops noted above were observed in two of the dogs with myocardial scars. The ST drops and the bradycardia (which was most pronounced in a male with the most severe lesions) were attributed to the heart muscle damage.

Weight Gains of the Male Dogs. The weights were measured in each case at the end of the experimental week.



BAY k 5552

Heart Rate (Beats per Minute)

(Average Values)

Dose mg/kg	Time of Investigation	Before Administration	1 Hour After Administration	% Deviation from 1-Hour Value	24 Hours After Administration
0	Preliminary Investigation	136			
	1st Administration	148	115	- 22	140
	11th Administration	118	108	- 8	125
	23rd Administration	123	123	0	118
1	Preliminary Investigation	158			
	1st Administration	143	253	+ 77	160
	11th Administration	120	235	+ 96	113
	23rd Administration	113	223	+ 97	110
3	Preliminary Investigation	128			
	1st Administration	133	195	+ 47	143
	11th Administration	113	213	+ 88	110
	23rd Administration	98	213	+ 117	90
10	Preliminary Investigation	133			
	1st Administration	133	210	+ 58	148
	11th Administration	68	223	+ 228	75
	23rd Administration	80	200	+ 150	81

Blood Pressure (mmHg)

(Average Values)

Dose mg/kg	Time of Investigation	Before Administration		1 Hour After Administration		% Deviation from 1-Hour Value		24 Hours After Administration	
		s	d	s	d	s	d	s	d
0	Preliminary Investigation								
	1st Administration	172	107	179	99	+ 4	- 7	181	101
	11th Administration	176	95	171	102	- 3	+ 7	174	110
	23rd Administration	181	78	191	96	+ 6	+ 24	187	97
1	Preliminary Investigation								
	1st Administration	171	95	115	63	- 33	- 34	116	83
	11th Administration	176	101	96	54	- 45	- 47	181	110
	23rd Administration	173	94	114	68	- 34	- 28	193	118
3	Preliminary Investigation								
	1st Administration	177	101	99	52	- 44	- 49	179	96
	11th Administration	178	106	78	49	- 56	- 54	176	109
	23rd Administration	178	99	107	53	- 40	- 46	188	96
10	Preliminary Investigation								
	1st Administration	177	94	114	51	- 36	- 47	149	74
	11th Administration	194	111	98	51	- 49	- 54	194	111
	23rd Administration	203	111	116	53	- 43	- 52	231	133

s = systolic pressure d = diastolic pressure

Histological Data

Oral, Dogs (2 Week Experiment)

Animal No.	Sex	Dose and Frequency of Administration	Heart	Lung	Liver	Spleen	Kidney	Adrenals
F 813	♂	Control (0 mg/kg)	0	Ici +	0	0	0	0
F 823	♂	"	0	Ici +	Ici +	0	0	0
F 800	♀	"	0	Ici +	Ici +	0	0	0
F 802	♀	"	0	Ici +	Ici +	0	0	0
F 807	♂	10 mg/kg	Fi2	Ici +	0	0	0	0
F 817	♂	"	0	Ici +	0	0	0	0
F 814	♀	"	Fi1-2	Ici1	Ici +	0	0	0
F 818	♀	"	Fi +	Ici2	Ici +	0	0	V+

List of Abbreviations

Histological Data

At	=	**Atrophy**
Cy	=	**Cyst**
Fi	=	**Focal fibrosis with isolated mononuclear cells (Figures 4 and 5)**
Ici	=	**Cellular or inflammatory-cellular infiltration**
P	=	**Parasitic lesion (bore hole, granuloma, eosinophilic infiltration)**
0	=	**Finding within the normal variability, which, in particular, corresponds to the species and to the age of the experimental animals and to their conventional housing conditions**
ø	=	**Not investigated (section missing)**
Pi	=	**Yellow-green (hematogenous) pigment**
Th	=	**Thrombus**
V	=	Cytoplasmic vacuoles

Intensity of the Changes

+	=	very slight, indicated
1	=	slight
2	=	moderate
3	=	severe

b. 13-Week Oral Administration Study in Dogs

Pharma-Report No: 10,380
Study No: B/K 5552/023

Performing Laboratory:

Dates Performed: 8/21/80 to 11/25/80

Quality Assurance: No statement of GLP compliance is included.

Test Animals: Purebred beagles, 3 males and 3 females per group, were used. At the start of dosing, the animals were 38 to 51 weeks old, with body weights of 6.8 to 10.8 kg.

Dose Levels/Mode of Administration: The test substance (Batch 576,923) was administered at doses of 0, 1, 2.5 and 6.25 mg/kg, once daily, 4 to 6 hours before feeding, in a vehicle of polyethylene glycol 400, glycerol and water, in gelatin capsules.

Observations/Measurements: Body weights were obtained before the start of treatment and weekly thereafter. Appearance, behavior, body posture, appetite and feces were checked daily. Neurological examinations (pupillary reflex, patellar reflex and extensor postural reflex), ophthalmoscopic examinations (direct) and body temperature measurements (rectal) were performed pretreatment and after 2, 5 and 12 weeks. Femoral artery blood pressure was measured at the time of the first dose, and in weeks 3, 6 and 13, before administration, and 1 and 24 hours after, via a Stratham element, Hellige measuring bridge and Hellige recorder; ECG measurements (Leads I, II and III) were recorded at the same time periods. Blood and 6 hour urine samples were obtained before treatment, then after 3, 6 and 13 weeks, for hematology, blood chemistry and urinalysis. Postmortem examination included weights of 12 (female) or 13 (male) organs, gross pathology and complete histopathology (31 or 32 organs for control and high dose, but all 3 doses for heart).

Mortality: There were no deaths.

Drug Associated Findings: Circumoral reddening of the skin and reddening of the conjunctiva in the mid and high dose groups, and ataxia in the high dose group, occurred regularly throughout the treatment period, around 1 hour after dosing. Blood pressure decreased (systolic decreased to a greater extent than the diastolic), and heart rate increased (data on heart rate not provided by sponsor) at 1 hour post dosing in all 3 treated groups. Neither of these two effects were considered to be dose related, and values returned to pretreatment levels by 24 hours post-treatment. No changes in ECG occurred at low and mid doses. One high dose male developed a ventricular tachycardia with a "bundle-branch-block-like deformation of the QRS complex",

diagnosed 1 hour after the first dose. For this animal, another ECG was taken on the following day 2 hours after dosing; the P wave was still elevated and the ST segment again showed sagging depression. "On the 19th day in this dog, no pathological finding in the ECG was observed" but this animal showed extra systoles and an elevated P wave. Serum chemistry effects included a small increase in GOT during week 6. The only compound related post-mortem finding noted was scaring of the left ventricular papillary muscles of 1 male and 1 female at the high dose, and 1 female at the mid dose. Histopathology revealed focal fibrosis with isolated mononucler cells and a cellular and inflammatory-cellular infiltration.

Study No. 5552/023

Blood Pressure (mm Hg) - Average Values

Dose mg/kg	Time of Examination	Before administration		1 hour after administration		% Deviation of 1 hour value		24 Hours after administration	
		s	d	s	d	s	d	s	d
0	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration	210	130	180	110	-14	-15	180	110
	in the 3rd week	200	120	190	120*	- 5	0	205	125**
	in the 6th week	225	135	170	90	-24	-33	165	105**
	in the 13th week	200	120	205	130	+ 2	+ 8	195	120*
1.0	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration	190	115	140	75*	-26	-35	175	115
	in the 3rd week	195	115	135	75	-31	-35	190	120
	in the 6th week	185	115	85	50	-54	-57	175	100**
	in the 13th week	190	120	130	80	-32	-33	195	115**
2.5	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration	190	105	145	70	-24	-33	180	105
	in the 3rd week	195	115	115	60	-41	-48	200	120
	in the 6th week	195	115	100	50	-49	-56	175	105
	in the 13th week	195	120	130	70	-33	-42	175	110
6.25	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration -	185	115	110	60	-41	-48	185	125
	in the 3rd week	190	115	110	55	-42	-52	170	110**
	in the 6th week	185	115	80	45	-57	-61	170	105
	in the 13th week	195	120	100	60**	-49	-50	190	125

s = systolic blood pressure; d = diastolic blood pressure *n = 5; **n = 4

5552/Study 023

Animal No.	Sex	Dose	Esophagus	Stomach	Intestine	Mesenteric Lymph Nodes	Thymus	Gall-bladder	Urinary Bladder
K 103	♂	Control	0	0	0	0	0	0	0
K 109	♂	Control	0	0	0	ø	At3	0	0
K 121	♂	Control	0	0	0	0	0	0	0
K 108	♀	Control	0	0	0	0	0	0	0
K 112	♀	Control	0	0	0	0	At2	0	0
K 120	♀	Control	0	0	0	0	ø	0	0
K 93	♂	6.25 mg/kg	0	0	0	0	ø	0	0
K 115	♂	6.25 mg/kg	0	0	0	0	0	0	0
K 117	♂	6.25 mg/kg	0	0	0	0	ø	0	0
K 102	♀	6.25 mg/kg	0	0	0	P/Ic1i	At1	0	0
K 104	♀	6.25 mg/kg	0	0	0	P/Ic12	0	0	0
K 118	♀	6.25 mg/kg	0	0	0	0	0	0	0

5552/Study 023

Animal No.	Sex	Dose	Heart	Animal No.	Sex	Dose	Heart
K 103	♂	Control	0	K 113	♂	2.5 mg/kg	0
K 109	♂	Control	0	K 119	♂	2.5 mg/kg	0
K 121	♂	Control	0	K 123	♂	2.5 mg/kg	0
K 108	♀	Control	0	K 122	♀	2.5 mg/kg	F11 P1+
K 112	♀	Control	0	K 124	♀	2.5 mg/kg	0
K 120	♀	Control	0	K 126	♀	2.5 mg/kg	0
K 93	♂	6.25 mg/kg	0	K 79	♂	1.0 mg/kg	0
K 115	♂	6.25 mg/kg	F12-3 Ic11	K 105	♂	1.0 mg/kg	0
K 117	♂	6.25 mg/kg	0	K 107	♂	1.0 mg/kg	0
K 102	♀	6.25 mg/kg	0	K 110	♀	1.0 mg/kg	0
K 104	♀	6.25 mg/kg	F11	K 114	♀	1.0 mg/kg	0
K 118	♀	6.25 mg/kg	0	K 116	♀	1.0 mg/kg	0

c. 52-Week Oral Administration Study in Dogs

Study No: T 20 10 506

Performing Laboratory:

Dates Performed: July 13, 1981 to July 11, 1982

Quality Assurance: No statement of GLP compliance is included.

Test Animals: Purebred beagles, 4 males and 4 females per group. At the start of dosing, the animals were 38 to 51 weeks old, with body weights of 6.9 to 11.2 kg.

Dose Levels/Mode of Administration: The test substance (batch 57 69 23) was administered at doses of 0, 0.3, 1.0 and 3.0 mg/kg, once daily, 7 days per week, 4 to 6 hours before feeding, in a vehicle consisting of 85.3% polyethylene glycol 400, 4.8% anhydrous glycerol and 9.9% water, contained in gelatin capsules.

Observations/Measurements: Body weights were obtained before the start of treatment and weekly thereafter. General appearance was checked "several times a day". Neurological exams were conducted and body temperatures were checked pretreatment and after 3, 6, 17, 29, 39 and 50 weeks. Ophthalmoscopy was performed pre-treatment and after 12, 31, 38 and 51 weeks. Blood pressure and ECG were measured pre-treatment and after 3, 6 and 17, 29, 39 and 50 weeks, before dosing, then 1 and 24 hours after dosing. The methods and instruments used were the same as in the preceding dog studies. Blood and 6 hour urine samples were obtained before treatment, then after 3, 6, 13, 26, 39 and 52 weeks, for hematology, blood chemistry and urinalysis. Postmortem examination included weights of 11 or 12 organs, gross pathology and complete histopathology (31 or 32 organs for all animals on test). Liver enzyme induction of O-demethylase, N-demethylase and cytochrome P_{450} content of liver homogenates were measured.

Mortality: No deaths occurred.

Drug Associated Findings: Slight reddening of the mucosa and skin, observed in all nisoldipine treated groups, was considered to be due to the vasodilator effect of the drug. Dose related decreases in blood pressure and resultant increases in heart rate were observed. Twenty-four hours after dosing, all values had returned to normal. Slight ST segment depression, T wave inversion and QT segment shortening were observed, which were all reversible (data on ECG could not be found) and considered to be due to increased heart rate.

Study No: T 20 10 506

BLOOD PRESSURE (mm Hg)

(Means n = 8)

DOSE	TIME	before admin.	1 h after admin.	% difference **	24 h after admin.
0.0 mg/kg	1st admin.	195/112	198/113	+1.5/+0.9	199/114
	17th admin.	194/112	193/119	-0.5/+6.3	
	38th admin.	184/102	187/106	+1.6/+3.9	
	114th admin.	210/116	205/116	-2.4/0.0	
	200th admin.	199/108	204/113	+2.5/+4.6	
	269th admin.	211/108	195/110	-7.6/+1.9	
	347th admin.	201/118	204/119	+1.5/+0.8	
0.3 mg/kg	1st admin.	208/119	*167/ 97	-19.7/-18.5	193/115
	17th admin.	203/111	164/ 98	-19.2/-11.4	
	38th admin.	180/ 98	163/ 88	- 9.4/-10.2	
	114th admin.	219/118	171/ 98	-21.9/-16.9	
	200th admin.	191/113	153/ 86	-19.9/-23.9	
	269th admin.	205/114	168/ 90	-18.0/-21.1	
	347th admin.	215/124	184/105	-14.4/-15.3	

* n = 7

** Calculation using unrounded figures

Study No: T 20 10 506

BLOOD PRESSURE (mm Hg)

(Means n = 8)

DOSE	TIME	before admin.	1 h after admin.	% difference**	24 h after admin.
1.0 mg/kg	1st admin.	179/104	141/ 76	-21.2/-26.9	173/104
	17th admin.	177/101	136/ 76	-23.2/-24.8	
	38th admin.	181/ 98	116/ 68	-35.9/-30.6	
	114th admin.	184/106	133/ 75	-27.7/-29.0	
	200th admin.	178/107	136/ 79	-23.6/-26.3	
	269th admin.	196/109	135/ 73	-31.1/-33.0	
	347th admin.	195/115	161/ 93	-17.6/-19.1	
3.0 mg/kg	1st admin.	195/114	116/ 64	-40.7/-43.9	194/115
	17th admin.	200/127	133/ 71	-33.5/-44.1	
	38th admin.	186/112	*106/ 59	-43.0/-47.3	
	114th admin.	212/126	124/ 66	-41.6/-47.6	
	200th admin.	197/119	133/ 76	-32.5/-36.1	
	269th admin.	218/123	115/ 62	-47.2/-49.6	
	347th admin.	206/124	134/ 69	-35.0/-44.4	

* n = 7

** Calculation using unrounded figures

Study No: T 20 10 506

HEART RATES (beats/min)

(means, n = 8)

(calculation using unrounded figures)

DOSE (mg/kg)	Initial Figure	TIME OF INVESTIGATION: 1st admin. before	1st admin. 1 h	1st admin. 24 h	17th admin. before	17th admin. 1 h	38th admin. before	38th admin. 1 h	114th admin. before	114th admin. 1 h	200th admin. before	200th admin. 1 h	269th admin. before	269th admin. 1 h	347th admin. before	347th admin. 1 h
0.0	134*	120	113*	134	128	132*	136	133	133	121	129	138	119	119	101**	112
Diff. %			-5.9		+3.1		-2.2		-9.0		+7.0		0.0		+10.9	
0.3	138	136	172	141	134	190	137	194	123	177	128	205	118*	178	113*	161
Diff. %			+26.5		+41.8		+41.6		+43.9		+60.2		+50.8		+42.5	
1.0	147	134	191	135	130	209	131	218	106	221	113	220	96	207	111	153
Diff. %			+42.5		+60.8		+66.4		+108.5		+94.7		+115.6		+37.8	
3.0	146	134	211	128	120	234	113	201	101	224	99	215	90	209	93	186*
Diff. %			+57.5		+95.0		+77.9		+121.8		117.2		+132.2		+100.0	

*n = 7
**n = 6

CHRONIC TOXICITY STUDY ON DOGS

B A Y K 5 5 5 2 STUD.T2 010 506
CHRONISCHER TOXIZITAETSVERSUCH AN HUNDEN

SYNOPSIS OF GROUP MEANS

ANLISTUNG ALLER MITTELWERTE

		HEART / HERZ	LUNG / LUNGE	LIVER / LEBER	KIDNEYS / NIEREN	SPLEEN / MILZ	TESTES / HODEN	OVARIES / OVARIEN	THYROID / SCHILD-DRUESE	ADREN. / NEBEN-NIEREN	THYMUS / THYMUS	PROSTA. / PRO-STATA	BRAIN / GEHIRN	PANC. / PAN-KREAS
ABSOLUTE ORGANWEIGHTS (G) / ABSOLUTE ORGANGEWICHTE (G)														
MALES / MN.TIERE														
CNTR./KONTR.		98.6	96.8	436.0	57.0	30.3	17.30	-	0.825	1.277	5.15	8.087	78.8	33.0
GROUP/GRUPPE	I	108.0	91.8	423.0	56.5	29.8	18.38	-	0.727	1.162	5.32	6.910	75.5	35.8
GROUP/GRUPPE	II	103.3	100.5	484.3	66.3	37.3	20.92	-	0.860	1.335	5.28	5.707	82.3	36.0
GROUP/GRUPPE	III	111.3	93.8	472.0	61.3	68.0	20.42	-	0.805	1.310	5.65	7.340	78.5	33.5
FEMALES / WB.TIERE														
CNTR./KONTR.		95.8	86.0	409.8	53.0	26.3	-	0.937	0.867	1.385	6.96	-	76.5	25.5
GROUP/GRUPPE	I	102.8	96.5	385.5	57.5	57.5	-	0.832	0.797	1.550	7.27	-	73.3	29.5
GROUP/GRUPPE	II	96.8	82.5	393.5	53.3	38.0	-	1.113	0.807	1.672	7.67	-	77.8	32.3
GROUP/GRUPPE	III	103.0	90.3	393.8	59.3	41.0	-	1.787	0.860	1.587	7.17	-	77.0	31.0
BOTH SEXES / ALLE TIERE														
CNTR./KONTR.		97.3	91.4	424.3	55.0	28.3	17.30	0.937	0.846	1.331	6.05	8.087	77.6	29.3
GROUP/GRUPPE	I	105.4	94.1	404.3	57.0	43.6	18.38	0.832	0.762	1.356	6.30	6.910	74.4	32.3
GROUP/GRUPPE	II	100.0	91.5	438.9	59.8	37.6	20.92	1.123	0.834	1.504	6.44	5.707	80.0	33.9
GROUP/GRUPPE	III	107.1	92.0	432.9	60.3	54.5	20.42	1.787	0.832	1.447	6.41	7.340	77.8	32.3
RELATIVE ORGANWEIGHTS (G/KG) / RELATIVE ORGANGEWICHTE (G/KG)														
MALES / MN.TIERE														
CNTR./KONTR.		9.12	9.00	40.25	5.25	2.85	1.667	-	0.0780	0.1200	0.480	0.7577	7.42	3.05
GROUP/GRUPPE	I	10.17	8.62	39.77	5.32	2.85	1.760	-	0.0687	0.1100	0.496	0.6585	7.15	3.30
GROUP/GRUPPE	II	9.15	8.87	42.67	5.85	3.30	1.842	-	0.0757	0.1175	0.462	0.5085	7.30	3.20
GROUP/GRUPPE	III	10.02	8.45	42.75	5.52	6.25	1.842	-	0.0730	0.1180	0.510	0.6627	7.10	3.05
FEMALES / WB.TIERE														
CNTR./KONTR.		9.50	8.55	40.77	5.25	2.50	-	0.0912	0.0865	0.1372	0.667	-	7.65	2.55
GROUP/GRUPPE	I	9.37	8.85	39.88	5.25	5.20	-	0.0755	0.0722	0.1415	0.652	-	6.70	2.65
GROUP/GRUPPE	II	9.72	8.25	44.17	5.32	3.77	-	0.0869	0.0812	0.1690	0.767	-	7.82	3.22
GROUP/GRUPPE	III	9.87	8.62	37.32	5.65	3.85	-	0.1642	0.0805	0.1535	0.670	-	7.45	3.05
BOTH SEXES / ALLE TIERE														
CNTR./KONTR.		9.31	8.77	40.51	5.25	2.67	1.667	0.0912	0.0822	0.1286	0.556	0.7577	7.54	2.80
GROUP/GRUPPE	I	9.77	8.74	41.18	5.29	4.02	1.760	0.0755	0.0705	0.1257	0.575	0.6585	6.92	2.97
GROUP/GRUPPE	II	9.44	8.56	43.42	5.59	3.54	1.842	0.0869	0.0785	0.1432	0.615	0.5085	7.56	3.21
GROUP/GRUPPE	III	9.94	8.54	40.04	5.59	5.05	1.842	0.1642	0.0767	0.1358	0.590	0.6627	7.27	3.05

REPRODUCTIVE TOXICITY STUDIES (S. Stolzenberg)

1. Fertility and Reproduction Ability in Wistar Rats

Bayer Study No: T0002152

This report is accompanied by a "first amendment to report no. 12691", dated 8/11/93. Tables in the original English translation of the report were of very poor quality, not legible, contained errors in translation and typing, and a few tables were not logically organized. Some of these faults are listed in the amendment under the "rationale for the first amendment".

Performing Laboratory:

Dates Performed: 2/81 to 9/81

Quality Assurance: No statement on GLP compliance was found in the original report. In the amendment, it is claimed, "...there were no legally binding GLP regulations in force" during the time that this study was performed.

Test Animals: Mura:WIST (SPF 67 HAN), 24 males and 60 females per group. At the start of dosing, males were 5-7 weeks old, and weighed 74-110 g, females were 8-10 weeks old and weighed 158-190 g.

Procedure: The test substance (batch 576 923) was administered at doses of 0, 3, 10 and 30 mg/kg, once daily, by oral gavage in a vehicle consisting of polyethylene glycol 400:glycerol:water in a ratio of 969:60:100. Males were dosed starting 10 weeks before mating and during the 3 week mating period, females were dosed for 3 weeks prior to mating until the 7th day of pregnancy. Except during mating and lactation, both the males and females were kept in individual Makrolon cages. Each male was paired with 2 or 3 females, which were placed together in a Makrolon cage each night and the females were examined for vaginal sperm in the morning. Half the pregnant females in each group, selected by "statistical methods", were C-sectioned on day 20 of gestation, the remaining half were allowed to litter and raise their young to postpatum day (PPD) 21. All C sectioned fetuses were examined for external anomalies, 1/3 from each dam were examined for soft tissue anomalies (modified Wilson method) and 2/3 for skeletal malformations (alizarin red S). In addition to examining the F_1 parents for reproductive performance, the F_0 females for lactational performance and the F_1 offspring for survival and weight gain during lactation, one male and one female from each litter of the control group and of the highest dose group were reared to sexual maturity to determine F_1 reproductive capacity. The mated F_1 dams were allowed to litter, and testicular weights for F_1 males were obtained after mating.

Test substance administered was Batch 576 923. It is claimed that the preparations for oral gavage were tested for stability and concentration but the data and details for these tests were not included in the report.

There is no statement on why these doses were selected for this study.

Effects on *F_0 Males*

All treated and control males survived to scheduled necropsy and no compound related clinical signs were evident in males of any treated group. There were no effects on weight gain, mating behavior or fertility in males of any treated group compared to controls. There were no effects on gross pathology observed at necropsy (presumably sacrificed after mating and while still on drug treatment). The drug had no effect on testicular weights (See page which follows).

STUDY ON FERTILITY T0002152

BODYWEIGHTS [G] OF THE MALES BEFORE MATING
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEEK 10	90.5	87.3	91.0	89.9
	9.6	7.7	7.9	7.4
WEEK 9	136.5	132.5	135.4	131.7
	12.7	11.0	10.0	9.2
WEEK 8	175.0	172.0	174.3	173.3
	16.8	14.8	14.3	13.6
WEEK 7	215.5	212.3	215.7	213.5
	22.0	17.0	16.7	17.8
WEEK 6	252.5	249.6	249.6	252.1
	26.4	19.5	18.6	20.2
WEEK 5	279.0	276.3	276.1	280.6
	29.9	21.6	21.9	23.4
WEEK 4	291.5	291.5	287.6	294.5
	31.9	23.4	26.1	23.6
WEEK 3	311.4	309.9	308.8	314.0
	33.5	25.2	25.1	26.0
WEEK 2	331.5	329.7	327.1	331.6
	34.8	27.1	28.5	28.5
WEEK 1	344.5	345.9	341.6	346.9
	35.0	28.8	29.8	30.0
WEEK 0	360.7	358.9	354.7	360.4
	35.6	29.5	31.4	30.8

TESTICLE WEIGHTS [G]

GROUP MEAN VALUES AND STANDARD DEVIATIONS

0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
3.23	3.26	3.17	3.26
0.30	0.20	0.31	0.25

Effects on F_0 Females

Mortality: Deaths are listed only in the narrative portion of this report. In both the original report and the amendment, there is no indication of the time of death; not even if the deaths occurred before mating, during pregnancy or lactation. Deaths occurred in two rats at 3 mg/kg, in one at 10 mg/kg and in two at 30 mg/kg, but none of the deaths were attributed to treatment. Based on scrutinization of tables in the original report, the 2 animals in the 30 mg/kg group which died had both been assigned to "rearing animals". Deaths were attributed to misintubation for a low and mid dose rat, "gastrointestinal disorders" for the second low dose rat, to a lung tumor and to pneumonia for the two high dose rats. In addition, one dam in the control group, which had littered 12 pups and died shortly after birth, was not included in the results because at necropsy only 4 nidation sites were found.

Even in the amended tables for individual animal data, there is no indication of which animals died and the time of the deaths. Numerous animals were dropped from the study for a variety of reasons, which included, "not inseminated", "not pregnant", and for a few, there is a statement "animal dropped from the study" but no reason is given. Most summary tables do not specify the number of animals per group upon which the data are based. Therefore, the following table lists the total number of females in each group that were included in the results, based on a count taken from the individual animal body weight data.

Dosage Group	# C-Sectioned*	# Littered*
Control	25	22
3 mg/kg/day	23	27
10 mg/kg/day	24	20
30 mg/kg/day	27	22

* There were 60 mated females per group at initiation of the study, 30 of which were designated for C-section or littering.

Body Weight and Body Weight Gain: Mean body weight gains and body weights of pregnant females, those that were C-sectioned and those that were allowed to litter, are given on the two pages which follow. Body weights 3 weeks before mating (prior to initiation of treatment) and during pregnancy, were significantly lower for the high dose group of the C-sectioned animals, but there was no effect on body weight gain. Although a small increase in body weight gain was noted for the low dose C-sectioned group between days 7 and 20 of gestation, there was obviously no effect that could be attributed to treatment. No effects on mean body weight or body weight gain were observed in the females selected for delivery of litters during the 3 weeks prior to gestation, during gestation or during lactation.

STUDY ON FERTILITY T0002152

WEIGHT DEVELOPMENT [G] OF THE FEMALES UNDERGOING CESAREAN SECTION
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEIGHT GAIN				
DAY 0 - 7 P.C.	22.2	23.9	21.7	23.0
	4.7	5.1	5.1	5.6
DAY 7 - 20 P.C.	73.3	82.1*	76.2	71.4
	10.1	12.6	10.8	17.1
DAY 0 - 20 P.C.	95.5	106.0*	97.9	94.4
	12.4	15.5	12.6	18.1
BODYWEIGHTS BEFORE MATING				
WEEK 3	173.6	172.3	170.3	168.6**
	7.0	6.8	6.8	6.3
WEEK 2	187.1	187.1	186.0	184.9
	8.7	9.5	8.7	9.1
WEEK 1	198.9	197.3	198.3	194.9
	10.0	9.3	9.5	9.7
WEEK 0	209.7	210.2	210.8	204.8
	10.9	11.3	11.6	9.7
BODYWEIGHTS DURING GESTATION				
DAY 0 P.C.	223.8	223.2	223.9	213.5**
	11.5	13.9	13.6	12.4
DAY 7 P.C.	245.9	247.0	245.6	236.5*
	13.2	14.3	13.4	13.5
DAY 20 P.C.	319.2	329.1	321.8	308.0
	17.2	23.2	17.4	23.3

* SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.01$
** SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.005$

T0002152

WEIGHT DEVELOPMENT [G] OF THE DAMS
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEIGHT GAIN				
DAY 0 - 7 P.C.	23.7	19.9	23.7	21.4
	6.2	5.1	5.2	4.4
DAY 7 - 20 P.C.	73.5	70.3	77.0	71.7
	13.3	13.8	13.0	14.2
DAY 0 - 20 P.C.	97.2	90.2	100.7	93.1
	14.6	14.5	11.7	15.0
BODYWEIGHTS BEFORE MATING				
WEEK 3	173.7	171.6	172.5	172.4
	8.2	7.9	8.2	8.9
WEEK 2	186.1	186.1	188.8	187.6
	9.2	10.2	10.1	10.1
WEEK 1	194.7	196.8	200.2	198.6
	11.1	11.1	10.3	12.4
WEEK 0	206.3	208.7	211.0	208.7
	13.1	12.8	10.8	14.2
BODYWEIGHTS DURING GESTATION				
DAY 0 P.C.	216.4	225.0	224.1	217.5
	16.4	16.8	13.6	18.7
DAY 7 P.C.	240.1	244.9	247.8	238.9
	19.4	16.5	11.0	20.1
DAY 20 P.C.	313.5	315.3	324.8	310.6
	25.3	26.1	19.6	31.2
BODYWEIGHTS DURING LACTATION				
DAY 1 P.P.	244.8	250.0	253.7	240.3
	18.9	18.1	15.1	21.3
WEEK 1 P.P.	272.3	277.8	281.6	267.1
	20.5	19.4	14.3	24.4
WEEK 2 P.P.	272.5	278.7	283.3	271.7
	18.8	16.8	14.0	20.1
WEEK 3 P.P.	258.6	265.5	266.7	259.4
	19.0	17.8	15.3	18.2

C-Section *F_0 Females*

As seen in the tables which follow, there were no effects of compound treatment on number or percentages of animals inseminated, with implantations and with live fetuses, mean corpora lutea count, nidations, average number of male or female live fetuses, sex ratio or fetal loss. From these data, it is evident that there were no effects on pre- or post-implantation losses.

The mean fetal weights were significantly increased (apparently dose related) in the 10 and 30 mg/kg groups, and the mean placental weight was slightly but significantly increased in the 3 mg/kg group. The investigators claimed that the mean placental weight increase was incidental, and that the mean fetal weights for the mid and high dose groups were within the norm for this strain (given as 3.5 ± 0.27, based on 268 litters).

There were no compound related effects on mean numbers of gross, visceral or skeletal malformations, nor were there any effects on "underdeveloped forms" (fetuses weighing <3 g). There were also no effects on minor skeletal variations.

STUDY ON FERTILITY

NUMBER OF ANIMALS - RESULTS OF THE STUDY

ANIMALS UNDERGOING CESAREAN SECTION

DOSE [MG/KG]	USED	NUMBER OF FEMALES WITH FOETUSES					
		N	% OF THOSE USED	N	% OF THOSE INSEMINATED	N	% OF THOSE WITH IMPLANTATIONS
0	30	27	90.0	25	92.6	25	100.0
3	29	27	93.1	23	85.2	23	100.0
10	30	26	86.7	25	96.2	24	96.0
30	30	28	93.3	27	96.4	27	100.0

STUDY ON FERTILITY

T0002152

RESULTS OF THE CESAREAN SECTION (MEAN VALUES)

DOSE (MG/KG)	WEIGHT GAIN (G) 0-20 P.C.	WEIGHT GAIN (G) 7-20 P.C.	NUMBER (PER DAM) OF CORP. LUTEA	IMPL.	MALE	FEM.	SUM	LOSS	MEAN-WEIGHT IN GRAMMS FETUSES	PLACENT.	NO. OF FOETUSES EXAMINED BY WILSON	DAWSON	FOETUSES WITH MINOR SKELETAL DEVIAT.	MALFORMATIONS	NO. OF RUNTS (<3G)
0	95.5	73.3	12.4	11.9	6.3	4.9	11.2	0.7	3.49	0.50	3.3	7.9	3.52	0.04	0.52
3	106.0**	82.1**	12.4	12.0	6.3	5.0	11.4	0.6	3.58	0.53*	3.5	7.8	3.48	0.09	0.30
10	97.9	76.2	11.4	11.0	5.6	5.0	10.6	0.4	3.60*	0.50	3.4	7.6	2.67	0.00	0.25
30	94.4	71.4	11.6	11.0	5.4	4.9	10.3	0.7	3.63**	0.52	3.0	7.3	3.22	0.00	0.19

* SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.025$
** SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.01$

F_0 Females Allowed to Litter

Pregnancy duration was slightly increased in all 3 treated groups (statistically significant for the 3 and 30 mg/kg groups; see table below). This effect was considered to be "incidental" because the mean durations for these groups were within the norm for this strain. No effects during lactation were noted.

Postpartum Examination of Pups: There were no significant effects on total number of live pups at birth per group, nor on number of viable pups after 1, 2 or 3 weeks postpartum (See table below). It was claimed there were no treatment related effects on number of stillborn pups, and on sex ratio at birth or at the 3 weekly intervals. Mean birth weight was slightly higher for all 3 treated groups (statistically significant for low and high dose), but mean weight and weight increase during the 3 weekly intervals were not influenced by treatment (See table below).

Maturational Development: There were no effects on age of pinna unfolding of the ears, hair coat, eye opening or normal gait.

Function Tests: There were no effects on sight or pupillary reflexes to light, hearing ("pinna twitch reflex", tested by means of a Galton whistle with a set frequency and duration). In a proprioreceptive reflex test (running roller brought from stationary position to 10 revolutions per minute) there was a decrease in performance at 30 mg/kg during the first test but no effect in the second or third test. The age of the animals when these tests were done was not indicated.

Fertility Test of F_1 Generation: There was no effect of treatment with 30 mg/kg on mating, fertility, duration of pregnancy, litter size, live and dead pups, sex ratio, mean weights of the pups or external anomalies at birth.

T0002152

'AMS

DOSE (MG/KG)	USED	NUMBER OF FEMALES							
		N	INSEMINATED % OF THOSE USED	WITH IMPLANTATIONS N	% OF THOSE INSEMINATED	THAT LITTERED N	% OF THOSE WITH IMPLANTATIONS	THAT REARED THEIR PUPS N	% OF THOSE THAT LITTERED
0	29	24	82.8	22	91.7	22	100.0	22	100.0
3	29	28	96.6	27	96.4	27	100.0	27	100.0
10	29	22	75.9	20	90.9	20	100.0	20	100.0
30	28	26	92.9	22	84.6	22	100.0	22	100.0

DURATION OF PREGNANCY IN DAYS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
21.9	22.2*	22.1	22.2**
0.5	0.6	0.6	0.4

* SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.05
** SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.025

NUMBER OF IMPLANTATIONS OF THE DAMS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
10.8	10.4	11.0	11.2
3.0	2.9	2.4	2.8

PRENATAL LOSS OF DAMS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
0.5	0.6	0.4	0.9
0.7	0.9	1.0	1.1

STUDY ON FERTILITY T0002152

NUMBER AND WEIGHT DEVELOPMENT OF THE VIABLE PUPS
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION		0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
NUMBER OF PUPS					
AT BIRTH	TOTAL	10.4 3.0	9.8 2.8	10.4 2.5	9.7 2.7
	MALES	5.2 1.8	5.2 2.3	5.2 1.6	4.9 1.8
	FEMALES	5.2 2.3	4.6 1.7	5.3 2.0	4.8 2.1
AFTER 1 WEEK	TOTAL	10.2 3.0	9.7 2.7	10.4 2.5	9.4 2.9
	MALES	5.0 1.9	5.2 2.3	5.2 1.6	4.8 1.8
	FEMALES	5.1 2.3	4.5 1.7	5.3 2.0	4.5 2.3
AFTER 2 WEEKS	TOTAL	10.0 3.0	9.6 2.7	10.3 2.5	9.3 3.0
	MALES	5.0 1.9	5.1 2.2	5.1 1.7	4.7 1.9
	FEMALES	5.0 2.3	4.5 1.7	5.1 2.0	4.5 2.3
AFTER 3 WEEKS	TOTAL	10.0 3.0	9.6 2.7	10.2 2.5	9.3 3.0
	MALES	5.0 1.9	5.1 2.2	5.0 1.7	4.7 1.9
	FEMALES	5.0 2.3	4.5 1.7	5.1 2.0	4.5 2.3
WEIGHT [G] OF THE VIABLE PUPS					
AT BIRTH		5.9 0.5	6.2** 0.5	6.1 0.6	6.2* 0.6
AFTER 1 WEEK		14.0 2.2	14.8 1.6	14.9 1.8	15.0 2.2
AFTER 2 WEEKS		24.7 4.4	26.1 3.5	26.1 3.3	26.4 4.4
AFTER 3 WEEKS		38.1 6.1	40.4 5.8	39.7 5.8	41.5 7.4

* SIGNIFICANT DIFFERENCE TO CONTROL, $P \leq 0.05$
** SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.025$

2. Embryotoxic and Teratogenic Action in Long-Evans Rats

Pharma Report No: 7596 Study No: T2012540

Performing Laboratory:

This study was originally presented as a translation from German with only a few brief summary tables; no individual animal data. Amendments received at CDER on 9/29/93 and 10/8/93 contain tables with individual animal findings and summaries. It is claimed that the study was carried out between January and May, 1977, "in accordance with FDA recommendations", but there is no statement of GLP compliance.

Procedure: Naturally inseminated Long-Evans female (strain FB 30) rats, 20 or 21 per group, 2.5 to 3.5 months of age and weighing 195 to 262 g prior to mating, received 0, 10, 30 or 100 mg nisoldipine/kg/day by oral gavage (batch 3/76, micronized), from days 6 to 15 of gestation. The drug was dissolved in polyethylene glycol 400/glycerol/water. A C-section was performed for each dam on day 20 of gestation and the fetuses were examined for external, visceral (Wilson technique) and skeletal (alizarin red stain) anomalies.

Effects on Survival and Body Weights of Dams: One control rat died on gestation day 13 or 14, due to improper intubation into lungs, and was excluded from results. There was no compound related effect on mortality, nor on "general appearance or behavior" of the dams, but there was a dose related decrease in mean weight gain (see table which follows).

Dose (mg/kg)	Weight Gain in Grams	
	Treatment Period	Total Pregnancy
0	62.3	152.4
10	56.1	140.3
30	53.0*	132.6*
100	49.6*	131.8*

*) Significant difference from the control, $P < 0.01$

C-Section of Dams: Of the 21 inseminated rats in each of the 3 compound treated groups, 20 were pregnant, and all 20 surviving rats in the control group were pregnant. All pregnant treated and control rats had live fetuses at necropsy on day 20 of gestation. Corpora lutea count for each rat was not determined in this experiment, but no statistically significant differences between the treated groups and control were found for mean number of implantations, mean number of fetuses, mean number of dead fetuses and resorbed embryos, mean fetal weight, underdeveloped forms (fetuses <3 g in weight), mean placental weight, frequency of fetuses with minor skeletal deviations, sex distribution (see page 94), nor on external, soft tissue or bone deformations (see table below on this page).

Group	Dam No.	Number of Malformed Fetuses	Malformation
Control	664	2	Rib dysplasia (hump formation)
10 mg/kg	651	1	Edematous head
30 mg/kg	—	—	None
100 mg/kg	605	1	Rib dysplasia (hump formation)
	639	1	Cryptorchidism
	647	1	Kinking of the tail
	683	1	Hydrops universalis, micrognathia, kinking of the tail

T2012540

Results of the Caesarean Section

Mean values of the groups and standard deviations

Note: The mean fetal and placental weights given in the report no. 7596 were calculated by adding all litter weights of the group and by dividing these sums by the number of fetuses or placentas per group. In the following table these mean values are marked with "a".
For the calculation of the standard deviation the mean fetal and placental weights per litter were calculated first and were used for further calculation. Mean fetal and placental weights obtained by this procedure are marked with "b".

Dose mg/kg	Weight gain [g] during pregnancy	Weight gain [g] during treatment period	Number [per dam] of fetuses impl.	male	female	total	res.**	Mean - weight [g] of fetuses	of placentas	Number of fetuses with minor skeletal deviations	with malformations	runts < 3 g
0	152.4	62.3	11.6	5.8	5.4	11.1	0.4	4.26[a] 4.27[b]	0.57[a] 0.58[b]	2.95	0.10	0.00
	18.5	11.7	1.4	2.3	2.3	1.7	0.7	0.29	0.06	2.11	0.45	0.00
10	140.3	56.1	11.0	5.5	5.0	10.5	0.5	4.07[a] 4.07[b]	0.59[a] 0.59[b]	2.60	0.05	0.00
	23.9	9.7	2.9	1.8	1.6	2.8	0.8	0.29	0.11	2.19	0.22	0.00
30	132.6*	53.0*	11.3	5.3	5.1	10.3	0.9	4.08[a] 4.09[b]	0.57[a] 0.57[b]	3.50	0.00	0.05
	19.9	8.1	2.5	2.4	2.5	2.6	1.3	0.33	0.05	2.61	0.00	0.22
100	131.8*	49.6*	11.9	5.5	5.2	10.7	1.1	4.12[a] 4.14[b]	0.57[a] 0.57[b]	4.10	0.20	0.00
	21.2	11.2	2.7	2.4	2.1	2.7	1.4	0.23	0.05	2.45	0.41	0.00

* significant difference to control, $p < 0.01$ (WILCOXON-MANN-WHITNEY-U-TEST)

** Res. is the abbreviation for resorptions, which the sponsor defined as the total of resorbed embryos and dead fetuses

3. Teratology Study in Sprague Dawley (CD) Rats

Study No: Not provided. LSR Report No. 87/0938

Performing Laboratory:

Sponsor.

Dates Performed: 8/5/87 to 12/2/87

Quality Assurance: A signed statement of GLP compliance is included.

Test Animals: Charles River CD (Sprague-Dawley derived) females, 9-10 weeks of age and weighing 200-248 g on the day of insemination, were mated on a 1:1 basis with stock males of the same strain.

Procedure: The test substance (batch number 500139) was administered to 32 inseminated females per group, once daily by oral gavage as a suspension in 0.5% aqueous Tylose, prepared fresh each day, from days 7 to 17 of gestation, at doses of 0, 10, 30 and 100 mg/kg. On day 20 of gestation, 21 dams per group were C-sectioned and the fetuses were examined for external anomalies; 1/2 from each dam were examined by free hand serial sectioning (Wilson technique) for soft tissue anomalies. The remaining half were first dissected (neck, thoracic and abdominal cavities) to evaluate for soft tissue anomalies, then were prepared by a modification of Dawson's alizarin staining technique for evaluation of skeletal malformations.

The remaining 11 dams/group were allowed to litter and raise their young to postpatum day 25. On PPD 4, litters with more than 8 were reduced to 8 by random culling, leaving, if possible, 4 of each sex per litter. After weaning, the offspring were housed on a litter basis, but the sexes were separated and there was a maximum of 5 of the same sex per cage. At approximately 5 weeks of age, following completion of behavioral and neuromuscular function tests, 20/sex/group were randomly selected for further assessment of physical, sexual maturation and reproductive performance; unselected ones were killed and grossly examined. At 9 or 10 weeks of age, F_1 males and females were paired 1:1 within treatment groups, avoiding sibling matings. All F_1 mated females were laparotomized on day 20 of gestation; the fetuses were examined only for external malformations and discarded. After gross examination of the F_1 females, F_1 males were killed, then examined externally and internally for macroscopic abnormalities.

Stability of Test Substance: Test formulations for all 3 concentrations, taken from the first and last weeks of treatment,

were generally found to be within approximately 82 to 90% of the target concentrations, and were stable for at least 4 hours after preparation.

Results

F_0 Females

Mortality and Clinical Signs: No data on mortality, and no statement in the text pertaining to mortality, were found; all rats apparently survived. It is claimed that one dam receiving 100 mg/kg showed flaccid muscle tone and piloerection during the early stages of treatment (possibly compound related), but other females in that group were not affected.

Body Weights/Food Consumption: Small reductions in body weight gain (not statistically significant) were evident in all 3 treated groups during the initial day or 2 of treatment (See table on the page which follows), and this was accompanied by significant reductions in food intake by the mid and high dose groups, limited to the first 3 days of treatment. Subsequent body weight gains in all 3 treated groups were not affected by treatment, but the body weights remained below control to the day of necropsy. The lower mean body weights of the mid and high dose groups compared to controls were statistically significant only on day 18 of gestation.

Laparotomy Observations: There were no effects on mean corpora lutea counts, total implantatiions, viable males or females. There was a slight increase in total resorptions ($P< 0.05$) predominently due to number of late resorptions, and in percent post-implantation loss ($P< 0.05$), in the high dose group. Fetal weights were depressed in all 3 treated groups; statistically significant and dose related at mid and high dose (See table two pages ahead).

Fetal Evaluation: There was an increased incidence of small fetuses (<2.7 g) and litters with one or more small fetuses in the 100 mg/kg group. An increased number of fetuses with slightly increased dilatation of lateral ventricles and/or space between the body wall and organs, occurred mainly in two litters, and this was associated with fetuses of low body weight in these two litters. The investigators considered this to be indicative of fetal immaturity. Also associated with the fetuses weighing <2.7 g in 2 litters of the high dose group and considered to be due to fetal immaturity, was an increased incidence of incomplete ossification of basisphenoid, first thoracic vertebral centrum, sacral vetebral arches, ischia, metacarpals and metatarsals.

Group mean bodyweights (g) of females during gestation

Group	:	1	2	3	4
Compound	:	Control	--		--
Dosage (mg/kg/day)	:	0	10	30	100

Group		Day of gestation														
		0	3	7	8	9	10	11	12	13	14	15	16	17	18	20
1	Mean	219	238	255	261	266	272	278	284	291	298	307	317	330	346	378
	SD	8	9	12	11	12	12	12	13	13	13	14	15	16	17	18
	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
2	Mean	217	237	251	256	261	267	273	279	285	292	300	312	323	338	368
	SD	10	12	14	12	13	12	14	13	14	14	15	16	16	17	18
	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
3	Mean	217	237	253	255	260	266	272	278	284	291	299	311	323	338*	367
	SD	11	13	14	13	14	14	14	15	15	15	17	17	18	19	21
	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
4	Mean	215	235	253	250	252	261	267	272	278	285	293	303	316	327***	361
	SD	8	9	12	12	12	13	12	12	13	13	13	16	17	18	20
	n	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31

SD Standard deviation.
n Number of pregnant animals.
* Bodyweight gain from Day 7 significantly different from Controls, $P<0.05$ (one way analysis of variance and Student's t-test).
*** Bodyweight gain from Day 7 significantly different from Controls, $P<0.001$ (one way analysis of variance and Student's t-test).

Group mean litter data - females killed on Day 20 of gestation

Group	: 1	2	3	4
Compound	: Control	---		-
Dosage (mg/kg/day)	: 0	10	30	100

Group	Number of pregnant animals		Corpora lutea count	Implant-ations	Viable young			Resorptions			Implantation loss (%)		Foetal weight (g)	Placental weight (g)
					M	F	Total	Early	Late	Total	Pre-	Post-		
1	21	Mean	17.1	15.6	8.0	6.9	14.9	0.5	0.1	0.7	9.4	4.3	3.50	0.53
		SD	1.7	1.3	2.3	1.9	1.1	0.7	0.4	0.8			0.06	0.01
2	21	Mean	16.9	14.9	7.0	7.0	14.0	0.8^{NS}	0.1	0.9^{NS}	11.6	6.1^{NS}	3.45	0.53
		SD	1.5	1.4	1.8	2.0	1.5	0.9	0.4	1.0			0.06	0.02
3	21	Mean	16.4	14.9	6.4	7.1	13.5	1.2^{NS}	0.1	1.4^{NS}	9.8	9.3^{NS}	3.34*	0.54
		SD	1.7	1.4	2.3	2.3	2.4	1.1	0.4	1.2			0.06	0.02
4	20	Mean	16.9	15.1	6.7	6.8	13.5	0.9^{NS}	0.8^{NS}	1.6^{+}	11.2	10.6^{+}	3.19***	0.52
		SD	1.9	3.3	2.3	2.6	3.6	0.9	0.9	1.3			0.09	0.03
Background control (159 studies)														
Mean			15.9	14.5	6.7	6.9	13.7	0.69	0.18	0.87	8.7	6.0	3.32	0.50
Low			13.9	12.0	5.2	5.6	11.1	0.05	0.00	0.25	1.6	1.7	3.00	0.43
High			19.0	16.7	8.2	8.7	15.3	1.68	0.58	1.79	16.5	12.7	3.55	0.57

SD Standard deviation.
* Significantly different from Control, P<0.05 (Nested analysis of variance and weighted t-test).
*** Significantly different from Control, P<0.001 (Nested analysis of variance and weighted t-test).
NS Not significant (Mann Whitney 'U'-test).
+ Significantly different from Control P<0.05 (Mann Whitney 'U'-test)

Summary of foetal observations at necropsy

Group	:	1	2	3	4
Compound	:	Control	-		--
Dosage (mg/kg/day)	:	0	10	30	100

Group :	1	2	3	4	Control data	
External examination						
Number of foetuses (litters) examined:	313(21)	294(21)	284(21)	269(20)	39809	159
Number of male : female foetuses:	168:145	147:147	135:149	134:135	foetuses	studies
Observations: % incidence[♦] (number of litters)					Mean	Study ranges
Small foetus (less than 2.70 g)	1.0(3)	0.7(2)	1.4(4)	11.9(6)	3.5	0 - 16.9
Large foetus (more than 4.00 g)	2.9(3)	1.4(3)	1.4(2)	-	1.3	0 - 9.7
Shiny pup	-	0.3(1)	-	1.1(1)	0.3	0 - 4.1
Pale pup	-	-	-	0.4(1)	0.02	0 - 1.1
Domed head	-	0.3(1)	-	-	0.01	0 - 0.4
Subcutaneous haemorrhage on chin	0.3(1)	-	-	-	0.1	0 - 0.7
Small placenta (less than 0.30 g)	-	-	-	0.4(1)	0.2	0 - 2.3
Large placenta (more than 0.70 g)	2.2(4)	2.0(6)	3.2(4)	3.0(3)	1.3	0 - 6.2
Conjoined placentae	-	0.3(1)	-	-	0.03	0 - 0.8
Dark green material surrounding placenta	-	0.3(1)	-	-	0.1	0 - 6.9
Short tail	-	-	-	0.4(1)	0.01	0 - 0.5
Threadlike tail	0.3(1)	-	-	-	0.02	0 - 0.8
Imperforate anus	0.3(1)	-	-	-	0.04	0 - 0.8

♦ One foetus may have more than one observation.

continued

Summary of foetal observations after free-hand serial sectioning

Group	:	1	2	3	4
Compound	:	Control	-		--
Dosage (mg/kg/day)	:	0	10	30	100

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined.*	156(21)	148(21)	143(21)	134(20)	12835	126
Number of males : females	82:74	78:70	70:73	68:66	foetuses	studies
Observations: % foetal incidence (number of litters affected)					Mean	Study ranges
Abdomen:						
Diaphragmatic hernia	0.6(1)	-	-	0.7(1)	0.1	0 - 1.9
Small additional liver lobe(s)	25.6(19)	32.4(18)	23.1(18)	28.4(17)	0.8	0 - 10.9
Hepatic haemorrhage(s)	9.0(8)	12.2(15)	7.7(7)	9.0(11)	10.3	0 - 27.7
Localised internal abdominal haemorrhage	1.9(3)	-	0.7(1)	2.2(3)	1.3	0 - 6.7
Haemorrhagic peritoneal fluid	0.6(1)	-	0.7(1)	-	2.4	0 - 18.0
Haemorrhagic abdomen	0.6(1)	0.7(1)	0.7(1)	-	1.7	0 - 8.0
Left kidney displaced slightly towards midline	-	-	-	0.7(1)	0.05	0 - 1.7
Small haemorrhage within capsule of right kidney	-	0.7(1)	-	-	0.02	0 - 1.0
Unilateral hydronephrosis	1.3(1)	3.4(3)	1.4(2)	1.5(2)	2.6	0 - 11.7
Bilateral hydronephrosis	-	1.4(1)	-	-	0.9	0 - 9.8
Unilateral hydroureter	13.5(12)	7.4(6)	7.7(6)	14.2(13)	6.7	0 - 24.2
Bilateral hydroureter	2.6(2)	7.4(4)	2.8(4)	4.5(4)	4.4	0 - 27.1
Testis(es) displaced slightly†	11.0(7)	12.8(7)	8.6(6)	10.3(7)	3.7	0 - 23.5
Fluid-filled vesicle at anal edge of genital tubercle	-	0.7(1)	-	-	*	*
Genital tubercle slightly elongated	-	-	-	3.0(2)	0.4	0 - 6.3
Blood in anus	-	1.4(2)	0.7(1)	1.5(2)	0.2	0 - 7.2
Threadlike tail; imperforate anus; displacement of adrenal glands and kidneys	0.6(1)	-	-	-	0.08	0 - 1.8
Tip of tail threadlike and hooked	0.6(1)	-	-	-	*	*

* One foetus may have more than one observation.
† Percentage calculated on number of male foetuses.
* No record in background control data.

continued

Summary of foetal observations after free-hand serial sectioning

Group	:	1	2	3	4
Compound	:	Control	---		-
Dosage (mg/kg/day)	:	0	10	30	100

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined.*	156(21)	140(21)	143(21)	134(20)	12835 foetuses	126 studies
Number of males : females	82:74	70:70	70:73	68:66		
Observations: % foetal incidence (number of litters affected)					Mean	Study ranges
Thorax:						
Oesophagus displaced to right of trachea	-	-	-	0.7(1)	0.01	0 - 1.2
Left lobe of thyroid gland very reduced in size/absent	-	-	-	0.7(1)	0.1	0 - 2.2
Space between bodywall and organs	-	2.0(2)	-	11.2(5)	10.9	0 - 47.4
Retro-oesophageal right subclavian artery and misshapen thymus gland	-	0.7(1)	-	-	0.02	0 - 1.2
Blood-filled thoracic lymph duct	0.6(1)	1.4(2)	0.7(1)	-	0.3	0 - 2.9
Innominate artery reduced in length/absent	-	1.4(2)	1.4(1)	0.7(1)	0.1	0 - 1.4
Gross cardiovascular abnormality with valve defects	-	-	-	0.7(1)	*	*
Gross cardiovascular abnormality with double outlet right ventricle	-	-	-	0.7(1)	*	*
Slightly increased amount of pericardial fluid	1.3(2)	0.7(1)	1.4(2)	0.7(1)	0.3	0 - 5.3
Slightly haemorrhagic pericardial fluid	-	0.7(1)	1.4(1)	-	0.2	0 - 9.1
Haemorrhages on edge of lung lobes	-	-	-	1.5(2)	0.1	0 - 2.2

* One foetus may have more than one observation.
* No record in background control data.

continued

Summary of foetal observations after free-hand serial sectioning

Group	:	1	2	3	4
Compound	:	Control	---		-
Dosage (mg/kg/day)	:	0	10	30	100

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined.*	156(21)	148(21)	143(21)	134(20)	12835	126
Number of males : females	82:74	78:70	70:73	68:66	foetuses	studies

Observations: % foetal incidence (number of litters affected)					Mean	Study ranges
Others:						
Subcutaneous haemorrhage(s):						
Lower/side of jaw	3.2(3)	7.4(4)	4.2(4)	5.2(3)	0.8	0 - 8.9
Submandibular	2.6(2)	3.4(5)	6.3(7)	6.0(6)	1.0	0 - 8.8
Nasal	1.3(2)	3.4(5)	0.7(1)	2.2(2)	1.1	0 - 5.9
Cranial	1.9(3)	4.1(6)	1.4(2)	2.2(3)	2.5	0 - 8.2
Ventral/dorsal cervical	0.6(1)	2.7(3)	1.4(2)	6.7(5)	1.1	0 - 5.8
Scapular	11.5(12)	17.6(11)	16.1(12)	15.7(11)	28.6	6.4 - 90.5
Lateral/ventral/dorsal thoracic	2.6(3)	5.4(7)	7.7(8)	5.2(5)	1.5	0 - 5.8
Fore-/hind-limb(s)	17.3(10)	13.5(7)	10.5(7)	16.4(12)	*	*
Lateral/dorsal abdominal	0.6(1)	2.0(3)	0.7(1)	0.7(1)	0.6	0 - 3.8
Anal region	-	0.7(1)	-	0.7(1)	0.5	0 - 10.0
Tail	-	-	0.7(1)	0.7(1)	0.7	0 - 10.6
Subcutaneous oedema - trunk	2.6(2)	6.1(4)	4.2(5)	9.0(8)	3.6	0 - 17.5

* One foetus may have more than one observation.
* No record in background control data.

continued

Summary of foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	---		-----
Dosage (mg/kg/day)	:	0	10	30	100

Group :	1	2	3	4	Control data	
Number of foetuses (litters) examined:	157 (21)	146 (21)	141 (21)	135 (20)	19316 foetuses	129 studies
Observations :Grand Mean % foetal incidence @ (number of litters)					Mean	Study ranges
Vertebrae, limbs and girdles						
Ossification of ventral arch of 1st cervical vertebra.	7.0 (7)	4.8 (6)	7.1 (5)	2.2 (3)	6.94	0.0 - 22.2
Incomplete ossification, one or more cervical vertebral arches.	0.6 (1)	1.4 (2)	0.7 (1)	0.0 (0)	0.50	0.0 - 5.2
1st thoracic vertebral centrum unossified.	0.6 (1)	1.4 (1)	0.7 (1)	7.4 (2)	1.10	0.0 - 5.5
Incomplete ossification, one or more thoracic vertebral centra.	27.4 (15)	19.9 (16)	25.5 (17)	27.4 (14)	26.66	8.6 - 58.3
Incomplete ossification of one or more lumbar vertebral centra.	0.0 (0)	0.7 (1)	0.0 (0)	0.7 (1)	0.41	0.0 - 2.5
Incomplete ossification of one or more lumbar vertebral arches.	0.0 (0)	1.4 (2)	0.0 (0)	0.0 (0)	0.12	0.0 - 1.8
Incomplete ossification of sacral vertebral centra.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	0.01	0.0 - 0.5
Incomplete ossification of one or more sacral vertebral arches.	1.9 (3)	2.1 (3)	3.5 (5)	9.6 (4)	1.17	0.0 - 6.2
Short tail, tip thickened.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	*	
25 pre-sacral vertebrae.	1.3 (2)	0.0 (0)	2.8 (4)	0.7 (1)	0.81	0.0 - 6.7
Incomplete ossification of caudal vertebrae (less than 5).	1.3 (2)	1.4 (2)	1.4 (2)	14.1 (5)	2.96	0.0 - 14.5
Metacarpals/metatarsals 3/4.	69.4 (20)	56.2 (20)	80.1 (21)	74.1 (19)	67.30	28.6 - 86.9
Metacarpals/metatarsals 4/4.	29.3 (14)	43.2 (16)	19.9 (10)	16.3 (12)	30.65	6.2 - 71.4
Metacarpals/metatarsals incompletely ossified or unossified.	6.4 (5)	4.8 (5)	5.0 (6)	14.1 (6)	3.05	0.0 - 10.8
One or more phalangeal bones ossified.	1.3 (1)	6.2 (5)	2.1 (2)	0.7 (1)	1.89	0.0 - 8.1
Inner corners of one or both scapulae unossified.	5.7 (6)	2.7 (2)	9.2 (9)	5.2 (4)	3.59	0.0 - 14.4
Pubic bones incompletely ossified or unossified.	7.6 (6)	4.1 (6)	7.1 (5)	14.8 (8)	7.43	0.0 - 18.6
Incomplete ossification of one or both ischial bones.	1.9 (3)	2.1 (3)	5.0 (3)	5.2 (5)	0.90	0.0 - 4.7
Asymmetric pelvis, ilial bones associated with different sacral vertebrae.	0.0 (0)	0.0 (0)	1.4 (2)	0.7 (1)	0.49	0.0 - 3.7

@ One foetus may have more than one observation
* New parameter, no control data available

- continued

Summary of foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	---		----
Dosage (mg/kg/day)	:	0	10	30	100

Group :	1	2	3	4	Control data	
Number of foetuses (litters) examined:	157 (21)	146 (21)	141 (21)	135 (20)	19316 foetuses	129 studies
Observations :Grand Mean % foetal incidence @ (number of litters)					Mean	Study ranges
Sternebrae and ribs						
Incomplete ossification of 1 sternebra.	10.5 (13)	19.2 (12)	9.2 (8)	11.9 (9)	13.53	0.0 - 40.0
Incomplete ossification of 2 sternebrae.	66.9 (21)	65.1 (21)	75.9 (21)	48.9 (18)	66.88	43.3 - 84.8
Incomplete ossification of 3 sternebrae.	7.6 (8)	11.6 (7)	11.3 (11)	23.0 (14)	11.80	1.1 - 23.3
Incomplete ossification of 4 sternebrae.	4.5 (4)	3.4 (4)	1.4 (2)	7.4 (9)	3.68	0.0 - 17.5
Incomplete ossification of 5 sternebrae.	0.0 (0)	0.0 (0)	2.1 (1)	3.0 (2)	0.00	0.0 - 3.8
Incomplete ossification of 6 sternebrae.	1.3 (2)	0.0 (0)	0.0 (0)	5.2 (2)	0.53	0.0 - 6.7
1st sternebra cleft.	0.6 (1)	0.7 (1)	0.7 (1)	5.2 (4)	0.86	0.0 - 7.6
One or more sternebrae offset.	2.5 (4)	1.4 (1)	2.1 (3)	0.0 (0)	1.32	0.0 - 5.2
Ribs 13/13.	100.0 (21)	97.3 (21)	98.6 (21)	97.0 (20)	98.17	92.5 -100.0
Ribs 13/14.	0.0 (0)	1.4 (2)	1.4 (2)	1.5 (2)	1.20	0.0 - 4.2
Ribs 14/14.	0.0 (0)	1.4 (2)	0.0 (0)	1.5 (2)	0.51	0.0 - 3.5
14th rib enlarged.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	*	
13th rib or ribs reduced in length.	1.3 (2)	2.7 (3)	2.8 (3)	2.2 (2)	2.31	0.0 - 13.4
Slight medial thickening of one or more ribs.	0.0 (0)	0.7 (1)	0.0 (0)	0.0 (0)	0.02	0.0 - 1.4

@ One foetus may have more than one observation
* New parameter, no control data available

Summary of foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	-----	.....	---
Dosage (mg/kg/day)	:	0	10	30	100

Group :	1	2	3	4	Control data	
Number of foetuses (litters) examined:	157 (21)	146 (21)	141 (21)	135 (20)	19316 foetuses	129 studies
Observations :Grand Mean % foetal incidence @ (number of litters)					Mean	Study ranges
Head						
Small anterior fontanelle.	1.9 (2)	0.0 (0)	0.7 (1)	0.0 (0)	1.33	0.0 - 11.2
Medium anterior fontanelle.	96.2 (21)	98.6 (21)	99.3 (21)	91.9 (19)	96.20	76.4 -100.0
Large anterior fontanelle.	1.9 (3)	1.4 (2)	0.0 (0)	8.1 (2)	2.46	0.0 - 23.6
Incomplete ossification of supra-occipital bone.	24.2 (12)	14.4 (10)	12.8 (11)	19.3 (11)	13.48	0.0 - 29.2
Incomplete ossification of interparietal bone.	49.0 (20)	40.4 (18)	40.4 (18)	41.5 (18)	28.40	4.9 - 91.0
Incomplete ossification of parietal bone.	0.6 (1)	1.4 (2)	0.0 (0)	2.2 (1)	1.30	0.0 - 7.1
Incomplete ossification of squamosal bone.	0.0 (0)	0.7 (1)	0.0 (0)	3.0 (2)	0.84	0.0 - 4.7
Incomplete ossification of frontal bone.	1.3 (2)	0.0 (0)	0.0 (0)	3.0 (2)	0.09	0.0 - 1.7
Discrete unossified area in frontal bone.	0.6 (1)	2.7 (3)	2.1 (3)	2.2 (2)	0.43	0.0 - 4.6
Incomplete ossification of nasal bone.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	0.02	0.0 - 0.6
Discrete unossified area in basioccipital bone.	0.0 (0)	0.0 (0)	0.7 (1)	0.0 (0)	0.12	0.0 - 2.4
Incomplete ossification of basioccipital bone.	0.0 (0)	0.7 (1)	0.0 (0)	0.0 (0)	0.01	0.0 - 0.6
Incomplete ossification of basisphenoid, craniopharyngeal canal enlarged.	0.0 (0)	0.0 (0)	1.4 (2)	2.2 (2)	0.19	0.0 - 14.3
Incomplete ossification of basisphenoid bone.	4.5 (7)	7.5 (8)	11.3 (9)	13.3 (8)	0.94	0.0 - 12.6
Presphenoid bone incompletely ossified or unossified.	0.6 (1)	0.0 (0)	0.0 (0)	4.4 (2)	0.12	0.0 - 5.4
Fronto-nasal suture enlarged.	1.9 (3)	0.7 (1)	0.7 (1)	4.4 (2)	1.13	0.0 - 5.3
Incomplete ossification of hyoid bone.	7.0 (6)	5.5 (6)	9.9 (3)	5.9 (4)	7.28	0.0 - 25.0
Hyoid bone unossified.	12.7 (8)	11.6 (9)	7.1 (8)	5.2 (6)	8.52	0.0 - 18.5

@ One foetus may have more than one observation

F_0 Dams; Postnatal Phase

There was a slight increase in gestation length in the 30 mg/kg (n.s.) and 100 mg/kg ($P< 0.05$) groups, from a mean of 22.5 days in control to a mean of 23.0 days in the 100 mg/kg group). Although body weights of the drug treated animals tended to be higher than control, no significant intergroup differences in body weight were observed during lactation.

F_1 Offspring to 5 Weeks of Age

There were no compound related effects at any dose level on number of stillbirths, litter size or sex ratio at birth, number of implantation sites, survival indices throughout lactation, body weight or body weight gain during lactation.

There were no compound related effects on physical development (time of pinna unfolding, hair growth, testis descent, tooth eruption, eye opening and vaginal opening).

There were no effects of compound treatment on auditory and visual function (tested on PPD 25), within-cage activity (measured by means of electronic detectors and infra-red light apparently on PPD 26 to 27), learning ability (water filled Y-maze on PPD 27) and neuromuscular function (traversing flat and round rods, rotorod treadmill, mid-air righting reflex, fore- and hind-limb wire hanging and grid-griping ability on PPD 28-30). There were no effects on body weight or body weight gain to 5 weeks of age.

F_1 Offspring Between 5 and 10 Weeks of Age

The 20 males and 20 females per group, selected at 5 weeks of age, were assessed primarily for physical and sexual maturation and reproductive performance.

There were no compound related effects in males or females on appearance, behavior, body weights, mating performance at 9 or 10 weeks of age or on fertility.

In F_1 females killed on day 20 of gestation, there was no evidence of effects on implantation, embryo/fetal survival, fetal weights or placental weights.

No gross pathology abnormalities were observed in F_1 males or females that were considered to be related to treatment of the F_0 females.

4. Embryotoxic and Teratogenic Effects in Rabbits

Report No: 7595
Study No.: Not given

Performing Laboratory:

Dates Performed: 10/77 to 1/78 (first test)
1/78 to 4/78 (second test)

Quality Assurance: These studies were performed prior to the time that GLP compliance was required. The investigators in Germany claim that to the best of their knowledge, the study was performed "according to the state of the art".

Test Animals: Sexually mature female Himalayan rabbits, around 2 to 3.5 kg body weight, inseminated twice by means of copulation with males of the same strain and similar age.

Procedure: There were two separate but related studies in which batch 1/77, micronized were used. In the first study, the test substance was administered once daily by stomach tube, as a suspension in a vehicle consisting of "60 g anhydrous glycerol, 100 g demineralized water and polyethylene glycol 400 to make up 1129 g". Twelve or 13 does/group were treated from days 6 to 18 of gestation with doses of 0, 3, 10 or 30 mg/kg. On day 29, a C-section was carried out and each doe was examined for number of implantations (no data on corpora lutea count), number of live and dead fetuses and embryos, weight of litter and placentae. Each fetus was sexed, then examined for external, visceral and skeletal anomalies. Because of diarrhea in 4 animals of the 30 mg/kg group and spontaneous abortion in 2 of these 4 does, a second study limited to 0 and 30 mg/kg (n = 12 or 13/group), with a vehicle consisting of 0.5% aqueous Tylose, was performed. It was suspected that the vehicle contributed to the diarrhea in the first study. In all other respects, the procedure was the same as in the first study.

Results of the First Study

Maternal Survival, Clinical Signs and Body Weight Gain

One death at high dose (day 27 p.c.) was attributed to diarrhea on multiple days; 1 death at mid dose (day 9 p.c.) was attributed to an intubation accident.

The 30 mg/kg dose "caused" diarrhea in 4 animals (1, with multiple days of diarrhea, died; the remaining 3 had diarrhea on only 1 day, either on day 16 or 17 p.c.). Spontaneous abortions occurred in 1 doe at 3 mg/kg (day 25 p.c.), 1 at 10 mg/kg (day 28 p.c.), and 2 at 30 mg/kg (days 18 and 27; both had diarrhea). None of the control does aborted. Although there was only one

more spontaneous abortion in the high dose group than in the low or mid dose groups, the investigators nevertheless suggested that the incidence in the high dose group was increased, and attributed this effect to diarrhea associated with the vehicle and treatment. The abortions at low and mid doses were considered to be neither treatment related nor significant because the observed rates were considered normal for the strain of rabbit used.

Only females found to be pregnant were included in calculation of mean body weight values (N = 13, 12, 12 and 10 for control, 3, 10 and 30 mg/kg groups). Decreases in body weight gain between days 6 and 18 and over the entire period of gestation in all 3 treated groups were not statistically significant (See table on page 105A).

Laparoscopic Observations

There were no significant effects on mean numbers of implantations or resorptions per doe. The mean number of live male fetuses per doe was reduced in the 30 mg/kg group vs control ($P< 0.05$) resulting in a reduction in ratio of males:females but it was considered to be a random occurrence. There was no effect on fetal or placental weights, and no increase in number of "underdeveloped forms" (i.e. fetuses lower than 2.5 g body weight). There was an increase in malformation rate in the high dose group; the high dose malformations occurred in the offspring of the three animals that had diarrhea and were suggested to be the result of maternal stress (see pages 104 to 105A).

Results of the Second Study:

Maternal Survival, Clinical Signs and Body Weight Gain

Diarrhea or other clinical signs did not occur in does of the 30 mg/kg group, but one doe on 30 mg/kg died of an intubation accident (sometime between days 18 and 29, p.c., based on individual animal weight gain tables). Final results were based on 11 surviving does in each group. There was a decrease in mean body weight in the treated group, compared to an increase in control, between days 6 and 18 (treatment period), resulting in a compound related decrease in body weight gain over the entire gestation period.

Laparoscopic Observations

There were no compound related effects on pregnancy rate, spontaneous abortion rate, mean number of implantations (corpora lutea were not counted) or live fetal count, but mean fetal weight and placental weight were lower ($P<0.05$) and the number of "underdeveloped forms" was higher in the treated group. One of the dams on 30 mg/kg had 5 fetuses with "reduced motility". In this second study, there was no effect on sex ratio at birth, as had been observed in the first study. There was no increase in

external, visceral or skeletal malformations in any treated group vs control (See tables on pages 105B, C & D).

Comment: There were indications of fetal toxicity at 30 mg/kg, a dose that was maternally toxic. For example, there was a reduction in live male fetuses per dam and suggestions of an increase in total number of fetuses with malformations and the total number of litters with fetuses that had malformations, compared to control, in the first study. The total number of runts was higher and mean fetal weights were lower than control in the second study. However, there was no increase in incidence of any specific form or class of terata and no clear indication that this substance was teratogenic in rabbits.

Report No. 7595

Incidence table of the findings of the fetuses#

Findings	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
MENINGOCELE	1(1)[a]			
TELENCEPHALON dysplasia	1(1)[a]			
CRAWN-HAND slight	1(1)[a]			
TONGUE small/sharp/thin		1(1)		
FORE-LIMB abnormal position				1(1)[a]
arthrogryposis	1(1)[b]			1(1)[a]/1(1)[b]
MULTIPLE MALFORMATION			1(1)	1(1)[a]/[illegible][b]
CLEFT PALATE				1(1)[a]
MOTILITY reduced				5(1)[b]

# on individual basis; values in () on litter basis
a = first study / b = second study

Report No. 7595

Individual clinical findings of the dams

Note: animals without findings are not listed

Dose [mg/kg]	Dam-No.	Findings
0 (1st study)	914	on day 26 p.c. no stool, from day 27 p.c. very reduced stool
3	899	abortion on day 25 p.c.: 3 placentas
10	876	abortion on day 28 p.c.: 4 fetuses with placentas
	896	found dead on day 9 p.c. (lung application)
30 (1st study)	877	abortion on day 27 p.c.: 3 fetuses and 3 placentas
	881	on day 13 p.c. red bordered eyes
		on day 16 p.c. scratch wounds
		on day 17 p.c. diarrhea
	885	on day 17 p.c. diarrhea
	905	on day 18 p.c. diarrhea
	921	on days 17 and 18 p.c. diarrhea
		from day 24 p.c sick, reduced stool
		on day 25 p.c. bloody urination
		found dead on day 27 p.c
	925	abortion on day 18 p.c.: 3 fetuses
0 (2nd study)	-	-
30 (2nd study)	950	from day 9 p.c. sick, decreased feed consumption, no stool
		on one day between day 18 and 25 p.c. diarrhea
		found dead on day 25 p.c

5. Teratogenicity Study in Cynomolgus Monkey

Bayer Study No: T 3 022 847

Performing Laboratory:

Sponsor:

Dates Performed: 2/23/87 to 6/16/87

Quality Assurance: A signed statement of GLP compliance was included. The statement notes two deviations from 21CFR 58: 1) "the stability of the test article/carrier mixture had not been determined at the time of the study", and 2) "the final report of the study does not contain all the information specified in subsection 58.185. In particular, no analytical data relating to the test article or test article/carrier mixture are included."

Justification for Species Selection: "...because of its similar hormonal profile during pregnancy to that of man and this particular non-human primate submits itself as a favourable species for reproductive toxicology studies."

Doses Tested: 0, 30 and 100 mg/kg

Procedure: Feral cynomolgus monkeys (Macaca fascicularis) were obtained from and from . The females were "sexually mature", quarantined for "at least 2 weeks" and acclimatized to laboratory conditions for 3 weeks, before initiation of the study. There were 10 (30 mg/kg) or 12 females (0 and 100 mg/kg) per group that had tested positive for pregnancy (by a mouse uterotropic test); these animals weighed 2.6 to 3.8 kg on day 20 post-coitum. Pregnancy was subsequently monotored by rectal palpation on specified days between GD 30 and 86. Test substance was administered by intragastric intubation between gestation days (GD) 20 and 50, and necropsies for C-section were done on GD 100 ±1 day. Examination for fetal malformations included a comprehensive external examination, including head and body size measurements and appearance of externally observable organs. Soft tissue examination consisted of "a full necropsy of each fetus with visual macroscopic inspection" of the organs and weight measurements of 12 organs. After weighing, the organs were fixed in formalin, but not further evaluated. The skeleton was cleared and stained and examined for skeletal anomalies.

Test Substance: Batch No. 828 305. The vehicle consisted of 9.9% demineralized water, 4.8% glycerine and 85.3% Lutrin (polyethylene glycol 400). Test mixtures, consisting of separate solutions for each dose level, were prepared daily. The report notes that "Analysis of formulations and proof of absorption were not required by the study sponsor."

Results:

Spontaneous Abortions, Clinical Observations and Mortality: One control and 1 high dose monkey were found to be not pregnant, thus leaving 11, 10 and 11 monkeys in control, low and high dose groups, respectively. Incidence of abortions and deaths are summarized below.

Dose mg/kg	#/Gp*	# Abortions	# Deaths
Control	11	6 (GD 26-56)	3 (GD 21-99)
30	10	7 (GD 28-53)	1 (GD 48)
100	11	8 (GD 33-59)	5 (GD 27-88)

*Includes only pregnant animals.

Symptoms included heavy bleeding (followed by abortion), reduced food intake, diarrhea and vomiting. Symptoms were observed only during intervals of treatment, and included animals in the control group. Although all of the symptoms were generally more frequent or of longer duration in drug treated monkeys, they are considered to be due to treatment with the vehicle.

Two of the 3 control animals that aborted, subsequently died. The increased incidence of spontaneous abortions and deaths in the 100 mg/kg dose treated group were considered treatment related. (See comments on next page.)

The death of an additional animal in the high dose group which was found to be not pregnant and is not included in these results, was also considered to be treatment related. Causes of death in the 3 pregnant control monkeys included gastro-enteritis, catarrhal enteritis and "signs of asphyxia". Of the drug treated animals which died, the one in the 30 mg/kg group, and 4 of the 5 in the 100 mg/kg group, each had a volvulus (an intestinal obstruction due to twisting of the bowel); 3 of these animals (all high dose) exhibited abdominal distention shortly before death, and one of these 3 had acute catarrhal enteritis. A volvulus was considered to be a compound related cause of death; no volvulus was seen in any control animals.

Blood Analyses: Blood samples were taken from each monkey on GD 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90 and 97. However, data on blood analyses of any kind could not be found.

Fetal Examinations: External, visceral and skeletal findings for each surviving fetus are shown on the page which follows. In spite of the very few surviving fetuses (3 control, 2 mid dose and 1 high dose), the investigators concluded that the external and skeletal malformations seen in the sole surviving high dose fetus were drug related and were due to severe maternal toxicity. It was claimed that these malformations had never

before been observed in controls.

Fetal Organ Weights: Although weights of 10 or 11 different fetal organs with means (and standard errors only for controls) are presented in Table 4 and Appendix III of the report, statistical comparisons were obviously not possible (only one or two surviving fetuses in the treated groups).

Comments: It should be noted that the control incidence of symptoms, mortality and spontaneous abortions were "unusually high". Although the investigators attributed the higher incidence of abortions and deaths in the high dose group to treatment, there are no indications that the differences were statistically significant. The animals used in the present study were feral monkeys. It seems reasonable to suggest that there may have been an interaction between disease or parasitic infestations (often inherent in feral monkeys), stress of handling or control vehicle administration, and treatment with the high dose, which would confound the outcome of this study. The monkey is not a commonly used model for reproductive toxicity tests and there is limited background information. The limited number of offspring (usually one per monkey) is considered to be a disadvantage for using this species for this type of study. The high mortality and abortion rates, even in control animals, further limits the usefulness of this study. However, the only surviving fetus in the in the high dose group showed malformations "which were never before observed in control fetuses".

Group 1 - 0 mg/kg

Female number	Fetus alive	Type of defect		
		External findings	Visceral findings	Skeletal findings
33797	+	bent tail end	left adrenal severely enlarged	6th to 10th and 12th rib on the right side and 7th to 9th rib on the left side of uneven thickness; 4th to 6th sternebra not ossified, 7th sternebra incompletely ossified
33383	+	no abnormalities detected	no abnormalities detected	5th to 11th rib on the right side and 4th to 11th rib on the left side of uneven thickness; 1st to 7th sternebra not ossified
28980	+	no abnormalities detected	no abnormalities detected	5th to 11th rib on the right side and 6th to 10th rib on the left side of uneven thickness; 6th sternebra not ossified and 7th sternebra incompletely ossified
149	+	no abnormalities detected	no visceral investigation due to caesarian section on day 76 p.c.	no skeletal investigation due to caesarian section on day 76 p.c.

Group 2 - 30 mg/kg

Female number	Fetus alive	Type of defect		
		External findings	Visceral findings	Skeletal findings
34738	+	prepuce not patent	no abnormalities detected	displaced zygostyle; 5th to 11th rib on the right side and 8th to 11th rib on the left side of uneven thickness; 1st to 2nd and 6th to 7th sternebra not ossified
34733	+	bent tail end	no abnormalities detected	parietals incompletely ossified; 6th to 13th rib on the right side and 2nd to 3rd, 7th to 11th and 13th rib on the left side of uneven thickness; 2nd and 6th to 7th sternebra not ossified

Group 3 - 100 mg/kg

Female number	Fetus alive	Type of defect		
		External findings	Visceral findings	Skeletal findings
34040	+	left forelimb appears thinner than normal; tail shortened and inwards curved tail end; only three fingers on the left side and 3rd finger with two nails	no abnormalities detected	additional ossification site between the metacarpals of the 2nd and 3rd finger, additional fingernail on the 3rd finger, proximal phalanx of the 2nd and 3rd finger and medial phalanx of the 3rd finger on the left side abnormally developed; the last three coccygeal vertebrae asymmetrically and incompletely ossified; 5th to 11th rib on the right side and 7th to 12th rib on the left side of uneven thickness; 1st to 3rd and 6th to 7th sternebra not ossified

6. Perinatal and Postnatal Effects Following Oral Administration to Rats

Study No: T1002153

This report is accompanied by a "first amendment to report no. 12801 A", dated 8/14/93. Tables in the original English translation of the report were of very poor quality, not well organized, not legible, and contained errors in translation and typing. Some of these faults are listed in the amendment under the "rationale for the first amendment".

Performing Laboratory:

Dates Performed: May 1981 to October 1981

Quality Assurance: No statement on GLP compliance was found in the original report. In the amendment, it is claimed, "...there were no legally binding GLP regulations in force" during the time that this study was performed.

Doses Tested: 0, 3, 10 and 30 mg/kg.

Test Animals: Mura:WIST (SPF 67 Han) female rats, 11-14 weeks of age and weighing 177-240 g, were mated with stock males of the same strain.

Procedure: Presumed pregnant females were dosed orally by gastric intubation from gestation day (GD) 16 to postpartum day (PPD) 21. Half the females in each group were C-sectioned on GD 20, the remainder were allowed to litter and rear their young to PPD 21. Of the C-sectioned animals, approximately 1/3 of all the fetuses in each group were examined for visceral anomalies by the Wilson technique, the remainder were evaluated for bone anomalies by staining with Alizarin red. The abdominal and thoracic organs of animals selected for bone anomaly examination were removed and "evaluated".

Test Substance: Batch No. 576 923. The vehicle consisted of Lutrol 400, anhydrous glycerol and demineralized water in a ratio of 969:60:100. A preparation of 0.6% nisoldipine was tested for stability and was found to be stable after 7 days.

Effects on All Pregnant Females: There were two deaths, one at 3 mg/kg (cause of death not determined), and one at 10 mg/kg (died of pneumonia), but no treatment related effects on mortality or clinical signs were evident. A small but significant decrease in body weight gain was found for the dams at 30 mg/kg (both the C-sectioned and rearing groups) between the first day of administration (GD 16) and GD 20 (See tables on pages 113 & 113A). Body weights of the low dose group of the C-sectioned rats were significantly higher than control throughout gestation, even on GD 0, but obviously this was not a compound related effect.

There were no compound related effects on reproduction parameters (percent inseminated, percent with implantations, etc.; see table on a page 114), nor were there any effects on gross pathology.

Examination of C-Sectioned Females: There were no effects found on mean number of implantation sites, live or dead fetuses or resorptions per dam. Furthermore, there were no effects of treatment on sex distribution, mean placental weight, number of runts (fetuses <3 g), or frequencies of external, visceral or bone deformations. A decrease ($P< 0.01$) in mean fetal weight at the 30 mg/kg dose was evident (See table on page 115). The table which follows immediately below is a summary of malformations found in all 4 groups, copied from the original report.

Group	Dam No.	Number of changed foetuses	Changes
Control	-	-	-
3 mg/kg	2995	1	no tail
10 mg/kg	2849	1	Otocephaly
	2852	2	slight dilation of the lateral ventricle of the brain
30 mg/kg	2991	1	Cryptorchism

Effects on F_0 Dams Allowed to Litter: There were no effects on duration of pregnancy at any dose level. The report indicates a significantly higher number of implantations per dam in the high dose group (probably incidental and not treatment related), and no effect on prenatal loss (implantations - surviving and dead pups). Complete litter losses were reported for 2 dams in the 30 mg/kg group (both did not suckle their young), and for 1 in the 10 mg/kg group (devoured its young during the 3rd week), but normal lactational behavior was observed in all other dams (not shown in tables but indicated narratively in the original report).

Effects on F_1 Offspring: There was an increase in number of stillborn pups, and a dose related increase in mortality of the newborn pups during the first week postpartum in the 10 and 30 mg/kg groups (See table on page 115B; no statistical analysis). The birth weight and the weight increase during the 3 weeks postpartum were both significantly reduced in the 30 mg/kg group vs control (See table on page 115C). The report claims no compound related effects on appearance or clinical signs of the F_1 offspring. In the maturational development tests, there were no effects of treatment on time to pinna unfolding, appearance of fur or eye opening, but there was a slight delay in time for normal walking in the 30 mg/kg group. For the functional tests, there was no effect on pupillary reflex ("following a light in a darkened room"; age when given is not stated), and no effect on hearing (stimuli from a Galton whistle "at the end of the lactation period"). Running performance on a running roller

(sensomotor behavior or proprioreceptor reflexes) was considerably reduced in the 3 mg/kg group, but this was considered an incidental finding because there was no effect at the 10 and 30 mg/kg dose levels. For the F_1 generation fertility test, 1 male and 1 female in each litter of the control and high dose groups were reared to 10 weeks of age, then mated. There were no differences between the 2 groups in rate of insemination or fertilization, duration of pregnancy, total number of live male or female pups at birth, body weight of pups or pups with external deformities.

PERI- AND POSTNATAL-STUDY T1002153

WEIGHT DEVELOPMENT (G) OF THE FEMALES UNDERGOING CESAREAN SECTION
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEIGHT GAIN				
DAY 0 - 16 P.C.	59.8	64.4	60.1	57.8
	9.6	9.9	11.1	10.3
DAY 16 - 20 P.C.	38.4	40.2	39.2	33.0*
	10.2	8.0	6.6	5.4
DAY 0 - 20 P.C.	98.3	104.5	99.3	90.8
	16.4	16.3	14.6	12.5
BODYWEIGHT DURING GESTATION				
DAY 0 P.C.	201.0	210.0*	201.8	203.1
	11.8	12.2	10.1	11.3
DAY 16 P.C.	260.8	274.4*	261.9	260.9
	12.9	16.3	16.9	16.5
DAY 20 P.C.	299.3	314.5*	301.1	293.9
	20.8	20.0	20.6	16.8

* SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.025$
** SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.01$

PERI- AND POSTNATAL-STUDY T1002153

NUMBER OF ANIMALS RESULTS OF THE STUDY

DAMS

DOSE (MG/KG)	USED	NUMBER OF FEMALES							
		INSEMINATED		WITH IMPLANTATIONS		THAT LITTERED		THAT REARED THEIR PUPS	
		N	% OF THOSE USED	N	% OF THOSE INSEMINATED	N	% OF THOSE WITH IMPLANTATIONS	N	% OF THOSE THAT LITTERED
0	25	25	100.0	21	84.0	20	95.2	20	100.0
3	25	25	100.0	20	80.0	20	100.0	19	95.0
10	25	25	100.0	23	92.0	23	100.0	22	95.7
30	25	25	100.0	20	80.0	20	100.0	17	85.0

ANIMALS UNDERGOING CESAREAN SECTION

DOSE (MG/KG)	USED	NUMBER OF FEMALES					
		INSEMINATED		WITH IMPLANTATIONS		WITH FOETUSES	
		N	% OF THOSE USED	N	% OF THOSE INSEMINATED	N	% OF THOSE WITH IMPLANTATIONS
0	25	25	100.0	20	80.0	20	100.0
3	25	25	100.0	20	80.0	19*	95.0
10	25	25	100.0	21	84.0	19*	90.5
30	25	25	100.0	23	92.0	22	95.7

* ONE FEMALE DIED BEFORE CESAREAN SECTION

PERI- AND POSTNATAL-STUDY T1002153

RESULTS OF THE CESAREAN, SECTION (MEAN VALUES)

DOSE [MG/KG]	WEIGHT GAIN [G] 0-20 P.C.	WEIGHT GAIN [G] 16-20 P.C.	NUMBER (PER DAM) OF IMPL.	MALE	FEM.	SUM	LOSS	MEAN-WEIGHT IN GRAMMS FETUSES	PLACENT.	NO. OF FOETUSES EXAMINED BY WILSON	DAWSON	FOETUSES WITH MINOR SKELE-TAL DEVIAT	MALFOR-MATIONS	NO. OF RUNTS [<3G]
0	98.3	38.5	11.0	5.1	5.2	10.3	0.7	3.50	0.50	3.1	7.1	4.80	0.00	0.60
3	104.5	40.2	11.4	5.1	5.5	10.6	0.8	3.52	0.52	3.3	7.2	6.11***	0.05	0.58
10	99.3	39.2	10.2	4.5	4.9	9..	0.8	3.54	0.53	2.8	6.9	4.68	0.05	0.32
30	90.8*	32.0*	10.7	5.3	4.6	9.9	0.8	3.38**	0.49	3.1	7.2	4.73	0.05	0.91

* SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.05$
** SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.01$
*** SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.002$

7. Supplemental Perinatal and Postnatal Study in Rats

Study No: T3008898

Sponsor:

Dates Performed: February 1984 to March 1984

Quality Assurance: A signed statement of GLP compliance was included.

Doses Tested: 0 and 30 mg nisoldipine/kg/day. On the last 2 days of treatment, "several animals" received only 20 mg/kg due to an error, but the study results were not considered to have been affected by this error.

Procedure: Bor:WISW (SPF Cpb) naturally inseminated female rats (25 per group) were 12-14 weeks of age and weighed 178-216 g at the start of treatment. They were dosed orally by gastric intubation from gestation day (GD) 16 to postpartum day (PPD) 14. All the pregnant dams were allowed to litter and rear their young to PPD 14. The dams and offspring were evaluated for tolerance of the compound, effects of nisoldipine on birth and lactation and influence on post-natal development (similar to the observations in the main study). However, this study differed from the main study because the treatment and observation period extended to PPD 21 in the main study and only to PPD 14 in the present study.

Test Substance: Batch No. 907437. The vehicle consisted of Lutrol, glycerol and water.

Effects on the Dams: At 30 mg/kg, 1 died during parturition (with 6 dead fetuses). Treatment related effects noted were a highly significant decrease in weight gain between GD 16 and GD 20 (initial part of the treatment period), which resulted in a reduced mean body weight up to PP day 7, no longer evident by PPD 14, lightly colored feces, no longer evident after littering, and prolonged gestation (from 21.6 days in control to 22.1 days in the treated group; $P < 0.001$).

Effects on the Offspring: There were no significant intergroup differences in number of pups per litter at birth or after 1 and 2 weeks. However, there was a higher incidence of stillbirths and a higher rate of neonatal mortality throughout the 2 week postpartum study period for offspring of nisoldipine treated dams. The body weights of pups in the nisoldipine treated group were lower than control at the time of birth, and after 1 and 2 weeks. There was no effect on sex ratio at birth or at PPD 14 (See table which follows).

DOSE (MG/KG)	TOTAL NO. OF YOUNG (ALIVE + DEAD)	TOT. NO. OF DEAD YOUNG AT BIRTH	UP TO TIME OF 1ST WEIGHING	AFTER 1 WEEK	2 WEEKS
CONTROL	208	2	2	7	11
30	227	22*	30**	42**	50**

* significant difference from the controls, $p < 0.01$
**significant difference from the controls, $p < 0.001$

Comment: The increase in mean duration of gestation, followed by a higher incidence of stillbirths and a higher rate of neonatal mortality throughout the 2 week postpartum study period, and the lower body weight at birth for offspring of nisoldipine treated dams, were observed in both the main study and in the present study. These observations are suggestive of dystocia. Dystocia is defined as abnormal labor, which is usually accompanied by increased duration of labor and an increaed incidence of stillbirths and neonatal mortality.

MUTAGENICITY STUDIES (S. Stolzenberg)

1. Salmonella/Microsome Test

Study No.: Not provided. Pharma Report No. 9634

Performing Laboratory:

Date Performed: August, 1980 to September, 1980

Quality Assurance: A signed statement of compliance with GLP is not included.

Background: This widely used mutagenicity assay detects point mutations (base pair by TA 1535 and TA 100 and frame shift by TA1537 and TA 98), caused by chemical agents, *in vitro*. The reversion rates to prototrophy of histidine requiring (his-) mutants to the wild type histidine independent strain (his+), are evaluated in a medium with a low content of histidine. In the presence of test compound, an increase in reversion rate, measured by an increase in colony growth on the agar plate, is an indication of mutagenicity.

Procedure: The evaluation was performed with and without metabolic activation (provided by S-9 fraction of livers of Aroclor pretreated rats). *S. typhimurium* strains TA1535, TA100, TA1537 and TA98 were used. Two studies with TA 1535 but only one with the remaining 3 strains were carried out with 4 plates per concentration of test substance, DMSO control or postive control substances. Concentrations tested were 0, 20, 100, 500, 2500 and 12500 ug/plate.

Positive Controls:

a). without S-9 activation
Endoxan (cyclphosphamide) for TA1535 and TA100
Trypaflavin for TA1537 and TA98

b). with S-9 activation
2- aminoanthracene for all 4 tester strains

Results: 2500 ug/plate was toxic to bacterial growth for all 4 strains, whereas 12500 was toxic and caused precipitation, making it not possible to evaluate colony growth. 500 ug/plate was toxic for TA1535. In the first test, the positive control for TA1535 without S-9 showed no response, and the negative controls, both and without S-9, were unusually low. Therefore, the test with TA1535 was repeated. In the second test with TA1535, no indication of mutagenicity was seen with or without S-9. Similarly, Bay k 5552 showed no mutagenic effects on TA100, TA1537 and TA98. Positive controls gave adequate responses; i.e. well over double those of the negative controls.

Salmonella/Microsome Test with BAY k 5552 on Salmonella typhimurium TA 1535.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment / M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
12500			Could not be evaluated		
2500	0	0.8	64.4**	-	0.80
500	8.5	6.5	126.9**	8.50[a]	6.50[a]
100	9.0	6.3	158.4	9.00[a]	6.30[a]
20	14.5	5.0	146.0	14.50[a]	5.00[a]
Negative Control 0	1.0	1.0	152.4	1.00	1.00
Positive Control Endoxan 435	0.5	1.0	163.7	0.50[a]	1.00[a]
Positive Control 2-Aminoanthracene 10	32.8	19.0	5.2**	32.80[a]	19.00[a]

** Bacteriotoxic effect
a) See the "Results" section

In this test system, was considered not mutagenic at non-toxic, soluble concentrations.

Salmonella/Microsome Test with . on Salmonella typhimurium TA 100.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
12500			Could not be evaluated		
2500	4.3	19.5	2.0**	0.06	0.04
500	80.5	34.5	4.3	1.18	0.71
100	72.3	62.0	4.7	1.06	1.27
20	53.5	49.3	6.2	0.78	1.01
Negative Control 0	68.5	48.8	4.7	1.00	1.00
Positive Control Endoxan 435	273.8	82.0	3.9	4.00*	1.68
Positive Control 2-Aminoanthracene 10	1136.0	81.8	0.6**	16.58*	1.68

* Mutagenic effect
** Bacteriotoxic effect

Salmonella/Microsome Test with on Salmonella typhimurium TA 1537.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
12500			Could not be evaluated		
2500	0	0.5	45.9**	-	0.13
500	3.3	1.5	49.1	0.66	0.38
100	7.3	2.0	51.3	1.46	0.50
20	5.0	1.5	51.5	1.00	0.38
Negative Control 0	5.0	4.0	50.9	1.00	1.00
Positive Control Trypaflavine 200	162.8	114.8	44.7**	32.56*	28.70*
Positive Control 2-Aminoanthracene 10	37.3	15.3	0.3**	7.46*	3.83*

* Mutagenic effect

** Bacteriotoxic effect

Salmonella/Microsome Test with on Salmonella typhimurium TA 98.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment / M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
12500			Could not be evaluated		
2500	3.0	1.3	100.0**	0,18	0,27
500	22.0	3,0	127.3	1.31	0.68
100	19.3	4,8	151.70	1.15	1,00
20	14.0	5,5	-	0,83	1.14
Negative Control 0	16,8	4,8	121.5	1,00	1.00
Positive Control Trypaflavine 200	460,0	4,3	144,3	27.38*	0.90
Positive Control 2-Aminoanthracene 10	845.3	8,0	70,7**	50.32*	1.57

* Mutagenic effect
** Bacteriotoxic effect

Salmonella/Microsome Test with on Salmonella typhimurium TA 1535. Repeat.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment / M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
2500	0	0	toxic	-	-
500	0	0	toxic	-	-
100	6.5	6.0	non-toxic	1.08	1.33
20	6.8	7.0	non-toxic	1.13	1.56
Negative Control 0	6.0	6.5	non-toxic	1.00	1.00
Positive Control Endoxan 435	427.3	24.3	non-toxic	71.22*	5.40*
Positive Control 2-Aminoanthracene 10	266.0	8.5	non-toxic	44.33*	1.89

* Mutagenic effect

2. Salmonella/Microsome Test

Study No.: T 5027709

Performing Laboratory:

Date Performed: 1/29/88 to 3/11/88

Quality Assurance: A signed statement of compliance with GLP is included.

Procedure: Tester strains used were *S. typhimurium* TA1535, TA100, TA1537 and TA98. Two different forms of S-9 were used; from livers of Aroclor 1254 pretreated male NMRI mice, and from livers of NMRI male mice that had been pre-treated for 28 days with 2000 ppm Bay k 5552 in the diet. All the studies were carried out with 4 plates per concentration of test substance, control or postive control substances. Initially, concentrations tested both without and with metabolic activation were 0, 20, 100, 500, 2500 and 12500 ug/plate. Subsequently, concentrations tested were 0, 75, 150, 300, 600, 1200 and 2400 ug per plate. There is no explanation as to why two methods of enzyme activation were employed.

Positive Controls:

a). without S-9 activation
Sodium azide for TA1535
Nitrofurantoin for TA100
4-nitro-1,2-phenylene diamine for TA 1537 and TA98

b). with S-9 activation
2- aminoanthracene for all 4 tester strains

Results: No indication of mutagenicity was observed at any concentration tested. However, 20 ug/plate, the lowest concentration tested, was the only concentration at which bacteriotoxic problems were not encountered. Starting at 150 ug/plate, precipitation problems were also encoutered. Positive controls gave adequate responses; i.e. well over double the colony count of the negative controls.

Bay k 5552 was considered not mutagenic in this test system, but this study is valid only at 20 ug/plate because it was the only concentration at which toxicity and/or precipitation problems were not encountered with all 4 bacterial strains.

3. CHO HGPRT Forward Mutation Assay

Study No.: T 1023114 (sponsor's number)

Performing Laboratory:

Sponsor:

Date Performed: 7/11/86 to 10/29/86

Quality Assurance: A signed statement of compliance with GLP is included.

Background: "The objective of this study was to evaluate the test article for its ability to induce forward mutation at the HGPRT locus in the CHO-K1-BH Chinese hamster cell line, as assessed by colony growth in the presence of 6-thioguanine (TG). Hypoxanthine guanine phosphoribosyl transferase (HGPRT) is a cellular enzyme that allows cells to salvage hypoxanthine and guanine from the surrounding medium for use in DNA synthesis. If a purine analog such as TG is included in the growth medium, the analog will be phosphorylated via the HGPRT pathway and incorporated into nucleic acids, eventually resulting in cellular death. The HGPRT locus is located on the X chromosome. Since only one of the two X chromosomes is functional in the female CHO cells, a single-step forward mutation from HGPRT+ to HGPRT- in the functional X chromosome will render the cell unable to utilize hypoxanthine, guanine, or TG supplied in the culture medium. Such mutants are viable as wild-type cells in normal medium because DNA synthesis may still proceed by de novo synthetic pathways that do not involve hypoxanthine or guanine as intermediates. The basis for the selection of HGPRT- mutants is the loss of their ability to utilize toxic purine analogs (e.g., TG), which enables only the HGPRT- mutants to grow in the presence of TG. Cells which grow to form colonies in the presence of TG are therefore assumed to have mutated, either spontaneously or by the action of the test article, to the HGPRT- genotype."

Procedure: In preliminary, range finding tests, concentrations of 50 and 100 ug/ml (batch #828305) without metabolic activation were found to be 100% cytotoxic to the cell culture, but in the presence of metabolic activation (provided by S-9 fraction of livers of Aroclor 1254 pretreated rats), 100 ug/ml caused only a 39% decrease in survival index.

Three tests without S-9 included duplicate cultures with concentrations between 10 to 40 ug/ml, and two tests with S-9 included duplicate cultures with concentrations of 5 to 85 ug/ml. Positive controls were 5-bromodeoxyuridine without S-9 and 3-methylcholanthrene in the presence of S-9.

Results: Decreases in relative cell survival were seen at 30 ug/ml or higher concentrations without S-9, and at 50 ug/ml and higher with S-9. There were sporadic, small but statistically significant increases in mutant frequencies in each of the 3 tests without S-9 and in one of the two tests with S-9. In every case the increase occurred in only one of the two duplicate cultures. In spite of a suggestion of a concentration relationship at 40 and 60 ug/ml without S-9 (see first 2 tables which follow), it was claimed that a concentration relationship did not exist. It should, however, be noted that even among the duplicate controls, there were wide variations in mutant frequencies in most of the tests.

was considered not mutagenic in this test system.

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: _____ ASSAY NO: E-9516

VEHICLE: DMSO TEST DATE: August 20, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants
Expression Time: 7 days

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES/DISH NUMBER: 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
NONACTIVATION:																		
Vehicle Control	258.0 ± 13.5	100.0	100.0	0	1	0	0	0	0	0	0	0	0	0	0	1	118.5 ± 8.7	0.4
	275.0 ± 1.0			2	2	0	1	0	1	2	1	2	3	0	4	18	108.8 ± 13.9	6.9*
Positive Control (50 µg/ml BrdU)[b]	145.7 ± 1.5	54.7	67.4	26	20	22	17	26	17	26	24	18	20	22	23	261	88.5 ± 1.3	122.9**
	149.3 ± 9.1	56.0	68.4	23	22	25	18	35	27	18	23	31	31	18	25	296	86.8 ± 7.3	142.1**
TEST ARTICLE																		
10.0 µg/ml	242.7 ± 6.5	91.1	169.6	NOT CLONED														
10.0 µg/ml	251.7 ± 12.7	94.4	159.6	NOT CLONED														
12.5 µg/ml	237.3 ± 7.6	89.1	131.4	NOT CLONED														
12.5 µg/ml	263.7 ± 32.0	98.9	139.6	NOT CLONED														
15.0 µg/ml	243.3 ± 7.5	91.3	127.3	0	2	0	0	0	0	0	0	1	C	0	0	3	83.5 ± 3.6	1.6
15.0 µg/ml	245.0 ± 22.6	91.9	164.6	0	0	1	0	0	0	0	0	0	0	0	0	1	80.5 ± 6.1	0.5
20.0 µg/ml	243.0 ± 21.7	91.2	143.7	NOT CLONED														
20.0 µg/ml	232.7 ± 11.6	87.3	136.2	NOT CLONED														
25.0 µg/ml	215.7 ± 10.2	80.9	124.1	1	1	1	1	3	1	2	0	2	0	1	0	13	84.0 ± 4.0	6.4
25.0 µg/ml	206.7 ± 28.6	77.5	115.2	2	0	1	1	0	0	1	1	1	1	2	0	10	100.3 ± 2.3	4.2
30.0 µg/ml	249.0 ± 6.6	93.4	99.7	C	C	C	C	C	C	C	C	C	C	C	C	-	100.0 ± 0.0†	-
30.0 µg/ml	227.3 ± 10.0	85.3	66.1	0	0	0	0	4	0	1	1	2	0	1	1	10	113.3 ± 4.6	3.7
35.0 µg/ml	237.7 ± 21.5	89.2	59.8	C	C	C	C	C	C	C	C	C	C	C	C	-	122.5 ± 6.4[x]	-
35.0 µg/ml	227.7 ± 11.7	85.4	55.1	0	0	0	0	2	0	0	0	0	1	0	1	4	133.7 ± 4.4	1.2
40.0 µg/ml	193.3 ± 9.3	72.5	39.6	4	2	1	1	4	3	3	0	0	3	0	0	21	105.2 ± 5.5	8.3**
40.0 µg/ml	193.0 ± 13.0	72.4	42.3	0	0	1	0	0	0	0	0	0	0	0	1	2	109.8 ± 3.3	0.8

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: _____ ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: September 19, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; 2×10^5/dish for mutants
Expression Time: 7 days

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES/DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E. ± S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
NONACTIVATION:																		
Vehicle Control	193.7 ± 6.0	100.0	100.0	0	3	1	1	0	2	2	0	1	0	0	0	10	102.3 ± 4.9	4.1
	187.0 ± 15.4			0	2	1	1	2	2	0	1	0	1	0	0	10	111.3 ± 13.7	3.7
Positive Control	148.3 ± 15.8	77.9	37.6	12	14	16	10	13	13	15	14	13	16	17	15	168	103.3 ± 4.0	67.8**
(50 µg/ml BrdU)[b]	139.7 ± 7.8	73.4	32.9	20	12	18	16	20	15	22	14	16	13	14	10	190	105.0 ± 6.6	75.4**
TEST ARTICLE																		
10.0 µg/ml	147.3 ± 12.1	77.4	43.6	0	4	0	1	0	1	0	0	0	1	0	0	7	129.0 ± 5.3	2.3
10.0 µg/ml	156.3 ± 11.8	82.1	59.5	3	0	2	2	1	3	3	0	3	3	1	0	21	125.8 ± 6.4	7.0
15.0 µg/ml	152.0 ± 17.3	79.9	44.0	1	1	1	1	1	1	1	0	2	0	1	2	12	127.0 ± 14.1	3.9
15.0 µg/ml	151.3 ± 11.7	79.5	39.8	1	0	1	0	0	4	1	0	3	1	3	0	14	142.7 ± 14.7	4.1
20.0 µg/ml	137.0 ± 23.1	72.0	44.8	0	0	2	0	1	0	0	0	1	0	2	0	6	112.3 ± 7.4	2.2
20.0 µg/ml	151.3 ± 11.2	79.5	31.8	1	2	4	5	2	2	1	2	1	0	1	1	22	141.3 ± 2.8	6.5
30.0 µg/ml	125.3 ± 3.2	65.8	42.9	0	2	1	1	0	0	0	1	2	0	2	0	9	110.2 ± 1.2	3.4
30.0 µg/ml	135.7 ± 8.6	71.3	49.9	3	1	0	1	1	2	2	2	3	5	2	0	22	115.5 ± 4.4	7.9*
40.0 µg/ml	42.7 ± 9.0	22.4	7.0	0	0	1	0	0	0	1	0	0	1	2	1	6	129.3 ± 15.7	1.9
40.0 µg/ml	42.3 ± 8.1	22.2	7.4	0	3	2	4	3	0	4	3	4	3	7	3	44	96.3 ± 1.5	19.0**
60.0 µg/ml	0.0 ± 0.0	0.0	< 0.1	NOT CLONED														
60.0 µg/ml	0.0 ± 0.0	0.0	< 0.1	NOT CLONED														

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: _____ ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: October 10, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants
Expression Time: 7 days

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES/DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
MONACTIVATION:																		
Vehicle Control	152.3 ± 11.2	100.0	100.0	1	2	0	0	1	2	0	0	0	0	0	3	9	112.8 ± 6.8	3.3
	172.0 ± 8.5			1	2	0	0	2	3	3	3	2	1	3	1	21	126.5 ± 11.0	6.9
Positive Control	84.7 ± 1.2	52.2	42.9	24	25	27	15	40	25	20	21	27	24	21	28	297	101.3 ± 11.5	122.2**
(50 µg/ml BrdU)[b]	84.7 ± 14.5	52.2	41.1	23	22	21	21	24	26	17	13	28	20	30	20	265	90.7 ± 5.5	121.7**
TEST ARTICLE																		
10.0 µg/ml	140.0 ± 6.1	86.3	76.0	2	2	1	3	5	2	2	3	1	2	2	0	25	108.7 ± 1.3	9.6*
10.0 µg/ml	145.7 ± 4.0	89.8	95.6	0	3	0	1	3	1	2	0	2	1	2	5	20	101.8 ± 11.3	8.2
15.0 µg/ml	146.7 ± 13.1	90.4	85.1	0	C	1	0	0	1	0	2	0	2	3	1	10	120.5 ± 1.7	3.5
15.0 µg/ml	136.7 ± 12.0	84.3	72.4	2	2	3	3	2	1	3	0	3	0	1	1	21	115.8 ± 13.8	7.6
20.0 µg/ml	138.7 ± 11.5	85.5	67.7	0	2	1	0	1	0	0	2	0	1	0	1	8	124.2 ± 6.6	2.7
20.0 µg/ml	120.0 ± 20.9	78.9	73.9	0	2	3	3	3	4	2	3	1	2	1	3	27	100.3 ± 9.4	11.2**
30.0 µg/ml	102.7 ± 7.4	63.3	65.8	1	2	1	1	1	3	2	1	1	1	3	3	20	104.2 ± 3.2	8.0
30.0 µg/ml	108.3 ± 9.0	66.8	57.7	T	T	T	2	2	1	1	5	2	1	4	0	18	122.3 ± 11.0	8.2
40.0 µg/ml	45.7 ± 12.1	28.2	8.2	6	0	0	1	2	4	4	0	1	0	3	1	22	116.8 ± 3.3	7.8
40.0 µg/ml	47.0 ± 5.3	29.0	9.1	1	0	1	0	1	1	3	0	1	1	2	0	11	113.5 ± 3.9	4.0
60.0 µg/ml	0.3 ± 0.6	0.2	< 0.1	NOT CLONED														
60.0 µg/ml	0.3 ± 0.6	0.2	< 0.1	NOT CLONED														
80.0 µg/ml	0.0 ± 0.0			NOT CLONED														
80.0 µg/ml	0.0 ± 0.0			NOT CLONED														
100.0 µg/ml	0.0 ± 0.0			NOT CLONED														
100.0 µg/ml	0.0 ± 0.0			NOT CLONED														

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: _____ ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: September 10, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; 2×10^5/dish for mutants

Expression Time: 7 days S9 batch: 1-186

TEST CONDITION	SURVIVAL TO TREATMENT: TRAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
S9 ACTIVATION:																		
Vehicle Control	294.3 ± 12.9	100.0	100.0	0	2	2	2	1	0	4	1	0	2	0	0	14	110.3 ± 5.1	5.3
	396.3 ± 122.1			0	0	0	2	1	1	0	0	0	0	0	0	4	110.2 ± 5.8	1.5
Positive Control	276.0 ± 20.2	79.9	55.4	69	55	53	88	53	70	69	60	61	63	61	52	754	97.2 ± 9.0	323.2**
(5 µg/ml 3-MCA)[b]	256.7 ± 12.0	74.3	37.8	86	81	65	64	80	64	54	62	63	77	68	83	841	117.3 ± 5.1	298.7**
TEST ARTICLE																		
10.0 µg/ml	272.7 ± 16.3	79.0	194.8	NOT CLONED														
10.0 µg/ml	267.3 ± 4.5	77.4	167.8	NOT CLONED														
20.0 µg/ml	295.3 ± 11.7	85.5	111.4	NOT CLONED														
20.0 µg/ml	324.7 ± 8.4	94.0	145.5	NOT CLONED														
35.0 µg/ml	305.0 ± 26.6	88.3	140.3	1	0	1	0	1	0	0	0	0	1	0	0	4	96.2 ± 10.2	1.7
35.0 µg/ml	293.0 ± 9.5	84.9	153.9	0	1	0	0	0	0	0	0	1	0	0	0	2	94.5 ± 5.9	0.9
50.0 µg/ml	680.3 ± 43.7	197.0	108.1	0	0	3	0	1	2	2	3	0	3	1	1	16	94.7 ± 3.8	7.0*
50.0 µg/ml	250.0 ± 11.5	72.4	94.4	1	0	2	0	0	1	0	0	0	0	0	0	4	87.8 ± 5.0	1.9
60.0 µg/ml	215.3 ± 13.6	62.4	77.2	0	0	0	0	0	0	0	0	0	0	0	0	0	91.7 ± 6.0	0.0
60.0 µg/ml	250.0 ± 13.0	72.4	37.5	0	0	0	0	1	0	1	0	3	2	0	0	7	95.7 ± 4.5	3.0
70.0 µg/ml	124.7 ± 2.1	36.1	17.3	0	2	0	0	0	0	0	0	0	0	1	0	3	81.3 ± 5.9	1.5
70.0 µg/ml	119.7 ± 12.7	34.7	17.8	0	0	0	0	1	0	1	2	0	1	0	0	5	79.8 ± 1.3	2.6
85.0 µg/ml	22.0 ± 1.7	6.4	2.9	0	0	0	0	0	0	0	0	0	0	0	0	0	102.2 ± 5.5	0.0
85.0 µg/ml	24.0 ± 3.6	7.0	3.0	0	0	0	0	0	0	0	0	0	0	0	0	0	98.3 ± 4.8	0.0
100.0 µg/ml	6.3 ± 1.5	1.8	0.3	NOT CLONED														
100.0 µg/ml	5.0 ± 2.6	1.4	0.3	NOT CLONED														

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIEN _____ TEST ARTICLE: _____ ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: October 1, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; 2×10^5/dish for mutants

Expression Time: 7 days S9 batch: I-186

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES/DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
S9 ACTIVATION:																		
Vehicle Control	287.3 ± 12.7	100.0	100.0	0	C	0	0	0	C	C	1	2	1	0	1	5	101.8 ± 3.5	2.7
	347.3 ± 31.5			1	0	0	1	1	1	2	1	3	2	2	1	15	125.8 ± 9.5	5.0
Positive Control	259.7 ± 1.5	81.8	49.4	C	71	C	94	C	80	69	93	71	72	73	73	696	80.2 ± 7.2	482.1**
(5 µg/ml 3-MCA)[b]	266.3 ± 24.0	83.9	46.4	80	84	87	89	79	82	70	70	73	62	65	74	915	85.8 ± 7.7	444.3**
TEST ARTICLE																		
20.0 µg/ml	332.0 ± 26.5	104.6	90.3	0	0	1	0	1	0	2	2	1	0	1	0	8	119.7 ± 4.0	2.8
20.0 µg/ml	283.0 ± 43.8	89.2	80.6	2	2	0	1	1	1	1	0	1	0	1	3	13	127.3 ± 2.1	4.3
35.0 µg/ml	341.7 ± 13.1	107.7	99.2	0	1	0	0	2	0	1	0	0	1	0	0	5	122.3 ± 6.8	1.7
35.0 µg/ml	266.0 ± 16.6	83.8	132.5	0	0	0	0	0	0	0	1	0	0	1	0	2	129.2 ± 10.2	0.6
50.0 µg/ml	268.7 ± 7.6	84.7	C	NOT CLONED														
50.0 µg/ml	272.0 ± 7.8	85.7	C	NOT CLONED														
60.0 µg/ml	278.7 ± 39.4	87.8	75.4	2	0	0	1	3	2	3	0	1	1	1	C	14	117.3 ± 3.8	5.4
60.0 µg/ml	280.7 ± 4.6	88.5	82.3	1	1	>0	0	1	0	0	0	1	0	1	1	6	141.5 ± 4.8	1.8
70.0 µg/ml	169.3 ± 14.5	53.4	22.9	1	1	0	1	0	1	0	0	0	1	0	0	5	116.8 ± 9.4	1.8
70.0 µg/ml	142.7 ± 10.1	45.0	31.6	0	0	1	0	2	0	0	1	0	0	0	0	4	99.5 ± 4.8	1.7
85.0 µg/ml	46.0 ± 5.6	14.5	3.3	3	1	0	2	1	2	0	2	1	0	1	0	13	109.5 ± 2.8	4.9
85.0 µg/ml	34.0 ± 3.6	10.7	2.3	2	0	1	0	0	0	0	0	0	0	0	0	3	125.7 ± 0.6	1.0
100.0 µg/ml	5.0 ± 0.0	1.6	0.2	NOT CLONED														
100.0 µg/ml	3.3 ± 0.6	1.0	0.2	NOT CLONED														

4. Mouse Hepatocyte Primary Culture DNA Repair Assay

Study No.: T2 023 386 (sponsor's number)

Performing Laboratory:

Sponsor:

Date Performed: 8/14/86 to 2/25/87

Quality Assurance: A signed statement of compliance with GLP is included.

Background: Freshly obtained rodent, metabolically active hepatocytes are capable of limited biotransformation activity. Chemically induced damage to nucleic acid of the mammalian cells results in an effort by the enzyme systems to repair the defect(s), resulting in unscheduled DNA synthesis.

Procedure: Freshly prepared hepatocyte primary cell cultures from adult $B6C3F_1$ males were used, according to the method of Williams et al, Cancer Letters 6: 119-306, 1982. Eight concentrations between 1 mg/ml and 5×10^{-4} mg/ml (listed in the table which follows) were tested in triplicate against $5X10^5$ hepatic cells. DNA repair was determined by ^3H-thymidine uptake, to determine the net increase in nuclear grains induced by the test compound. Benzo(a)pyrene served as positive control and DMSO and pyrene served as negative controls. A test compound was considered positive when the mean net nuclear grain count exceeded that of the DMSO control by more than 2 standard deviations.

Results: Cytotoxicity was observed at concentrations of 1 and $5X10^{-1}$ mg/ml. No net increase in nuclear grain count was observed at concentrations between $5X10^{-4}$ and 10^{-1} mg/ml or with pyrene and DMSO (negative controls); the positive control, benzo(a)pyrene, was highly genotoxic, indicating that the hepatocytes were capable of metabolic transformation and DNA repair.

Under the conditions of this test system, Nisoldipine was considered to be not genotoxic at concentrations up to 10^{-1}M

NDI/IN VITRO Systems Facility
Report on: UDS/DNA Repair Assay

Date:	November 21, 1986	Assay method	Autoradiography
Expt #	NII2186	Species/strain/sex/age/wt	House/B6C3F$_1$/Male/Adult/28-32g
Chemical	Nisoldipine	Organ or tissue/condition	Liver
Molecular wt		Cells/primary or line	Primary
Source/Purity		Medium used:	Williams Medium E
Lot #		Duration of chemical exposure	18 hrs.
Solvent/vehicle	DMSO	Chemical dose range	1 to 5 x 10^{-6} mg/ml
Volatility		Exposure method	In Situ
Precautions		Label/uCi/ml or Ci/mM. final	^{3}H-thymidine, 10uCi/ml
Positive control	Benzo(a)pyrene (BaP)	Interval between exp. and label	Simultaneous
Negative control	Pyrene	Duration of label	18 hrs.

CONTROLS			TEST RESULTS				COMMENTS:
Positive	Conc.	Autoradiog. grains/nucleus (NET)*	Conc. mg/ml	Autoradiog. grains/nucleus (NET)*	Cytoxicity	Evaluation	* Mean ± standard deviation of triplicate coverslips.
BaP	10^{-5}M	43.7 ± 5.0	1		Toxic		**Mean of duplicate coverslips.
			5×10^{-1}		Toxic		
			10^{-1}	-11.6 ± 1.7	Subtoxic	Negative	
			5×10^{-2}	-15.2**	Subtoxic	Negative	
			10^{-2}	-16.5 ± 1.8	Nontoxic	Negative	
			5×10^{-3}	-15.3 ± 2.4	Nontoxic	Negative	
Negative	conc.	grains/nucleus (NET)	10^{-3}	-16.5 ± 2.1	Nontoxic	Negative	
Pyrene	10^{-5}M	-11.1 ± 4.2	5×10^{-4}	-12.8 ± 0.9	Nontoxic	Negative	
DMSO	1%	-13.6 ± 1.3					
Cell Control		-13.9 ± 3.7					

5. Micronucleus Test in Mice

Study No.: T 1010 875

Performing Laboratory:

Date Performed: 11/6/81 to 12/1/81

Quality Assurance: A signed statement of compliance with GLP is not included.

Background: The micronucleus test permits the recognition of a mutagenic action on a somatic tissue, the femoral bone marrow, of an intact animal. Erythrocytes are normally anuclear. The increased occurrence of micronuclei (chromosome fragments) in polychromatic erythrocytes, compared to negative controls, indicates that the test substance brings about chromosome breaks, or has effects on the spindles in the erythroblasts.

Procedure: Bor: NMRI (SPF Han) mice, 8 to 12 weeks of age, weighing 23 to 32 g, 5 of each sex per group, received 0, 100 and 200 mg Bay k 5552/kg/day, p.o., on two consecutive days; positive controls received cyclophosphamide (Endoxan®) orally. All animals were sacrificed 6 hours after the second dose and femoral bone marrow samples were removed for the purpose of determining the frequency of micronuclei observed, by examination of 1000 polychromatic erythrocytes per animal.

Results: Since there was no difference between males and females, the results obtained for both sexes were pooled. No deaths or clinical signs were observed. The significant decrease in normochromatic erythrocytes in the 100 mg/kg group was considered "not biologically significant" (see table on page which follows). It is therefore claimed that produced no inhibition of erythropoiesis.

There was no increase in polychromatic erythrocytes with micronuclei. The positive control caused a high level of response.

It was concluded that there was no indication of a mutagenic effect by this test.

NDA 20356

Subj. No.	Ae B1 [ug]	Ae B1 [%]	Ae B2 [ug]	Ae B2 [%]
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
MEAN	3437.79	8.594	.	.
SDEV	3962.24	9.906	.	.
CV [%]	1.15	1.153	.	.
MEDIAN	1203.74	3.009	.	.
MIN	64.21	0.161	.	.
MAX	11002.43	27.506	.	.
COUNT	7.00	7.000	2.00	2.000

B1: p.o. adm. of 2 x 20 mg Nisoldipine CC tablets
One tablet containing 20 mg of Nisoldipine.
B2: p.o. adm. of 1 x 40 mg Nisoldipine CC tablets
One tablet containing 40 mg of Nisoldipine.

Table 3

Feces: Excreted amount of Nisoldipine [ug] and percentage of dose.

Single Values, Arithmetic Means, Standard Deviations, Coefficients of Variation, Medians, Minimum, Maximum and Number of Cases.

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TABLE 4

90% CONFIDENCE LIMITS AND TEST OF BIOEQUIVALENCE

Parameter	MS Error (ANOVA)	geo.mean 100*B1/B2	lower limit	upper limit	bioeq. decision
Cmax,norm	0.156329	115.109332	94.623699	140.030019	false
AUC(0-tn,norm)	0.081927	96.526425	83.759172	111.239766	true
AUC(0-∞,norm)	0.076920	97.826364	85.261817	112.242477	true

Acceptable range of bioequivalence: 80 to 125

Student's t for a=0.05 (one tailed) and DF=22 : 1.717
24 subjects

B1 : Nisoldipine, following p.o. adm. of 2 x 20 mg
Nisoldipine CC tablets
One tablet containing 20 mg of Nisoldipine
B2 : Nisoldipine, following p.o. adm. of 1 x 40 mg
Nisoldipine CC tablets
One tablet containing 40 mg of Nisoldipine

Table 4

A controlled, double blind, two way single dose crossover study of the bioequivalence and tolerability of 3x20 mg vs 2x30 mg nisoldipine C.C. tablets in healthy volunteers.

STUDY #: D90-020-01 VOLUME: 1-32-1-33 PAGES: 902-1838.

INVESTIGATOR:

OBJECTIVES:

To determine the bioequivalence and single dose pharmacokinetics of 3x20 vs 2x30 mg nisoldipine C.C. tablets and to examine the safety and tolerability of a 60 mg dose of nisoldipine C.C.

FORMULATIONS:

-20 mg nisoldipine C.C tablets batch # 523272.
-30 mg nisoldipine C.C tablets batch # 523274.
-Placebo tablets batch # 523167.

STUDY DESIGN:

30 healthy male volunteers between the ages of 18 and 40 years weighing between 140 and 220 lbs participated in this randomized, placebo controlled double blind crossover study. 24 subjects were assigned to the active treatment group while 6 subjects were assigned to the placebo group. The nisoldipine group was to be given, in a random order, either 3x20 mg or 2x30 mg dose of the C.C. at period 1, then crossed to the alternate dose at period 2. The placebo group was to be given nisoldipine placebo tablets at both periods. There was a one week washout period between treatments. 5 mls blood samples were collected predose and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 16, 23, 24, 28, 32, 36, 48 and 72 hours post-dose.
A pre-dose and up to 72 hours post dose urine and feces collections were to be performed.

RESULTS:

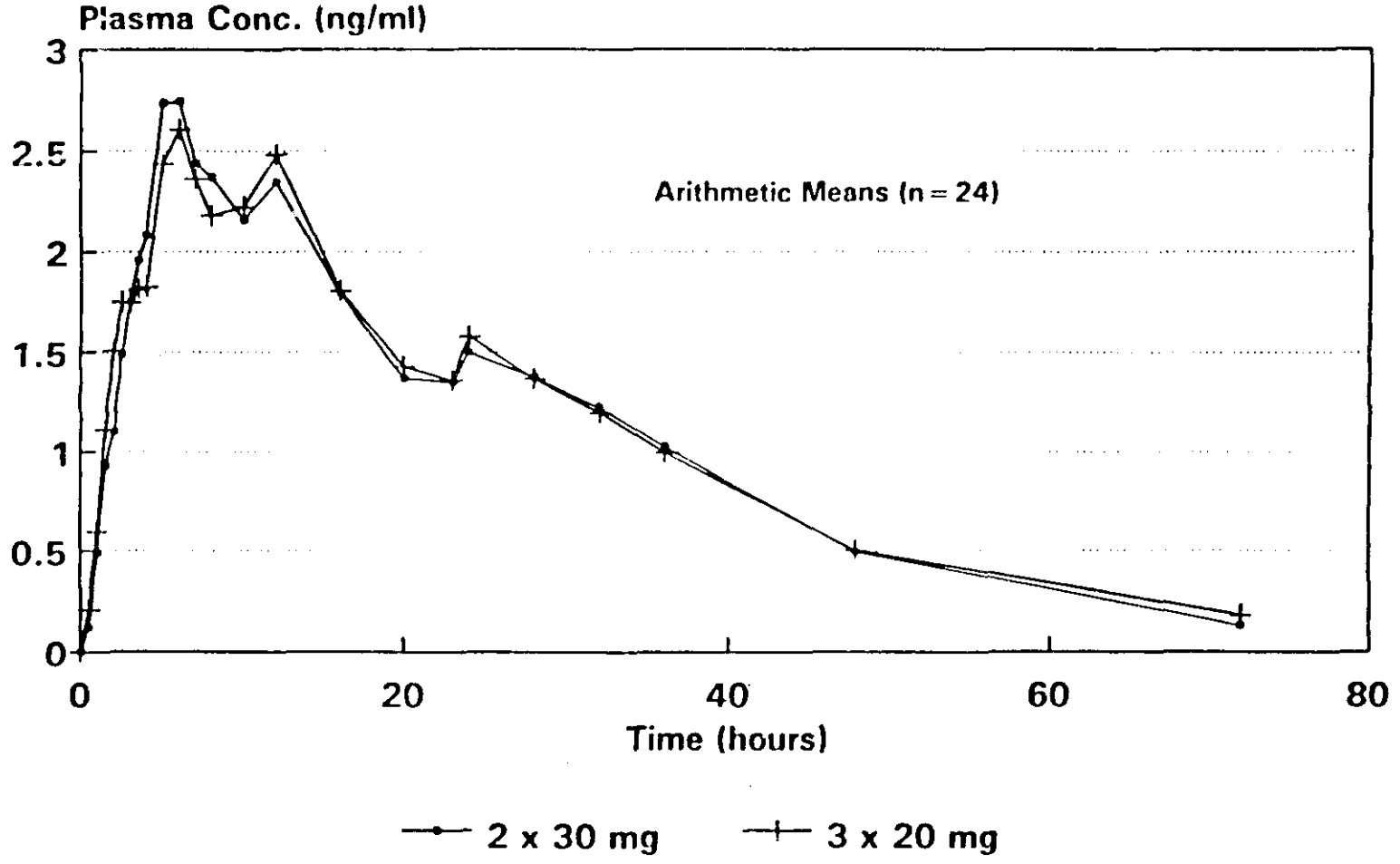
Mean plasma profiles for both treatments are presented in Figure 1.The 90 % confidence intervals for AUC and CMAX ratios are presented in Table 1.

	AUC (ng.hr/ml)	Ratio	90 % CI	CMAX (ng/ml)	Ratio	90 % CI
2x30 mg	81.98	1.0482	95-115	3.4	0.9587	83.64-109.87
3x20 mg	80.39			3.66		

Conclusion:

2x30 mg nisoldipine C.C. tablets are bioequivalent to 3x20 mg C.C. tablets.

Figure 1. Nisoldipine Concentrations After 60 mg Given as Either 2x30 mg of 3x20 mg CC Tablets



Study D90-020

Pharmacokinetics and tolerability of nisoldipine after single administration of retard tablets with the old and new core composition.

STUDY # KF 715 VOLUME: 1-31 PAGES: 489-613.

INVESTIGATOR:

OBJECTIVES:

The objectives of the present study is to evaluate the bioequivalence of the new C.C formulation in doses of 1x20, 2x10 and 4x5 mg with the 20 mg old C.C. formulation after single administration.

FORMULATION:

-Nisoldipine coat core (C.C.) tablets 20 mg (old formulation) batch # 523081, release date october 7th 1988, expiration date: december 12, 19889.

-Nisoldipine C.C. tablets 10 mg (new formulation), batch # 523230, release date october 7 1988, expiration date: december 3, 1989.

-Nisoldipine C.C. tablets 5 mg (new formulation), batch # 523228, release date october 7, 1988, expiration date: december 3, 1989.

-Nisoldipine C.C. tablets 20 mg (new formulation), batch # 523232, release date october 7, 1988, expiration date: december 31, 1989.

STUDY DESIGN:

16 healthy male volunteers (average age 35.3 years, weight 75.2 Kg) participated in this randomized, non-blind four way cross-over study. A washout period of at least six days was allowed between individual treatments.
The test substances were administered to the fasting volunteers in the morning with 100 ml of water on an empty stomach. Food was administered 2 hours post drug administration.
Each subjects received each of the following treatments:
-20 mg tablet of the old formulation of nisoldipine C.C. tablet.
-4x5 mg tablets of the new formulation of nisoldipine C.C. tablet.
-2x10 mg tablets of the new formulation of nisoldipine C.C. tablet.
-20 mg tablet of the new formulation of nisoldipine C.C. tablet.
6 ml blood samples were taken at the following times: 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 20, 24, 36 and 48 hours post administration.
Urine was collected over the periods 0-24 hours and 24-48 hours after drug administration for

the determination of nisoldipine metabolites if needed.

RESULTS:

Table 1 shows the pharmacokinetic parameters after administration of the 4 different treatments while Table 2 gives the 90 % confidence for the parameters of interest in relation to the old 20 mg C.C. formulation. Figure 1 shows the mean pharmacokinetic profile for all the 4 different treatments. It can be seen that the 4x5 mg tablets of the new C.C formulation gave higher plasma levels than the three remaining formulations. The plasma levels for the 4x5 mg tablets were on the average 20 % higher resulting in bioinequivalence (as can be seen from table 2). Moreover, the 2x10 mg tablets of the new formulation passed the 90 % confidence interval as far as AUC is concerned, however, it failed in terms of CMAX since the 90 % CI was 91 to 132 %.

Conclusion:

Only the 20 mg new C.C formulation was considered to be bioequivalent to the old the 20 mg formulations. 2x10 and 4x5 mg of the new formulation are not **bioequivalent** to the 20 mg old formulation.

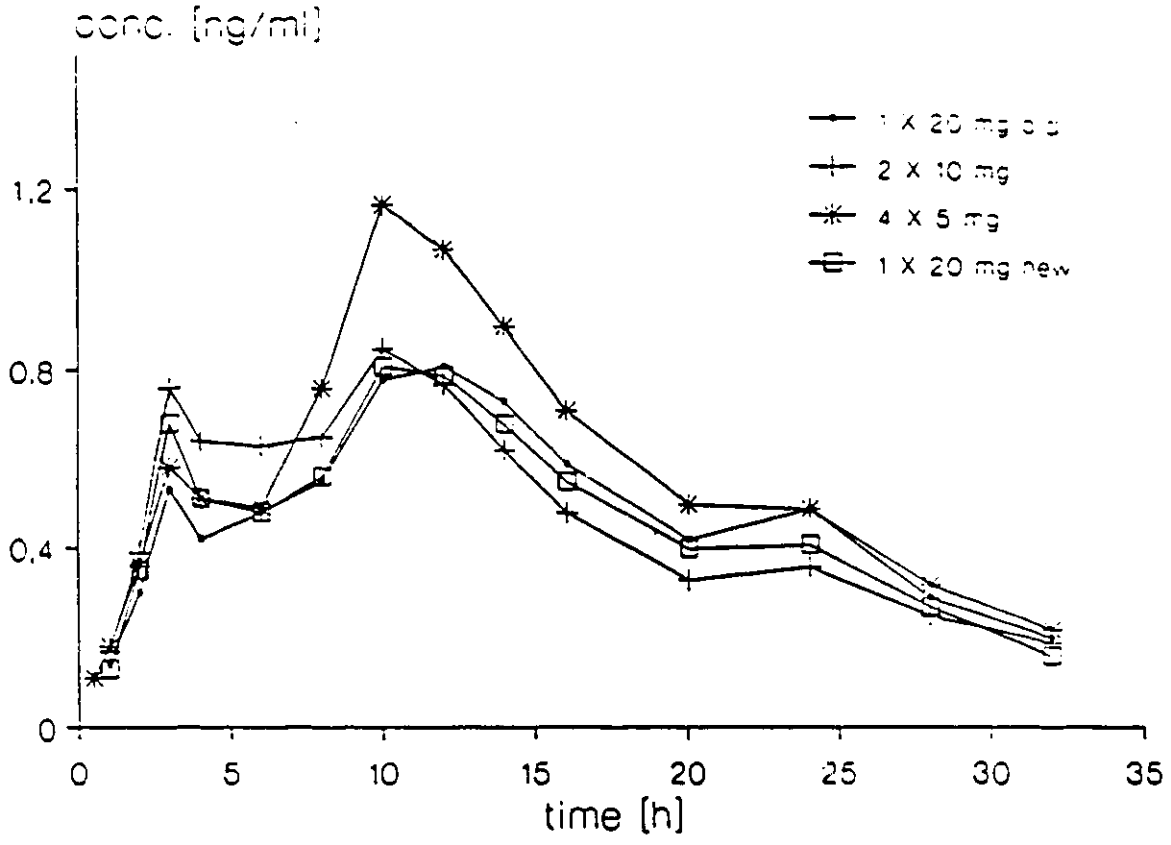


Fig. 1: Mean plasma concentrations after single administration of 20 mg nisoldipine as different retard tablets (1 x 20 mg, 2 x 10 mg and 4 x 5 mg with new core composition and 1 x 20 mg with old core composition); means without volunteer no. 4

Table 1: Comparison of pharmacokinetic parameters after single administration of 20 mg nisoldipine as different retard tablets (1 x 20 mg, 2 x 10 mg and 4 x 5 mg with new core composition and 1 x 20 mg with old core composition); geometric means and S.D. without volunteer no. 4.

Parameter	1 x 20 mg (old)		2 x 10 mg		4 x 5 mg		1 x 20 mg (new)	
AUC(0-48)	17.26	1.54	16.85	1.61	20.18	1.60	16.84	1.50
AUC-norm	64.46	1.51	62.90	1.55	75.34	1.62	62.89	1.46
MRT(0-48)	16.20	1.21	14.74	1.28	15.13	1.23	15.49	1.28
Cmax	1.04	1.62	1.08	1.58	1.31	1.50	0.99	1.79
Cmax-norm	3.90	1.57	4.04	1.54	4.89	1.47	3.69	1.74
C24h	0.49	1.56	0.36	2.27	0.49	1.89	0.41	1.52
Cmax/C24h	2.13	1.75	3.00	2.34	2.65	1.76	2.41	1.82
tmax	8.67	1.78	8.31	2.03	9.50	1.42	7.01	1.88
f	102.49	1.29	100.01	1.34	119.80	1.37		

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WITHOUT VOL.NO.4 / STUDY 656 (RF715) 19JUN89

TABLE 9: ESTIMATES OF PHARMACOKINETIC PARAMETERS
QUOTIENTS TO RESULT OF 1*20 MG TABL.NEW IN %
GEOMETRIC MEAN, GEOMETRIC SD, 90%-CONFIDENCE INTERVALS
REFERRING TO ANALYSIS OF VARIANCE AND DUNNETT-TESTS.

PARAMETER		QUOTIENT (%) 1*20 MG TABL.OLD /1*20 MG TABL.NEW		2*10 MG TABL. /1*20 MG TABL.NEW		4*5 MG TABL /1*20 MG TABL.NEW	
AUC (H*NG/ML)	MEAN, SD	102.5	1.29	100.0	1.34	119.8	1.37
	90% CONFID.INTERV.	87.7 -	119.7	85.6 -	116.8	102.6 -	139.9
AUC NORM (H*MG/ML)	MEAN, SD	102.5	1.29	100.0	1.34	119.8	1.37
	90% CONFID.INTERV.	87.7 -	119.7	85.6 -	116.8	102.6 -	139.9
CMAX (NG/ML)	MEAN, SD	105.8	1.40	109.7	1.37	132.7	1.54
	90% CONFID.INTERV.	87.8 -	127.5	91.0 -	132.2	110.1 -	160.0
CMAX NORM (MG/ML)	MEAN, SD	105.8	1.40	109.7	1.37	132.7	1.54
	90% CONFID.INTERV.	87.8 -	127.5	91.0 -	132.2	110.1 -	160.0
MRT (H)	MEAN, SD	104.6	1.19	95.2	1.25	97.7	1.24
	90% CONFID.INTERV.	93.2 -	117.3	84.9 -	106.8	87.1 -	109.6
PLASM.CON.24H NG/ML	MEAN, SD	120.0	1.39	88.3	1.87	120.7	1.50
	90% CONFID.INTERV.	87.4 -	164.7	64.3 -	121.2	87.9 -	165.7
CMAX / CONC.24 H	MEAN, SD	88.2	1.46	124.3	2.02	110.0	1.58
	90% CONFID.INTERV.	61.8 -	125.7	87.2 -	177.2	77.1 -	156.8

Pilot study on the pharmacokinetics and tolerability of three different controlled release formulations of nisoldipine in comparison to the IR tablet after single oral administration.

STUDY # 632. VOLUME: 1-38 PAGES: 3250-3560.

INVESTIGATOR:

OBJECTIVES:

The objective of the present study is to assess the pharmacokinetics and tolerability of three controlled release formulations of nisoldipine in comparison to the immediate release tablet.

FORMULATION:

-Nisoldipine coat core (C.C.) tablets 20 mg dev #034, pt # 523082.

-Nisoldipine C.C. tablets 20 mg dev #029, pt# 523081.

-Nisoldipine C.C. tablets 20 mg dev # 023, pt # 523028.

-Nisoldipine IR tablets 10 mg dev # E126, pt # 520210.

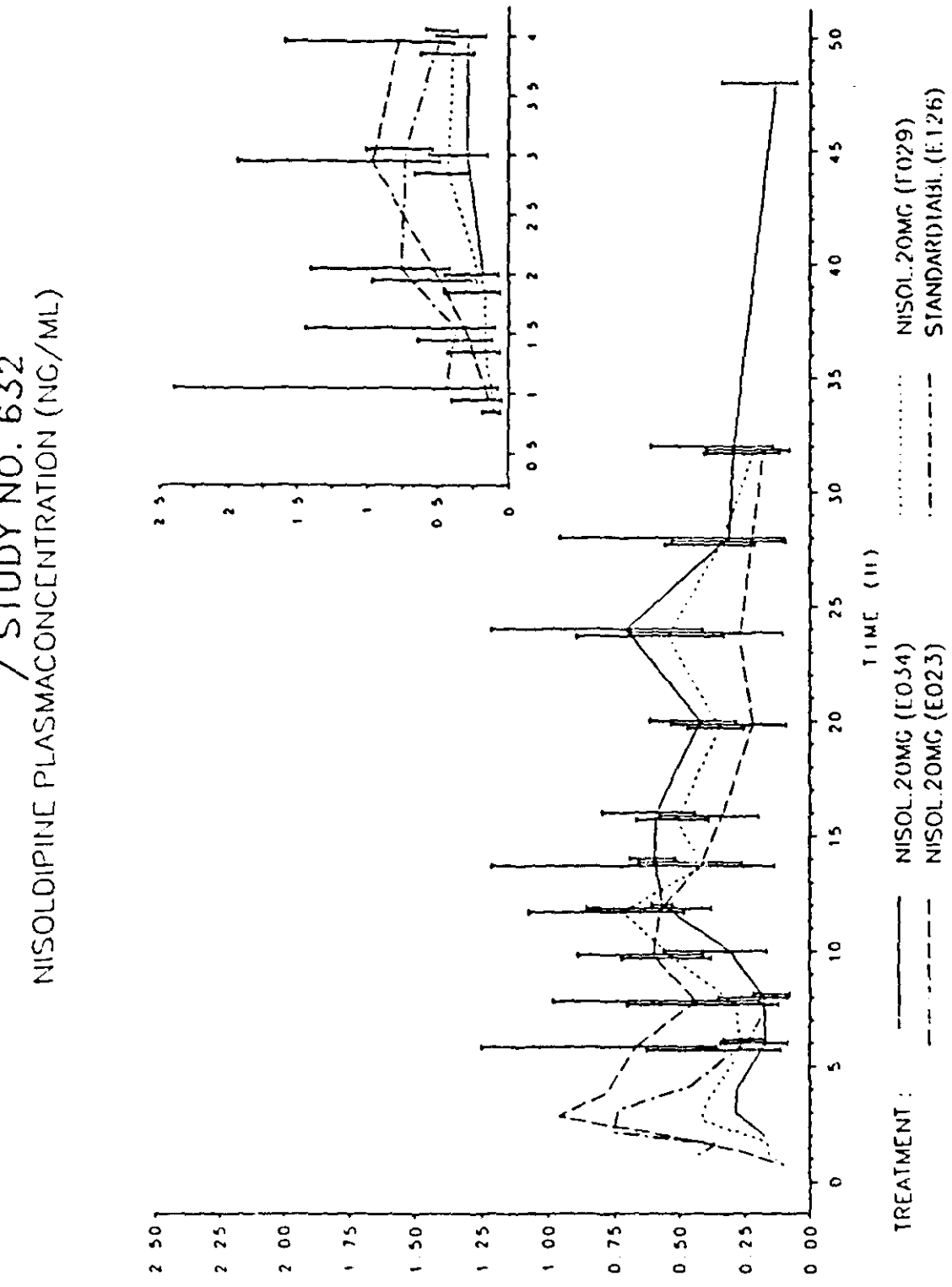
STUDY DESIGN:

6 healthy male volunteers between the ages of 18 and 45 years participated in this non blind randomized triple crossover study for the C.C. tablet followed by a single dose administration of the immediate release formulation with washout phases of 6 days.
Plasma samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16, 20, 24, 28, 32 and 48 hours (56 hrs at period 2 and 3) post drug administration. Heart rate and blood pressure measurements were taken prior to blood sampling.

RESULTS:

Figure 1 shows the mean plasma concentration time profile for the 4 treatments while table 1 shows the corresponding pharmacokinetic parameters while Table 2 gives the 95 % confidence for the parameters of interest in relation to the 10 mg IR tablet.
The sponsor concluded from this study that slowing the release rate of nisoldipine increases its bioavailability as reflected by an increase in AUC, MRT and the duration during which the plasma concentration was above 0.3 ng/ml. This increase in bioavailability was accompanied by a significant decrease in CMAX.
Based on the result of this study, the sponsor thought that formulation E 029 gave the most favorable results and chose this formulation for further development.

Fig. 1


/ STUDY NO. 632
NISOLOIPINE PLASMACONCENTRATION (NG/ML)
TIME (H)
TREATMENT :
NISOL.20MG (E034)
NISOL.20MG (E023)
NISOL.20MG (F029)
STANDARDIABL.(E126)

/ STUDY 0632

26NOV92

TABLE 1 ESTIMATES OF PHARMACOKINETIC PARAMETERS
GEOMETRIC MEAN, GEOMETRIC SD BY TREATMENT

PARAMETER	N	NISOL.20MG (34)		NISOL.20MG (29)		NISOL.20MG (23)		NISOL.10MG (STD)	
AUC NORM (G*H/L)	6	68.673	1.78	57.153	1.40	52.949	1.72	31.347	1.36
CMAX (NG/ML)	6	0.800	1.56	0.857	1.51	1.060	1.80	1.547	2.12
MRT (H)	6	27.332	1.27	21.194	1.11	16.565	1.38	4.226	1.48
DURATION C>=0.3 (H)	6	24.008	1.65	23.912	1.29	17.961	1.92	4.409	1.24

/ STUDY 0632

26NOV92

TABLE 2 ESTIMATES OF PHARMACOKINETIC PARAMETERS
QUOTIENTS TO RESULT OF NISOL.10MG (STD) IN %
GEOMETRIC MEAN, GEOMETRIC SD, 95%-CONF.INTERV. ACCORDING TO MODEL
REFERRING TO ANALYSIS OF VARIANCE WITHOUT ALPHA ADJUSTMENT.

PARAMETER	N SD(MODEL)		QUOTIENT (%) NISOL.20MG (34) /NISOL.10MG (STD)	QUOTIENT (%) NISOL.20MG (29) /NISOL.10MG (STD)	QUOTIENT (%) NISOL.20MG (23) /NISOL.10MG (STD)
AUC NORM (G*H/L)	6 1.48	MEAN, SD 95% C.I.	219.1 1.52 156.0 - 307.7	182.3 1.43 129.8 - 256.1	168.9 1.50 120.3 - 237.2
CMAX (NG/ML)	6 1.74	MEAN, SD 95% C.I.	51.7 1.66 31.9 - 83.7	55.4 2.22 34.2 - 89.7	68.5 1.67 42.3 - 111.0
MRT (H)	6 1.52	MEAN, SD 95% C.I.	646.7 1.59 448.4 - 932.8	501.5 1.61 347.7 - 723.3	392.0 1.87 271.7 - 565.3
DURATION C>=0.3 (H)	6 1.66	MEAN, SD 95% C.I.	544.5 1.64 349.6 - 848.2	542.3 1.48 348.2 - 844.8	407.4 2.13 261.5 - 634.6

Clinical study on the influence of food and time of administration on the pharmacokinetics of controlled release nisoldipine.

Study: 666 Volume: 42-43 Pages: 4802-5361.

Investigators:

Clinical:

Objectives:

The objective of this study was the evaluation of the influence of food and time of administration on the pharmacokinetics of nisoldipine in a controlled release formulation.

Formulation

20 mg nisoldipine C.C. tablets batch # 52332.

Study Design:

Twelve healthy male volunteers between the ages of 24 and 33 years participated in this study. A three factorial 3x3x6 latin square design with repeated measurements on Factor A, Period B Treatment and randomization on Factor C sequence with 2 subjects per sequence was applied. Single doses of 20 mg nisoldipine C.C. tablets were applied at 8 AM in fasted state (2 h before an american standard breakfast), together with an american standard breakfast and in fed state (1 hr after an american standard breakfast) during the first three periods.
During the additionally performed 4th period, all volunteers received single doses of 20 mg nisoldipine C.C. tablets at 8 PM together with a continental dinner.
There was a washout period of at least one week between the four treatments. The 4 treatments were as follows:
b_1: administration in fasted state (2 hr before an American standard breakfast).
b_2: administration together with an American standard breakfast.
b_3: administration in a fed state (1 hr after an American standard breakfast).
b_4: administration together with a continental dinner.
The American standard breakfast with an average calorie content of 575 Kcal consisted of:
-2 cups (0.2 l) of decaffeinated coffee.
-1 glass(0.2 l) of orange juice.
-20 ml of evaporated milk (7.5 % fat).
-2 slices of toast.

-2 slices of boiled ham (40 g).
-20 g butter.
-20 g jam.

The continental dinner consisted of:
-1 glass (0.2 l) of orange juice.
-4-5 slices of toast.
-50 g butter.
-boiled ham, cheese and sausages.
-mineral water ad libitum.
12 healthy male volunteers between the ages of 18 and 35 participated in this study.
For Periods I-III blood samples were withdrawn at the following time points: -0.17, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 14, 16, 20, 24, 28, 32, 36, and 48 hr after administration.
For Period IV blood samples were withdrawn at -.17, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 11, 12, 14, 16, 20, 24, 28, 36 and 48 hours after administration.

Results:

Figure 1 shows the mean plasma profile for all the 4 different treatments while Table 1 gives the mean pharmacokinetic parameters.
It can be seen that AUC normalized for dose and weight were as follows:
-fasted state: 61.69 g*h/l
-together with breakfast: 61.03 g*h/l (ratio 99% with 90 % CI= 78-126 %).
-1 hr after breakfast: 50.3 g*hr/l (ratio 82 % with 90 % CI= 64-104 %).
-together with dinner: 59.22 g*hr/l (ratio 96 % with 90 % CI= 77-120 %).

CMAX following administration together with meals are remarkably higher as compared to CMAX following administration in a fasted state (1.35 ng/ml).
The geometric means, quotients and the 90 % CI compared to the fasted state were as follows:
-together with breakfast: 2 ng/ml 148 % with a 90 % CI of 103 to 212 %.
-1 hr after breakfast: 1.87 ng/ml 138 % with a 90 % CI of 97 to 198 %.

-together with dinner: 1.7 ng/ml 126 % with a 90 % CI of 93 to 171 %.

Conclusion:

1-The lowest bioavailability related to AUCnorm was found following administration 1 hr after breakfast.

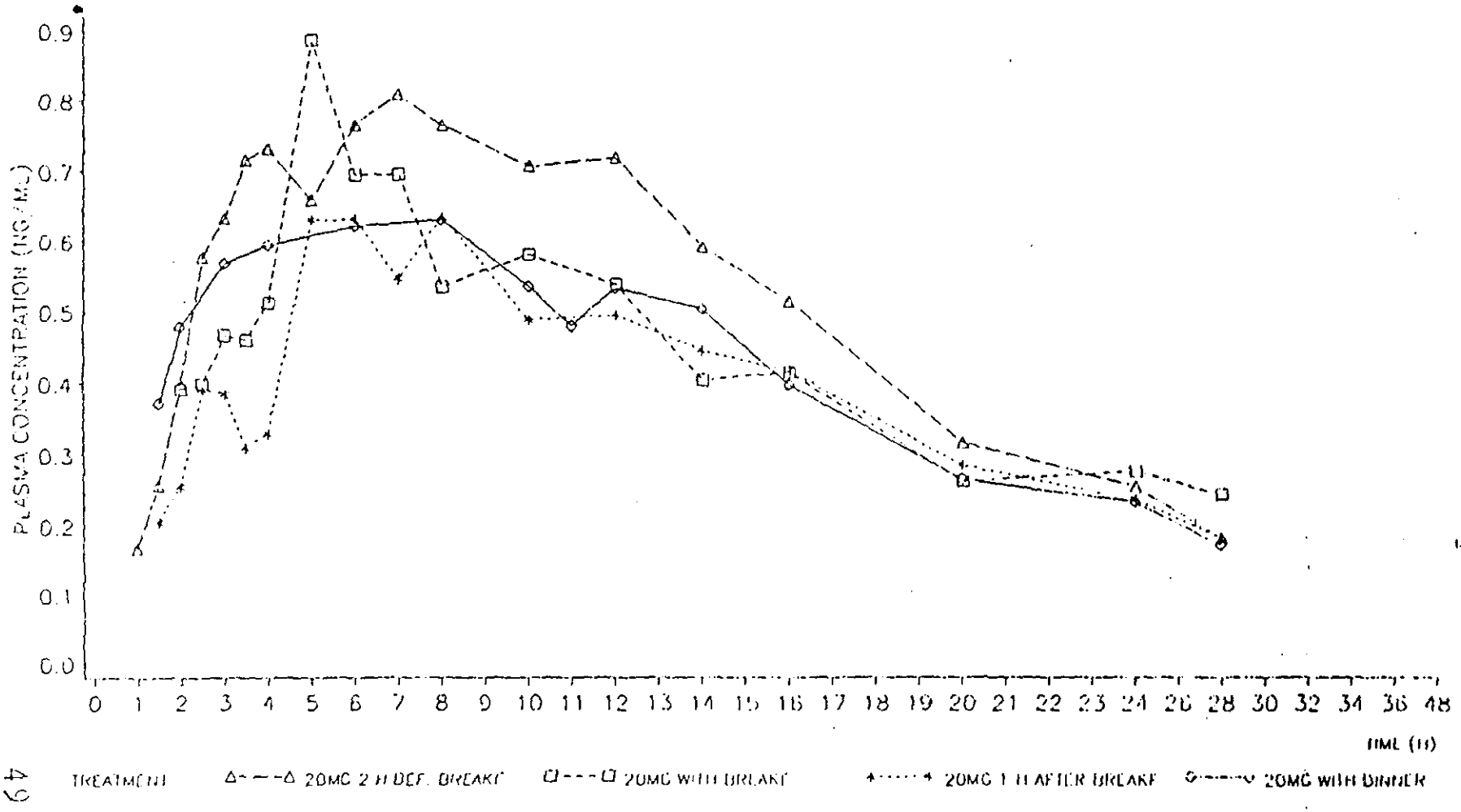
2- CMAX was higher by about 26 to 48 % higher when nisoldipine was administered with food.

3-Intake of food shortened TMAX by about 2 to 3 hours.

STUDY NO. 0666

PLASMA CONCENTRATIONS (NG/ML),GEOMETRIC MEANS

FIGURE 1:



STUDY NO. 666

TABLE 1: ESTIMATES OF PHARMACOKINETIC PARAMETERS
GEOMETRIC MEAN, GEOMETRIC SD BY TREATMENT

PARAMETER	N	FASTING		WITH BREAKFAST		1 H AFTER BREAKFAST		WITH DINNER	
AUD_{0-tn} (NG•H/ML)	12	15.65	1.45	15.48	1.74	12.76	2.30	15.02	1.95
$AUC_{0-\infty}$ (NG•H/ML)	12	18.40	1.39	18.35	1.56	15.44	1.91	17.81	1.92
AUD_{NORM} (G•H/L)	12	61.69	1.49	61.03	1.80	50.30	2.37	59.22	2.01
C_{MAX} (NG/ML)	12	1.35	1.67	2.00	1.72	1.87	1.95	1.70	2.01
$C_{MAX,NORM}$ (G/L)	12	5.33	1.67	7.87	1.78	7.37	1.98	-	-
$T_{1/2}$ (H)	12	7.46	1.66	7.03	1.72	6.75	1.68	9.81	1.86
T_{MAX} (H)	12	6.87	1.74	3.86	1.56	3.24	1.97	4.89	1.93
Duration C ≥ 0.3 NG/ML	12	20.43	1.45	16.97	1.77	15.00	2.52	-	-

Pharmacokinetics and tolerability of nisoldipine taken after breakfast and on an empty stomach.

Study: 0323 Volume: 43 Pages: 5362-5515.

Investigators:

Clinical:

Objectives:

1-To examine the effect of the timing of intake of a meal on the plasma concentration/time curve for nisoldipine in order to establish guidelines for intake where appropriate.

2-Examine in healthy subjects whether the intake of a meal has an effect on the relative bioavailability of nisoldipine.

Formulation:

-20 mg nisoldipine tablets batch # 929490.

Study Design:

8 healthy male volunteers participated in 2 way randomized crossover study. The 2 treatments were 20 mg nisoldipine either before or 1.5 hours after administration of a standardized breakfast. Blood samples were withdrawn at 0, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 24 hours after drug administration. Urine samples were collected for the following time intervals: 0-4 hr, 4-8 hrs, 8-24 hrs and 24 to 48 hours.
Blood pressure and heart rate were measured at the same time points when blood samples were withdrawn.
The standard breakfast consisted of one egg, 80 g of boiled ham, three rolls with butter and jam and 2 cups of caffeine free coffee.

Results:

Figure 1 shows the mean plasma levels after the fasted and fed treatments while Table 1 shows the main pharmacokinetic parameters with the corresponding 95 % Confidence intervals.
The results show that coadministration with food results in 28 % increase in AUC and 31 % increase in CMAX.
Table 2 shows a summary of the changes in systolic and diastolic blood pressure and heart rate. It can be seen from the results that when nisoldipine was taken with breakfast, the change in heart rate took place at a later time point but was more pronounced.

Conclusion:

1-Coadministration of food with nisoldipine resulted in both an increase in AUC and CMAX most probably due to a decrease in liver blood flow resulting in decreased first pass metabolism.

2-The results show that there was a great deal of inter-individual variability in the effect of food as can be observed in the very wide confidence intervals for the pharmacokinetic parameters.

3-The increase in plasma concentration of nisoldipine resulted in a more pronounced effect in heart rate since the change was more pronounced compared to when nisoldipine was given in a fasted state even though there was no difference in the effect on either the systolic or diastolic blood pressure between the fed and fasted state.

FIGURE 1

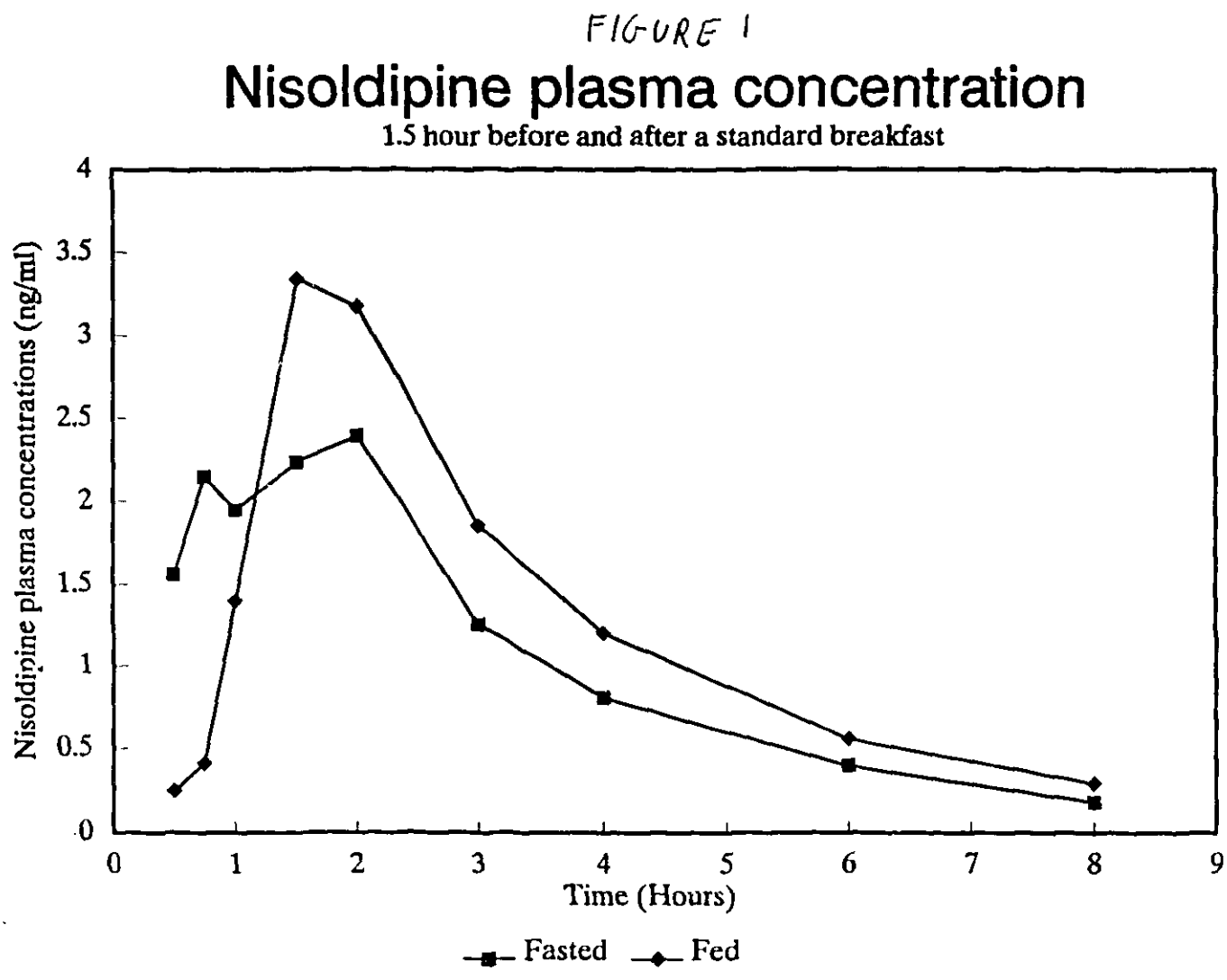

Nisoldipine plasma concentration
1.5 hour before and after a standard breakfast
Nisoldipine plasma concentrations (ng/ml)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
6
7
8
9
Time (Hours)
Fasted
Fed

Table 1:

variable	geometric	fasting	after meal	MQ · 1000 error	treatm. F period F	p	after meal / fasting mean (%)	95%-conf.limit (%)
AUC_{norm} (kg·h/1000 l)	mean	41.841	53.602	30.618	1.51	0.27	128.1	76.3, 215.1
	sd	1.644	1.318		0.82	0.40		
C_{max} (ng/ml)	mean	4.211	5.520	32.578	1.69	0.24	131.1	76.7, 223.8
	sd	1.539	1.614		3.97	0.10		
MRT	mean	4.049	4.159	19.681	0.02	0.88	102.7	67.7, 155.6
	sd	1.476	1.211		1.63	0.91		

STUDY NO.323
DIFFERENCES TO PREVALUES

TREATMENT=STAND.MEAL/DRUG VARIABLE=BP SYSTOLIC (MM HG)

TIME	N	MEAN	SD	MINIMUM	MEDIAN	MAXIMUM
AFT. 30 MIN - PRE	8	3.375	9.9562	-16	3.0	18
AFT. 45 MIN - PRE	8	0.625	11.7951	-26	2.0	12
AFT. 60 MIN - PRE	8	4.000	9.7980	-9	1.5	20
AFT. 90 MIN - PRE	8	3.125	11.7527	-15	2.0	23
AFT. 2 H - PRE	8	3.625	10.7429	-17	9.0	16
AFT. 3 H - PRE	8	-0.750	16.0424	-26	-3.0	22
AFT. 4 H - PRE	8	-5.125	13.3249	-24	-4.0	17
AFT. 6 H - PRE	8	7.125	5.1944	1	6.5	15
AFT. 8 H - PRE	8	3.500	10.0000	-15	6.0	14
AFT. 24 H - PRE	8	3.625	9.8116	-14	7.5	13

TREATMENT=STAND.MEAL/DRUG VARIABLE=BP DIASTOLIC (MM HG) - SITTING

TIME	N	MEAN	SD	MINIMUM	MEDIAN	MAXIMUM
AFT. 30 MIN - PRE	8	-0.750	8.2071	-16	2.5	7
AFT. 45 MIN - PRE	8	2.500	9.2273	-13	2.0	16
AFT. 60 MIN - PRE	8	-0.625	8.6510	-12	-0.5	16
AFT. 90 MIN - PRE	8	-3.125	3.4821	-7	-4.0	2
AFT. 2 H - PRE	8	0.625	7.9810	-10	-2.0	12
AFT. 3 H - PRE	8	-0.375	5.7802	-9	-0.5	8
AFT. 4 H - PRE	8	-2.250	11.3484	-21	3.0	8
AFT. 6 H - PRE	8	8.125	11.4447	-5	6.0	30
AFT. 8 H - PRE	8	5.125	7.1001	-2	3.0	17
AFT. 24 H - PRE	8	2.500	6.6762	-5	0.5	13

TREATMENT=STAND.MEAL/DRUG VARIABLE=HEART RATE (/MIN)

TIME	N	MEAN	SD	MINIMUM	MEDIAN	MAXIMUM
AFT. 30 MIN - PRE	8	5.125	7.3957	-3	5.0	18
AFT. 45 MIN - PRE	8	8.875	10.2461	-6	9.0	22
AFT. 60 MIN - PRE	8	14.875	12.5861	1	13.5	38
AFT. 90 MIN - PRE	8	14.750	11.0551	2	12.5	38
AFT. 2 H - PRE	8	11.875	12.1824	-1	9.5	39
AFT. 3 H - PRE	8	-0.125	8.9990	-13	-0.5	16
AFT. 4 H - PRE	8	-4.500	7.6904	-13	-5.5	13
AFT. 6 H - PRE	8	10.000	10.0854	-3	10.5	30
AFT. 8 H - PRE	8	9.250	13.7087	-6	7.5	35
AFT. 24 H - PRE	8	-4.125	8.3911	-16	-5.5	8

STUDY NO.323
DIFFERENCES TO PREVALUES

TREATMENT=DRUG /STAND.MEAL VARIABLE=UP SYSTOLIC (MM HG)

TIME	N	MEAN	SD	MINIMUM	MEDIAN	MAXIMUM
AFT. 30 MIN - PRE	8	-4.250	11.8894	-25	-2.5	9
AFT. 45 MIN - PRE	8	-2.750	12.4757	-17	-4.5	13
AFT. 60 MIN - PRE	8	-1.750	12.9367	-28	2.5	12
AFT. 90 MIN - PRE	8	0.000	12.6829	-23	0.5	19
AFT. 2 H - PRE	8	3.625	8.2451	-8	7.5	12
AFT. 3 H - PRE	8	2.875	16.4789	-18	3.0	26
AFT. 4 H - PRE	8	1.375	11.5504	-13	0.0	21
AFT. 6 H - PRE	8	12.750	10.0250	-6	16.0	24
AFT. 8 H - PRE	8	4.125	16.9068	-26	6.0	29
AFT. 24 H - PRE	8	3.500	11.4268	-14	7.5	15

TREATMENT= DRUG/STAND.MEAL VARIABLE=DP DIASTOLIC (MM HG) - SITTING

TIME	N	MEAN	SD	MINIMUM	MEDIAN	MAXIMUM
AFT. 30 MIN - PRE	8	-0.500	9.5169	-20	-0.5	10
AFT. 45 MIN - PRE	8	-2.875	11.2686	-26	-1.0	8
AFT. 60 MIN - PRE	8	-4.750	9.3618	-18	-7.0	10
AFT. 90 MIN - PRE	8	-2.125	11.0897	-20	-2.5	18
AFT. 2 H - PRE	8	-1.250	8.4134	-16	2.5	7
AFT. 3 H - PRE	8	-3.375	10.9013	-20	-2.5	12
AFT. 4 H - PRE	8	2.250	11.6466	-20	1.0	16
AFT. 6 H - PRE	8	4.750	9.6325	-13	6.5	15
AFT. 8 H - PRE	8	2.750	5.9702	-5	4.0	12
AFT. 24 H - PRE	8	4.500	8.7831	-9	6.0	16

TREATMENT= DRUG/STAND.MEAL VARIABLE=HEART RATE (/MIN)

TIME	N	MEAN	SD	MINIMUM	MEDIAN	MAXIMUM
AFT. 30 MIN - PRE	8	9.375	7.9451	1	6.5	22
AFT. 45 MIN - PRE	8	7.000	9.8125	-4	5.0	21
AFT. 60 MIN - PRE	8	6.250	7.8513	-7	8.5	16
AFT. 90 MIN - PRE	8	5.875	8.1141	-4	4.5	21
AFT. 2 H - PRE	8	13.125	12.1707	-5	11.0	34
AFT. 3 H - PRE	8	13.875	12.9553	-3	15.0	32
AFT. 4 H - PRE	8	6.750	11.7565	-7	8.5	22
AFT. 6 H - PRE	8	13.000	10.3233	-6	17.0	23
AFT. 8 H - PRE	8	13.375	11.4385	-1	12.5	28
AFT. 24 H - PRE	8	-0.750	13.1230	-27	1.5	15

The effect of food on the pharmacokinetics of 30 and 40 mg nisoldipine C.C. tablets in healthy male volunteers.

Study: D92-045-02 Volume: 2 Pages: 1-671.

Investigators:

Clinical:

Objectives:

The aim of the study is to evaluate the effect of food on the pharmacokinetics of 30 and 40 mg Nisoldipine C.C. tablets.

Formulation:

Coat-Core tablet: 30 mg nisoldipine batch # 526245.
Coat-Core tablet: 40 mg nisoldipine batch # 526069.

Study Design:

28 healthy male volunteers between the ages 18 and 45 years participated in this open, randomized, 2 period crossover study. Two dose levels 30 and 40 mg were studied concurrently, each in a 2 way crossover design in a different set of 14 subjects.

At each dose level, the subjects received a single dose (30 or 40 mg) of nisoldipine C.C. either in the fasting state or immediately after a standardized breakfast. There was a 1 week washout period between each single dose at each dose level.
Blood samples were collected according to the following time table:0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 24, 30, 36, 48, 60 and 72 hours post-administration.
Breakfast was consumed in 20 minutes and the drug was given immediately (within 5 minutes) after the end of the meal.

The breakfast consisted of 1 cup of caffeinated or decaffeinated coffee (optional), 2 slices of buttered toast; jelly optional, 2 eggs fried in butter, 2 strips bacon, 4 ounces of hash brown potatoes and 8 ounces of whole milk.

Results:

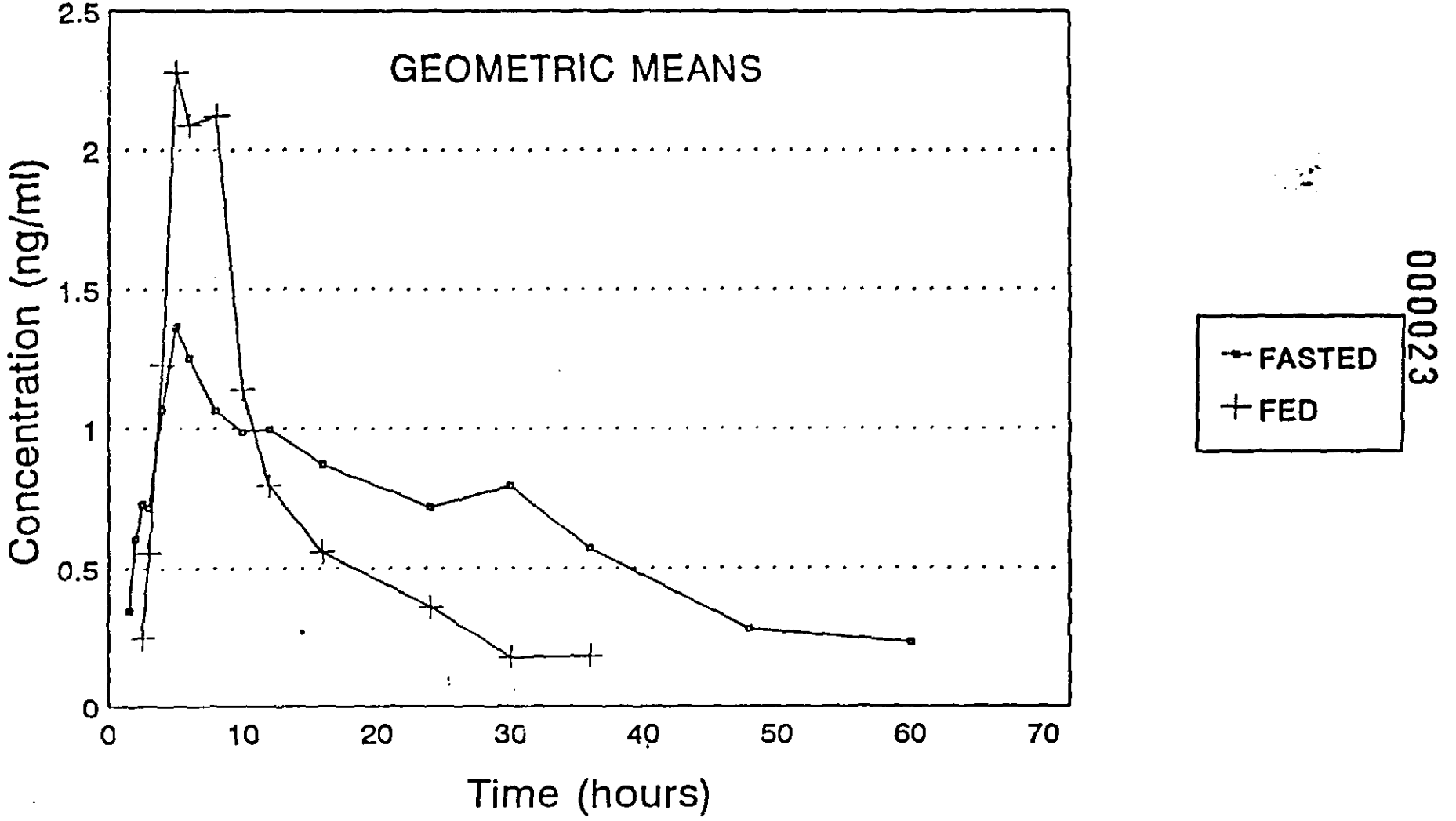
Figure 1 shows the geometric mean plasma profile for the 30 mg nisoldipine CC under fed and fasted conditions while Figure 2 shows the corresponding profile for the 40 mg tablet. Table 1 gives a comparison of the CMAX geometric means for all the 4 different treatments while Table 2 gives the same comparison for AUC.

Conclusion:

Administration of nisoldipine C.C. tablets immediately following a high fast breakfast resulted in greater peak plasma drug concentrations than were seen when the same dose was given on an empty stomach. CMAX on the average increased 250 to 300 %. However AUC was decreased on the average by 26 % in the fed state as compared to the fasted state.

SINGLE 30 MG DOSE GIV. .N FED & FASTED
(STUDY D92-045-02)

FIGURE 1

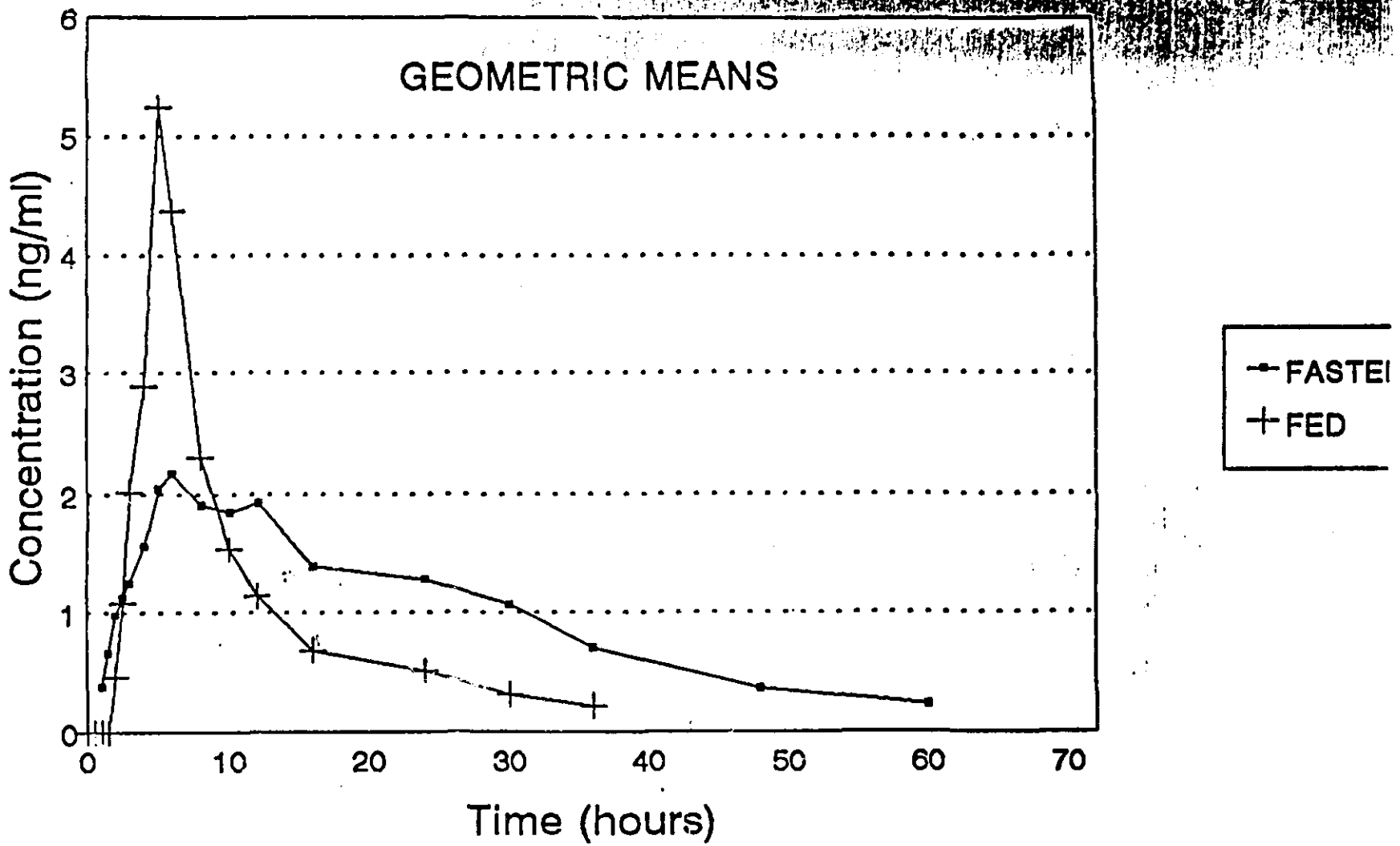


000023

N = 14

Fig. 12

SINGLE 40 MG DOSE REGIMEN FED & FASTED
(STUDY D92-045-02)



N = 14

TABLE 1

Geometric Mean (approximate CV) C_{max}

	30 mg	40 mg
C_{max} (ng/ml) Fasted	1.9 (62%)	2.7 (53%)
C_{max} (ng/ml) Fed	4.5 (74%)	7.5 (72%)
Ratio (Fed/Fasted)	2.35	2.75
90% CI	1.65 - 3.36	1.92 - 3.92

TABLE 2

Geometric Mean (approximate CV) $AUC_{0-\infty}$

	30 mg	40 mg
$AUC_{0-\infty}$ (ng*h/ml) Fasted	49.2 (42%)	70.4 (50%)
$AUC_{0-\infty}$ (ng*h/ml) Fed	35.4 (46%)	53.0 (51%)
Ratio (Fed/Fasted)	0.72	0.75
90% CI	0.58 - 0.89	0.61 - 0.93

A study to determine the single dose and steady-state pharmacokinetics of nisoldipine coat-core tablet 20 mg in elderly and young volunteers and in elderly hypertensives.

Study #: 712. VOLUME: 1-44-47 PAGES: 6-01-0001-1337

INVESTIGATOR:

OBJECTIVES:

To determine and compare the acute and steady-state pharmacokinetic parameters of the nisoldipine C.C. tablet in healthy young and elderly volunteers and in elderly hypertensive patients and to evaluate the safety of nisoldipine in these subjects.

FORMULATIONS:

Nisoldipine 20 mg C.C. tablets batch # 524526 expiration date June 30 1992.

STUDY DESIGN:

This was an open, multiple dose, non randomized study. The study population was selected according to the following criteria:
-healthy young male volunteers between the ages of 18 and 30 years.
-healthy elderly male and female volunteers aged 65 years or more.
-male and female elderly patients with a history of mild to moderate essential hypertension aged 65 years or more.
20 mg nisoldipine C.C. tablet was administered at 8 AM on days 1, 3, 4, 5, 6 and 7. Days 1 and 7 being the profile days.
5 ml blood samples were collected according to the following schedule: 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 14, 16, 20, 24, 28, 32, 36 and 48 hours after drug administration. Blood samples for trough level assessment were collected on days 5 and 6 before drug administration. Measurements of systolic and diastolic blood pressure and heart rate with volunteers in the supine position having rested for at least ten minutes were made just prior to the drawing of each blood sample.
21 healthy elderly volunteers, 23 healthy young volunteers and 11 hypertensive elderly patients of both sexes completed the study.

RESULTS:

Figure 1 shows the geometric mean nisoldipine plasma concentrations after single dose (day 1) and multiple dose (day 7) of a 20 mg nisoldipine C.C. in young healthy volunteers, while Figure 2 and 3 show the same plasma concentrations obtained in healthy elderly volunteers and elderly hypertensive patients. Figure 4 gives the comparative mean plasma profiles after single dose administration in the three different populations while Figure 5 gives the comparative profiles after multiple dose administration. Table 1 to 3 give a comparison of the pharmacokinetic parameters among the three different populations while Table 4 to 6 gives the same comparison for the pharmacokinetic parameters after multiple dose administration. Figure 6 gives the mean trough plasma nisoldipine concentrations.

The results show that after single dose administration of 20 mg nisoldipine C.C., the elderly normal and hypertensive patients tended to have higher AUC than young healthy volunteers by about 50 %. CMAX in the elderly hypertensives was also 50 % higher than in either the healthy young or elderly volunteers.

However upon multiple dose administration, there was a greater tendency for increase in AUC and CMAX in both the healthy and hypertensive subjects as compared to the young subjects. This resulted in a greater degree of accumulation in the elderly compared to the young. This is most probably due to lower clearance rates in the elderly as compared to the young.

It is to note that there was essentially no accumulation in the young however in the elderly healthy and hypertensives, the accumulation ratio was around 2.

FIGURE 1

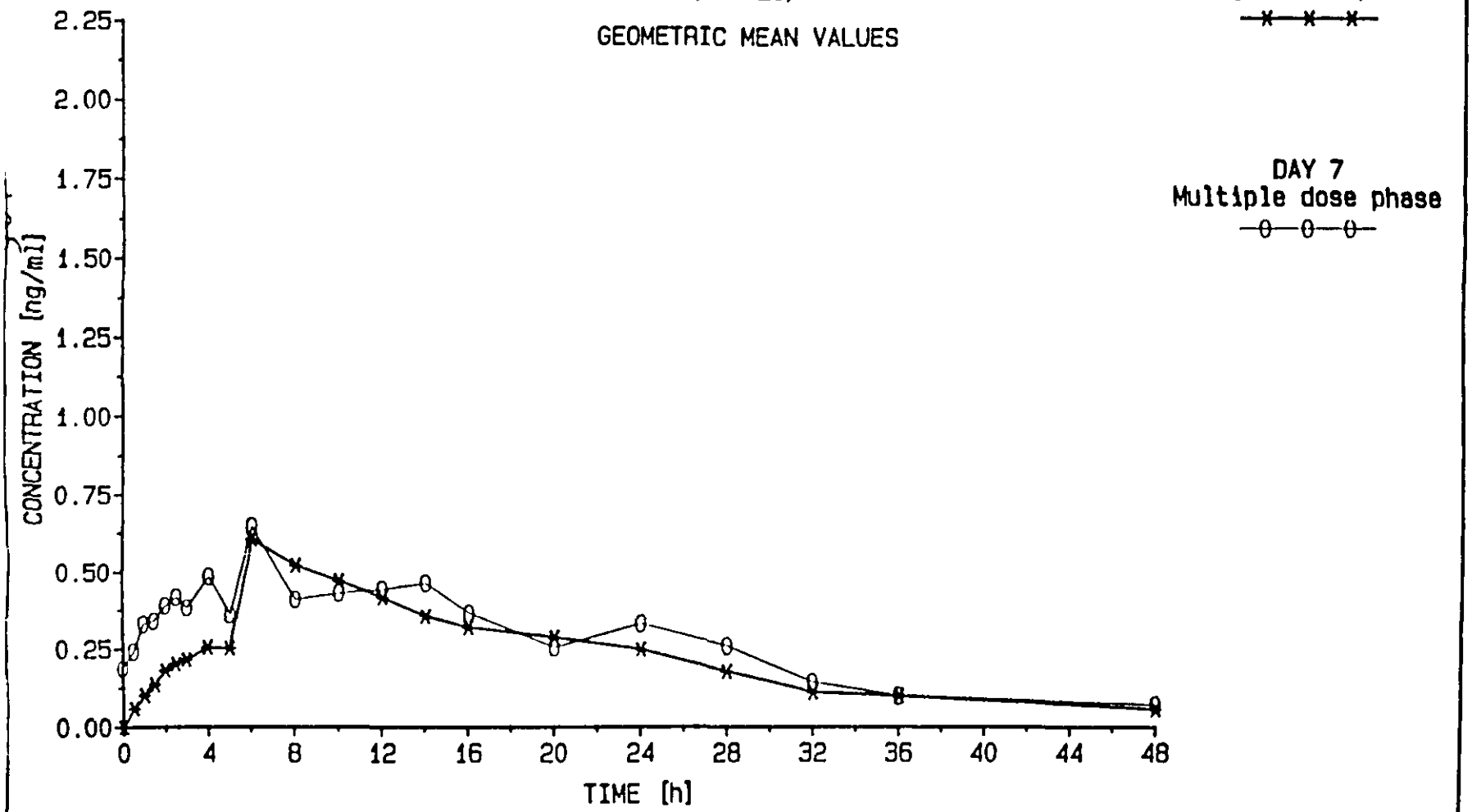
/ 0712

PLASMA NISOLDIPINE CONCENTRATIONS

Dose : 20 mg nisoldipine cc tablet

1 : Healthy young volunteers
(n = 20)

GEOMETRIC MEAN VALUES



000211

FIGURE 3

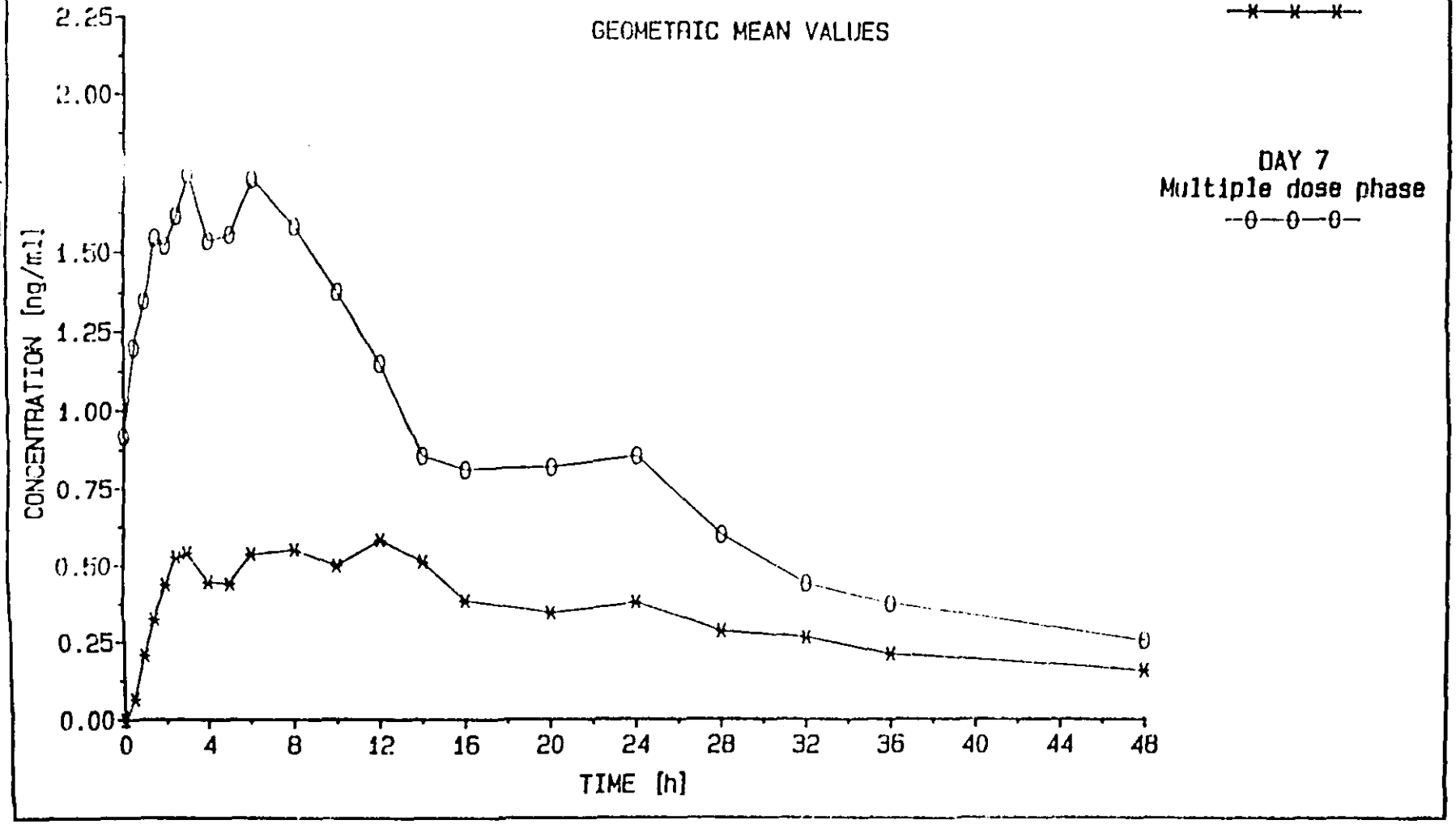
/ 0712

PLASMA NISOLDIPINE CONCENTRATIONS

Dose : 20 mg nisoldipine cc tablet

2 : Healthy elderly volunteers
(n = 21)

GEOMETRIC MEAN VALUES



717000

FIGURE 3

/ 0712

PLASMA NISOLDIPINE CONCENTRATIONS

Dose : 20 mg nisoldipine cc tablet

3 : Elderly hypertensive patients
(n = 11)

GEOMETRIC MEAN VALUES

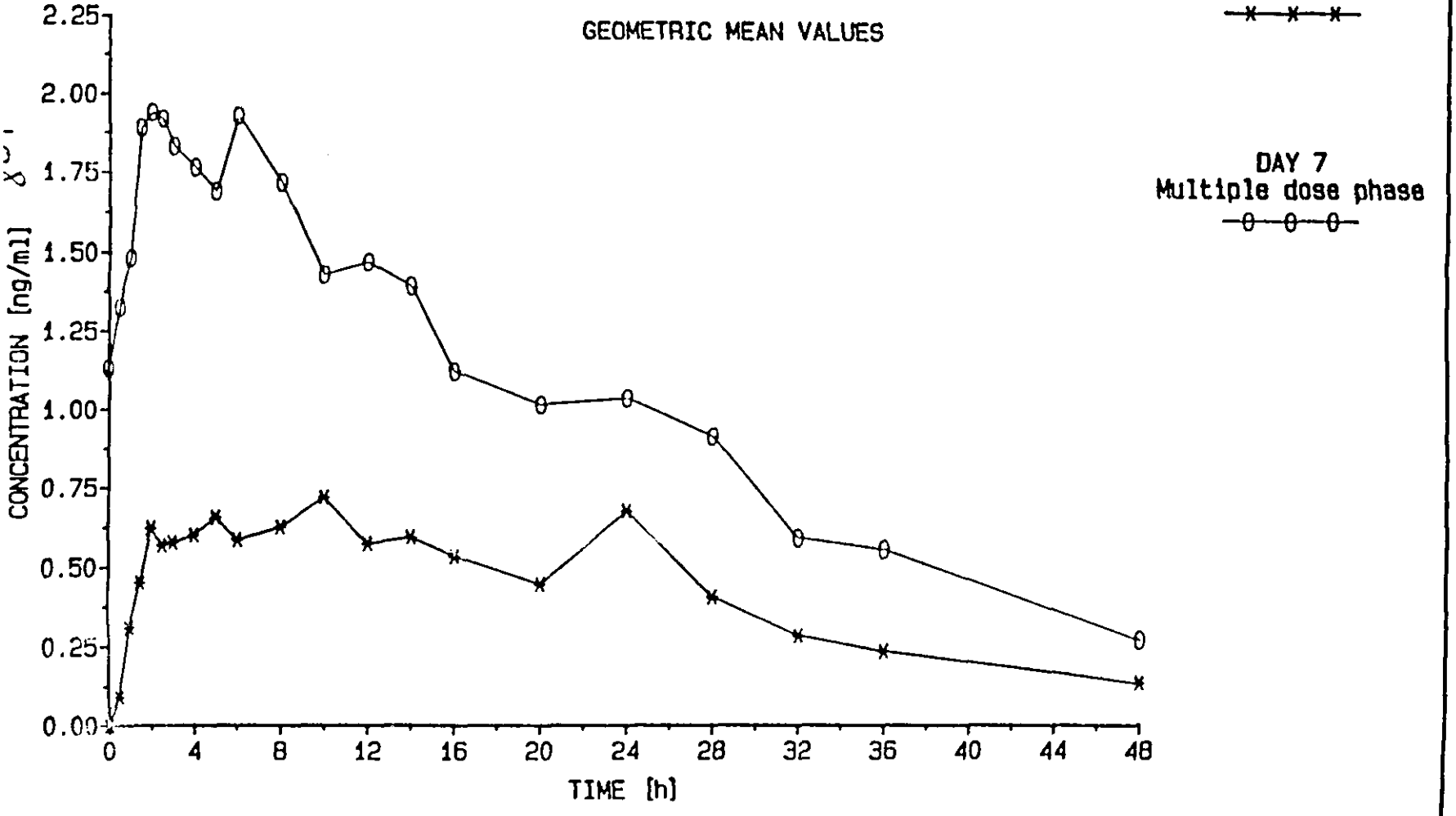


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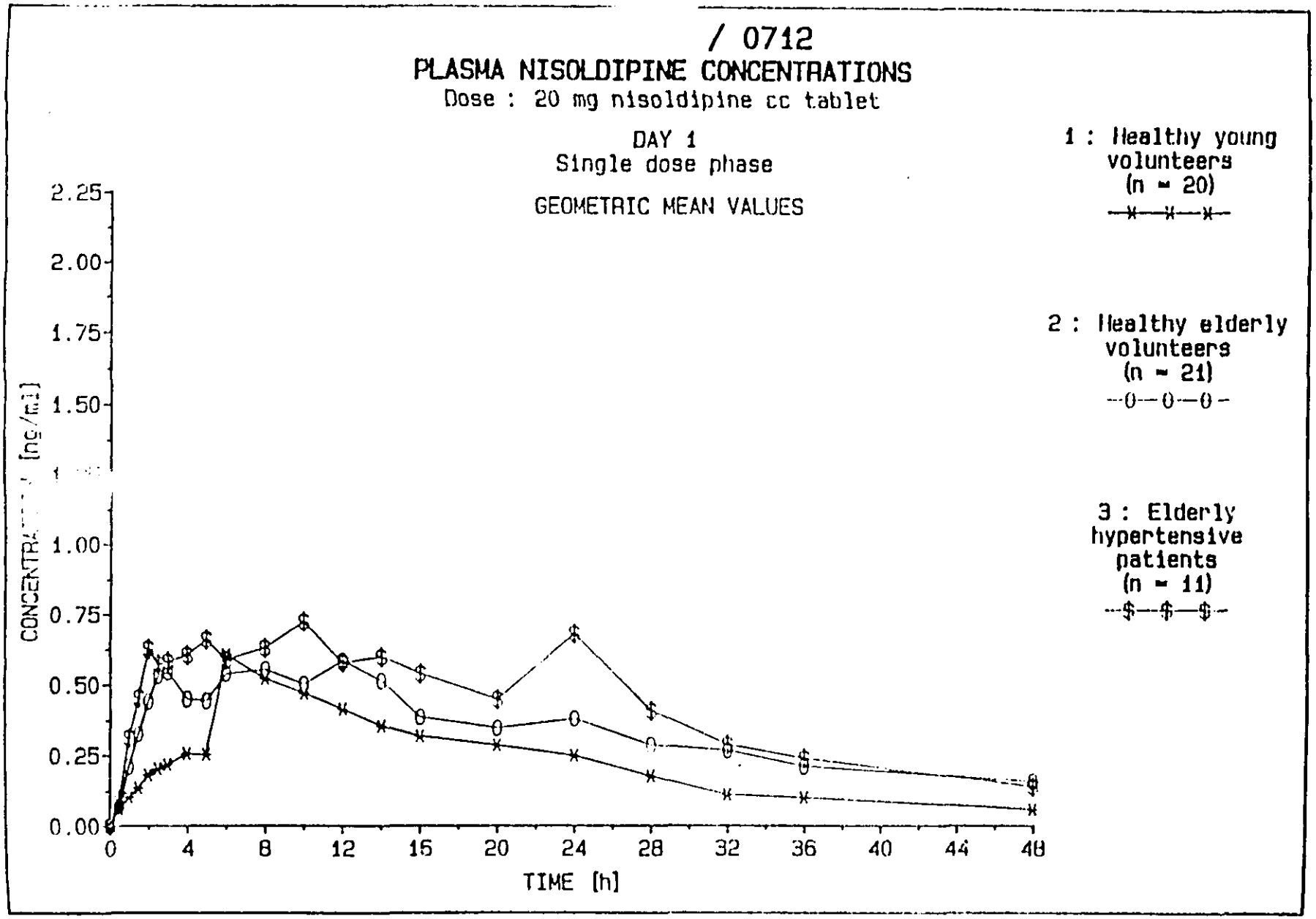
FIGURE 4



bc1

FIGURE 5

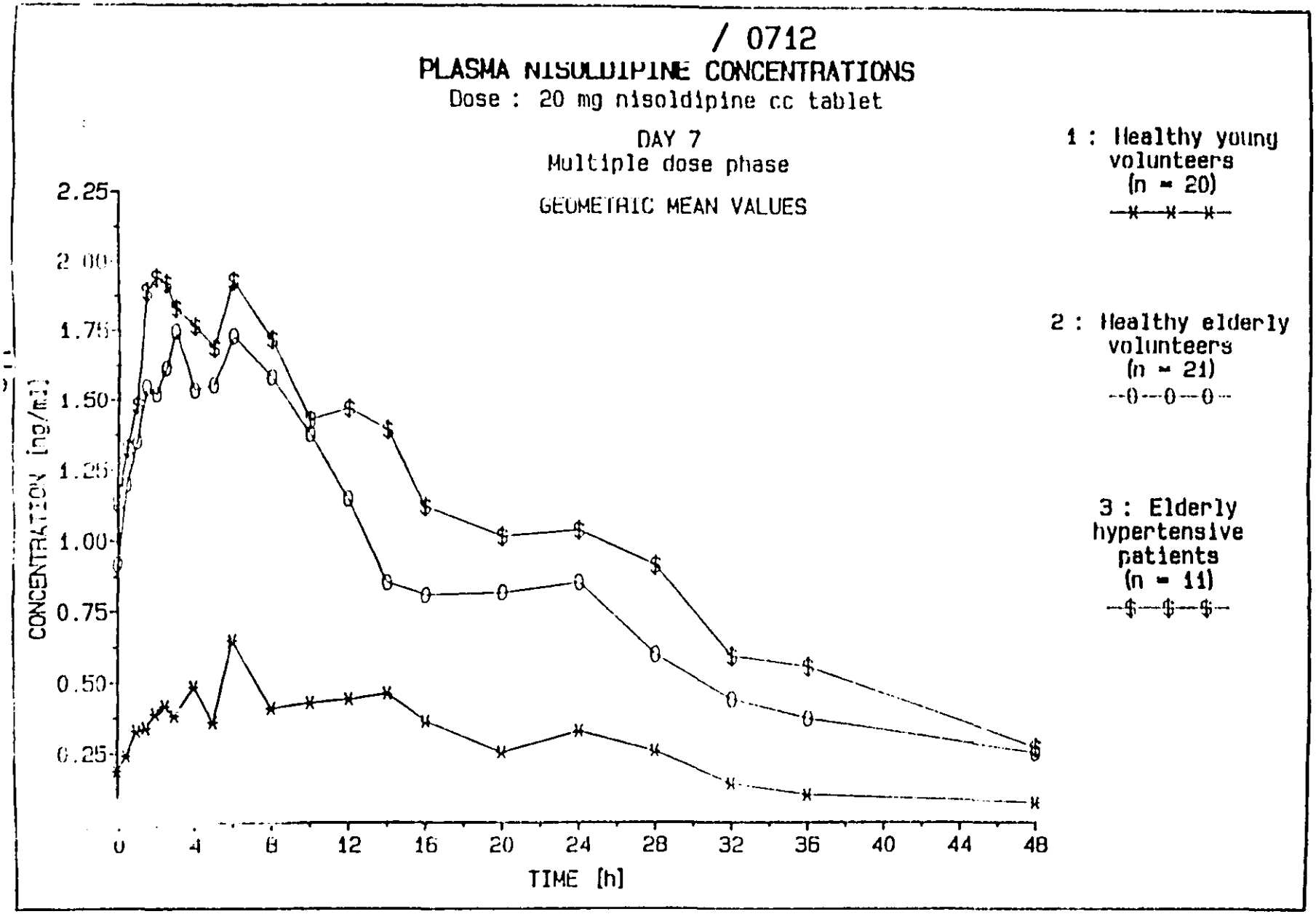
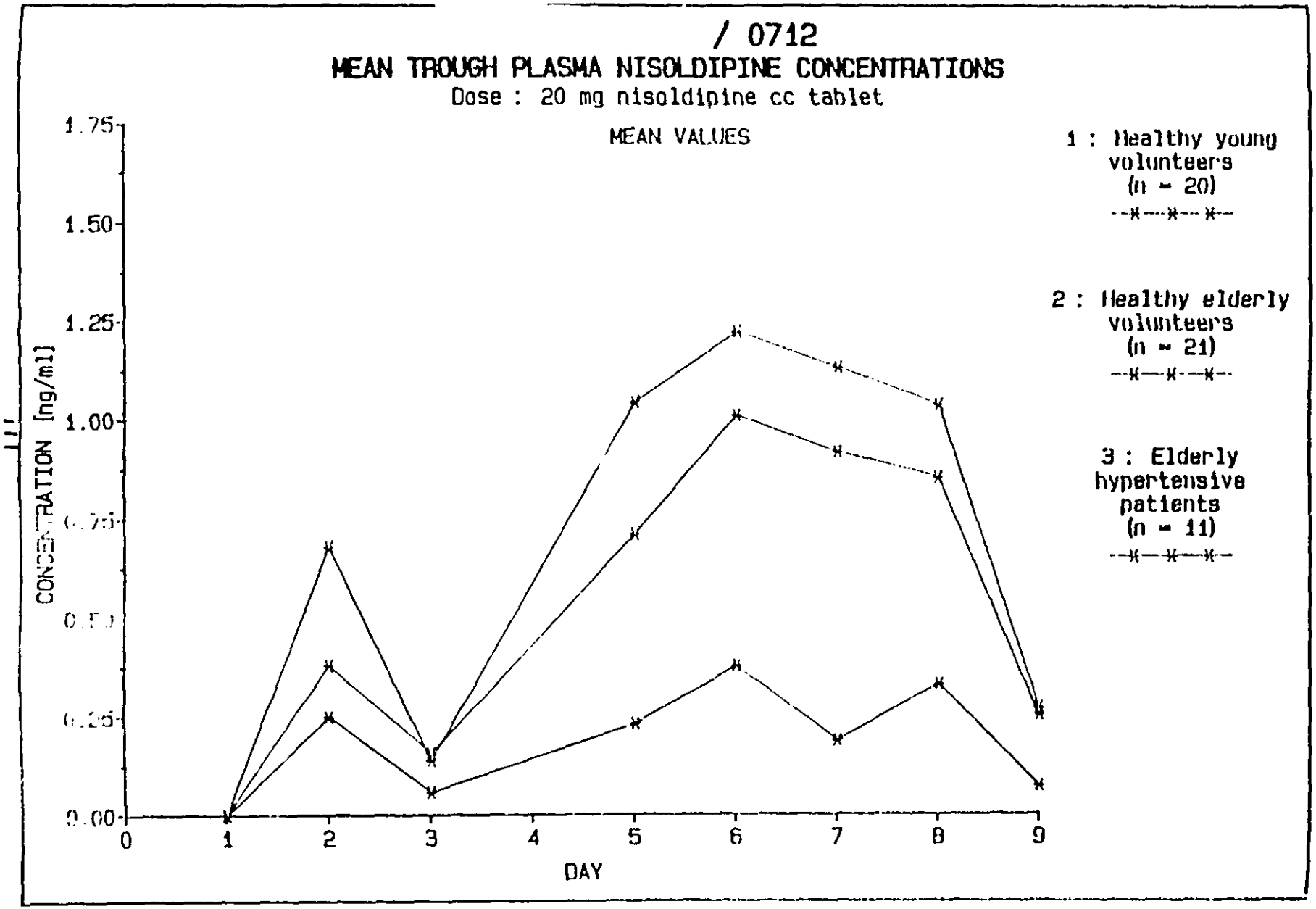


FIGURE 6



222040

TABLE 3

Table 1: **SUMMARY OF PHARMACOKINETIC DATA FOR NISOLDIPINE**

Day 1 - **Single dose phase** (Dose : 1 x 20 mg nisoldipine cc tablet)

		Healthy young volunteers			Healthy elderly volunteers				
VARIABLE	UNIT	*n*	MEDIAN	RANGE	*n*	MEDIAN	RANGE	MEAN RATIO (%)*	90% CONFIDENCE INTERVAL (%)**
C_{max}	(ng/ml)	20	0.92		21	1.07			
t_{max}	(h)	19	6.00		21	6.00		[0.0	-3.0-2.5]#
AUC(0-t_n)	(ng.h/ml)	20	15.0		21	23.3			
AUC(0-24h)	(ng.h/ml)	20	11.0		21	15.5			
AUC(0-∞)	(ng.h/ml)	20	18.1		21	26.0			
$C_{max,norm}$+	(mg/ml)	20	3.34		21	3.65		97	71-135
AUC(0-24h)$_{norm}$+	(mg.h/ml)	20	40.0		21	56.6		131	85-186
AUC(0-∞)$_{norm}$+	(mg.h/ml)	20	68.9		21	93.5		158	100-252
$t_{1/2}$	(h)	13	10.5		17	13.6		150	106-212
MRT	(h)	13	19.7		17	28.5			
CL/f	(l/h)	20	1105		21	770			
CL/f_{norm}++	(l/h/kg)	20	14.6		21	10.7			
V_{ss}/f	(l)	13	21186		17	17651			
V_{ss}/f_{norm}++	(l/kg)	13	323		17	259			

*: Nonparametric estimate of "elderly/young" mean ratio

**: Nonparametric 90% confidence interval for the "elderly/young" mean ratio

#: Nonparametric estimate of "elderly-young" mean difference and 90% confidence interval for the "elderly-young" mean difference

+: Normalized for dose per body mass.

++: Normalized for body mass.

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022215

TABLE 4

Table 2: **SUMMARY OF PHARMACOKINETIC DATA FOR NISOLDIPINE**

Day 1 - **Single dose phase** (Dose : 1 x 20 mg nisoldipine cc tablet)

		Healthy young volunteers			Elderly hypertensive patients				
VARIABLE	UNIT	n	MEDIAN	RANGE	n	MEDIAN	RANGE	MEAN RATIO (%)*	90% CONFIDENCE INTERVAL (%)**
C_{max}	(ng/ml)	20	0.92		11	1.40			
t_{max}	(h)	19	6.00		11	6.00		[0.0	-2.0-3.0]#
AUC(0-t_z)	(ng.h/ml)	20	15.0		11	19.5			
AUC(0-24h)	(ng.h/ml)	20	11.0		11	18.1			
AUC(0-∞)	(ng.h/ml)	20	18.1		11	24.9			
$C_{max,norm}$+	(ng/ml)	20	3.34		11	5.88		152	86-228
$AUC(0\text{-}24h)_{norm}$+	(ng.h/ml)	20	40.0		11	75.1		174	102-286
$AUC(0\text{-}\infty)_{norm}$+	(ng.h/ml)	20	68.9		11	95.8		202	117-360
$t_{1/2}$	(h)	13	10.5		10	10.4		107	81-189
MRT	(h)	13	19.7		10	21.8			
	(l/h)	20	1105		11	805			
CL/f_{norm}++	(l/h/kg)	20	14.6		11	10.4			
V_{ss}/f	(l)	13	21186		10	14291			
V_{ss}/f_{norm}++	(l/kg)	13	323		10	174			

*: Nonparametric estimate of "elderly/young" mean ratio

**: Nonparametric 90% confidence interval for the "elderly/young" mean ratio

#: Nonparametric estimate of "elderly-young" mean difference and 90% confidence interval for the "elderly-young" mean difference

+: Normalized for dose per body mass.

++: Normalized for body mass.

TABLE 5

Table 3: **SUMMARY OF PHARMACOKINETIC DATA FOR NISOLDIPINE**

Day 1 - Single dose phase (Dose : 1 x 20 mg nisoldipine cc tablet)

		Healthy elderly volunteers			Elderly hypertensive patients				
VARIABLE	UNIT	n	MEDIAN	RANGE	n	MEDIAN	RANGE	MEAN RATIO (%)*	90% CONFIDENCE INTERVAL (%)**
C_{max}	(ng/ml)	21	1.07		11	1.40			
t_{max}	(h)	21	6.00		11	6.00		10.5	-2.0-4.0]#
AUC(0-t_z)	(ng.h/ml)	21	23.3		11	19.5			
AUC(0-24h)	(ng.h/ml)	21	15.5		11	18.1			
AUC(0-∞)	(ng.h/ml)	21	26.0		11	24.9			
$C_{max,norm}$+	(mg/ml)	21	3.65		11	5.88		152	95-227
AUC(0-24h)$_{norm}$+	(mg.h/ml)	21	56.6		11	75.1		135	89-204
AUC(0-∞)$_{norm}$+	(mg.h/ml)	21	93.5		11	95.8		126	79-231
$t_{\frac{1}{2}}$	(h)	17	13.6		10	10.4		78	52-127
MRT	(h)	17	28.5		10	21.8			
CL/f	(l/h)	21	770		11	805			
CL/f_{norm}+ +	(l/h/kg)	21	10.7		11	10.4			
V_{ss}/f	(l)	17	17651		10	14291			
V_{ss}/f_{norm}+ +	(l/kg)	17	259		10	174			

*: Nonparametric estimate of "elderly patients/elderly volunteers" mean ratio

**: Nonparametric 90% confidence interval for the "elderly patients/elderly volunteers" mean ratio

#: Nonparametric estimate of "elderly patients-elderly volunteers" mean difference and 90% confidence interval for the "elderly patients-elderly volunteers" mean difference

+: Normalized for dose per body mass.

+ +: Normalized for body mass.

TABLE 1

SUMMARY OF PHARMACOKINETIC DATA FOR NISOLDIPINE

Day 7- **Multiple dose phase** (Dose : 20 mg nisoldipine ec tablet once daily for 5 days)

		Healthy young volunteers			Healthy elderly volunteers				
VARIABLE	UNIT	n	MEDIAN	RANGE	n	MEDIAN	RANGE	MEAN RATIO (%)*	90% CONFIDENCE INTERVAL (%)**
$C_{ss\,max}$	(ng/ml)	20	1.06		21	2.07			
$C_{ss\,min}$	(ng/ml)	20	1.10		21	0.33			
$t_{ss\,max}$	(h)	20	6.00		21	3.00		[-3.0	-4.0 0][#]
AUC_{ss}	(ng.h/ml)	20	12.5		21	32.0			
AR		20	1.12		21	2.29		206	156-274
$C_{ss\,max,norm}$+	(mg/ml)	20	3.74		21	7.25		193	144 261
$AUC_{ss,norm}$+	(mg.h/ml)	20	47.0		21	122		265	178-383
$t_{1/2}$	(h)	13	10.4		20	13.8		160	106-238
CL/f	(l/h)	20	1597		21	624			
CL/f_{norm}++	(l/h/kg)	20	21.3		21	8.22			

*: Nonparametric estimate of "elderly/young" mean ratio

**: Nonparametric 90% confidence interval for the "elderly/young" mean ratio

#: Nonparametric estimate of "elderly-young" mean difference and 90% confidence interval for the "elderly-young" mean difference

+: Normalized for dose per body mass.

++: Normalized for body mass.

TABLE 2

Table 6: SUMMARY OF PHARMACOKINETIC DATA FOR NISOLDIPINE

Day 7- Multiple dose phase (Dose : 20 mg nisoldipine cc tablet once daily for 5 days)

		Healthy elderly volunteers			Elderly hypertensive patients				
VARIABLE	UNIT	n	MEDIAN	RANGE	n	MEDIAN	RANGE	MEAN RATIO (%)*	90% CONFIDENCE INTERVAL (%)**
$C_{ss,max}$	(ng/ml)	21	2.07		11	2.42			
$C_{ss,min}$	(ng/ml)	21	0.33.		11	0.32			
$t_{ss,max}$	(h)	21	3.00		11	2.50		[-0.5	-3.0-0.5]#
AUC_{ss}	(ng.h/ml)	21	32.0		11	38.0			
AR		21	2.29		11	1.92		84	70-117
$C_{ss,max,norm}$+	(mg/ml)	21	7.25		11	11.0		139	102-188
$AUC_{ss,norm}$+	(mg.h/ml)	21	122		11	135		139	94-200
$t_{1/2}$	(h)	20	13.8		11	14.1		97	62-142
CL/f	(l/h)	21	6.24		11	526			
CL_{norm}++	(l/h/kg)	21	8.22		11	7.42			

*: Nonparametric estimate of "elderly patients/elderly volunteers" mean ratio

**: Nonparametric 90% confidence interval for the "elderly patients/elderly volunteers" mean ratio

#: Nonparametric estimate of "elderly patients-elderly volunteers" mean difference and 90% confidence interval for the "elderly patients-elderly volunteers" mean difference

+: Normalized for dose per body mass.

++: Normalized for body mass.

9.1

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TABLE 6

Table 5: SUMMARY OF PHARMACOKINETIC DATA FOR NISOLDIPINE

Day 7- Multiple dose phase (Dose : 20 mg nisoldipine cc tablet once daily for 5 days)

		Healthy young volunteers			Elderly hypertensive patients				
VARIABLE	UNIT	n	MEDIAN	RANGE	n	MEDIAN	RANGE	MEAN RATIO (%)*	90% CONFIDENCE INTERVAL (%)**
$C_{ss,max}$	(ng/ml)	20	1.0[illegible]		11	2.42			
$C_{ss,min}$	(ng/ml)	20	1.10		11	0.32			
$t_{ss,max}$	(h)	20	6.00		11	2.50		[-3.5	-5.0--0.5]#
AUC_{ss}	(ng.h/ml)	20	12.5		11	38.0			
AR		20	1.12		11	1.92		186	138-288
$C_{ss,max,norm}$+	(mg/ml)	20	3.74		11	11.0		275	202-264
$AUC_{ss,norm}$+	(mg.h/ml)	20	47.6		11	135		347	254-549
$t_{1/2}$	(h)	13	10.4		11	14.1		149	104-220
Cl/f	(l/h)	20	1597		11	526			
Cl/f_{norm}++	(l/h/kg)	20	21.3		11	7.42			

*: Nonparametric estimate of "elderly/young" mean ratio

**: Nonparametric 90% confidence interval for the "elderly/young" mean ratio

#: Nonparametric estimate of "elderly-young" mean difference and 90% confidence interval for the "elderly-young" mean difference

+: Normalized for dose per body mass.

++: Normalized for body mass.

A study to investigate the acute and short term pharmacokinetic profiles of nisoldipine in elderly and young normotensive volunteers.

STUDY #: 563. VOLUME: 1-48 PAGES: 6-02-001-0219

INVESTIGATORS:

OBJECTIVES:

To investigate the pharmacokinetics of nisoldipine 10 mg PO in one group of young normotensive volunteers and one group of elderly volunteers after acute (1 day) and chronic (7-8 days treatments).

FORMULATION:

Nisoldipine immediate release 10 mg tablets (batch #: 929488).

STUDY DESIGN:

12 elderly (age greater than 65 years) healthy volunteers and 9 young healthy volunteers (age 20-28 years) participated in this open non-randomized study. Subjects took a 10 mg nisoldipine tablet for 8 days. On day 1 and day 8 plasma samples were collected at 0, 1, 1.5, 2, 3, 5, 7 and 24 hours after dosing. Blood pressure and heart rate measurements were taken at the same time schedule as for the plasma samples.

ASSAY: No description of the assay was presented in the study.

1

RESULTS:

Figure 1 shows the plasma concentration for day 1 in the young and elderly volunteers while Figure 2 shows the corresponding profile for day 8. Table 1 summarizes the mean pharmacokinetic parameters for both types of population.
It can be seen from the results that the plasma concentrations of nisoldipine tended to be somewhat higher in the elderly as compared to the young. However, there was no difference in the single dose and multiple dose pharmacokinetics of nisoldipine in both the young and the elderly. Moreover, no accumulation was observed upon multiple dose administration.

FIGURE 2

FIGURE 2 : MEAN SEMILOGARITHMIC PLOT OF PLASMA NISOLDIPINE IN YOUNG AND ELDERLY SUBJECTS - DAY 8

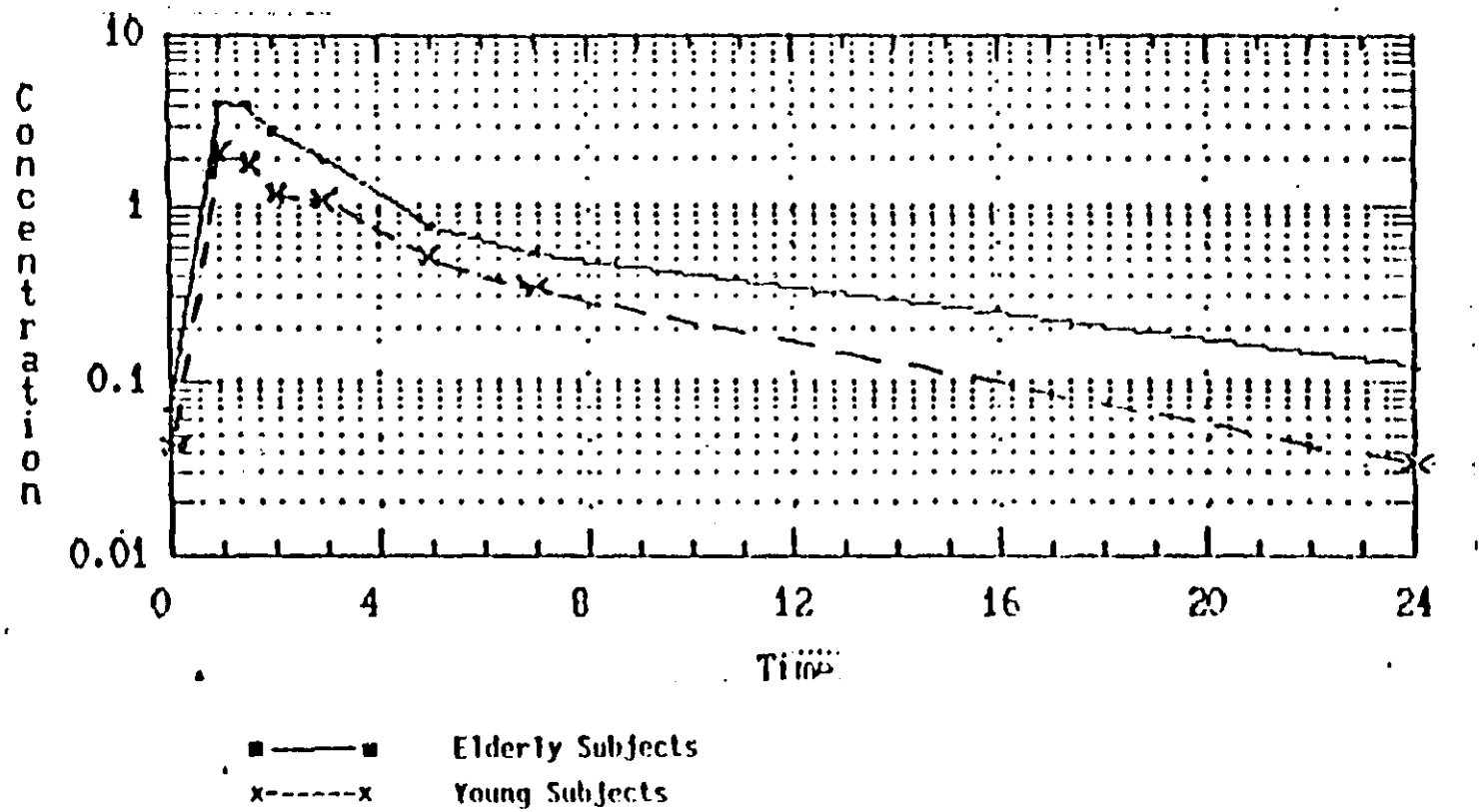
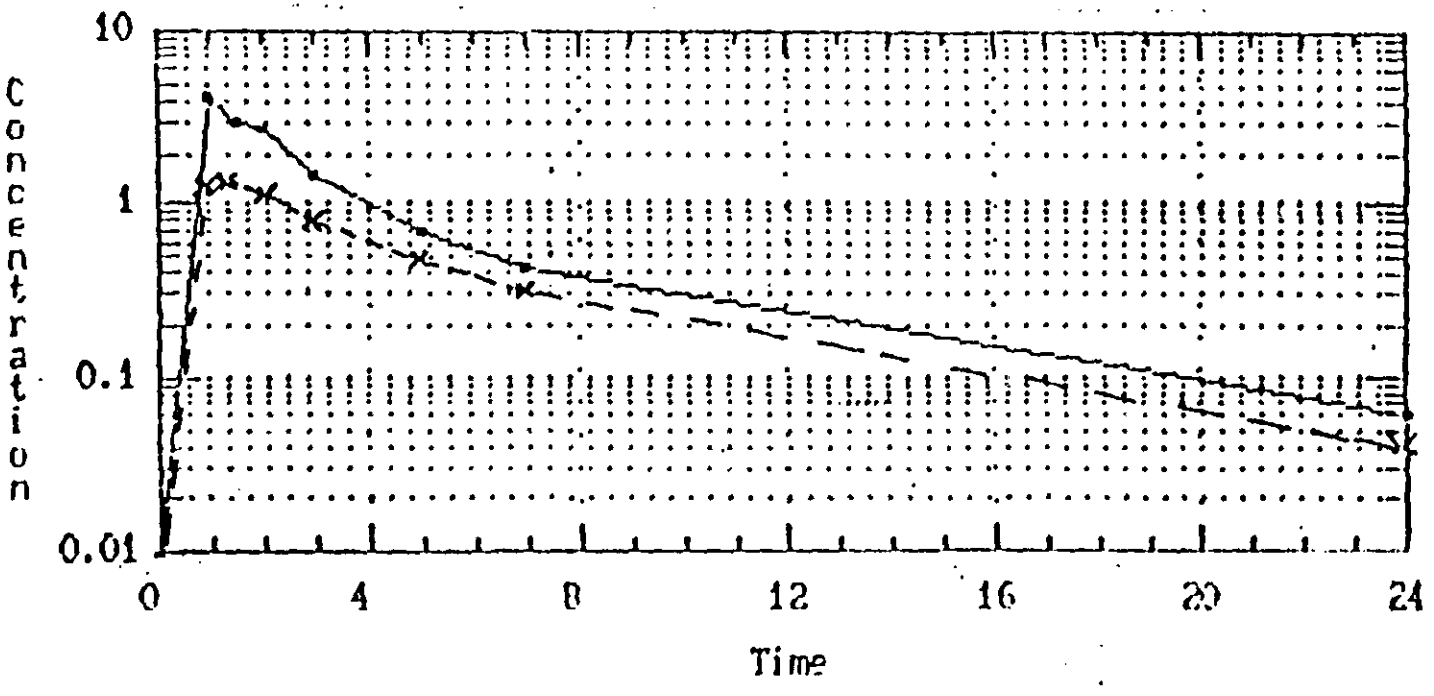


FIGURE1

FIGURE 1 : MEAN SEMILOGARITHMIC PLOT OF PLASMA NISOLDIPINE IN YOUNG AND ELDERLY SUBJECTS - DAY 1



120

The effect of cirrhosis on the steady state pharmacokinetics of nisoldipine coat-core sustained release tablets.

Study #: D90-026-01 Volume: 1-49. Pages: 06-02-0220-0704.

Investigator:

Objectives:

The aim of the study is to:

1-to compare the pharmacokinetic profile of nisoldipine C.C. following a single dose 10 mg and multiple doses (10 mg qd for 7 days) in cirrhotic subjects vs a control group of healthy subjects.

2-to assess the general safety and tolerability of the above dose regimens in cirrhotic subjects vs healthy subjects.

Formulation:

-Nisoldipine 10 mg coat-core tablets batch # 526022.

Study Design:

This was a single center non randomized, non blinded study in which a total of 16 subjects participated (8 healthy subjects and 8 with mild to moderate hepatic impairment, 4 males and 4 females in each group). The study was conducted in 2 stages with a period of 8 days between stage I and stage II. In the first stage all subjects received a single 10 mg nisoldipine C.C. tablet. In stage II, each subject received 10 mg nisoldipine C.C. once a day for 7 days. Each healthy subject was to be matched to a subject in the hepatically impaired group with respect to sex age and weight (less than 10 % deviation was allowed).
Blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 hours post dose administration in stage I day 1 and stage II day 7.
In stage II day 1 blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 10, 12 and 16 hours. On days 7 of stage II an additional blood sample was collected at 72 hours.
Blood pressure and heart rate measurements were taken at the following times relative to dose: Stage I day 1 and stage II day 1 and day 7: predose, 1, 2, 3, 4, 6, 8, 10, 12, 16 and 24 hours. stage II, days 2-6, pre-dose, 4 and 8 hours.

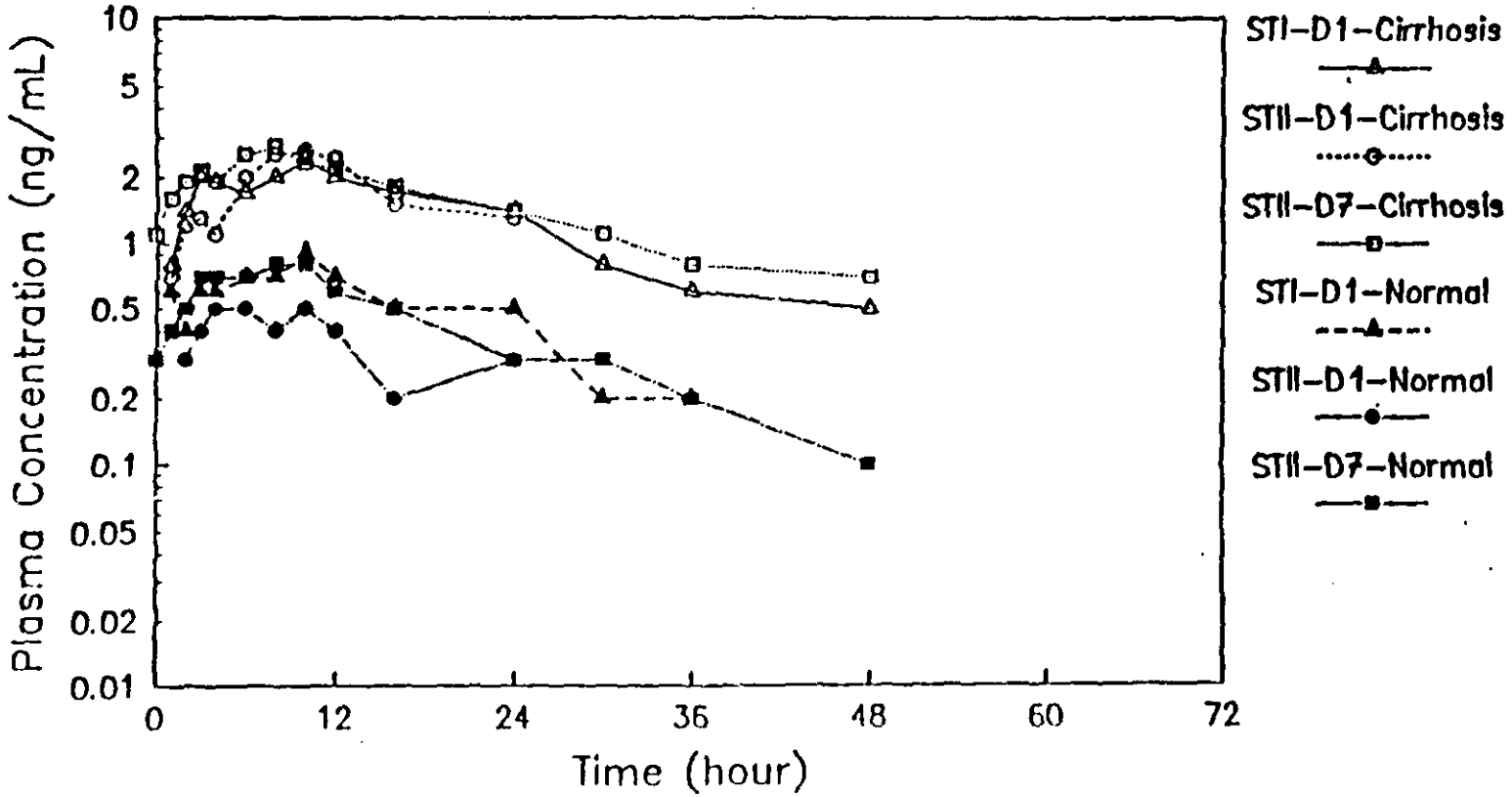
Results:

Figure 1 shows the means for the plasma concentration for both single and multiple dose administration of 10 mg nisoldipine C.C. in both the healthy volunteers and the liver impaired patients while Table 1 and 2 give a summary of the most important pharmacokinetic parameters for both stage I and II of this study.
The results show that there is an increase in both AUC and CMAX in the liver cirrhosis patients as compared to the healthy volunteers. This increase was almost three to four fold. Additionally, there was a slight nisoldipine accumulation in both the cirrhotic patients and the healthy volunteers since the accumulation ratio was 1.1 and 1.3 respectively.

Conclusion:

From the results of this study as shown in Table 1 and 2, liver impairment seems to have a pronounced effect on the pharmacokinetics of nisoldipine since both AUC and CMAX were increased four fold as compared to normal volunteers. Therefore extra care should be exercised when giving this drug to patients with impairment liver function, the initial dose should be lowered and the patient monitored to avoid any unwarranted side effects or toxicities.

Mean Plasma Nisoldipine Concentrations Following Single or Multiple Dose of 10 mg Coat Core Tablets in Normal and Cirrhotic Subjects



Mean Plot

Study D90-026-01

Table 1
Pharmacokinetic Parameters for Nisoldipine Following
A Single Dose of 10 mg Coat-Core Tablet-Stage I
[Arithmetic Mean (Coefficient of Variation)]

PARAMETERS (UNITS)	NORMAL (N=8)	CIRRHOTIC (N=8)
C_{max} (ng/mL)	1.03 (76)	2.89 (51)
T_{max} (hr)	8.13 (91)	7.25 (48)
AUC_{0-48} (ng·hr/mL)	19.0 (72)	58.2 (63)

Table 2
Pharmacokinetic Parameters Following Multiple Doses of
Nisoldipine 10 mg Coat-Core Given Once Daily for Seven Days
[Arithmetic Mean (Coefficient of Variation)]

PARAMETER (UNITS)	DAY	NORMAL (N=8)	CIRRHOTIC (N=8)
C_{max} (ng/mL)	1 7	0.73 (48) 0.94 (58)	2.98 (69) 3.62 (91)
T_{max} (hr)	1 7	6.5 (55) 4.6 (42)	10.0 (59) 6.9 (71)
AUC_{0-24} (ng·hr/mL)	1 7	7.55 (48) 11.9 (77)	39.9 (71) 46.7 (82)
AUC_{0-72} (ng·hr/mL)	7	17.7 (80)	76.3 (89)

Influence of renal function on the pharmacokinetics of nisoldipine C.C. tablets after single and multiple dosing.

STUDY #: D92-001 VOLUME: 1-50-52 PAGES: 06-02-797-2134.

INVESTIGATOR:

OBJECTIVES:

To determine the effect of impaired renal function on the pharmacokinetics of nisoldipine C.C. after single and multiple dosing.

FORMULATIONS:

-20 mg nisoldipine C.C. tablets batch # 524652.

STUDY DESIGN:

This was a multi-center, non-blinded, non randomized comparative study among four groups. 3 centers were to enrol 12 subjects each for a study total of 36 subjects. There were to have been 3 subjects per center in each of the 4 renal function groups. 27 seven subjects were to have renal impairment and 9 subjects were to serve as the control group. The 4 renal function groups were as follows:
-Group 1 (normal): 9 subjects with creatinine clearance of > 90 ml/min.
-Group 2 (mild): 9 subjects with creatinine clearance between 61 and 90 ml/min.
-Group 3 (moderate): 9 subjects with creatinine clearance between 30 and 60 ml/min.
-Group 4 (severe): 9 subjects with creatinine clearance of < 30 ml/min.
Patients on hemodialysis were not allowed to participate in the study.
On day 1 of the study, following an overnight fast, subjects were given a 20 mg dose of nisoldipine C.C. one hour prior to breakfast. Beginning on day 3, subjects were dosed once a day with 20 mg nisoldipine C.C. for 6 days. The final dose of nisoldipine was given the morning of day 8.
On day 1 and 8, blood samples were collected at: 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 16 hours. On day 2, samples were to be drawn at 20, 24, 28, 32 and 36 hours after the first dose of study drug. On day 3, a 48 hour post dose sample was drawn prior to the Day 3 dose. On day 5, a predose sample was drawn immediately before that morning dose. On day 9, samples were to be drawn at 20, 24, 28, 32 and 36 hours. On day 10, a 48 hour post dose sample was drawn. On day 1 and 2, urine was collected 0-12, 12-24, and 24-36 hours post-dose. Beginning on day 8, urine was collected 0-12, 12-24 and 24-36 hours post dose.

DATA ANALYSIS:

Data analysis was performed using standard pharmacokinetic techniques.

RESULTS:

Table 1 summarizes some of the pharmacokinetic parameters of nisoldipine after single and multiple dose administration in the 4 groups of patients while Figure 1 and Figure 2 show the corresponding plasma profiles for all 4 groups on day 1 and day 8 respectively. It can be seen from the results that the plasma concentrations of nisoldipine in patients with severe renal impairment (group 4) are slightly higher than the control group. Figure 3 and 4 give the corresponding plasma concentrations for These plots indicate that the plasma concentrations on day 1 of this metabolite were significantly higher in the subjects with moderate and severe renal failure. However on day 8 there were no significant differences between the 4 groups even though the levels were still higher in the moderate to severe renal impairment group.

Figure 5 and 6 shows the plasma concentrations of metabolite L which is the most abundant metabolite with plasma concentrations ten times the parent compound while table 2 summarizes the most important pharmacokinetic parameters. The same trend of results was observed as with metabolit ' with the moderate to severe renal impairment patients showing much higher plasma concentrations on day 1 that the control group with the differences being less pronounced on day 8.

Figure 7 and 8 show the mean plasma concentration of metabolit the only active metabolite of nisoldipine (10 times less active than the parent drug) and is the least abundant

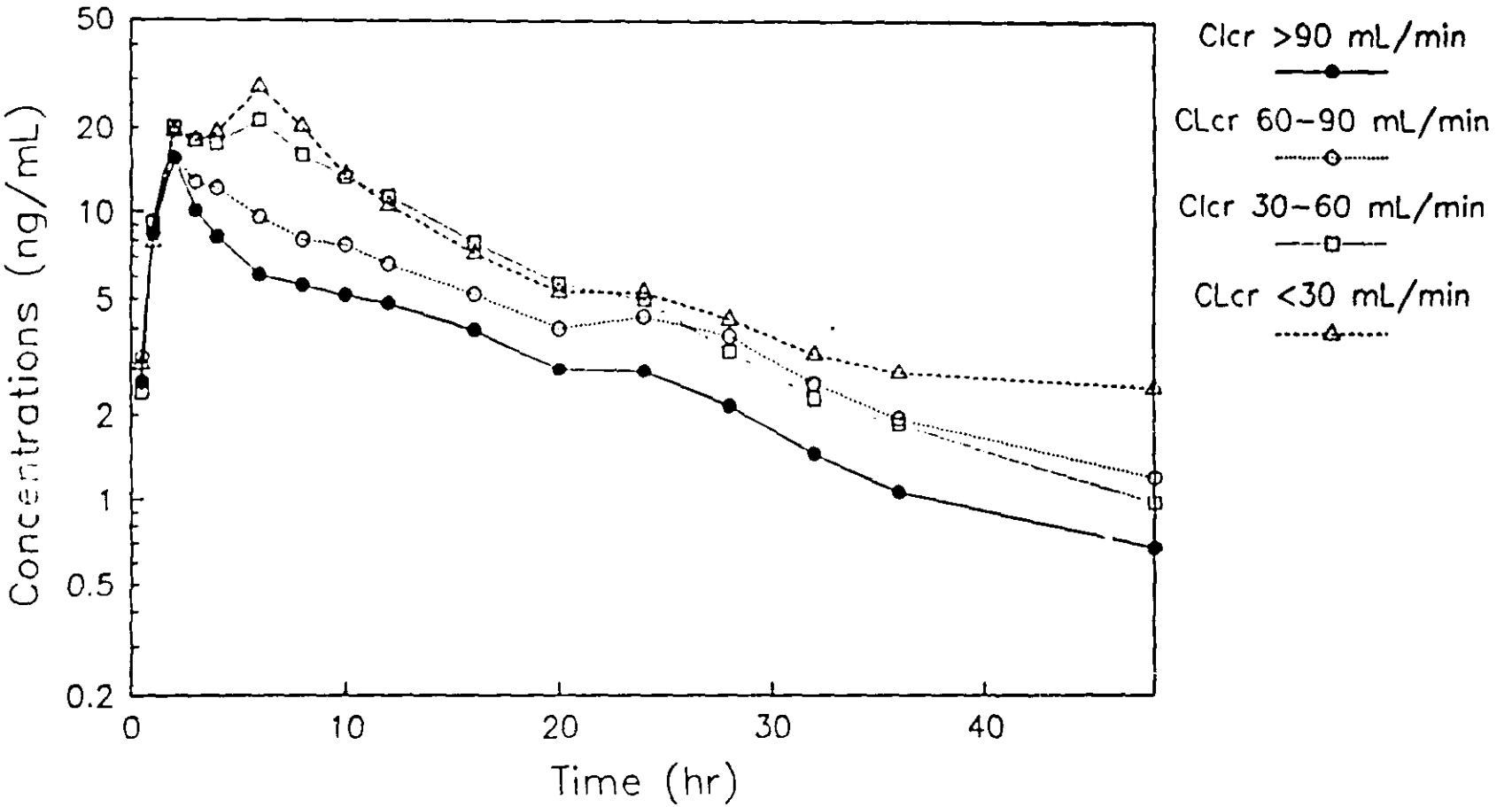
among the metabolites. Table 3 summarizes the major pharmacokinetic parameters for this metabolite. The results show that the moderately impaired patients but not the severely impaired, had modestly higher AUC and CMAX relative to the normal subjects. However these differences tended to disappear on day 8.

Conclusion:

In patients with severe renal impairment (creatinine clearance less than 30 ml/min, the nisoldipine plasma concentrations were higher by as much as 2 fold than the control group, however this difference seem to have subsided by day 8. Therefore, renal impairment does not seem to significantly alter the pharmacokinetics of nisoldipine and its metabolites. Patients should be closely monitored and titration to higher doses should be based on clinical response.

FIGURE 5

Plasma Concentrations For Following Single Dose of 20 mg Nisoldipine Coat-Core Tablet

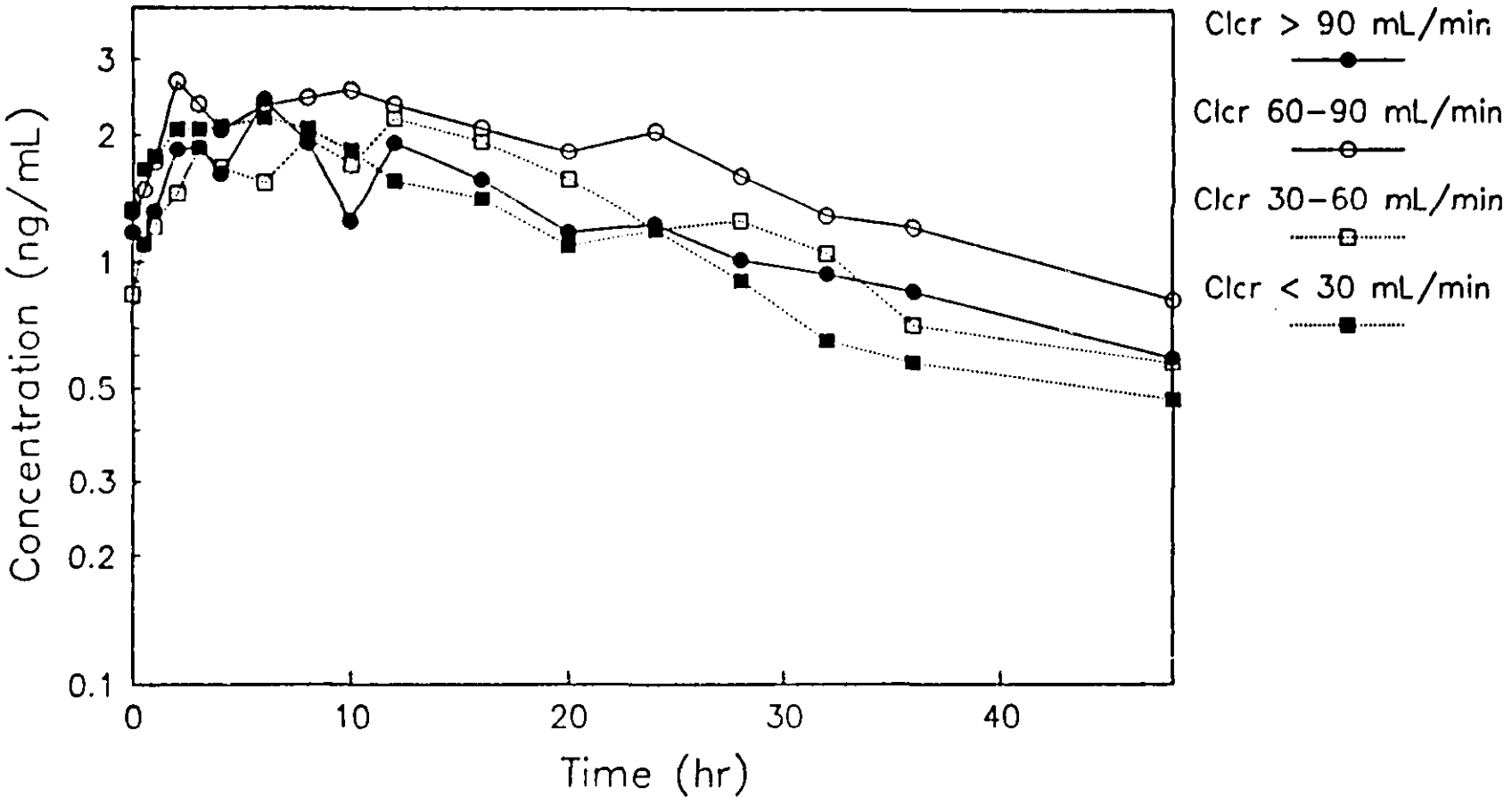


D92-001
FIGURE 5

Mean Plot
Day 1

FIGURE 2

Mean Plasma Concentrations For Nisoldipine Following a 20 mg Coat-Core Tablet Once Daily For Eight Days



Mean Plot
Day 8

Study D92-001
FIGURE 2

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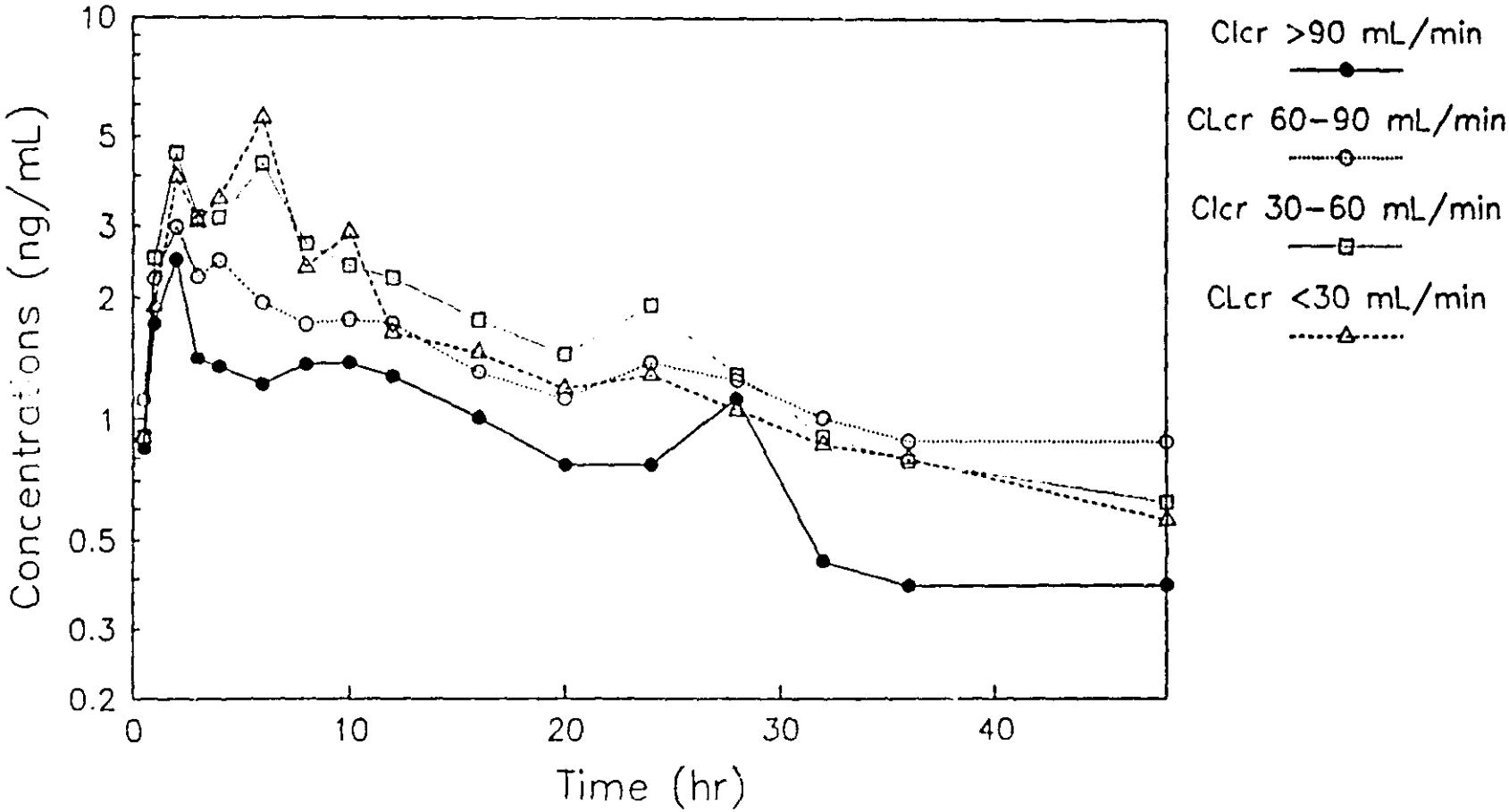
000705

FIGURE 3

Plasma Concentrations For Following a Single Dose of 20 mg Nisoldipine Coat-Core Tablet

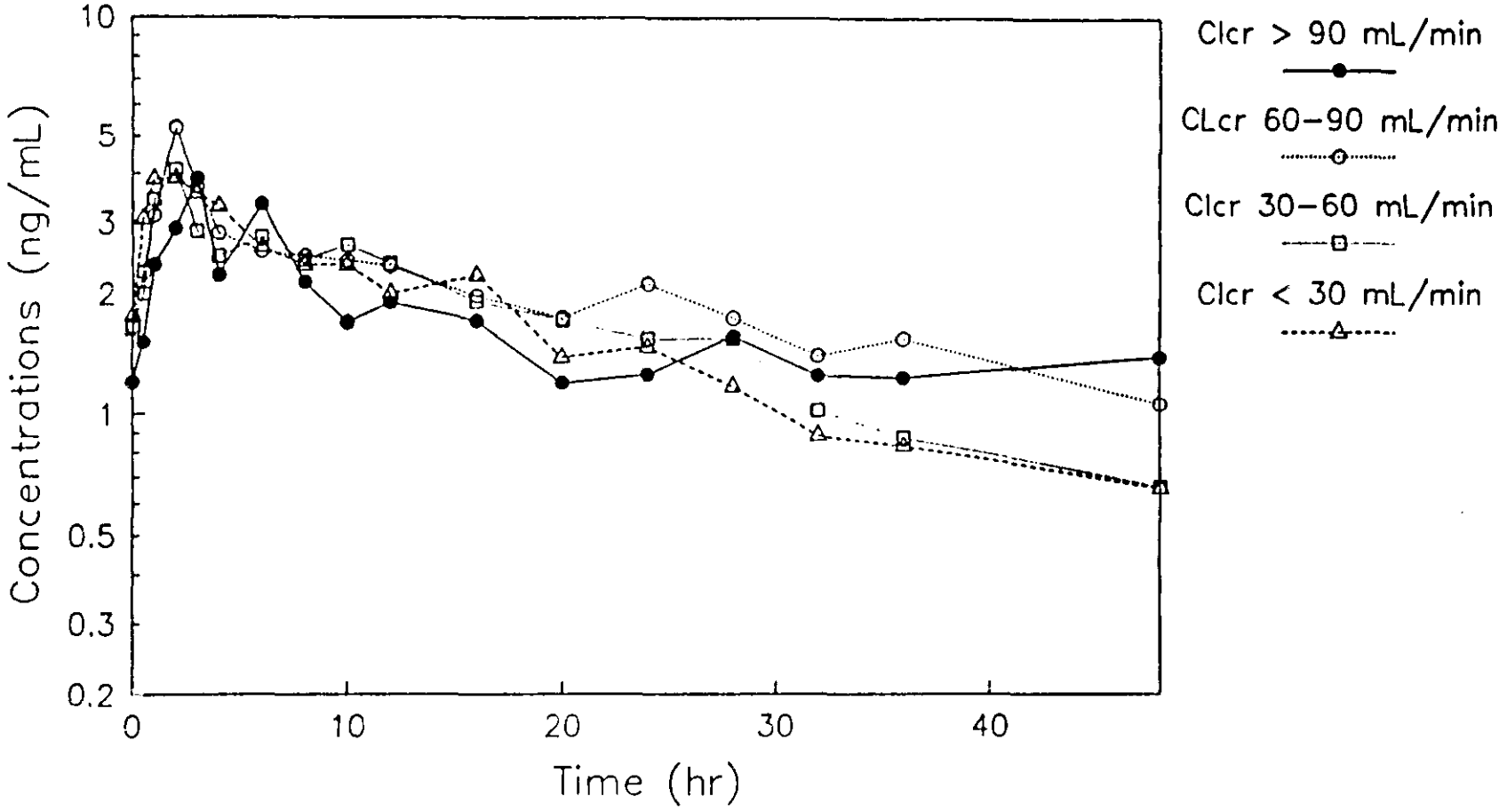


Mean Plot
Day 1

D92-001
FIGURE 3

FIGURE 4

Plasma Concentrations For Following a Single Daily Dose of 20 mg Nisoldipine Coat-Core Tablet For Eight Days



Mean Plot
Day 8

D92-001
FIGURE 4

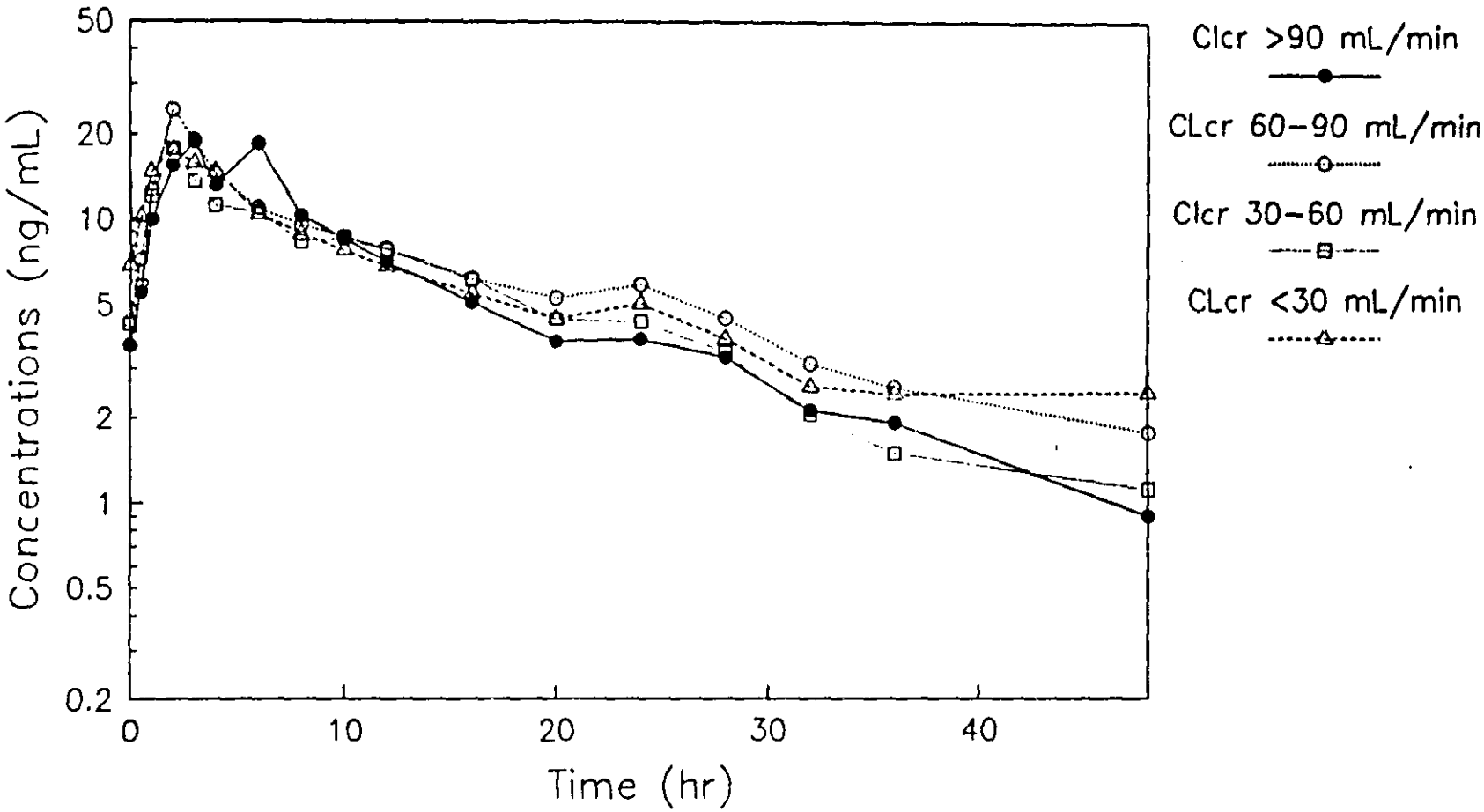
132

08 02 1502

000707

FIGURE 5

Plasma Concentrations For Following
Single Dose of 20 mg Nisoldipine
Coat-Core Tablet

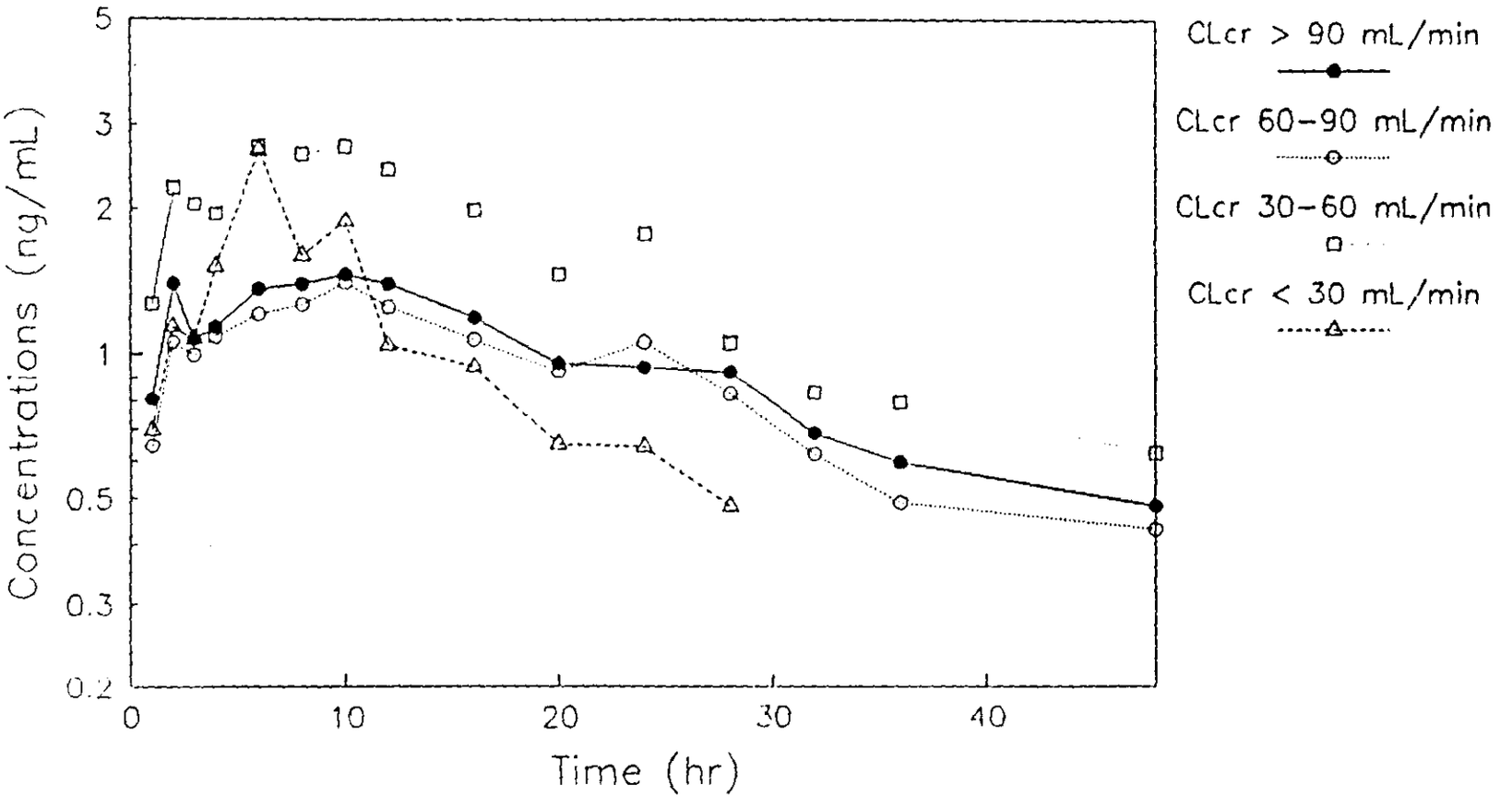


Mean Plot
Day 8

D92-001
FIGURE 6

FIGURE 6

Plasma Concentrations For [redacted] Following a Single Dose of 20 mg Nisoldipine Coat-Core Tablet

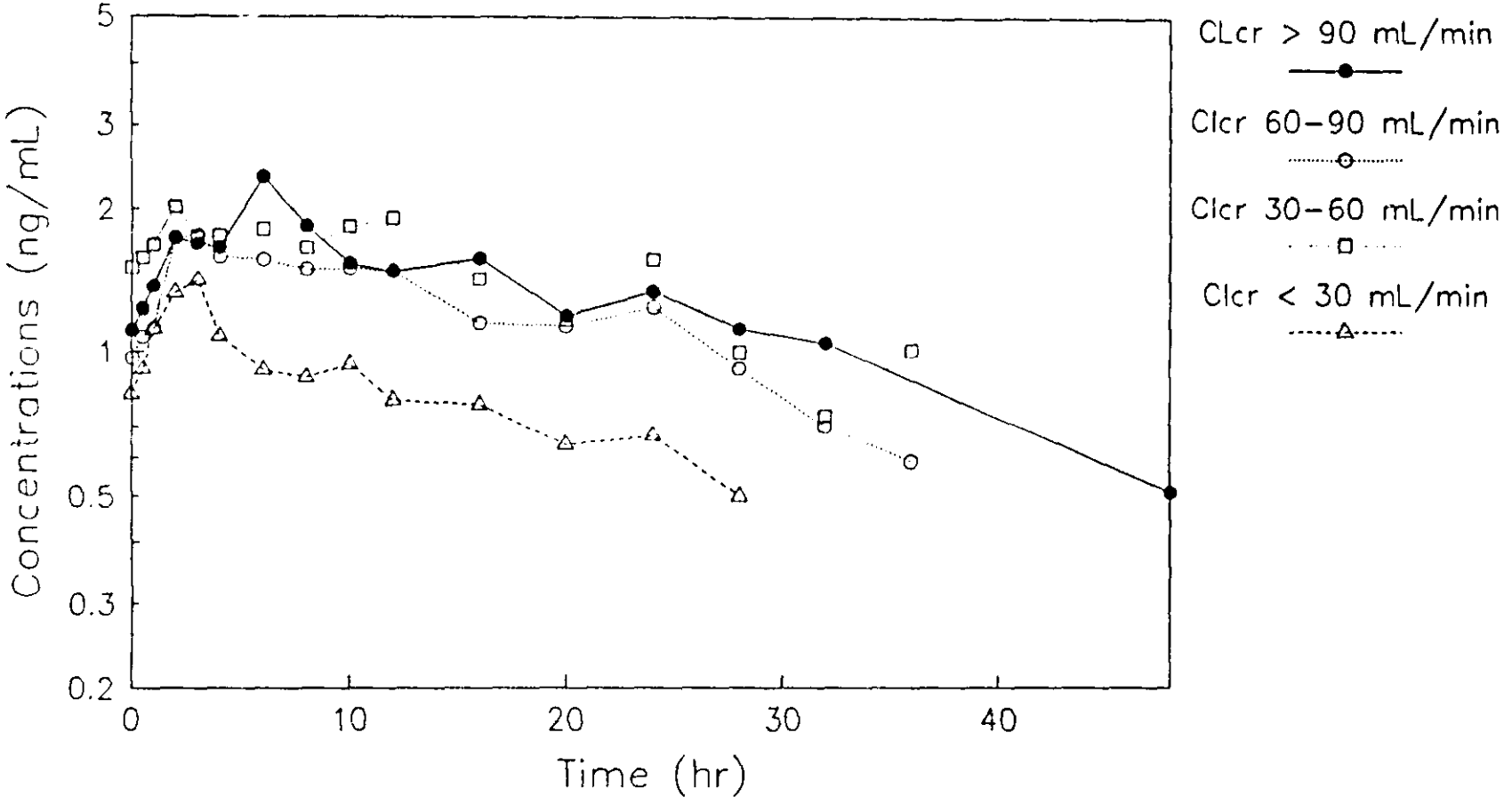


Mean Plot
Day 1

D92-001
FIGURE 7

FIGURE 7

Plasma Concentrations For Following a Single Daily Dose of 20 mg Nisoldipine Coat-Core Tablet For Eight Days



Mean Plot
Day 8

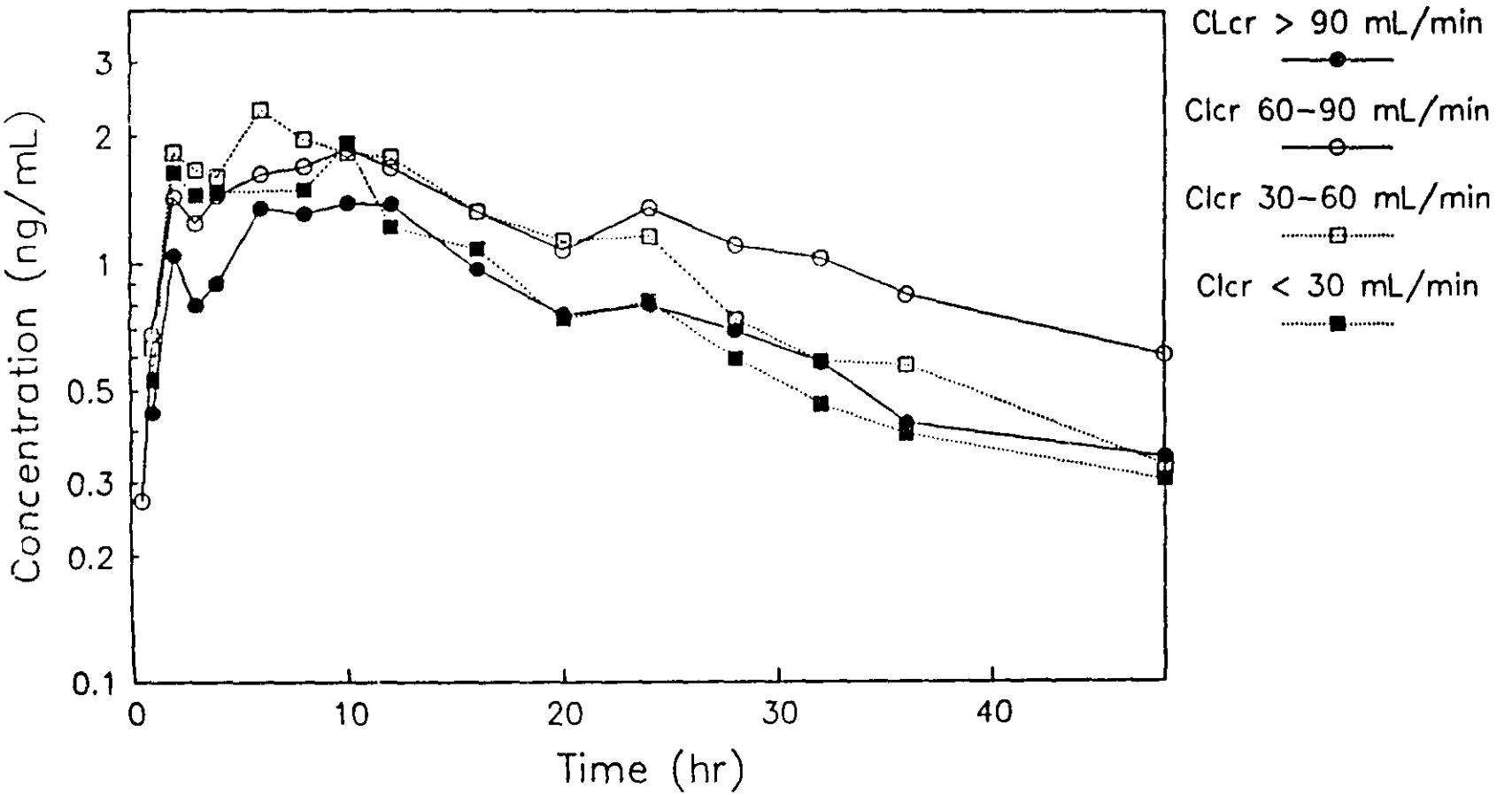
92-001
GURE 8

135

000711

FIGURE 8

Mean Plasma Concentrations For Nisoldipine Following a Single 20 mg Coat-Core Tablet



Mean Plot
Day 1

136

06 02 1499

Study D92-001
FIGURE 21

0007014

TABLE 1

Geometric Means (Geometric Standard Deviations)

	GROUP 1	GROUP 2	GROUP 3	GROUP 4
DAY 1 AUC (ng-h/ml)	20.7 (1.95)	29.0 (1.74)	30.1 (2.00)	25.0 (2.19)
DAY 8 AUC (ng-h/ml)	30.4 (2.28)	41.7 (2.00)	34.6 (1.63)	38.6 (1.74)
DAY 1 C_{max} (ng/ml)	1.5 (1.75)	2.0 (1.94)	2.2 (2.07)	2.0 (2.28)
DAY 8 C_{max} (ng/ml)	2.4 (2.36)	2.7 (1.83)	2.2 (1.71)	2.6 (1.84)
DAY 1 C_{av}* (ng/ml)	0.86 (1.95)	1.21 (1.74)	1.25 (2.00)	1.04 (2.19)
DAY 8 C_{av} (ng/ml)	1.27 (2.28)	1.74 (2.00)	1.44 (1.63)	1.61 (1.74)
24HR ACCUMULATION RATIO**	1.47 (1.76)	1.49 (1.46)	1.15 (1.83)	1.54 (1.27)
AUC Linear Accumulation≠	1.08 (1.77)	0.95 (1.34)	0.80 (1.76)	1.07 (1.19)

* C_{av} = AUC/24 hours

** $AUC_{DAY\ 8}/AUC_{DAY\ 1}$

≠ $AUC_{0\text{-}24,\ DAY\ 8}/AUC_{0\text{-}tn,\ DAY\ 1}$; tn = time of last observed concentration

TABLE 2

VARIABLES	GROUP 1	GROUP 2	GROUP 3	GROUP 4
DAY 1 C_{max}*	16.7	15.8	25.1	28.2
DAY 8 C_{max}*	19.8	22.3	22.6	26.6
DAY 1 C_{max} ratio**		0.94 (0.59-1.49)	1.50 (0.90-2.49)	1.68 (0.95-2.98)
DAY 8 C_{max} ratio**		1.13 (0.76-1.68)	1.14 (0.74-1.78)	1.35 (0.82-2.20)
DAY 1 AUC_{norm}*	590.9	573.3	987.2	1029.8
DAY 8 AUC_{norm}*	758.0	701.6	759.6	999.9
DAY 1 AUC ratio**		0.97 (0.64-1.48)	1.67 (1.05-2.66)	1.74 (1.03-2.94)
DAY 8 AUC ratio**		0.93 (0.61-1.41)	1.00 (0.63-1.59)	1.32 (0.79-2.20)

* Geometric least squares means

** Ratios are values for a given group divided by Group 1 value; parentheses contain 90% Conf. interval

TABLE 3

9.4.3

This metabolite, the only active metabolite of nisoldipine, is approximately 10 times less active than the parent drug.[8] the least abundant of the metabolites, also appeared to be the least influenced by renal impairment. The data are presented in Appendix 13.8.4 and summarized below.

VARIABLES	GROUP 1	GROUP 2	GROUP 3	GROUP 4
DAY 1 C_{max}*	1.73	1.56	3.16	1.80
DAY 8 C_{max}*	2.63	1.81	2.64	1.51
DAY 1 C_{max} ratio**		0.90 (0.57-1.41)	1.82 (1.10-3.01)	1.04 (0.59-1.82)
DAY 8 C_{max} ratio**		0.69 (0.46-1.03)	1.00 (0.64-1.58)	0.58 (0.35-0.95)
DAY 1 AUC_{norm}*	117.4	87.6	172.7	85.1
DAY 8 AUC_{norm}*	143.7	102.5	143.6	79.3
DAY 1 AUC ratio**		0.75 (0.47-1.18)	1.47 (0.89-2.44)	0.72 (0.41-1.28)
DAY 8 AUC ratio**		0.71 (0.44-1.16)	1.00 (0.58-1.71)	0.55 (0.30-1.00)

* Geometric least squares means

** Ratios are values for a given group divided by Group 1 value; parentheses contain 90% Conf. interval

A randomized double blind, placebo controlled study to investigate the possible influence of nisoldipine on quinidine plasma levels.

STUDY: 384. VOLUME: 1-53 PAGES: 06-02-2199-2264.

INVESTIGATOR:

OBJECTIVES:

1-To assess the possible influence of nisoldipine on quinidine plasma levels in African and Caucasian volunteers.

FORMULATIONS:

-nisoldipine 10 mg tablets, batch # 929488, expiration date 11-18 1986.
-nisoldipine placebo tablets, batch # 929270, expiration date 09-15 1986.
-quinidine bisulphate 250 mg tablets, Astra batch # KA1089, expiration date 2-1989.

STUDY DESIGN:

6 healthy male volunteers between the ages of 21 and 65 years (3 Caucasians and 3 Africans) participated in this randomized, double-blind, placebo controlled cross-over study, comparing the pharmacokinetic profile of quinidine bisulphate when on nisoldipine therapy 10 mg bid for seven days, with the pharmacokinetic profile when on placebo. Each subject received 2x250 mg quinidine twice a day on days 5 and 6 and once on day 7 of the study. There was a one week washout period between treatments.
Blood samples were collected at 0, 30, 60 and 90 minutes and 2, 3, 4, 6, 8, 10 and 24 hours after drug administration.

RESULTS:

Figure 1 shows the mean quinidine plasma concentrations with and without nisoldipine

coadministration while Table 1 and 2 summarize the most important pharmacokinetic parameters. The results show that only quinidine's AUC was increased upon coadministration of nisoldipine. This increase was in the order of 25 % and was statistically significant. No significant differences on quinidine CMAX, TMAX and MRT were observed.

CONCLUSION:

Coadministration of nisoldipine with quinidine, seems to increase quinidine plasma levels since the AUC increased by 25 %. However, the effect of quinidine on nisoldipine pharmacokinetics could not be measured since the dihydropyridine plasma concentrations were not measured.

TABLE 1

PHARMACOKINETIC DATA

AUC (0-24 hr) ng/ml hr

Volunteer No	PLACEBO		NISOLDIPINE
1			
2			
3			
4			
5			
6			
$\bar{X}$	17940.46		22637.19
SD	4823.01		6091.82
SE	1968.98		2486.99
t		2.66	
p		p<0.05	

AUC (0 - 10 hr) ng/ml hr

Volunteer No	PLACEBO		NISOLDIPINE
1			
2			
3			
4			
5			
6			
$\bar{X}$	14801.25		17348.21
SD	3339.94		5384.49
SE	1363.53		2198.21
t		1.13	
p		ns	

AUC (0-∞) ng/ml hr

1			
2			
3			
4			
5			
6			
$\bar{X}$	22420.59		28033.68
SD	9667.34		9546.75
SE	3931.93		3897.45
t		1.92	
p		ns	

TABLE 2

PHARMACOKINETIC DATA

MRT (mean resonance time) hr

	1	2	3	4	5	6
$\bar{X}$	14.32					14.86
SD	4.54					3.67
SE	1.85					1.50
t			0.27			
p			ns			

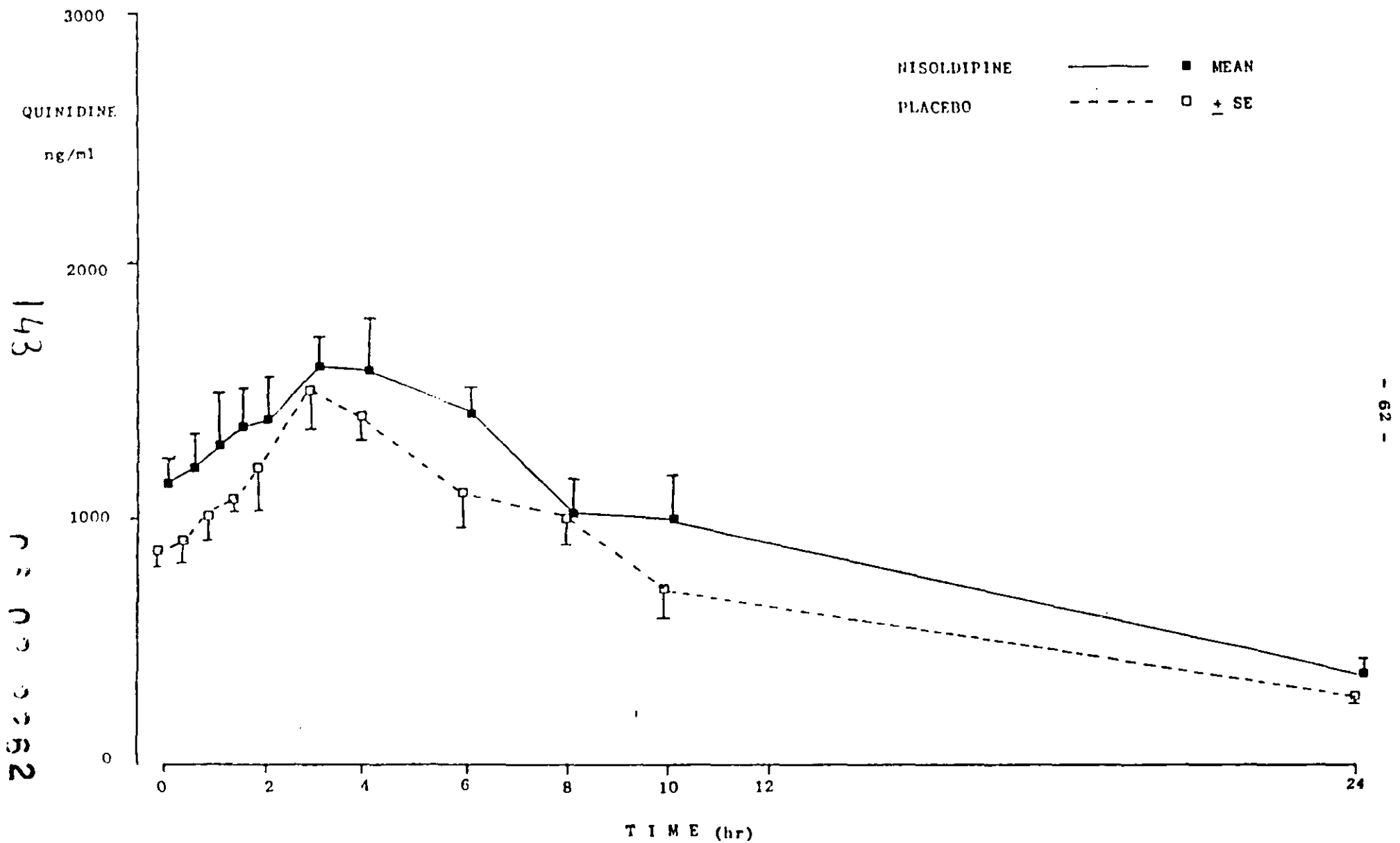
C MAX ng

	1	2	3	4	5	6
$\bar{X}$	1703.67					1814.00
SD	459.64					362.75
SE	187.65					148.09
t			0.78			
p			ns			

T MAX hr

	1	2	3	4	5	6
$\bar{X}$	3.33					3.17
SD	0.82					1.17
SE	0.33					0.48
t			-0.31			
p			ns			

FIG 1


3000
2000
1000
0
QUINIDINE
ng/ml
NISOLDIPINE
PLACEBO
MEAN
± SE
0
2
4
6
8
10
12
24
T I M E (hr)

To investigate the existence of a possible interaction between nisoldipine and warfarin.

Study #: 349. VOLUME: 1-53 PAGES: 6-02-2265-2367.

INVESTIGATOR:

OBJECTIVES:

To investigate whether nisoldipine alters the steady state total plasma levels of warfarin and to examine the effects of nisoldipine on the clotting profile of warfarin treated patients.

FORMULATIONS:

-Nisoldipine 10 mg tablets batch # 929488 expiration date November 11, 1986.
-Nisoldipine placebo.
-Warfarin (Marevan^{R}) batch # b/225.

STUDY DESIGN:

This was a placebo-controlled, randomized, double blind group comparison study in which 16 patients between the ages of 21 and 65 years who were receiving 3 to 10 mg warfarin following myocardial infarction or valve replacement.
After a placebo run in period of 7 days, the group of 16 patients was divided into 2 groups of 8 each. 1 group received 10 mg nisoldipine bid and the other group received placebo tablets bid for 21 days. Warfarin was continued at the steady-state throughout the trial.
10 ml blood samples were collected before and two hours after medication.
Clotting profiles were obtained on the following days: -7, -5, -3, 1, 3, 7, 14 and 21.

RESULTS:

Figure 1 shows the warfarin plasma concentrations for the 2 groups while Figure 2 shows the clotting profile for the nisoldipine and control group. Table 1 summarizes the mean clotting parameters as well as the mean of the 2 plasma warfarin concentrations (b1 which was before drug administration and b2 which was 2 hours post dose).

Conclusion:

The results show that the anticoagulant effect as well as the steady-state warfarin plasma concentrations were not affected by concomitant administration of a 10 mg nisoldipine IR tablet administered bid for 21 days.

13

TABLE 1

Warfarin Treatment

Parameter	Placebo Mean	Nisoldipine Mean	
PA(%)	12,8	8,7	NS
PT (Sec)	19,7	21,3	NS
PTT (Sec)	40,1	46,2	*S
Warfarin (B1) (ug/mℓ)	1,38	1,29	NS
Warfarin (B2) (ug/mℓ)	1,75	1,67	NS

* After baseline correction the difference for PTT was also not significant)

11.2.2 Analysis of variance to compare the mean baseline values with each of the mean values obtained as from Day 1 in a within-treatment comparison for all variables described in 11.1.1.

Clotting Profile

Warfarin-Placebo Treatment

Time	PA% Mean		PT(sec) Mean		PTT(sec) Mean	
Baseline	11,3	-	20,7	-	42,3	-
Day 1	12,9	NS	20,1	NS	40,3	NS
Day 3	12,0	NS	20,5	NS	39,5	NS
Day 7	11,8	NS	20,1	NS	43,5	NS
Day 14	13,1	NS	19,3	S	40,3	NS
Day 21	14,3	S	18,5	S	37,0	S

NS : Not Significant

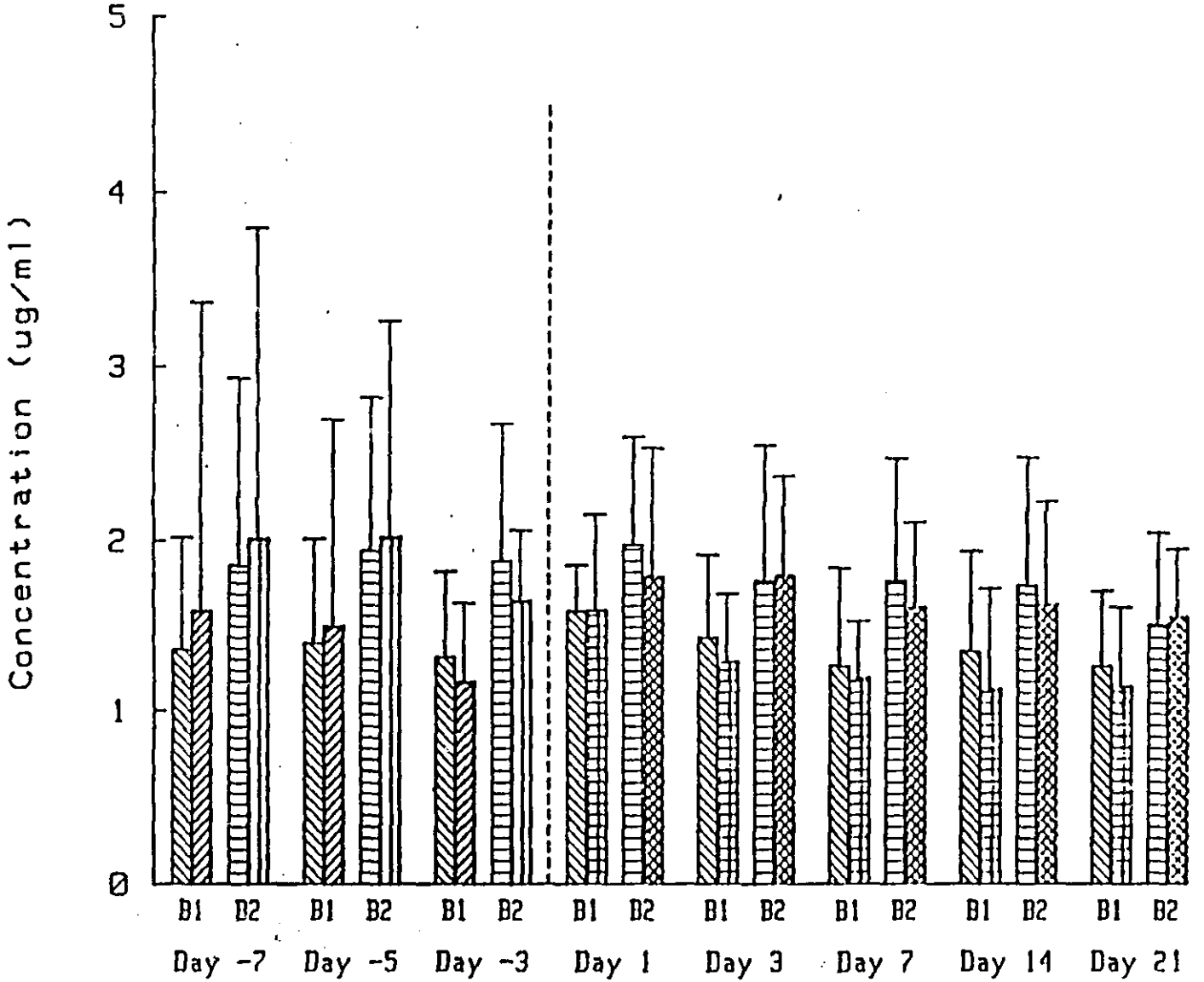
S : Significant

FIGURE 1

UOFS 5/84

Annexure 6 (a)

WARFÁRIN PLASMA CONCENTRATION



PLACEBO

PLACEBO

NISOLDIPINE

PLACEBO

PLACEBO

NISOLDIPINE

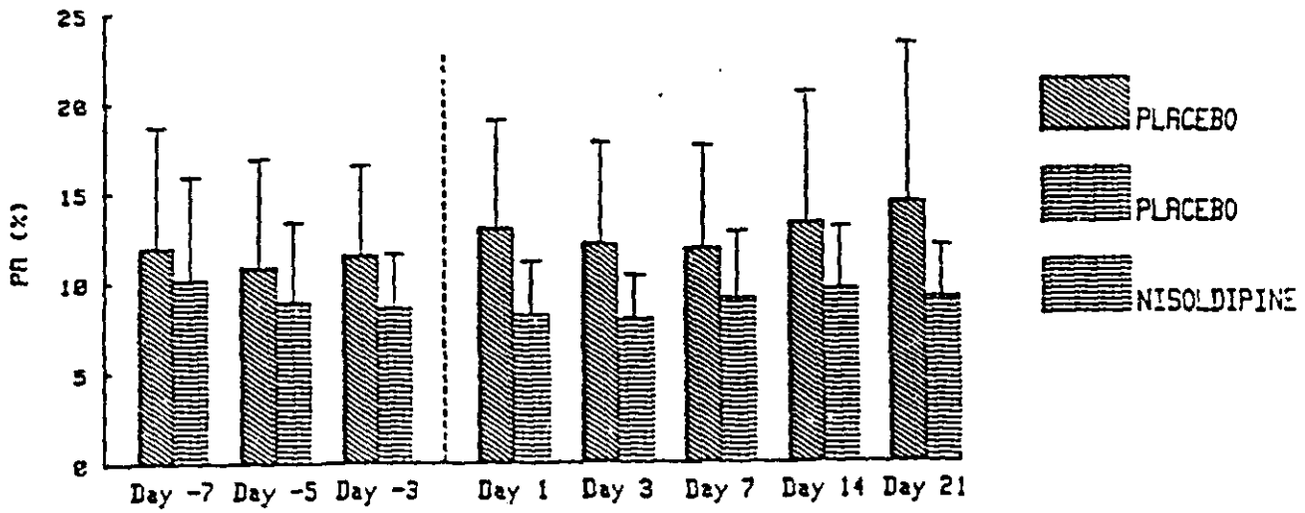
B1 = Blood sample (premedication)

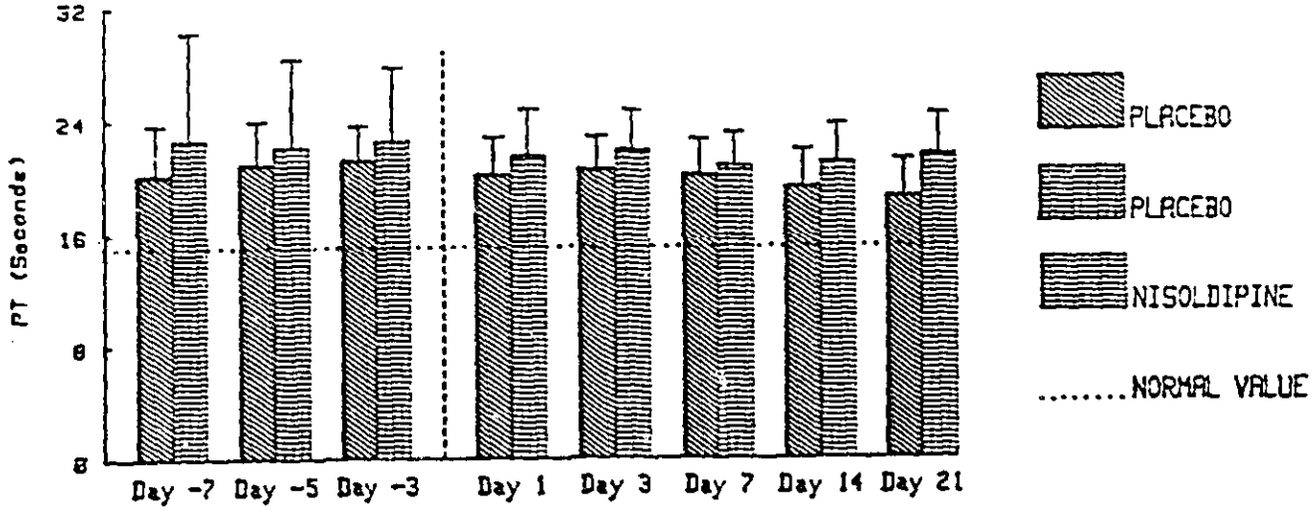
B2 = Blood sample (post medication)

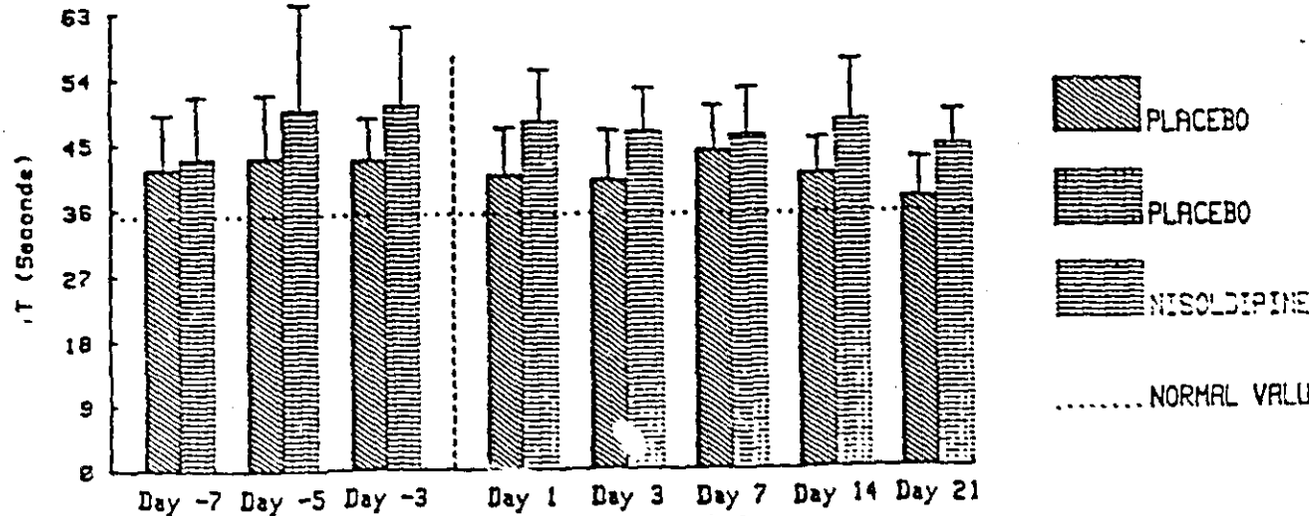
Annexure 6 (b)

FIGURE 2

UOFS 5/84
CLOTTING PROFILE







Comparison of pharmacokinetics and tolerability of nisoldipine C.C. given in combination with placebo, cimetidine or ranitidine.

STUDY #: 738. VOLUME: 1-53-54 PAGES: 6-02-2368-2961

INVESTIGATORS:

OBJECTIVES:

To investigate the pharmacokinetic drug/drug interaction and tolerability of nisoldipine C.C. given in combination with placebo, cimetidine, or ranitidine respectively.

FORMULATION:

-Nisoldipine 20 mg C.C. tablets (batch #: 523341/9).
-400 mg Tagamet[R] tablet batch # 888/90H30.
-150 mg Zantac[R] tablet batch # ON 448.
-0.5 mg placebo tablet containing lactose, corn starch and microcrystalline cellulose, batch # 523 196/20.

STUDY DESIGN:

12 healthy male volunteers between the ages of 24 to 35 years participated in this randomized complete block design. Each subject received one of the following treatments:
-treatment b1: 400 mg Tagamet bid for 6 days (days 1-6).
-treatment b2: 150 mg Zantac bid for 6 days.
-treatment b3: 0.5 gm placebo administered for 6 days.
Single oral doses of 20 mg Nisoldipine C.C. was administered on study day 5 of each period. There was a one week washout period between treatments.
6 ml blood samples were taken starting on day 5 of each study period at the following times post dose administration: 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 14, 16, 20, 24, 28, 32, 36 and 48 h. Further blood samples were drawn on study days 1, 4, 5 predose to determine trough levels of cimetidine or ranitidine and on study days 1 and 42 hours post administration for peak levels.

RESULTS:

Figure 1 shows the geometric mean plasma concentration for nisoldipine alone, with cimetidine and with ranitidine while Table 4 to 6 summarize the main pharmacokinetic parameters for nisoldipine for the 3 different treatments. Table 7 shows the geometric mean peak and trough concentrations for cimetidine and ranitidine.
The results show that cimetidine had a more pronounced effect on the pharmacokinetics of nisoldipine as compared to ranitidine. Cimetidine increased nisoldipine's AUC by 55 % and CMAX by 65 % while it decreased TMAX from 9.11 to 4.25 hours. On the other hand, ranitidine caused a small but statistically significant decrease in The AUC (12 %) and CMAX (15 %) of nisoldipine compared to placebo.

Conclusion:

Cimetidine seem to have a pronounced effect on nisoldipine pharmacokinetic parameters (since there was more than 50 % increase in some parameters of interest). Therefore great caution should be exercised when both of these drugs are administered concomitantly, the patients should be monitored and dose adjustments made as necessary. However, the interaction with ranitidine seem to be small enough and therefore is not expected to have any clinical significance.

TABLE 1

Nominal Concentration [µg/l]	Day 1	Day 2	Day 3	mean [µg/l]	SD	CV [%]	Q* actual/nominal
19.60	21.14	20.95	20.98	21.01	0.102	0.48	1.07
7.37	7.96	7.64	7.50	7.70	0.230	3.06	1.04
0.49	0.46	0.45	0.45	0.45	0.005	1.28	0.91

Table 3: Data for inter-assay variation for nisoldipine in plasma.

Nominal Concentration [µg/l]	Sample 1	Sample 2	Sample 3	mean [µg/l]	SD	CV [%]	Q* actual/nominal
19.60	20.97	22.28	19.70	20.98	1.29	6.15	1.07
7.37	7.69	7.46	7.36	7.50	0.17	2.26	1.02
0.49	0.44	0.46	0.46	0.45	0.01	2.55	0.93

Table 4: Data for intra-assay variation for nisoldipine in plasma.

TABLE 2

Nominal Concentration [µg/l]	Sample 1	Sample 2	Sample 3	$\bar{x}$ [µg/l]	SD	CV [%]	Q $\frac{\bar{x}_{actual}}{nominal}$
13.74	12.35	13.44	12.49	12.76	0.59	4.65	0.93
176.44	186.08	194.86	184.91	188.62	5.44	2.88	1.07
373.72	366.10	355.19	351.31	357.53	7.67	2.14	0.96

Table 3a: Data for intraassay variation for cimetidine in plasma

Nominal Concentration [µg/l]	Means of Triplicates Day 1	Day 2	Day 3	$\bar{x}$ [µg/l]	SD	CV [%]	Q $\frac{\bar{x}_{actual}}{nominal}$
13.74	16.15	12.76	15.49	14.83	1.84	12.38	1.08
176.44	185.37	188.62	206.46	193.48	11.36	5.87	1.10
373.72	410.89	357.53	395.65	388.02	27.49	7.08	1.04

Table 3b: Data for interassay variation of cimetidine in plasma

TABLE 3

Nominal Concentration [µg/l]	Sample 1	Sample 2	Sample 3	$\bar{x}$ [µg/l]	SD	CV [%]	Q $\frac{\bar{x}_{actual}}{nominal}$
1104.52	1114.58	1036.45	962.44	1037.82	76.08	7.3	0.94
393.95	400.32	391.58	373.66	388.52	13.59	3.5	0.99
112.82	115.98	113.69	130.16	119.94	8.92	7.4	1.06

Table 8: Data for intra-assay variation for ranitidine in plasma (Study No. 36/90-02.03.NE)

Nominal Concentration [µg/l]	Means of Triplicates Day 1	Day 2	Day 3	$\bar{x}$ [µg/l]	SD	CV [%]	Q $\frac{\bar{x}_{actual}}{nominal}$
1104.52	1042.40	1037.82	1061.37	1047.20	12.49	1.2	0.95
393.95	369.92	388.52	349.08	369.17	19.73	5.3	0.94
112.82	112.92	119.94	137.10	123.32	12.44	10.1	1.09

Table 9: Data for inter-assay variation of ranitidine in plasma (Study No. 36/90-02.03.NE)

NONCOMPARTMENTAL PHARMACOKINETIC PARAMETERS OF NISOLDIPINE

Parameter: / Unit :	Cmax [ug/l]	tmax [h]	AUC(0-tn) [ug*h/l]	Cmax,norm [g/l]	AUC(0-tn,norm) [g*h/l]
MEAN	1.05	9.11	14.97	3.82	54.17
SDEV	0.29	4.81	4.48	0.79	12.74
GEO.MEAN	1.01	7.51	14.37	3.73	52.87
GEO.SDEV	1.33	2.07	1.35	1.27	1.26
LOW.CON.	0.86	4.98	12.15	3.26	46.48
UPP.CON.	1.19	11.32	17.00	4.27	60.14
MEDIAN	1.00	10.00	13.82	3.94	48.56
MIN	0.64	2.00	9.73	2.01	39.33
MAX	1.64	16.00	22.08	5.22	79.04
COUNT	12.00	12.00	12.00	12.00	12.00

Table 13

Noncompartmental pharmacokinetic parameters of Nisoldipine following p.o. administration of respectively one tablet 20 mg Nisoldipine CC (o.a.d. on day 5) and Placebo coated tablet (b.i.d. on days 1 to 6) (treatment B3).

Arithmetic Means, Standard Deviations, Geometric Means, Geometric Standard Deviations, 95% Confidence Limits of Geometric Mean, Medians, Minimum, Maximum and Number of Cases.

NONCOMPARTMENTAL PHARMACOKINETIC PARAMETERS OF NISOLDIPINE

Parameter: Unit :	Cmax [ug/l]	tmax [h]	AUC(0-tn) [ug*h/l]	Cmax,norm [g/l]	AUC(0-tn,norm) [g*h/l]
MEAN	1.74	4.25	23.20	6.43	86.04
SDEV	1.08	3.28	12.70	3.98	47.75
GEO.MEAN	1.47	3.42	19.02	5.41	70.03
GEO.SDEV	1.87	1.91	2.18	1.91	2.24
LOW.CON.	1.03	2.37	12.23	3.76	44.39
UPP.CON.	2.09	4.94	29.60	7.79	110.48
MEDIAN	1.48	2.75	20.56	5.65	73.22
MIN	0.37	1.50	2.25	1.23	7.47
MAX	4.49	12.00	48.88	16.07	175.00
COUNT	12.00	12.00	12.00	12.00	12.00

Table 11

Noncompartmental pharmacokinetic parameters of Nisoldipine
following p.o. administration of respectively one tablet 20 mg Nisoldipine CC (o.a.d. on day 5) and Tagamet (R) (400 mg Cimetidine b.i.d. on days 1 to 6) (treatment B1).

Arithmetic Means, Standard Deviations, Geometric Means, Geometric Standard Deviations, 95% Confidence Limits of Geometric Mean, Medians, Minimum, Maximum and Number of Cases.

NONCOMPARTMENTAL PHARMACOKINETIC PARAMETERS OF NISOLDIPINE

Parameter: / Unit :	Cmax [ug/l]	tmax [h]	AUC(0-tn) [ug*h/l]	Cmax,norm [g/l]	AUC(0-tn,norm) [g*h/l]
MEAN	0.90	8.37	13.30	3.26	48.81
SDEV	0.41	3.90	5.97	1.30	22.43
GEO.MEAN	0.84	7.25	12.20	3.07	44.59
GEO.SDEV	1.47	1.84	1.54	1.42	1.55
LOW.CON.	0.68	5.13	9.57	2.51	34.75
UPP.CON.	1.04	10.26	15.55	3.74	57.21
MEDIAN	0.81	8.99	11.76	3.18	46.35
MIN	0.50	2.50	6.53	1.91	24.97
MAX	1.98	12.02	24.98	6.56	93.34
COUNT	12.00	12.00	12.00	12.00	12.00

Table 12

Noncompartmental pharmacokinetic parameters of Nisoldipine
following p.o. administration of respectively one tablet 20 mg Nisoldipine CC (o.a.d. on day 5) and Zantic (R) (150 mg Ranitidine b.i.d. on days 1 to 6) (treatment B2).

Arithmetic Means, Standard Deviations, Geometric Means, Geometric Standard Deviations, 95% Confidence Limits of Geometric Mean, Medians, Minimum, Maximum and Number of Cases.

Cimetidine and Ranitidine

The following Table 4 shows the geometric mean peak and trough concentrations for cimetidine and ranitidine.

Analyt	Day 1		Day 4		Day 5
	0 h	2 h	0 h	2 h	0 h
Cimetidine	n.d.	747.75	66.06	768.65	60.75
Ranitidine	n.d.	468.70	100.42	542.02	93.72

Table 4: Geometric mean cimetidine and ranitidine concentrations [µg/l] (peak/trough) on study days 1 and 4

FIGURE 1

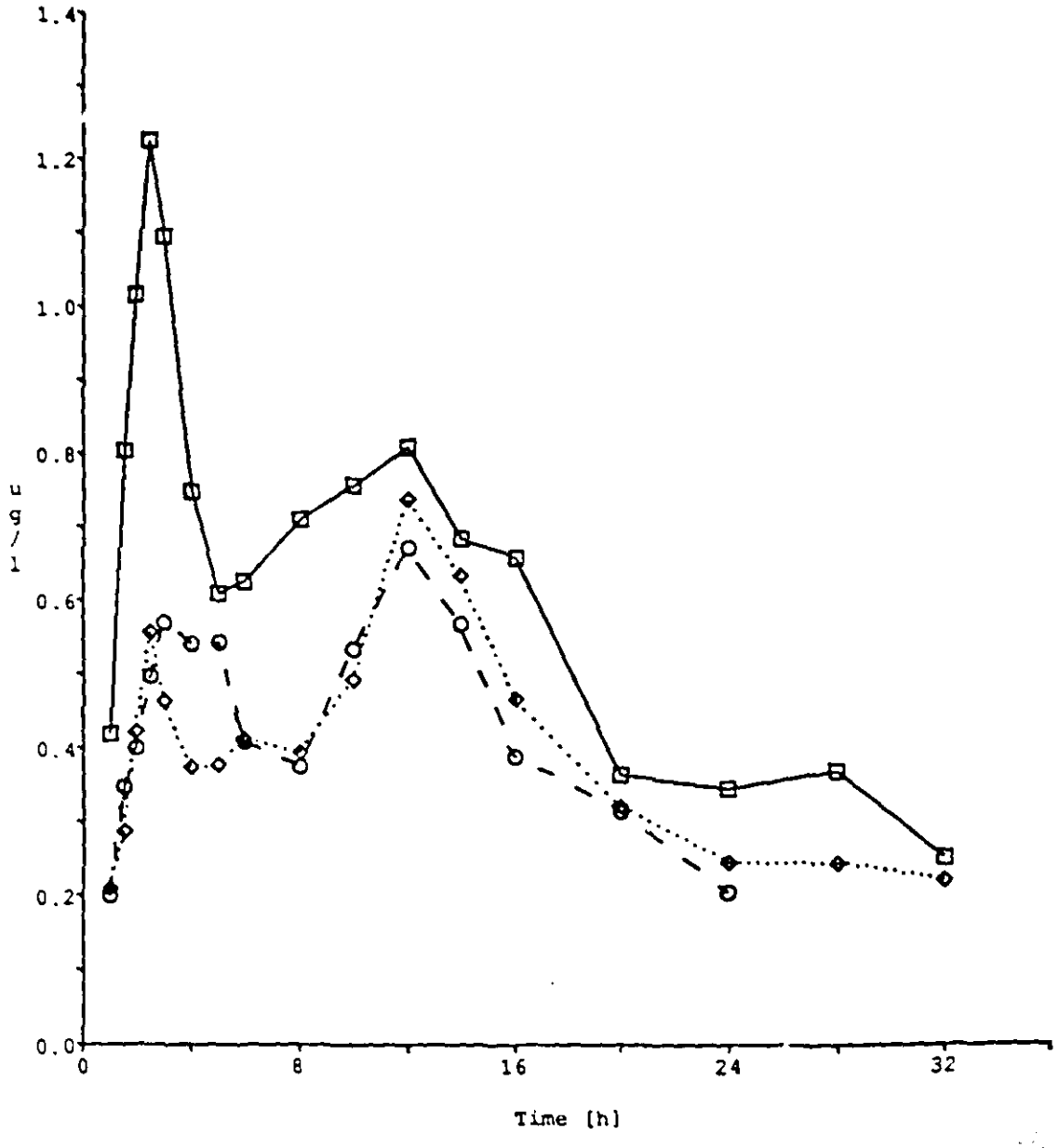


Figure 1

Syncptic plot of geometric mean concentrations of Nisoldipine [ug/l] vs time [h].

Geometric mean not calculated if more than 1/3 single concentrations are <0.18 or no sample. Concentrations <0.18 calculated as 0.09.

To investigate the existence of a possible interaction between nisoldipine and ranitidine.

STUDY: 385. VOLUME: 1-55 PAGES: 06-02-2962-3520.

INVESTIGATOR:

OBJECTIVES:

1-To determine if ranitidine alters nisoldipine kinetics.

2-to determine if a pharmacodynamic interaction exists either as a result of a pharmacokinetic interaction or independent from such an interaction.

FORMULATIONS:

-nisoldipine 20 mg tablets, batch # 929490, expiration date 10-03 1986.
-ranitidine tablets 150 mg.

STUDY DESIGN:

16 healthy male volunteers between the ages of 18 and 45 years participated in this placebo controlled, double blind crossover study.
300 mg ranitidine (single dose) or placebo were given for 3 days preceeding the test days (when 20 mg nisoldipine was given).
Venous blood samples were collected according to the following schedule: 0, 30, 60, 90, 120, 150, 180 minutes and 4, 8, 10 and 24 hours after drug administration.
Supine blood pressure, heart rate and systolic time intervals were measured at 0, 0.5, 1.5, 2, 3, 4, and 24 hours after medication.

RESULTS:

Figure 1 shows the mean nisoldipine plasma concentrations with and without ranitidine while Table 1 gives CMAX and AUC for the 2 treatments.
According to the sponsor,the results show that there was no difference in CMAX between the two treatments but the AUC was significantly greater when ranitidine was given with nisoldipine compared to when nisoldipine was given alone. However, the sponsor claims that when subject 13 was omitted, there was no longer any differences between the two treatments.

COMMENTS:

If one examines Figure 1, it can be seen that the CMAX was higher with the placebo treatment as compared to the ranitidine treatment. However, both CMAX values appear less than the stated values in Table 1. Additionally, ranitidine seems to have delayed and lowered the CMAX value when nisoldipine was given with ranitidine compared to when it was given with placebo.
The sponsor is asked to explain the discrepancy between the results of Figure 1 and the mean values presented in Table 1.

CONCLUSION:

No conclusion can be made from this study whether the pharmacokinetics of nisoldipine are altered by the coadministration of ranitidine.

FIGURE 1

U.O.F.S. 3/85 (STUDY No. 0385)

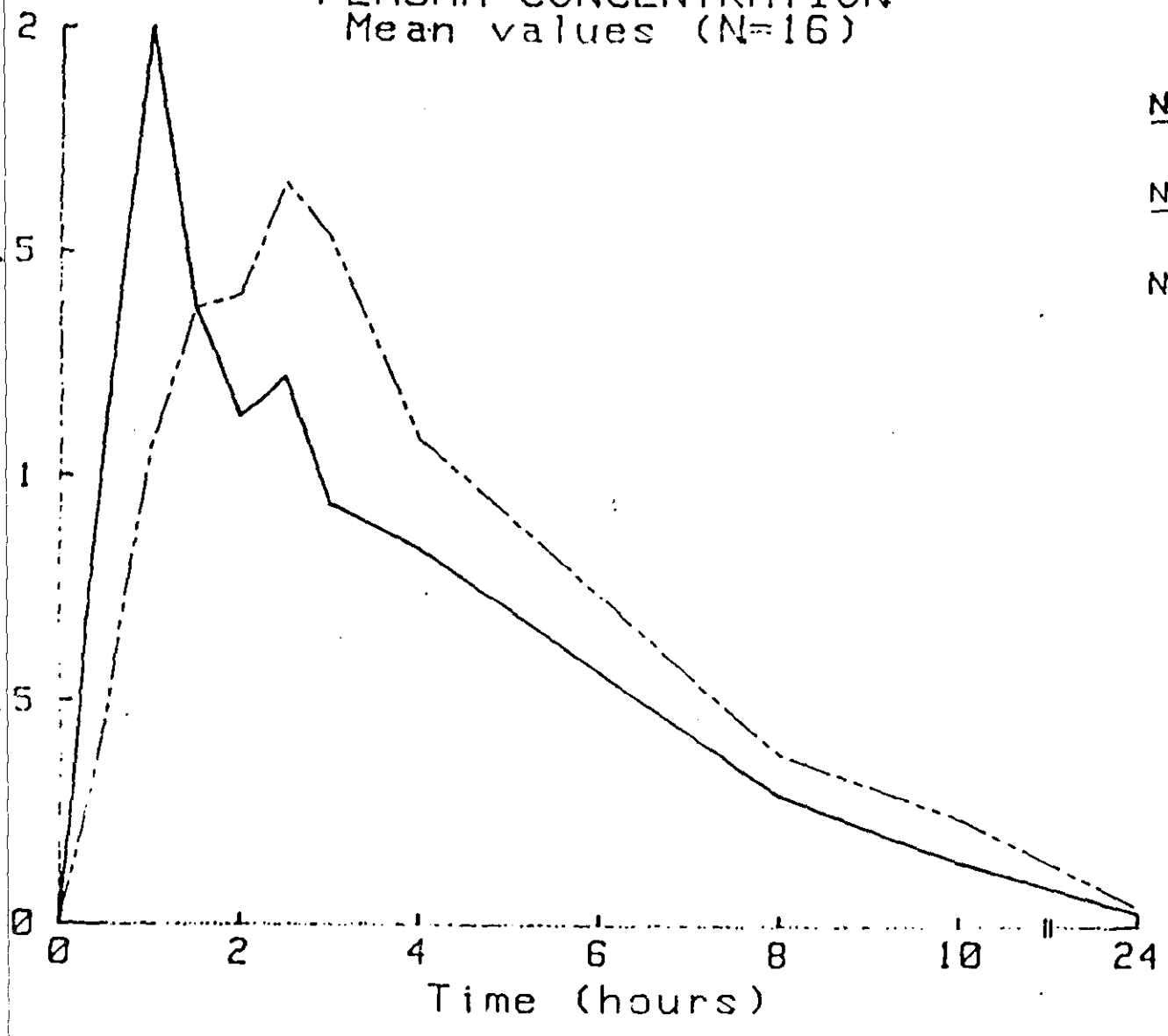


TABLE I

12.1 Pharmacokinetic variables

The results are summarised below:

	C_{max} (ng/mℓ)					T_{max} (h)			
	Mean	SD	1	2	B	Mean	SD	1	2
1	2,833	2,245	-	NS	1	2,250	1,966	-	NS
2	2,791	1,858	-	-	2	2,375	1,688	-	-
	Mean	SD	AUC			Mean	SD	MRT (h)	
1	8,145	4,112	-	*	1	4,749	2,655	-	NS
2	10,100	3,343	-	-	2	5,695	3,041	-	-

1 : Nisoldipine plus placebo

2 : Nisoldipine plus Ranitidine^{R}

Interaction study. Comparative investigation of safety, tolerability, pharmacodynamics and pharmacokinetics of Nisoldipine C.C. and propranolol given alone and in combination at steady state.

Study #: 704. VOLUME: 1-56 PAGES: 6-02-3623-4013.

INVESTIGATOR:

OBJECTIVES:

Comparative investigation of safety, tolerability, pharmacodynamics and pharmacokinetics of nisoldipine C.C. and propranolol given alone and in combination at steady state.

FORMULATIONS:

-Nisoldipine CC 20 mg tablets batch # 523231.
-Dociton^{R}= 40 mg propranolol.

STUDY DESIGN:

This was a randomized, 3 fold crossover multiple dose interaction study. 12 healthy male volunteers between the ages of 23 and 40 years participated in this 5 day study. Nisoldipine C.C. 20 mg once a day or/and Dociton tablets with 40 mg propranolol (tid) were given together with 100 mls of water.
Blood samples in nisoldipine treatments were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 20, 24, 28, 32, 36 and 48 hours after drug administration.
Blood samples in the propranolol treatment were collected at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 8.5, 9, 10, 12, 14, 16, 16.5, 17, 18, 20, 22, 24, 26, 28, 32 and 48 hours post dose.
Trough plasma concentrations were measured in the morning (8 AM) on study days 2 to 4.

RESULTS:

Figure 1 shows the mean geometric mean nisoldipine plasma concentration with and without propranolol administration while Table 1 gives the main pk parameters for both the 2 treatments. Figure 2 shows the mean geometric propranolol concentratrion for both corresponding treatments while Table 2 summarizes the most important pharmacokinetic parameters for propranolol.

Conclusion:

The results show that the nisoldipine plasma concentrations were slightly increased by propranolol coadministration while propranolol plasma concentrations were slightly decreased. This very small interaction between these 2 drugs is not expected to have any consequences from the clinical point of view.

FIGURE 1

0704

Plasmaconcentration vs. timeprofiles of Nisoldipine
with and without concomitant Propranolol treatment

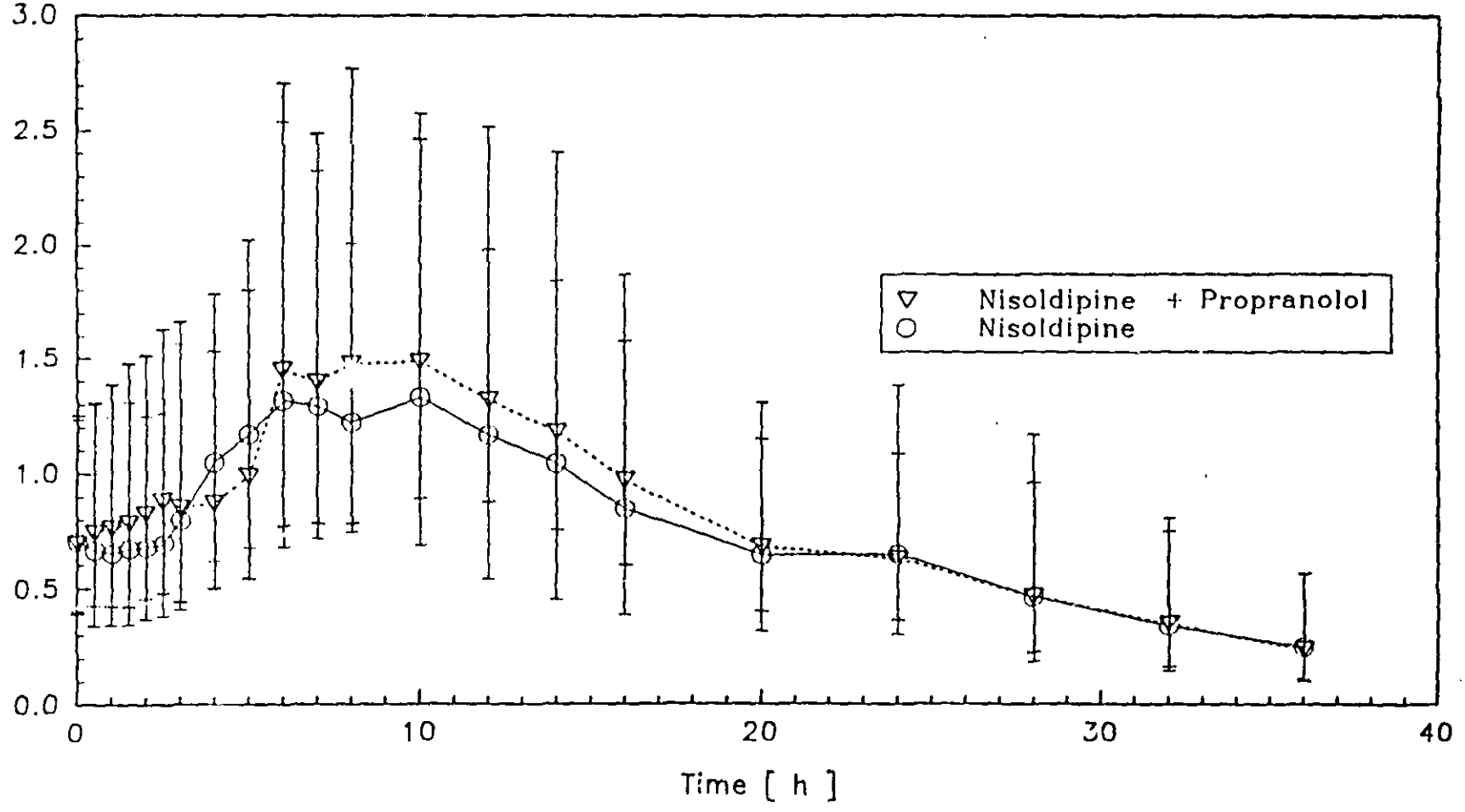


Fig. 6.1.1

FIGURE 2

/ 0704

Plasmaconcentration vs. timeprofiles of Propranolol
with and without concomitant Nisoldipine treatment

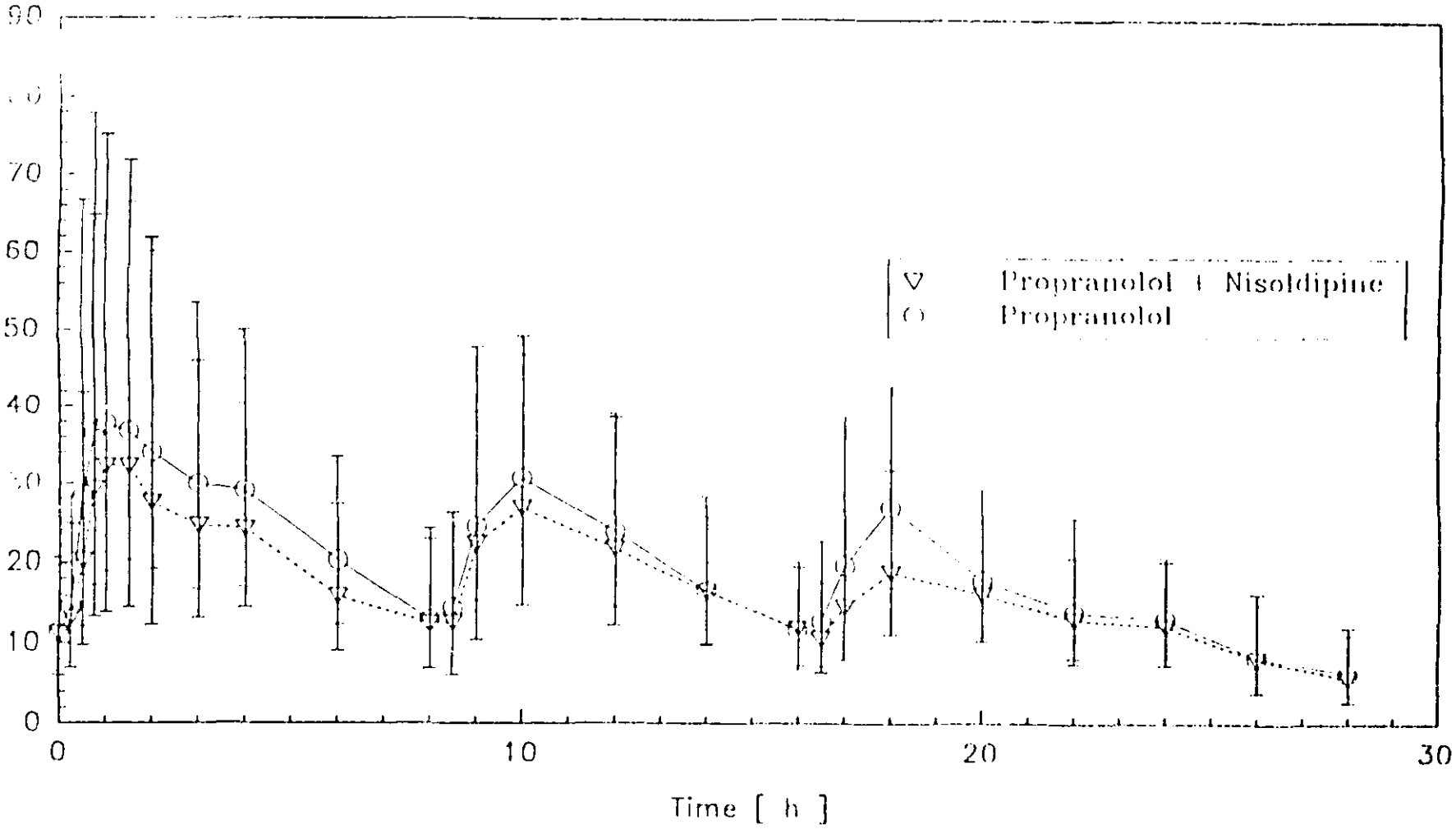


fig. 6.1.2

TABLE 1

/ STUDY NO.704

Estimates of Pharmakokinetic Parameters of Nisoldipine
(Geometric Means and SD) and Quotients to Results of Nisoldipine in %
(Geometric mean and SD,90% Conf.Interv.Referring to Analysis of Variance and Dunnett–Test)

Parameter Nisoldipine		Treatment Nisoldipine	Nisoldipine +Propranolol	Quotient Nisoldipine+Propranolol / Nisoldipine geo.mean	geo S.D.	90% C.I.
$AUC_{norm\ (0-24)}$	geo.mean [g*h/l]	96.26	103.24	107.3		90.6 – 127
	geo.SD	*1.71*	*1.51*		*1.38*	
C_{max}	geo.mean [ng/ml]	1.87	2.01	107.1		87.0 – 131
	geo.SD	*1.81*	*1.86*		*1.49*	
$t_{1/2}$	geo.mean [h]	13.02	10.53	80.9		66.8 – 97.9
	geo.SD	*1.63*	*1.29*		*1.45*	
fluctuation $_{(0-24)}$	geo.mean	1.33	1.40	105.2		81.9 – 135.
	geo.SD	*1.56*	*1.37*		*1.62*	

06 02 3868

TABLE 2

/ STUDY NO.704

Estimates of Pharmakokinetic Parameters of Propranolol
(Geometric Means and SD) and Quotients to Results of Propranolol in %
(Geometric mean and SD,90% Conf.Interv.Referring to Analysis of Variance and Dunnett–Test)

Parameter Propranolol		Treatment		Quotient Propranolol+Nisoldipine / Propranolol		
		Propranolol	Propranolol +Nisoldipine	geo.mean	geo S.D.	90% C.I.
$AUC_{norm\ (0-24)}$	geo.mean [g*h/l]	1072.47	923.02	86.1	1.76	64.3 – 115.
	geo.SD	1.61	1.65			
$AUC_{norm\ (0-8)}$	geo.mean [g*h/l]	421.20	356.49	84.6	2.03	58.6 – 122.
	geo.SD	1.75	1.76			
c_{max}	geo.mean [ng/ml]	46.50	39.94	85.9	2.45	53.9 – 136.
	geo.SD	1.87	1.81			
	geo.mean [h]	3.50	4.37	124.7	1.45	102.7 – 151.
	geo.SD	1.32	1.40			
Fluctuation $_{(0-24)}$	geo.mean	1.77	1.70	95.8	1.57	75.9 – 121.
	geo.SD	1.42	1.35			
Fluctuation $_{(0-8)}$	geo.mean	1.34	1.33	99.2	1.48	80.9 – 121.
	geo.SD	1.37	1.31			

Studies of the hemodynamic and pharmacokinetic interactions between the beta blockers atenolol and propranolol and the calcium antagonist nisoldipine in normotensive subjects.

Study: 3982. Volume: 112. Pages: 06-04-7235-7341.

Investigators:

Objectives:

1- to examine the pharmacokinetic and hemodynamic interactions of atenolol and propranolol with nisoldipine in normotensive patients.

Formulation:

-20 mg nisoldipine capsules.
-100 mg atenolol (Stuart Pharmaceuticals Ltd).
-160 mg propranolol (ICI Ltd).

Study Design:

This study was a randomized double blind placebo controlled study of 2 groups of 8 normotensive subjects between the ages of 19 and 40 years.
One group of subjects received 100 mg atenolol while the other group received propranolol 160 mg once daily. These active treatments were taken once daily for three weeks and matching placebo tablets were taken once daily during a fourth week. There was a 1 week washout period between weeks 2 and 3. For 2 weeks out of the total period additional treatment was administered: either nisoldipine 20 mg once a day or matching placebo capsules each for 1 week. The following treatment combinations were evaluated:
1-beta blocker + first dose nisoldipine.
2-beta blocker + steady state nisoldipine.
3-placeoo + placebo.
4-beta blocker + placebo.

Blood pressure and heart rate erect and supine were measured at baseline and after 0.5, 1, 1.5, 2, 3, 4, 6 and 8 hours.

The extent of beta blockade was assessed by the heart rate response to sub-maximal exercise (75 % of a pre-determined maximum) for 5 minutes on a bicycle ergometer at two times:2.5 (morning) and 5 hours (afternoon).
Plasma for drug assays were collected at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6 and 8 hours.
Hepatic blood flow was assessed 1 hour after drug administration by measurement of the clearance of indocyanine green.
Effective renal plasma flow and glomerular filtration rate were determined 1 hour after drug administration from the clearance of radiopharmaceuticals.

Results:

1-Pharmacokinetics:

Figure 1 shows the mean plasma concentrations for atenolol with and without nisoldipine while Figure 2 shows the plasma profile for propranolol with and without the calcium channel blocker. Table 1 gives a summary of the derived pharmacokinetic for atenolol while Table 2 gives the same pk parameters for propranolol.
The results show that coadministration of nisoldipine with atenolol sharply increased the atenolol CMAX (from 455 to 540 ng/ml) and to a lesser extent the AUC. The same trend of results was observed for propranolol whereby both the CMAX and AUC for acute and chronic propranolol were increased. However, there was no significant difference in propranolol AUC comparing the combination with acute and chronic calcium antagonist.

2-Liver blood flow and renal clearance:

The results shown in Table 3 and 4 respectively show that both atenolol and propranolol when given alone cause a slight decrease in the apparent liver blood flow. However the addition of nisoldipine to either atenolol or propranolol caused a significant increase in the apparent liver blood flow. This increase was slightly attenuated with the chronic administration of nisoldipine.

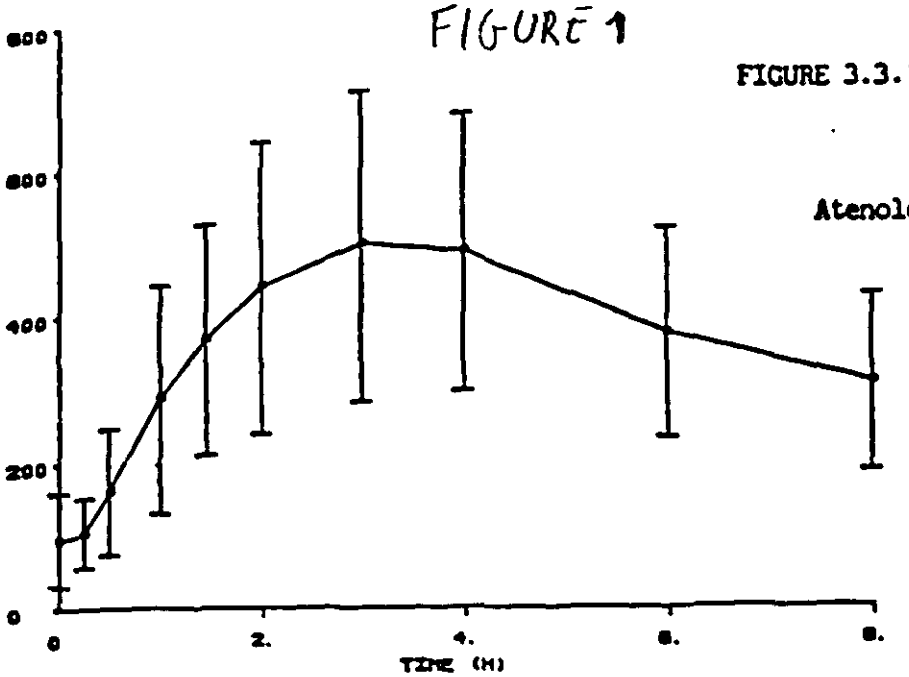
As for the renal function, it was not affected by any of the treatments.

3-Blood pressure and heart rate:

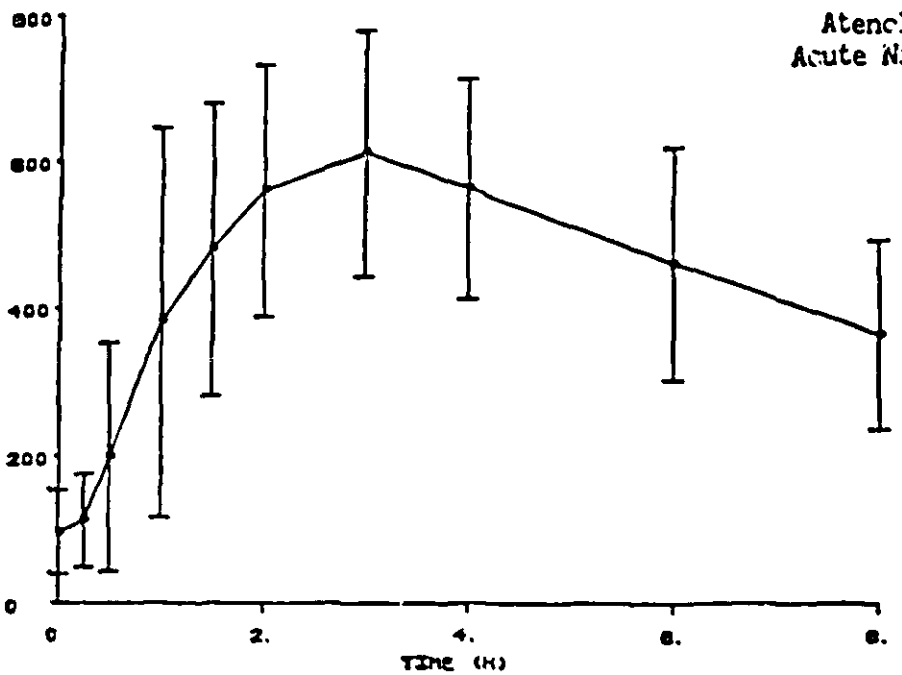
Figure 3, 4 and 5 show the supine systolic blood pressure, supine diastolic blood pressure and supine heart rate for the atenolol treatment while Figure 6, 7 and 8 show the corresponding graphs for the propranolol treatment.
The results show that both atenolol and propranolol caused a significant decrease in both supine and erect systolic and diastolic blood pressure as compared to placebo. The addition of nisoldipine caused a further slight reductions in blood pressures. This further reductions in blood pressures were generally accompanied by a slight increase in heart rate.

FIGURE 1

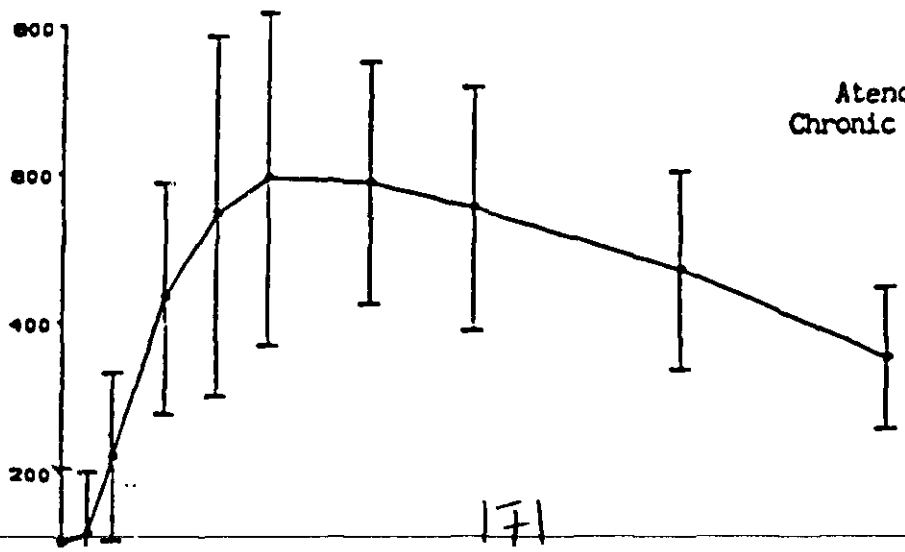
FIGURE 3.3.1



Atenolol alone



Atenolol +
Acute Nisoldipine

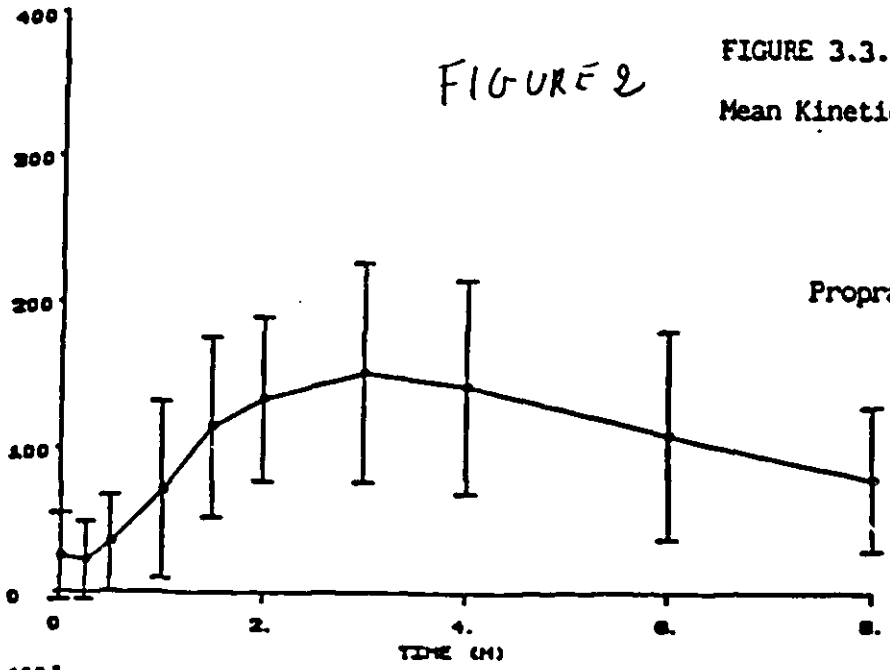


Atenolol +
Chronic Nisoldipine

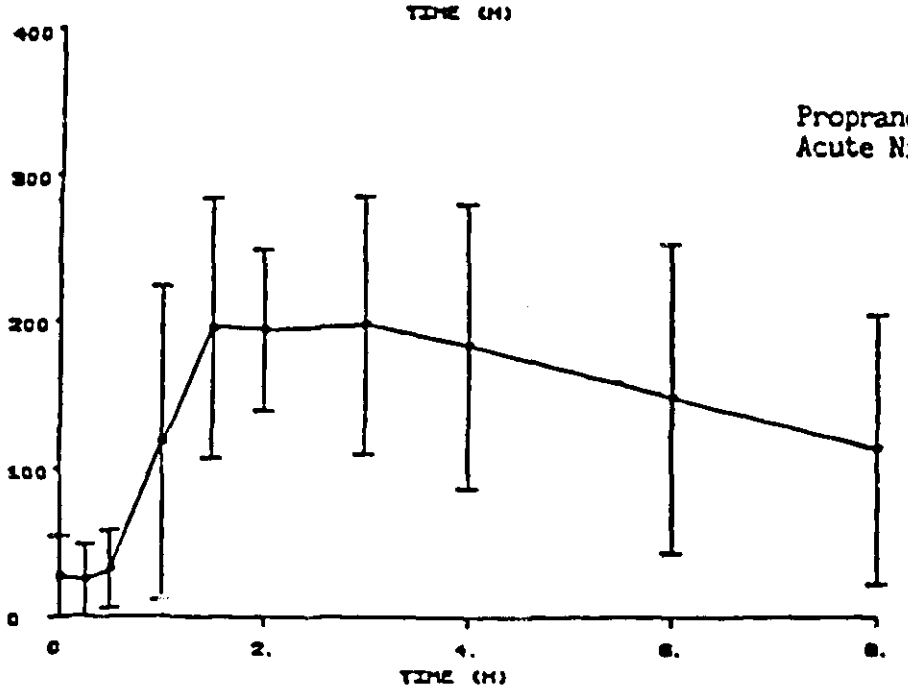
FIGURE 2

FIGURE 3.3.2

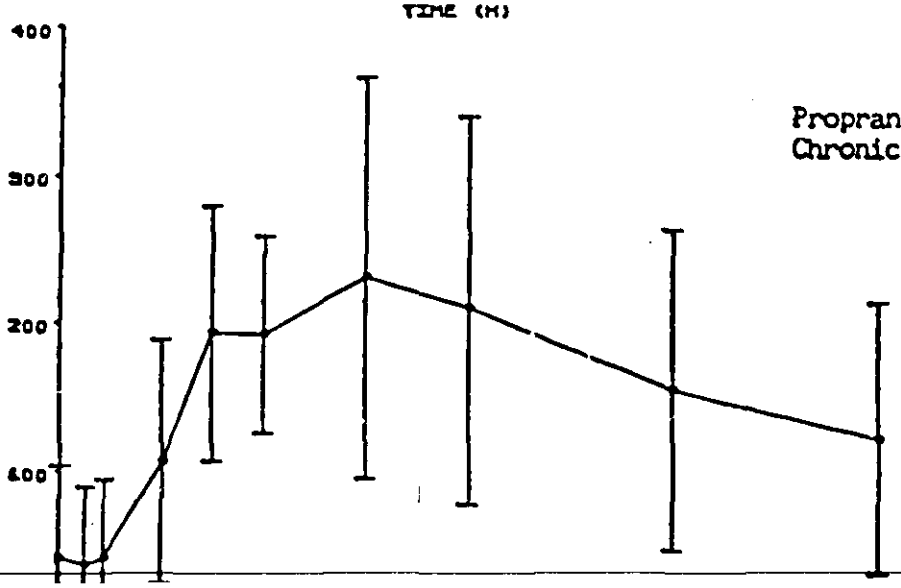
Mean Kinetic Profiles



Propranolol Alone

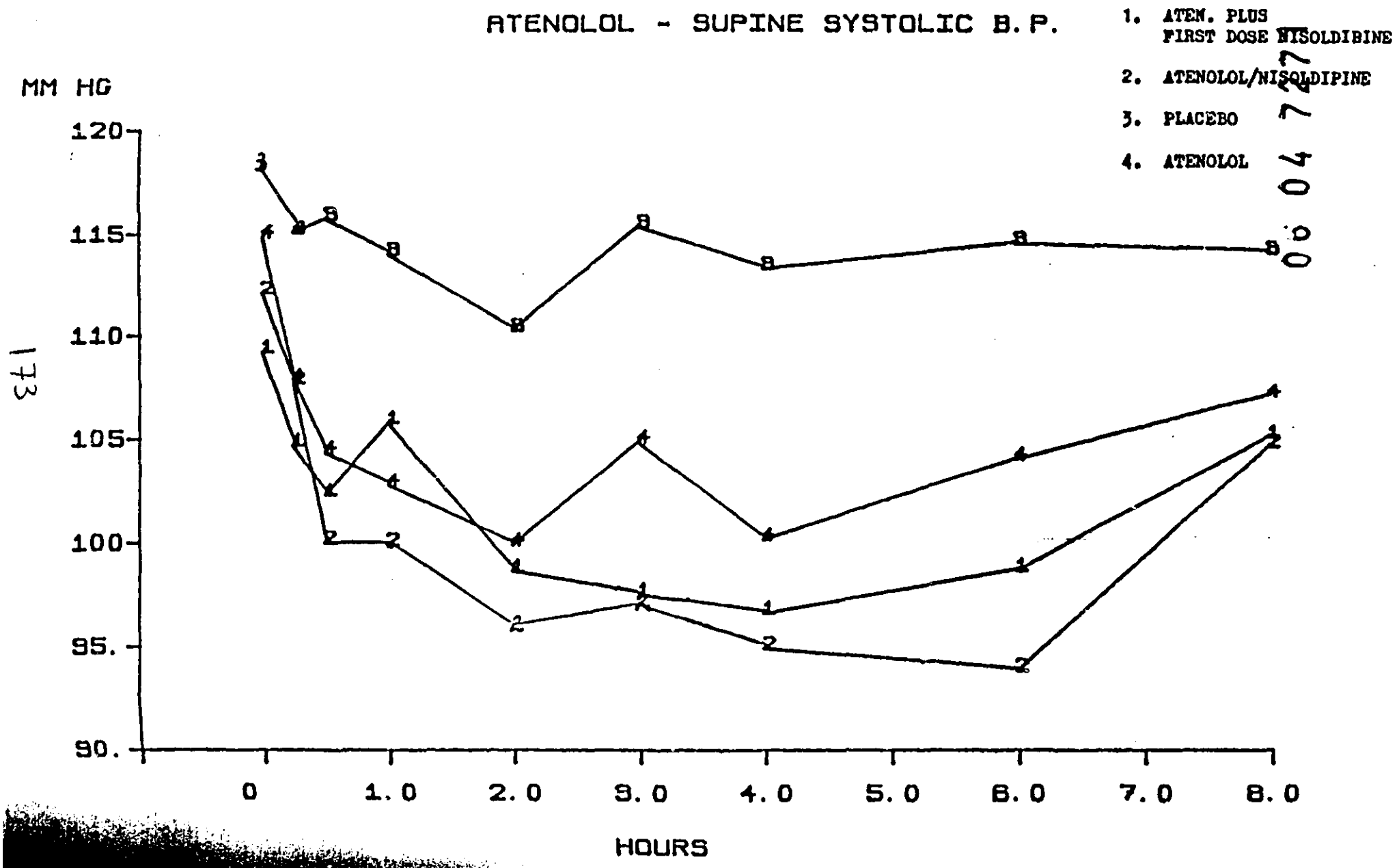


Propranolol +
Acute Nisoldipine

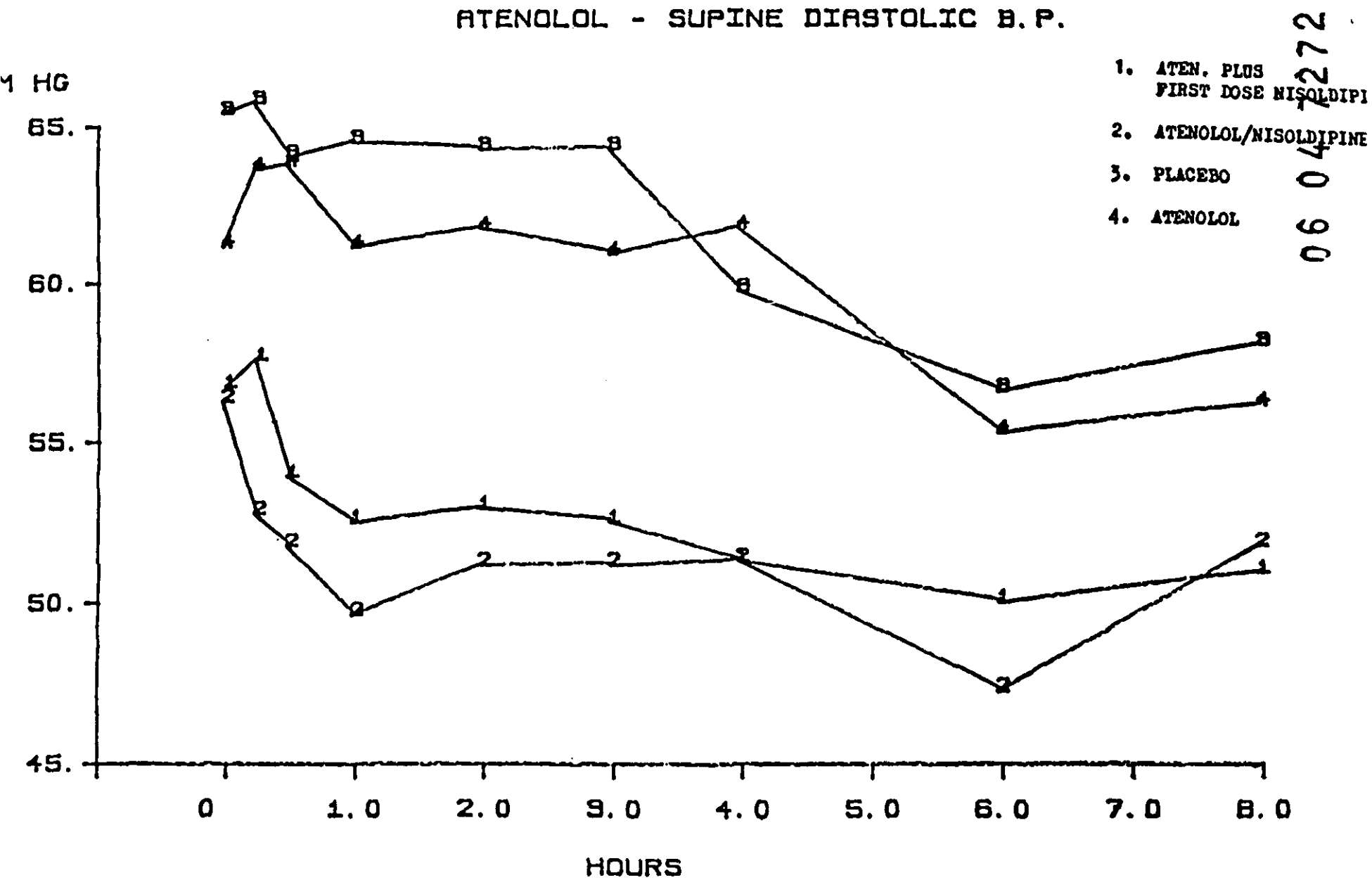


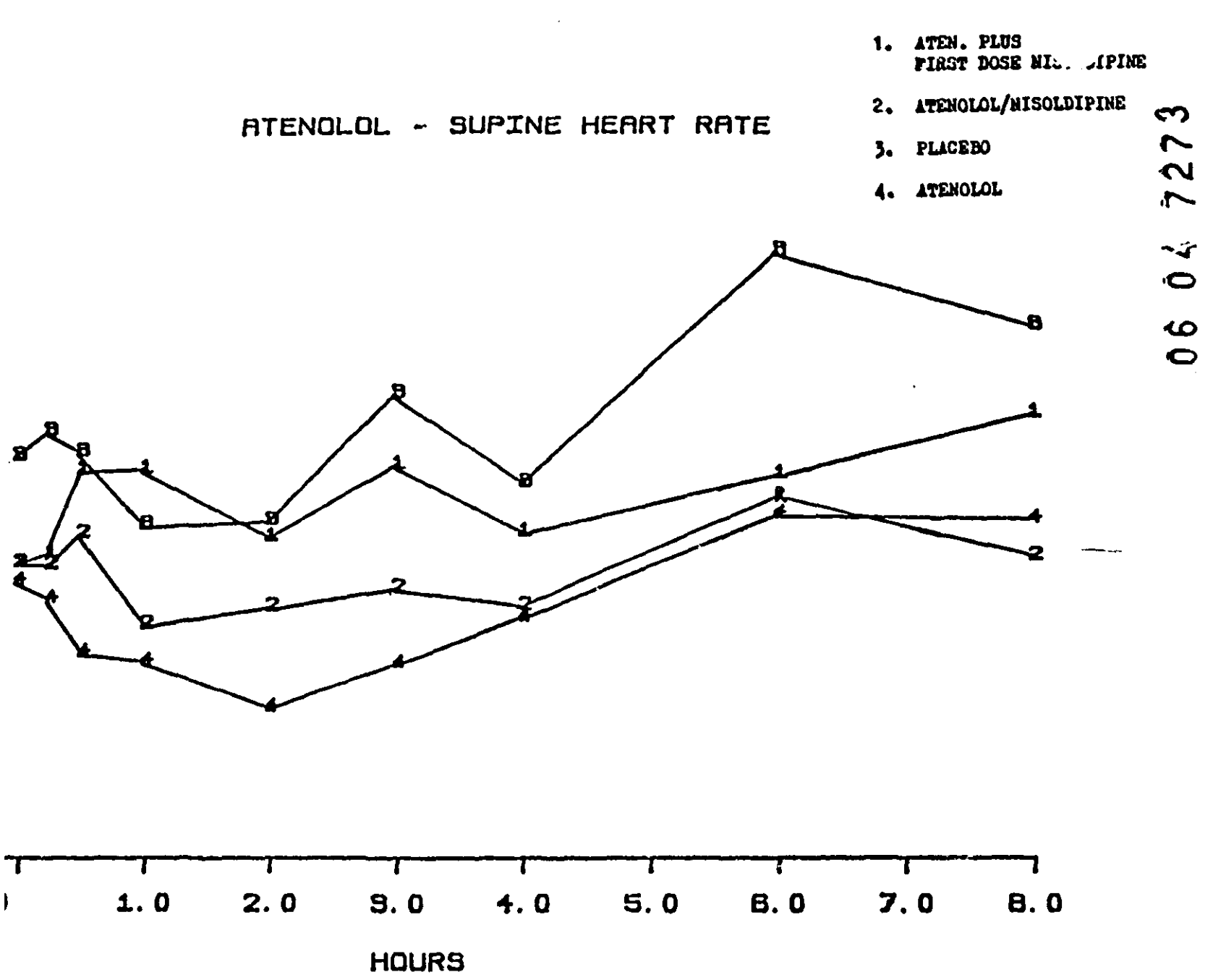
Propranolol +
Chronic Nisoldipine

FIGURE 3.[illegible]

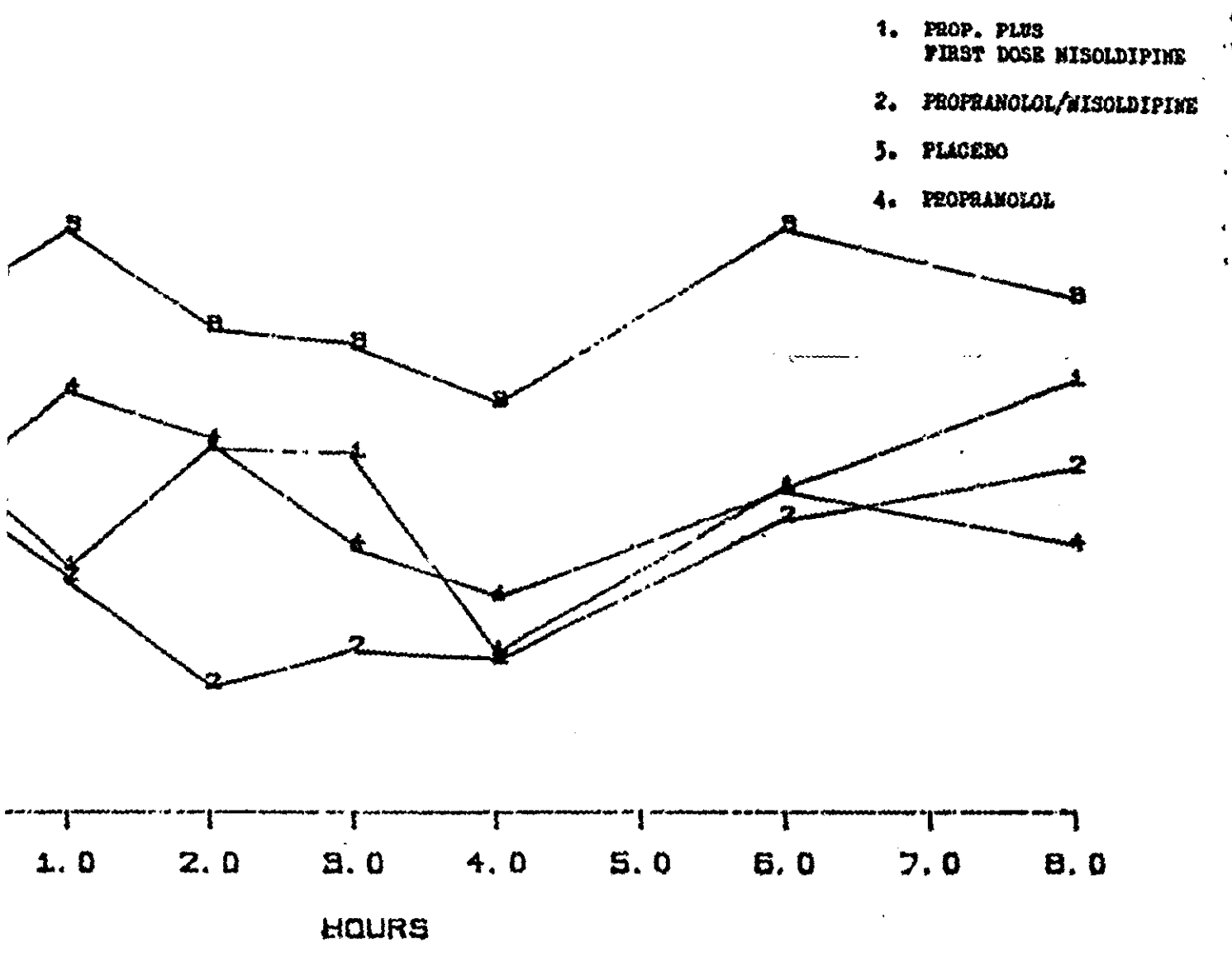


JURE 4.202

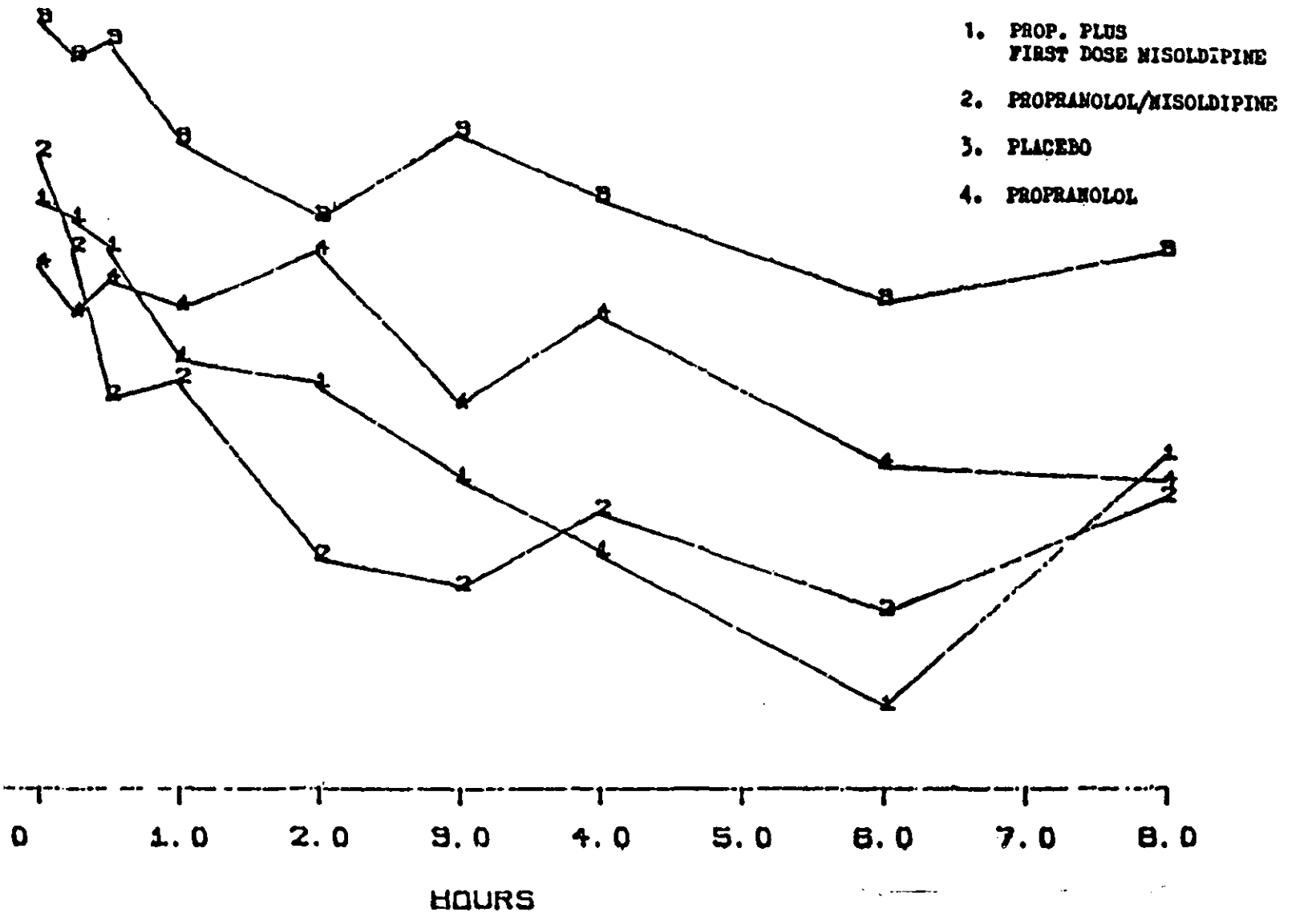



1. ATEN. PLUS
FIRST DOSE NI.. .IPINE
2. ATENOLOL/NISOLDIPINE
3. PLACEBO
4. ATENOLOL
ATENOLOL - SUPINE HEART RATE
06 04 7273
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
HOURS

PROPRANOLOL - SUPINE SYSTOLIC B.P.



PROPRANOLOL - SUPINE DIRSTOLIC B.P.



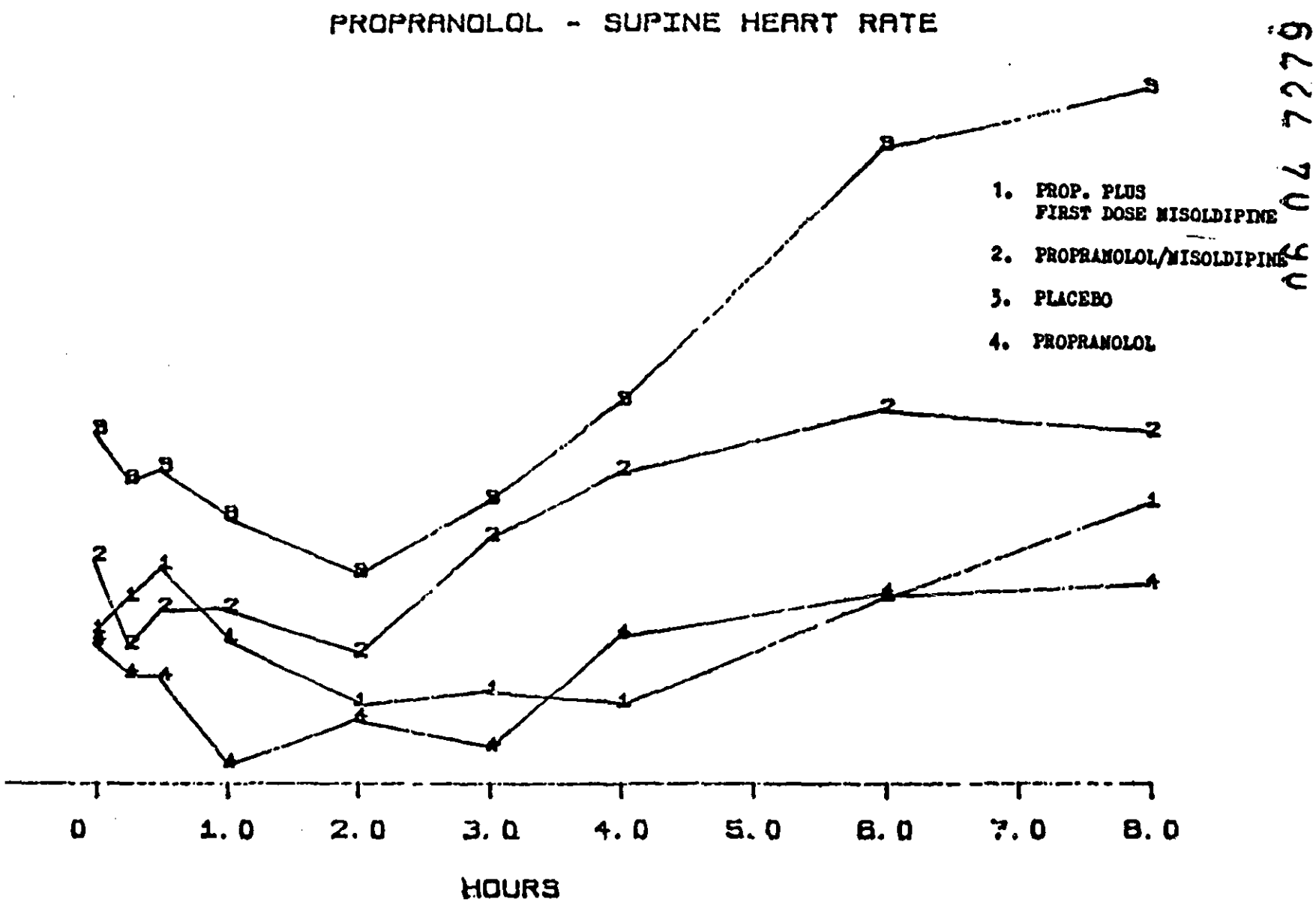

PROPRANOLOL - SUPINE HEART RATE
1. PROP. PLUS FIRST DOSE MISOLDIPINE
2. PROPRANOLOL/MISOLDIPINE
3. PLACEBO
4. PROPRANOLOL
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
HOURS

TABLE 1.3.1. DERIVED PHARMACOKINETIC PARAMETERS: ATENOLOL

Subject	AUC (ng.h/ml) CA	CA + AN	CA + CN	$\beta t_{1/2}$ (h) CA	CA + AN	CA + CN	t_{max} (h) CA	CA + AN	CA + CN	Δ (n CA
1	7584	8373	7832	5.4	6.7	7.3	4.1	3.0	3.0	580
2	7981	6003	6488	11.6	10.5	6.0	3.5	1.3	3.0	411
3	3553	4557	3975	9.1	4.8	5.7	2.5	2.2	2.7	463
4	2181	6079	4745	7.4	11.0	7.0	1.0	2.4	1.9	192
5	5658	7351	7091	5.9	4.9	3.4	3.2	4.4	3.8	479
6	5677	4267	5432	8.1	5.5	6.7	2.2	3.6	1.2	422
7	5190	9076	7992	7.0	7.6	8.9	4.2	3.3	2.9	441
8	9006	10189	11195	8.2	8.0	8.1	2.3	1.6	2.0	651
Mean ± S.D.	5854 ± 2291	6987 ± 2130	6844 ± 2269	7.8 ± 1.9	7.4 ± 2.4	6.6 ± 1.7	2.9 ± 1.1	2.7 ± 1.0	2.6 ± 0.8	455 ± 135
	ns*	ns		ns	ns		ns	ns		p<0.
	ns			ns			ns			

ns* = (0.1 > p > 0.05)

TABLE 3.3.2. DERIVED PHARMACOKINETIC PARAMETERS: PROPRANOLOL

Subject	AUC (ng.h/ml) CA	CA + AN	CA + CN	β t1/2 (h) CA	CA + AN	CA + CN	t_{max} (h) CA	CA + AN	CA + CN	CA
9	739	806	1378	2.7	2.2	3.4	2.6	1.8	3.1	125
10	1470	2074	2002	4.1	6.0	5.6	1.8	0.9	1.4	195
11	4235	5460	7441	10.7	8.1	13.9	3.8	4.5	2.5	225
12	1336	1272	1901	4.3	5.3	6.6	3.3	3.3	2.0	134
13	1283	1821	1452	7.2	3.9	4.4	0.7	1.5	1.7	121
14	570	828	643	3.4	2.5	2.6	1.7	1.2	1.0	101
15	1297	1829	2469	4.1	5.2	6.0	5.4	3.6	4.2	108
16	1521	2693	2567	6.2	8.1	6.1	3.3	1.1	1.6	133
Mean ± S.D.	1556 ± 1135	2098 ± 1501	2482 ± 2099	5.4 ± 2.6	5.2 ± 2.3	6.1 ± 3.6	2.8 ± 1.5	2.2 ± 1.4	2.2 ± 1.0	143 ± 44.
	$p<0.01$	ns		ns	ns		ns	ns		p<0.
	$p<0.03$			ns			ns			

TABLE 3.[redacted] APPARENT LIVER BLOOD FLOW: ATENOLOL PLUS NISOLDIPINE

Subject		Placebo	CA	CA + AN	CA + CN
1	LBF	2.17	1.50	2.55	2.69
	Vd	4.95	4.28	3.78	5.37
2	LBF	2.35	1.95	3.33	2.47
	Vd	5.60	4.60	6.01	4.65
3	LBF	1.61	1.77	1.71	1.98
	Vd	3.37	3.02	2.15	2.78
4	LBF	1.32	1.06	1.74	1.75
	Vd	3.41	2.65	2.33	2.96
5	LBF	1.61	1.88	2.04	1.69
	Vd	2.53	4.77	3.38	2.21
6	LBF	1.65	1.23	3.05	2.76
	Vd	3.33	2.93	4.47	4.97
7	LBF	0.94	0.83	1.56	1.32
	Vd	2.98	2.68	3.92	3.91
8	LBF	1.66	1.13	3.07	2.05
	Vd	3.34	2.22	4.34	3.85
Mean ± S.D.	LBF	1.66 ± 0.44	1.42 ± 0.42	2.38 ± 0.71	2.16 ± 0.42
		ns			
		p<0.01			
		p<0.01			
Mean ± S.D.	Vd	3.69 ± 1.04	3.40 ± 1.00	3.79 ± 1.24	3.83 ± 1.12

TABLE [redacted] APPARENT LIVER BLOOD FLOW: PROPRANOLOL PLUS NISOLDIPINE

Subject		Placebo	CP	CP + AN	CP + CN
9	LBF	2.12	1.73	3.04	3.24
	Vd	3.34	3.88	4.33	4.84
10	LBF	1.74	1.58	2.22	2.79
	Vd	3.16	2.63	4.99	4.83
11	LBF	1.89	1.14	2.36	2.87
	Vd	5.44	3.14	4.50	4.60
12	LBF	1.75	1.54	3.02	2.44
	Vd	3.48	3.34	4.21	3.97
13	LBF	1.40	1.10	2.39	2.64
	Vd	3.39	3.13	3.67	4.79
14	LBF	1.94	1.72	2.53	2.63
	Vd	3.68	3.01	3.23	2.91
15	LBF	0.96	1.15	1.25	1.28
	Vd	2.34	3.82	2.25	2.78
16	LBF	0.83	0.71	1.35	0.96
	Vd	1.18	2.28	2.77	1.53
Mean	LBF	1.58	1.33	2.27	2.36
± S.D.		0.47	0.36	0.67	0.80
			p<0.05		
			p<0.01		
				p<0.01	
Mean	Vd	3.25	3.15	3.74	3.78
± S.D.		1.21	0.54	0.94	1.24

Clinicopharmacological investigations on interaction of nisoldipine and digoxin.

STUDY #: 413. VOLUME: 1-57 PAGES:6-02-4014-4166

INVESTIGATORS:

OBJECTIVES:

To investigate the possibility of pharmacodynamic and/or pharmacokinetic interaction taking place between nisoldipine and digoxin.

FORMULATION:

-Nisoldipine 10 mg tablets (batch #: 974386).
-0.1 mg Beta acetyldigoxin (Novodigal mite^{R}).

STUDY DESIGN:

8 healthy male volunteers participated in this non controlled observational study which was statistically a fixed sequence study.
Each subject received on days 1 and 2, 0.6 mg/day of Novodigal mite tablets. Starting on day 3 the daily dose was reduced to 0.3 mg of acetyldigoxin. This doses was maintained up to the last study day (day 22). Nisoldipine was additionally taken in the morning and the evening before meals starting with day 9 until the last study day.
The plasma and urine sampling schedule was not given in this study.

RESULTS:

Table 1 shows the main pharmacokinetic parameters for digoxin. Unfortunately, no pharmacokinetic parameters could be calculated for nisoldipine because only peak and trough levels were measured. The sponsor concluded that coadministration of nisoldipine did not have any effect on the pharmacokinetics of digoxin.

Comments:

The results of the study could not be considered definitive as to whether an interaction with nisoldipine C.C. and digoxin occurs, since the immediate release formulation was used and no assay validation was provided. Therefore, one cannot judge whether the results of the study are valid and capable of detecting an interaction if it exists.

table C: pharmacokinetics of Digoxin, mean values of 6th to 8th day (Digoxin), 20th to 22th day (Digoxin + Nisoldipine) and Quotient of 20th to 22th day/6th to 8th day nonparametric 95%-confidence limits of quotient

		Digoxin	Digoxin + Nisoldipine	quotient (%)	95% confidence limit
plasma concentration [mg/ml]	median	0.564	0.625	106.4	106.9
	min,max	0.381, 0.714	0.393, 0.828	101.1, 134.6	102.7, 120.3
renal elimination [%]	median	54.0	62.7	110.7	112.2
	min,max	47.2, 63.5	51.6, 73.4	92.4, 139.4	100.8, 124.6
Cl - total [ml/min/kg]	median	6.04	5.64	94.9	94.2
	min,max	4.84, 7.75	4.43, 6.95	74.0, 98.6	83.7, 97.4
Cl - renal [ml/min/kg]	median	4.06	4.65	103.5	103.7
	min,max	3.55, 4.70	3.16, 4.85	81.4, 137.5	89.2, 119.5

NDA
203356

APPROVAL LETTER

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 20-356

FEB 2 1995

Miles Inc.
Pharmaceutical Division
Attention: Nancy Motola, Ph.D.
400 Morgan Lane
West Haven, CT 06516-4175

Dear Dr. Motola:

Please refer to your March 31, 1993 new drug application resubmitted on August 3, 1994 under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Nisocor (nisoldipine) Tablets, 10, 20, 30 and 40 mg.

We acknowledge receipt of your amendments and correspondence dated May 31, June 20 and 27, July 18 and 29 (two), September 8 and 16, October 19, November 8, 9, 17, 18 and 21, and December 16, 20 (two), 22 (three) and 28, 1994; and January 20, 23, and 27, 1995.

This new drug application provides for the use of Nisocor in the treatment of hypertension.

We have completed the review of this application including the submitted draft labeling and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the enclosed marked-up draft labeling. Accordingly, the application is approved effective on the date of this letter.

The final printed labeling (FPL) must be identical to the enclosed marked up draft labeling. Marketing the product with FPL that is not identical to this draft labeling may render the product misbranded and an unapproved new drug.

The approved dissolution specifications are as follows:

3 hours
6 hours
12 hours

Please submit fifteen copies of the FPL as soon as it is available, in no case more than 30 days after it is printed. Please individually mount ten of the copies on heavy weight paper or similar material. For administrative purposes this submission should be designated "FINAL PRINTED LABELING" for approved NDA 20-356. Approval of this labeling by FDA is not required before it is used.

Should additional information relating to the safety and effectiveness of the drug become available, revision of that labeling may be required.

In addition, please submit three copies of the introductory promotional material that you propose to use for this product. All proposed materials should be submitted in draft or mock-up form, not final print. Please send one copy to the Division of Cardio-Renal Drug Products and two copies of both the promotional material and the package insert directly to:

Food and Drug Administration
Division of Drug Marketing, Advertising and Communications, HFD-240
5600 Fishers Lane
Rockville, Maryland 20857

Validation of the regulatory methods is ongoing. At the present time, it is the policy of the Office not to withhold approval because the methods are being validated. Nevertheless, we expect your continued cooperation to resolve any deficiencies that may occur.

Please submit one market package of the drug when it is available.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any questions, please contact:

Mr. David Roeder
Consumer Safety Officer
(301) 594-5300

Sincerely yours,

Robert Temple, M.D.
Director
Office of Drug Evaluation I
Center for Drug Evaluation and Research

Enclosure

cc:
Original NDA
HF-2/MedWatch (with labeling)
HFC-130/JAllen
HFD-2/MLumpkin
HFD-80 (with labeling)
HFD-100 (with labeling)
HFD-110
HFD-110/CSO
HFD-240 (with labeling)
HFD-638 (with labeling)
HFD-735/DBarash (with labeling)
HFD-110/DRoeder/12/28/94
sb/12/28/94;12/29/94
R/D: RWolters/12/28/94
SChen/12/28/94
GBuehler for NMorgenstern/12/29/94

APPROVAL

DRAFT OF
APPROVED
LABELING

NISOCOR

(nisoldipine)

Extended Release Tablets

For Oral Use

DESCRIPTION

NISOCOR (nisoldipine) is an extended release tablet dosage form of the dihydropyridine calcium channel blocker nisoldipine. Nisoldipine is 3,5-pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, methyl 2-methylpropyl ester, $C_{20}H_{24}N_2O_6$, and has the structural formula:

Supply formula →

Nisoldipine is a yellow crystalline substance, practically insoluble in water but soluble in ethanol. It has a molecular weight of 388.4. NISOCOR tablets consist of an external coat and an internal core. Both coat and core contain nisoldipine, the coat as a slow release formulation and the core as a fast release formulation. NISOCOR tablets contain either 10, 20, 30, or 40 mg of nisoldipine for once-a-day oral administration.

Inert ingredients in the formulation are: hydroxypropylcellulose, lactose, corn starch, crospovidone, microcrystalline cellulose, sodium lauryl sulfate, povidone and magnesium stearate. The inert ingredients in the film coating

are: hydroxypropylmethylcellulose, polyethylene glycol, ferric oxide, and titanium dioxide.

CLINICAL PHARMACOLOGY

Mechanism of Action

Nisoldipine is a member of the dihydropyridine class of calcium channel antagonists (calcium ion antagonists or slow channel blockers) that inhibit the transmembrane influx of calcium into vascular smooth muscle and cardiac muscle. It reversibly competes with other dihydropyridines for binding to the calcium channel. Because the contractile process of vascular smooth muscle is dependent upon the movement of extracellular calcium into the muscle through specific ion channels, inhibition of the calcium channel results in dilation of the arterioles. *In vitro* studies show that the effects of nisoldipine on contractile processes are selective, with greater potency on vascular smooth muscle than on cardiac muscle. Although, like other dihydropyridine calcium channel blockers, nisoldipine has negative inotropic effects *in vitro*, studies conducted in intact anesthetized animals have shown that the vasodilating effect occurs at doses lower than those that affect cardiac contractility

The effect of nisoldipine on blood pressure is principally a consequence of a dose-related decrease of peripheral vascular resistance. While nisoldipine, like other dihydropyridines, exhibits a mild diuretic effect, most of the antihypertensive activity is attributed to its effect on peripheral vascular resistance

Pharmacokinetics and Metabolism

Nisoldipine pharmacokinetics are independent of the dose in the range of 20 to 60 mg, with plasma concentrations proportional to dose. Nisoldipine accumulation, during multiple dosing, is predictable from a single dose.

Nisoldipine is relatively well absorbed into the systemic circulation with 87% of the radiolabeled drug recovered in urine and feces. The absolute bioavailability of nisoldipine is about 5%. Nisoldipine's low bioavailability is due, in part, to pre-systemic metabolism in the gut wall and this metabolism decreases from the proximal to the distal parts of the intestine. Food with a high fat content has a pronounced effect on the release of nisoldipine from the coat-core formulation and results in a significant increase in peak concentration (C_{max}) by up to 300%. Total exposure, however, is decreased about 25%, presumably because more of the drug is released proximally. This effect appears to be specific for nisoldipine in the controlled release formulation, as a less pronounced food effect was seen with the immediate release tablet. Concomitant intake of a high fat meal with NISOCOR should be avoided.

Maximal plasma concentrations of nisoldipine are reached 6 to 12 hours after dosing. The terminal elimination half-life (reflecting post absorption clearance of nisoldipine) ranges from 7 to 12 hours. C_{max} and AUC increase by factors of approximately 1.3 and 1.5 respectively from first dose to steady state. After oral administration, the concentration of (+) nisoldipine, the active enantiomer, is about 6 times higher than the (-) inactive enantiomer. The

plasma protein binding of nisoldipine is very high, with less than 1% unbound over the plasma concentration range of 100 ng/mL to 10 mcg/mL. Nisoldipine is highly metabolized; 5 major urinary metabolites have been identified. Although 60 - 80% of an oral dose undergoes urinary excretion, only traces of unchanged nisoldipine are found in urine. The major biotransformation pathway appears to be the hydroxylation of the isobutyl ester. A hydroxylated derivative of the side chain, present in plasma at concentrations approximately equal to the parent compound, appears to be the only active metabolite, and has about 10% of the activity of the parent compound. Cytochrome P_{450} enzymes are believed to play a major role in the metabolism of nisoldipine. The particular isoenzyme system responsible for its metabolism has not been identified, but other dihydropyridines are metabolized by cytochrome P_{450} IIIA4. Nisoldipine should not be administered with grapefruit juice as this has been shown, in a study of 12 subjects, to interfere with nisoldipine metabolism, resulting in a mean increase in C_{max} of about 3-fold (ranging up to about 7-fold) and AUC of almost 2-fold (ranging up to about 5-fold). A similar phenomenon has been seen with several other dihydropyridine calcium channel blockers.

Special Populations:

Renal dysfunction: Because renal elimination is not an important pathway, bioavailability and pharmacokinetics of NISOCOR were not significantly different in patients with various degrees of renal impairment. Dosing adjustments in patients with mild to moderate renal impairment are not necessary.

Geriatric: Elderly patients have been found to have 2 to 3 fold higher plasma

concentrations (C_{max} and AUC) than young subjects. This should be reflected in more cautious dosing (See Dosage and Administration).

Hepatic Insufficiency: In patients with liver cirrhosis given 10 mg NISOCOR, plasma concentrations of the parent compound were 4 to 5 times higher than those in healthy young subjects. Lower starting and maintenance doses should be used in cirrhotic patients (See Dosage and Administration).

Gender and Race: The effect of gender or race on the pharmacokinetics of nisoldipine has not been investigated.

Disease States: Hypertension does not significantly alter the pharmacokinetics of nisoldipine.

Pharmacodynamics

Hemodynamic Effects

Administration of a single dose of nisoldipine leads to decreased systemic vascular resistance and blood pressure with a transient increase in heart rate. The change in heart rate is greater with immediate release nisoldipine preparations. The effect on blood pressure is directly related to the initial degree of elevation above normal. Chronic administration of nisoldipine results in a sustained decrease in vascular resistance and small increases in stroke index and left ventricular ejection fraction. A study of the immediate release formulation showed no effect of nisoldipine on the renin-angiotensin-aldosterone system or on plasma norepinephrine concentration in normals. Changes in blood pressure in hypertensive patients given NISOCOR were dose related over the range of 10 - 60 mg/day.

Nisoldipine does not appear to have significant negative inotropic activity in intact animals or humans, and did not lead to worsening of clinical heart

failure in three small studies of patients with asymptomatic and symptomatic left ventricular dysfunction. There is little information, however, in patients with severe congestive heart failure and all calcium channel blockers should be used with caution in any patient with heart failure.

Electropyhysiologic Effects

Nisoldipine has no clinically important chronotropic effects. Except for mild shortening of sinus cycle, SA conduction time and AH intervals, single oral doses up to 20 mg of immediate release nisoldipine did not significantly change other conduction parameters. Similar electrophysiologic effects were seen with single iv doses, which could be blunted in patients pre-treated with beta-blockers. Dose and plasma level related flattening or inversion of T-waves have been observed in a few small studies. Such reports were concentrated in patients receiving rapidly increased high doses in one study; the phenomenon has not been a cause of safety concern in large clinical trials.

Clinical Studies in Hypertension

The antihypertensive efficacy of NISOCOR was studied in 5 double-blind, placebo-controlled, randomized studies, in which over 600 patients were treated with NISOCOR as monotherapy and about 300 with placebo; 4 of the five studies compared 2 or 3 fixed doses while the fifth allowed titration from 10 - 40 mg. Once daily administration of NISOCOR produced sustained reductions in systolic and diastolic blood pressures over the 24 hour dosing interval in both supine and standing positions. The mean placebo-subtracted reductions in supine systolic and diastolic blood pressure at trough, 24 hours post-dose, in these studies, are shown below. Changes in standing blood

pressure were similar:

MEAN SUPINE TROUGH SYSTOLIC AND DIASTOLIC

BLOOD PRESSURE CHANGES (mm Hg)

NISOCOR Dose (mg/day)	10 mg	20 mg	30 mg	40 mg	60 mg	10-40mg titrated
Systolic:	8	11	11	14	15	15
Diastolic	3	5	7	7	10	8

In patients receiving atenolol, supine blood pressure reductions with NISOCOR at 20, 40 and 60 mg once daily were 12/6, 19/8 and 22/10 mm Hg, respectively. The sustained antihypertensive effect of NISOCOR was demonstrated by 24 hour blood pressure monitoring and examination of peak and trough effects. The trough/peak ratios ranged from 70 to 100% for diastolic and systolic blood pressure. The mean change in heart rate in these studies was less than one beat per minute. In 4 of the 5 studies, patients received initial doses of 20 - 30 mg NISOCOR without incident (excessive effects on blood pressure or heart rate). The fifth study started patients on lower doses of NISOCOR

Patient race and gender did not influence the blood pressure lowering effect of NISOCOR. Despite the higher plasma concentration of nisoldipine in the elderly, there was no consistent difference in their blood pressure response except that the 10 mg dose was somewhat more effective than in non-elderly patients. No postural effect on blood pressure was apparent and there was no

evidence of tolerance to the antihypertensive effect of NISOCOR in patients treated for up to one year.

INDICATIONS AND USAGE

NISOCOR is indicated for the treatment of hypertension. It may be used alone or in combination with other antihypertensive agents.

CONTRAINDICATIONS

NISOCOR is contraindicated in patients with known hypersensitivity to dihydropyridine calcium channel blockers.

WARNINGS

Increased angina and/or myocardial infarction in patients with coronary artery disease: Rarely, patients, particularly those with severe obstructive coronary artery disease, have developed increased frequency, duration and/or severity of angina, or acute myocardial infarction on starting calcium channel blocker therapy or at the time of dosage increase. The mechanism of this effect has not been established. In controlled studies of NISOCOR in patients with angina this was seen about 1.5% of the time in patients given nisoldipine compared with 0.9% in patients given placebo.

PRECAUTIONS

General:

Hypotension: Because nisoldipine, like other vasodilators, decreases

peripheral vascular resistance, careful monitoring of blood pressure during the initial administration and titration of NISOCOR is recommended. Close observation is especially important for patients already taking medications that are known to lower blood pressure. Although in most patients the hypotensive effect of NISOCOR is modest and well tolerated, occasional patients have had excessive and poorly tolerated hypotension. These responses have usually occurred during initial titration or at the time of subsequent upward dosage adjustment.

Congestive Heart Failure: Although acute hemodynamic studies of nisoldipine in patients with NYHA Class II-IV heart failure have not demonstrated negative inotropic effects, safety of NISOCOR in patients with heart failure has not been established. Caution therefore should be exercised when using NISOCOR in patients with heart failure or compromised ventricular function, particularly in combination with a beta-blocker.

Patients with Hepatic Impairment: Because nisoldipine is extensively metabolized by the liver and, in patients with cirrhosis, it reaches blood concentrations about 5 times those in normals, NISOCOR should be administered cautiously in patients with severe hepatic dysfunction (See Dosage and Administration)

Information for Patients: NISOCOR is an extended release tablet and should be swallowed whole. Tablets should not be chewed, divided or crushed. NISOCOR should not be administered with a high fat meal. Grapefruit juice, which has been shown to increase significantly the bioavailability of nisoldipine and other dihydropyridine type calcium channel

blockers, should not be taken with NISOCOR

Laboratory Tests: NISOCOR is not known to interfere with the interpretation of laboratory tests.

Drug Interactions: A 30 to 45% increase in AUC and C_{max} of nisoldipine was observed with concomitant administration of cimetidine 400 mg twice daily. Ranitidine 150 mg twice daily did not interact significantly with nisoldipine (AUC was decreased by 15 - 20 %). No pharmacodynamic effects of either H_2 antihistamine were observed.

Pharmacokinetic interactions between nisoldipine and beta-blockers (atenolol, propranolol) were variable and not significant. Propranolol attenuated the heart rate increase following administration of immediate release nisoldipine. The blood pressure effect of NISOCOR tended to be greater in patients on atenolol than in patients on no other antihypertensive therapy.

Quinidine at 648 mg bid ~~increased~~ decreased the bioavailability (AUC) of nisoldipine by 26%, but not the peak concentration. The immediate release, but not the coat-core, formulation of nisoldipine increased plasma quinidine concentrations by about 20 %. This interaction was not accompanied by ECG changes and its clinical significance is not known.

No significant interactions were found between nisoldipine and warfarin or digoxin.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Dietary administration of nisoldipine to male and female rats for up to 24 months

(mean doses up to 82 and 111 mg/kg/day, 16 and 19 times the maximum recommended human dose (MRHD) on a mg/m^2 basis, respectively) and female mice for up to 21 months (mean doses of up to 217 mg/kg/day, 20 times the MRHD on a mg/m^2 basis) revealed no evidence of tumorigenic effect of nisoldipine. In male mice receiving a mean dose of 163 mg nisoldipine/kg/day (16 times the MRHD of 60 mg/day on a mg/m^2 basis), an increased frequency of stomach papilloma, but still within the historical range, was observed. No evidence of stomach neoplasia was observed at lower doses (up to 58 mg/kg/day). Nisoldipine ~~tested~~ was when tested negative in a battery of genotoxicity assays, including the Ames test and the CHO/HGRPT assay for mutagenicity ~~mutagenicity and clastogenicity tests.~~ and the in vivo mouse micronucleus test and in vitro CHO cell test for clastogenicity. When administered to male and female rats at doses of up to 30 mg/kg/day (about 5 and 6 times the MRHD on a mg/m^2 basis respectively) nisoldipine had no effect on fertility.

Pregnancy Category C Nisoldipine was neither teratogenic nor fetotoxic at doses that were not maternally toxic. Nisoldipine was fetotoxic but not teratogenic in rats and rabbits at doses resulting in maternal toxicity (reduced maternal body weight gain). In pregnant rats, increased fetal resorption (post implantation loss) was observed at 100 mg/kg/day and decreased fetal weight was observed at both 30 and 100 mg/kg/day. These doses are respectively, about 5 and 16 times the MRHD when compared on a ~~body~~ mg/m^2 basis surface area basis. In pregnant rabbits, decreased fetal and placental weights were observed at a dose of 30 mg/kg/day, about 10 times the MRHD when compared on a ~~body surface area~~ mg/m^2 basis. In a study in which pregnant monkeys (6 per treated and control) had high rates of abortion and mortality, the only surviving fetus from a group exposed to a maternal dose of 100 mg nisoldipine/kg/day (about 30 times the MRHD when compared on a ~~body~~ mg/m^2 basis

~~surface area basis~~) presented with forelimb and vertebral abnormalities not previously seen in control monkeys of the same strain. There are no adequate and well controlled studies in pregnant women. NISOCOR should be used in pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers: It is not known whether nisoldipine is excreted in human milk. Because many drugs are excreted in human milk, a decision should be made to discontinue nursing, or to discontinue NISOCOR, taking into account the importance of the drug to the mother.

ADVERSE EXPERIENCES

More than 6000 patients world-wide have received nisoldipine in clinical trials for the treatment of hypertension, either as the immediate release or the NISOCOR extended release formulation. Of about 1,500 patients who received NISOCOR in hypertension studies, about 55% were exposed for at least 2 months and about one third were exposed for over 6 months, the great majority at doses of 20 to 60 mg daily.

NISOCOR is generally well-tolerated. In the U.S clinical trials of NISOCOR in hypertension, 10.9% of the 921 NISOCOR patients discontinued treatment due to adverse events compared with 2.9% of 280 placebo patients. The frequency of discontinuations due to adverse experiences was related to dose, with a 5.4% discontinuation rate at 10 mg daily and a 10.9% discontinuation rate at 60 mg daily

The most frequently occurring adverse experiences with NISOCOR are those related to its vasodilator properties; these are generally mild and only

occasionally lead to patient withdrawal from treatment. The table below, from U.S. placebo-controlled parallel dose response trials of NISOCOR using doses from 10 - 60 mg once daily in patients with hypertension, lists all of the adverse events, regardless of the causal relationship to NISOCOR, for which the overall incidence on NISOCOR was both >1% and greater with NISOCOR than with placebo.

Adverse Event	Nisoldipine (%) (n=663)	Placebo (%) (n=280)
Peripheral Edema	22	10
Headache	22	15
Dizziness	5	4
Pharyngitis	5	4
~~Asthenia~~	4	4
Vasodilation	4	2
Sinusitis	3	2
Palpitation	3	1
Chest Pain	2	1
Nausea	2	1
Rash	2	1

not greater on Nisocor

Only peripheral edema and possibly dizziness appear to be dose related.

Adverse Event	Placebo	NISOCOR 10 mg	NISOCOR 20 mg	NISOCOR 30 mg	NISOCOR 40 mg	NISOCOR 60 mg
(Rates in %)	N=280	N=30	N=170	N=105	N=139	N=137

Peripheral Edema	10	7	15	20	27	29
Dizziness	4	7	3	3	4	10

The common adverse events occurred at about the same rate in men as in women, and at a similar rate in patients over age 65 as in those under that age, ~~with the exception~~ except that headache was much less common in older patients. Except for peripheral edema and vasodilation, which were more common in whites, adverse event rates were similar in blacks and whites.

The following adverse events occurred in ≤1 % of all patients treated for hypertension in U.S. and foreign clinical trials, or with unspecified incidence in other studies. Although a causal relationship of NISOCOR to these events cannot be established, they are listed to alert the physician to a possible relationship with NISOCOR treatment.

Body As A Whole: cellulitis, chills, facial edema, fever, flu syndrome, malaise.

Cardiovascular: atrial fibrillation, cerebrovascular accident, congestive heart failure, first degree AV block, hypertension, hypotension, jugular venous distension, migraine, myocardial infarction, postural hypotension, ventricular extrasystoles, supraventricular tachycardia, syncope, systolic ejection murmur, T wave abnormalities on ECG (flattening, inversion, nonspecific changes), venous insufficiency,

Digestive: abnormal liver function tests, anorexia, colitis, diarrhea, dry mouth, dyspepsia, dysphagia, flatulence, gastritis, gastrointestinal

hemorrhage, gingival hyperplasia, glossitis, hepatomegaly, increased appetite, nausea, mouth ulceration,

Endocrine: diabetes mellitus, thyroiditis,

Hemic and Lymphatic: anemia, ecchymoses, leukopenia, petechiae,

Metabolic and Nutritional: gout, hypokalemia, increased serum creatine kinase, increased nonprotein nitrogen, weight gain, weight loss,

Musculoskeletal: arthralgia, arthritis, leg cramps, myalgia, myasthenia, myositis, tenosynovitis,

Nervous: abnormal dreams, abnormal thinking and confusion, amnesia, anxiety, ataxia, cerebral ischemia, decreased libido, depression, hypesthesia, hypertonia, insomnia, nervousness, paresthesia, somnolence, tremor, vertigo,

Respiratory: asthma, dyspnea, end inspiratory wheeze and fine rales, epistaxis, increased cough, laryngitis, pharyngitis, pleural effusion, rhinitis, sinusitis

Skin and Appendages: acne, alopecia, dry skin, exfoliative dermatitis, fungal dermatitis, herpes simplex, herpes zoster, maculopapular rash, pruritus, pustular rash, skin discoloration, skin ulcer, sweating, urticaria,

Special senses: abnormal vision, amblyopia, blepharitis, conjunctivitis, ear pain, glaucoma, itchy eyes, keratoconjunctivitis, otitis media, retinal detachment, tinnitus, watery eyes, taste disturbance, temporary unilateral loss of vision, vitreous floater, watery eyes,

Urogenital: dysuria, hematuria, impotence, nocturia, urinary frequency, increased BUN and serum creatinine, vaginal hemorrhage, vaginitis

In addition to NISOCOR, there is extensive experience with the immediate release formulation of nisoldipine. Adverse events were generally similar to

those seen with NISOCOR. Unusual events observed with immediate release nisoldipine but not observed with NISOCOR, were one case each of angioedema and photosensitivity. Spontaneous reports from postmarketing experience with the immediate release formulation of nisoldipine have not revealed any additional adverse events not identified in the above listings.

OVERDOSAGE

There is no experience with nisoldipine overdosage. Generally, overdosage with other dihydropyridines leading to pronounced hypotension calls for active cardiovascular support including monitoring of cardiovascular and respiratory function, elevation of extremities, judicious use of calcium infusion, pressor agents and fluids. Clearance of nisoldipine would be expected to be slowed in patients with impaired liver function. Since nisoldipine is highly protein bound, dialysis is not likely to be of any benefit; however, plasmapheresis may be beneficial.

DOSAGE AND ADMINISTRATION

The dosage of NISOCOR must be adjusted to each patient's needs. Therapy usually should be initiated with 20 mg orally once daily, then increased by 10 mg per week or longer intervals, to attain adequate control of blood pressure. Usual maintenance dosage is 20 to 40 mg once daily. Blood pressure response increases over the 10 - 60 mg daily dose range but adverse event rates also increase. Doses beyond 60 mg once daily are not recommended. NISOCOR has been used safely with diuretics, ACE inhibitors, and beta-

blocking agents.

Patients over age 65, or patients with impaired liver function are expected to develop higher plasma concentrations of nisoldipine. Their blood pressure should be monitored closely during any dosage adjustment. A starting dose not exceeding 10 mg daily is recommended in these patient groups.

NISOCOR tablets should be administered orally once daily. Administration with a high fat meal can lead to excessive peak drug concentration and should be avoided. Grapefruit products should be avoided before and after dosing. NISOCOR is an extended release dosage form and tablets should be swallowed whole, not bitten or divided.

HOW SUPPLIED

NISOCOR extended release tablets are supplied as 10 mg, 20 mg, 30 mg, and 40 mg round film coated tablets. The different strengths can be identified as follows:

Strength	Color	Markings
10 mg	Oyster	891 on one side and MILES 10 on the other side.
20 mg	Yellow Cream	892 on one side and MILES 20 on the other side.
30 mg	Mustard	893 on one side and MILES 30 on the other side.
40 mg	Burnt Orange	894 on one side and MILES 40 on the other side.

NISOCOR Tablets are supplied in:

	Strength	NDC Code
Bottles of 30	10 mg	0026-8911-30
	20 mg	0026-8921-30
	30 mg	0026-8931-30
	40 mg	0026-8941-30
Bottles of 100	10 mg	0026-8911-51
	20 mg	0026-8921-51
	30 mg	0026-8931-51
	40 mg	0026-8941-51
Unit Dose Packages of 100	10 mg	0026-8911-48
	20 mg	0026-8921-48
	30 mg	0026-8931-48
	40 mg	0026-8941-48

The tablets should be protected from light and moisture and stored below 86°F (30°C). Dispense in tight, light-resistant containers.

Distributed by:

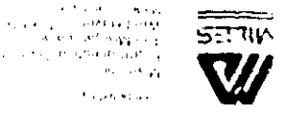
Miles Inc.

Pharmaceutical Division

400 Morgan Lane

West Haven, CT 06516 USA

Made in Germany



NDC

10 mg

For institutional use only

RECOMMENDED STORAGE

Nisocor

10 mg

NO SBA
WILL BE
PREPARED

Drug Interaction Studies

As noted above in the kinetic sections, drug interactions were separated into the following three groups

Effect of other drugs on pharmacokinetics of nisoldipine
Effect of other drugs on pharmacodynamics of nisoldipine.
Effect of nisoldipine on other drugs' pharmacokinetics/dynamics.

The first category has been described in the Pharmacokinetic Sections. For the remaining two limitations of individual studies, such as single/multiple doses, duration of treatment and use of control groups, have been described in tables summarizing the results. Studies 382 and 399 have been commented above in this section

CSO OVERVIEW

FEB 2 1995

CSO Application Overview

Application: NDA 20-356
Nisoldipine Coat Core Tablets

Sponsor: Miles Pharmaceuticals

NDA Receipt Date: April 1, 1993

NDA Resubmission Date: August 3, 1994

User Fee Goal Date: February 3, 1995

Date of Overview: November 28, 1994

Background

NDA 20-356 provides for the use of a sustained release (once-daily) formulation of nisoldipine in the treatment of hypertension. No formulation of nisoldipine is currently approved in the U.S.

A non-approval letter was issued on March 25, 1994, that listed deficiencies in the Chemistry, Pharmacology, and Clinical sections. The firm responded fully to this letter on August 3, 1994.

Review

Chemistry

Reviewer: Danute Cunningham

Reviews: 6/7/93 11/29/93 2/4/94 9/16/94

Ms. Cunningham's review of the application has been completed.

The trade name "Nisocor" has been approved by the Nomenclature Committee.

The facility inspection has been completed and was found to be satisfactory. We received a satisfactory response to our FUR on November 22, 1994.

The deficiencies outlined in the environmental assessment review were sent to the firm on October 27, 1994. The response from the firm has not been received yet.

Pharmacology

Reviewers: Xavier Joseph, D.V.M.
Sidney Stolzenberg, Ph.D.

Review: September 2, 1994

The reviewers comments have been incorporated into the draft labeling. The application went before the CAC, and the recommendations from that committee have also been incorporated into the labeling. The minutes of the CAC meeting have not been completed yet.

Biopharmaceutics

Reviewer: Patrick Marroum, Ph.D.

The Biopharm Day was held for this application, revisions have been made, and the draft is now under supervisory review.

Dr. Marroum has made a number of comments including extensive revisions of the labeling (see pp 13-16 of Dr. Marroum's review). The labeling recommendations have been incorporated into the draft package insert. He has also recommended that the dissolution specifications be revised as follows:

from:		to:	
	3 hours		3 hours
	6 hours		6 hours
	12 hours		12 hours

Statistical

Reviewer: Nancy Smith

Review (hypertension): 1/4/94

Dr. Smith has reviewed only the hypertension indication. There were no serious problems identified in the review.

Clinical

Reviewers: Shaw Chen, M.D., Ph.D. (Clinical Pharmacology): 2/16/94
Phil Dern, M.D. (Safety): 9/27/93
Cristobal Duarte, M.D. (hypertension): 8/4/93
Norman Stockbridge, M.D., Ph.D. 8/4/93

The reviewers recommend approval for the hypertension indication.

The final safety update is under review.

DSI Audits

Four of the seven requested DSI audits are completed. There have been no problems so far.

Labeling

Dr. Chen has provided a marked up copy of the package insert.

David Roeder
Consumer Safety Officer

dr/9 4-94/9-27-94/10-28-94/11-23-94/11-28-94

cc: NDA 20-356
HFD-110
HFD-111/DRoeder

MEDICAL OFFICE REVIEW

Preeter

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

Date: 12/22/94

From: Shaw T. Chen, M.D., Ph.D., Medical Group Leader, HFD-110
Through: Director, Division of Cardiorenal Drug Products, HFD-110 Typed by
To: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: NDA 20-356, Nisoldipine Core-Coat for Hypertension, Labeling

We did not get to see your memo in final prior to responding to your memo. There was one question that you asked that was not answered in our response of 12/17/94.

There were 2 "food studies" conducted by Miles. One of them was not the FDA "high fat" and studied only the 20 mg tablet (Study 666, it was more than average but not high) and in that study there was no evidence of "dose-dumping". The other study was conducted using the FDA "high fat" meal and in that study there was dose dumping. However, in this study (D92-045-02), 30 and 40 mg tabs were used and the 20 mg dose was not repeated. The food effect on the C_{max} was average 3 fold, and 5 of the 28 subjects had 5-11 fold changes. Thus either the fat content in food is important or dose-dumping by food may be dose-related.

Miles did conduct a pharmacokinetic/pharmacodynamic study, a review of that study was done by Dr. Marroum, in which they found that the pharmacodynamic effects (lowering of blood pressure) was a function of the log of the plasma concentration. So ten-fold changes in plasma concentration make a sizable difference, three-fold changes do not make a big difference. The slope of the concentration-response curve goes over 2 order of magnitude from beginning of effect to definitely over the maximum effect.

Although Dr. Marroum's review criticized the analysis of the study, the qualitative statements above are not materially affected by the quantitative problems that Dr. Marroum found.

So, it seems to us that there is considerable latitude that can be given with respect whether nisoldipine **must** be taken fasting. We do not think it must be taken fasting. To be silent about fasting or fed in the Dosage and Administration section is reasonable but since there are more than 5-fold changes in C_{Max} in 18% of subjects, the Dosage and Administration should probably say "..., preferably in a fasted state (see Clinical Pharmacology)". In Clinical Pharmacology the 11-fold increase in C_{Max} should be stated to be an upper limit when a High fat meal is ingested.

or even other alternatives as are discussed in the FM of 12/22/94. SZ

Shaw T. Chen, M.D., Ph.D.

cc:
ORIG: NDA- 20-356
HFD-110
HFD-110/CSO
HFD-110/SChen/12/22/94

10.3 Display and Analysis of All Adverse Events (Continued)

except for two episodes of moderate headache, one occurring in the fed state and the other in the fasted state. Overall, there was a similarity in adverse events reported in the fed and fasted condition. Headache was the most common adverse event (42.9% fasted, 35.7% fed) followed by dizziness (3.6% fasted and fed) and flushing (3.6% fasted and fed). There was no suggestion of a dose relationship in the incidence or adverse events for the Nisoldipine CC 30 mg and 40 mg doses either in the fed or the fasted state.

The relationship of C_{max} values to the adverse events, was evaluated. Geometric mean C_{max} concentrations at the 30 mg dose were 1.9 ng/ml (range: 0.7-4.3) in the fasted state and 4.5 ng/ml (range: 1.7-13.3) in the fed state. Geometric mean C_{max} concentrations at the 40 mg dose were 2.7 ng/ml (range: 1.2-8.1) in the fasted state and 7.5 ng/ml (range: 2.1-26.7) in the fed state (Section 13.9.5.2). Inspite of the much higher mean C_{max} concentrations, in the fed state as compared to the fasted state, there were no notable differences in the incidence rates or intensities of the adverse events in the fed state as compared to the fasted state (Table 1, Section 13.9.6).

Even though the overall incidence of adverse events was similar between the fed and fasted states, it was of interest to define whether the subjects with especially high fed/fasted C_{max} ratios showed a higher propensity for adverse events in the fed state as compared to the fasted state. Adverse events in subjects with a fed/fasted C_{max} ratio of ≥ 5 are shown below:

SUBJECT #	DOSE (mg)	C_{max} (ng/ml)		FED/FASTED C_{max} RATIO	ADVERSE EVENTS*	
		FASTED	FED		FASTED	FED
2103	30				NONE	NONE
2104	30				NONE	NONE
2110	30				HEADACHE	HEADACHE
2202	40				PERIPHERAL EDEMA HEADACHE	DIZZINESS PERIPHERAL EDEMA PALPITATION
2204	40				HEADACHE	NONE

* All adverse events in the fasted and fed states were mild in intensity.

DEC 19 1994

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

Date: 12/15/94

From: Shaw T. Chen, M.D., Ph.D., Medical Group Leader, HFD-110
Through: Director, Division of Cardiorenal Drug Products, HFD-110
To: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: NDA 20-356, Nisoldipine Core-Coat for Hypertension, Approvability

This is in response to the comments and questions raised in your draft memo of 12/13/94 regarding some labeling issues for the above application.

1. As stated in the Secondary Review (Dose Response), we also think that the effective dosage range is 20-60 mg/day and the recommended doses should include 60 mg. Usual maintenance doses in the labeling were changed to 20-40 mg in the Division's draft, which were concurred in your memo. We agree with your reasoning that dose titration should start at 20 mg.

3. We understand that the effects of food and grapefruit on the kinetics of nisoldipine CC are different. They were put together only in "Information for Patients", as they are both "food". The wording in your marked-up draft certainly described the problem much clearer.

4. A cleaned up draft of package insert with your mark-ups has been prepared.

Shaw T. Chen, M.D., Ph.D.

cc:
ORIG NDA 20-356
HFD-110
HFD-110/CSO
HFD-110/SChen/12/15/94

Kraeler

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

Date: 10/25/94

From: Shaw T. Chen, M.D., Ph.D., Medical Group Leader, HFD-110
Through: Director, Division of Cardiorenal Drug Products, HFD-110 NOV 21 1994
To: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: NDA 20-356, Nisoldipine Core-Coat for Hypertension, Approvability

OVERVIEW

This memorandum and the attached material constitute the Division's recommendation that NDA 20-356, Nisoldipine Core Coat (referred to as CC formulation) Tablets be approved for treatment of hypertension.

This package is being transmitted with a draft Summary Basis of Approval (SBA) prepared by the sponsor, which has not been edited by the Division but appears to be accurate in its contents to serve as one of the references for secondary/tertiary reviews of the application. In the draft SBA, any description or interpretation of the data different from that of this memo should be disregarded.

As one of the new team approaches, the primary medical review of the NDA were conducted in parallel by the following medical officers:

Clinical Pharmacology:	Dr. Chen
Hypertension -Efficacy:	Dr. Duarte
Hypertension -Safety:	Dr. Dern

Pharmacology sections of the application were also reviewed concurrently by two reviewers (Drs Joseph and Stolzenberg); a synoptic summary of all pharmacologic issues has been prepared by Dr. Joseph. As of the date of this memo, the chemistry, biopharmaceutical, pharmacological and statistical reviews have been completed. There are no major, unresolved preclinical issues which may affect the action recommended. Related labeling have been suitably edited.

Nisoldipine is a new calcium channel blocker of the dihydropyridine type and structurally related to nifedipine. It appears to be a less active inotrope than nifedipine *in vitro* but the two were not distinguishable in intact animals. There are no major efficacy or safety issues that should preclude the approvability of this drug for the hypertension.

The adverse experiences in the NDA have been amended with the First Safety Updates of 08/17/93. Selected major trials should be inspected before final approval of the application.

PRECLINICAL EVALUATIONS

Chemistry

There are no outstanding issues regarding the manufacturing and analytical controls. Final inspection will be scheduled.

Preclinical Pharmacology

Nisoldipine has been adequately characterized with respect to its preclinical pharmacokinetic and pharmacodynamic properties. There are no outstanding issues related to animal toxicity or carcinogenicity which may affect approvability of the drug.

Changes in proposed labeling, as recommended by the pharmacology reviewers, are summarized and commented below. They have been adopted with minor modification.

- Negative findings in carcinogenic studies should be qualified with the dosages studied, comparison with human dose should be based on both body weight and surface area calculations.
- Fetotoxicities in animals are suggestive, not conclusive. However, detailed description of the problematic monkey studies is not necessary. Again, basis of safety margin (toxic animal dose vs maximal human dose) should be specified (body weight and surface area).
- The pharmacology reviewers do not think malformation is increased in rabbits. Other recommendations related to fetotoxicity in rats/rabbits, sections of *Labor and Delivery*, and *Nursing Mothers* are all appropriate (Pharmacology Review, p 145).

CLINICAL PHARMACOLOGY

Pharmacokinetics/Pharmacodynamics

At the proposed dosages of 10-40 mgs, the **pharmacokinetic** profile of nisoldipine CC formulation supports a once-daily regimen. Compared with the immediate release (IR) form, availability of nisoldipine from the CC tablets was prolonged with lower C_{max} and higher AUC over 24 hrs. Bioavailability of nisoldipine CC was low for the unchanged drug but linear and dose-proportional over the range of 10-60 mg; it accumulates moderately after multiple oral dosing (7 days). While nisoldipine is extensively metabolized, the only active metabolite contributes about 10% of the pharmacologic effects.

The states of both hepatic and renal functions are potentially important for pharmacokinetics of the active drug, since nisoldipine is extensively metabolized and excretion of the metabolites is predominantly renal. Bioavailability of the parent drug was indeed increased by 4-5 fold in patients with hepatic failure, but changes in AUC and C_{max} due to various degree of renal impairment were only transient and diminished with multiple dosing. Plasma levels of nisoldipine were also higher in the elderly but dosage adjustment may not be required (see Efficacy -Hypertension). Nisoldipine metabolism probably involves P_{450} cytochrome system (as nifedipine), but no attempt to identify isozyme has been documented. Modest changes in bioavailability of CC nisoldipine were observed with

concomitant use of ranitidine (decreased 15-20%), cimetidine (increased 30-45%), quinidine (reduced by 25%), and propranolol ($t_{1/2}$ shorter by 20%). These interactions are probably of no significant clinical consequences, but labeling has been edited accordingly.

As noted in the Clinical Pharmacology and Biopharmaceutical Reviews, the problem of dose dumping when nisoldipine CC is administered in a non-fasted state or with grapefruit juice (see biopharm review of Study 770) can not be ignored. Nisoldipine CC should not be administered concomitantly with meal or grapefruit juice, but instead after overnight fast and 1-2 hours before breakfast. Appropriate instructions to avoid dose administration in such settings have been included in the labeling.

Nisoldipine is a vasodilating antihypertensive with **pharmacodynamic** activities similar to other approved calcium channel blockers. Cardiovascular and hemodynamic effects of nisoldipine have been fully well established. Correlation between nisoldipine dose, plasma level and blood pressure reduction was good over the recommended dosage range.

Nisoldipine has no appreciable inotropic effects, but its clinical advantages over nifedipine has not been demonstrated. Except for T wave changes mostly at high doses (see Safety), nisoldipine had minimal electrophysiology activities. There is some evidence that iv nisoldipine improves coronary blood flow, but its anti-ischemic effect was not established in clinical pharmacology studies.

Nisoldipine did not affect renal blood flow in kidney or liver, and has no significant pharmacologic effects on pulmonary or cardiovascular systems.

Biopharmaceutics

Issues raised in the Biopharmaceutical Review are commented as follows:

- The [redacted] metabolites and other metabolites) in special patient groups were [redacted]. Since no supportive clinical effects were observed in these patients which [redacted] required explanation, such data are not relevant for approval or [redacted] instruction.
- Nisoldipine is metabolized by the P-450 enzyme system, but the specific iso-enzyme involved has not been identified. While metabolism of nisoldipine is not expected to be significantly different from that of nifedipine, the study should be [redacted] approved, however.
- Inconsistent C_{max} (by 2 folds) obtained after a 30 mg dose in two small pharmacology studies may be related to the variability in dissolution and need further clarification, as different blood pressure reductions from placebo were also noted in the efficacy trials (see below). Since no efficacy/safety problems were attributed to this variation, approval is not affected.
- Assay validation was described in most of the studies, but missing in a few reports. It is reasonable to assume that same assay was used in all trials.
- Variations in dose proportionality in two Phase II studies were small and of no [redacted] significance.
- Pharmacokinetic and metabolism sections of the labeling have been [redacted] to comply with the recommendation of Biopharmaceutic Review.

CLINICAL: EFFICACY

Major Trials Supporting Approval

Nisoldipine has been evaluated as an antihypertensive treatment in 1,914 patients at dosages up to 80 mg/day. The efficacy data supporting approval were derived from the results of 7 double-blind, parallel placebo-controlled studies in 1,360 patients with hypertension, 886 of whom received nisoldipine. Long-term efficacy was supported by five open-label, 6-12 month follow-up of 584 patients (Studies X89-039, X90-019, X90-606, 678, 6000).

The primary efficacy endpoint in each of these studies was the change in supine diastolic blood pressure (SDBP) from baseline at the end of dosing interval (trough effect) after 4-9 weeks of therapy. Data from five of the seven studies should be considered for major evidence of efficacy.

Study	Doses, mg/day	Duration		Remarks
D88-085	10, 20, 40	QD	8 wks	fixed dose
D89-039	10-40	QD	9 wks	dose titrated per response
D90-019	30, 60	QD	6 wks	fixed dose (after week 1)
D89-085	20, 40	QD	8 wks	fixed dose (after week 1)
D90-606	10, 20, 5	QD	6 wks	fixed dose (after week 1)

In the last three trials listed above, high doses were phased in after one week of low dose treatment. It should be noted that in Study D89-039, another group of 15 patients were randomized to receive nisoldipine CC 80 mg qd, but the arm was terminated due to safety concerns before collection of efficacy data. Nisoldipine was also compared with verapamil 240 mg (additional group of 78 patients) in this parallel placebo controlled study.

The remaining two controlled studies may provide instructions on how to use nisoldipine in clinical setting, but are not very useful as primary evidence for assessing efficacy of nisoldipine vs placebo in general population who are not treated with other concurrent antihypertensive agents(s). Nisoldipine was evaluated in patients all receiving atenolol 50 mg qd as background therapy in one study (D89-029), and in the other (Study D90-029), lisinopril, hydrochlorothiazide (HCTZ) and placebo were compared in the presence of nisoldipine in all groups. Results of these studies will be commented in the Section of "Comparative Combination with other Antihypertensives".

Overall Treatment Effects vs Placebo

The primary efficacy data in Table 1 on Page 6 demonstrate that nisoldipine, at 20-60 mg qd, is a consistently and significantly more effective antihypertensive agent than placebo with adequate duration of activity for once daily treatment. At this dose range, the placebo subtracted net decreases in SDBP at trough ranged from 3.6 to 9.9 mmHg after 4 weeks of therapy. Treatment effects were less consistent for the 10 mg dose, but was superior to placebo in the larger trial (D90-606) with a decent drop in SDBP. Similar results were obtained for supine systolic blood pressures (SSBP) (same Table and Figure). Net pressures, excluding the smallest dose (D88-085), results not [illegible] in the [illegible]

The **percentages of responders** (SDBP reduction of ≥ 10 mmHg at trough or to ≤ 90 mmHg) are also summarized in Table 2 on next page. In the major trials, the response rates for 20-60 mg/day were in the range of 17-45% more than that of placebo. Again, less patients (around 17% over placebo) responded to 10 mg dose.

With respect to blood pressure changes and response rate, there were no significant differences between various statistical analyses, i.e. per-protocol or intent-to-treat (final visit).

Dose Response

The **dose-response** relationship, at trough, has been examined within the range of 10-60 mg once daily (Tables 1 & 2, in the associated Figure 1, % response curve was shifted on the dose axis for clarification). While dose of 10 mg/day was not consistently better than placebo as monotherapy, it is appears that blood pressure reduction may increase further at doses above 60 mg (but not for % responders). Although there are evidence from small pilot studies of hypertensive patients that dose-response for 30-90 mg/day was rather flat (Study D90-022, see Clinical Pharmacology Review), the finding should be accepted with reservation because dosages were forced-escalated rapidly in that study. There was a concern, also in the same early phase study, of asymptomatic T-wave changes at high doses (see Safety below). However, when doses were increased slowly as in efficacy trials, less patients reported the same abnormality. Besides, such ECG changes were common in hypertensive patients and their clinical meaning are not yet clear. Thus, effective doses of nisoldipine CC range from 20 to 60 mg once daily, with a weak support for the high-end limit.

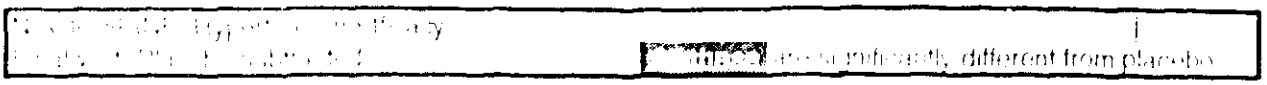
Correlation between blood nisoldipine level and blood pressure reduction has been demonstrated at trough in several clinical trials.

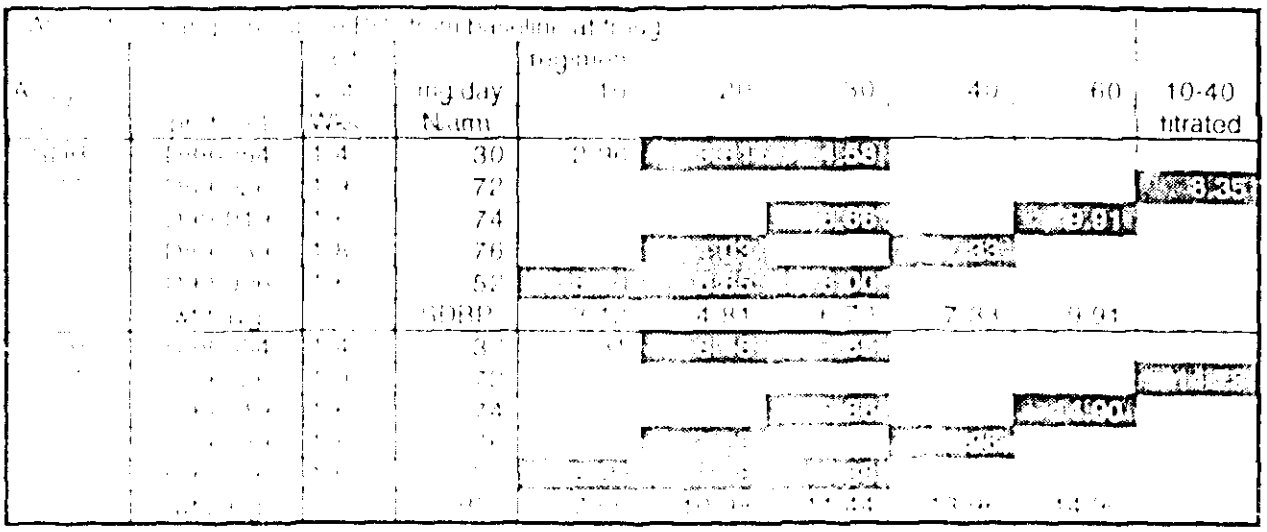
Time-Effect Relationship

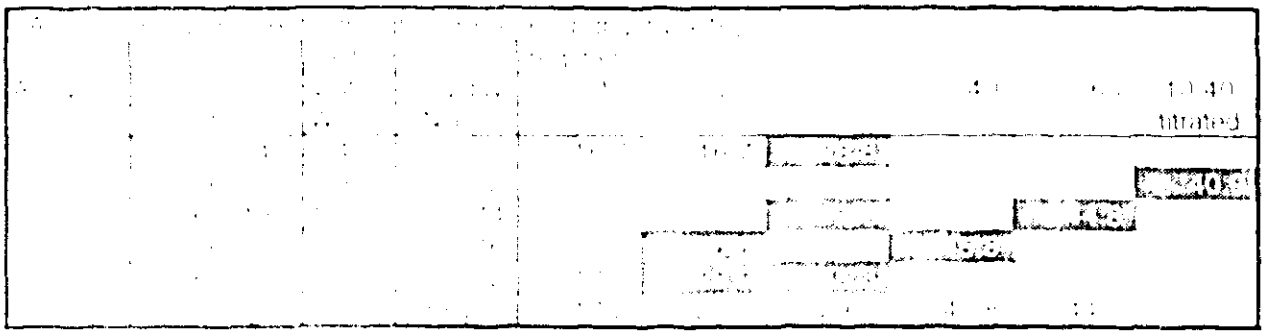
While only once-daily regimen was used in clinical trials and no direct comparison with other dosing schedule was performed, appropriate **dosing interval** for nisoldipine was established in the following studies:

	Studies	
Peak/Trough Effect	D88-054, D90-019, D89-039, D90-006	10-60 mg/day, QD
24 hour BPs	D88-054, D90-019, D89-039, D90-006	10-60 mg/day, QD

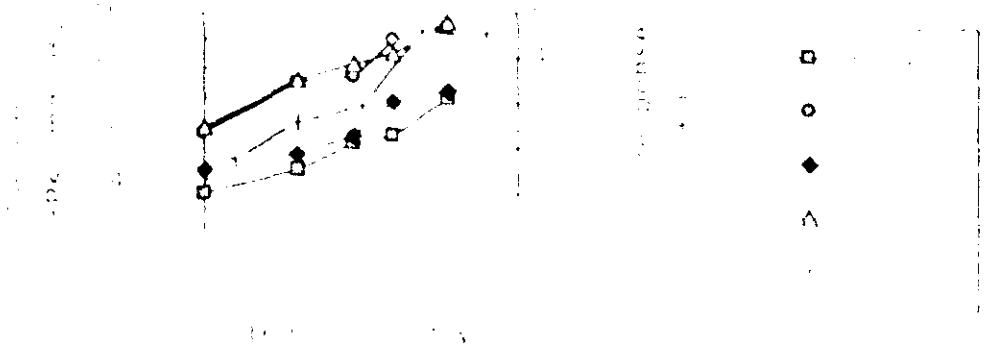
For the doses studied, the placebo-subtracted **trough-to-peak** ratios appeared to be acceptable, ranging from 70 to 100% for SDBP and SSBP.







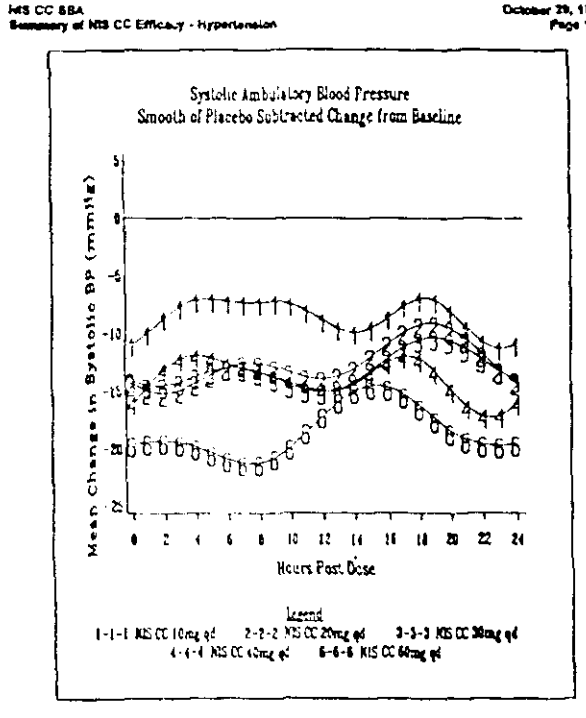
Dose-response

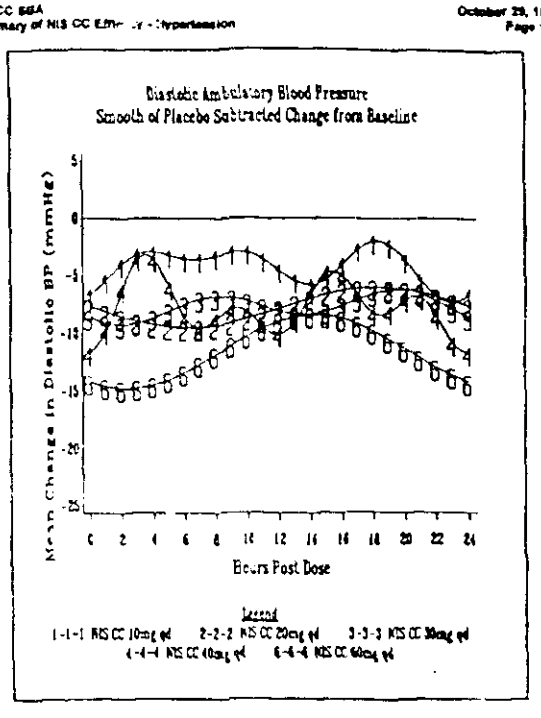


Total of 359 patients from the listed studies were pooled for analysis of **24 hours** ambulatory blood pressure change, the majority were white (65%) and male (60%). As shown by the 24-hour blood pressure curves, treatment effects of at least 5 mmHg reduction in SDBP over placebo were maintained during 24 hours for doses 20 mg and above (see Figure 2 below).

It is concluded that although other dosing schedules have not been evaluated, once-daily treatment with nisoldipine CC 20-60 mg per day appeared to be adequate to cover the dosing period.

Figure 2





Responses in Demographic Groups

In post hoc analyses (see draft SBA), nisoldipine appeared to be equally effective in **male/female**, with a slightly more pronounced dose-response relationship in the male patients. Despite increased bioavailability in the **elderly**, dose-response was less evident in such patients and there were no significant differences in blood pressure reduction between groups of age below and above 65 years. While blood pressure responses to nisoldipine were numerically greater in **black** than in white patients, such retrospective finding should not be described in the labeling or used in promotion. Not surprisingly, response to nisoldipine was greater in patients with higher **baseline blood pressure**, with a more significant dose-response relation.

Comparison/Combination with other Antihypertensives

Nisoldipine was compared or combined with the following antihypertensive agents in 3 controlled trials.

Comparison groups			Studies
nisoldipine+atenolol	vs	placebo+atenolol	D89-029
nisoldipine+lisinopril	vs	nisoldipine+placebo	D90-029
nisoldipine+HCTZ	vs	nisoldipine+placebo	D90-029

While results of the first study listed above indicated that concomitant atenolol did not affect the efficacy of nisoldipine CC, the second study suggested that some patients may have further response when a diuretic or ACE inhibitor is added. Up to one third of all patients received additional antihypertensive therapies in long-term, uncontrolled, follow-up studies. Overall, not much weight can be placed on these active controlled data for the efficacy claim.

Long-Term Efficacy

Long-term effectiveness of nisoldipine was evaluated in five open-label studies up to one year. Without a placebo control, reductions in supine blood pressures from baseline appeared to be sustained in more than 80% of 554 patients treated with nisoldipine for 6 months to one year.

CLINICAL: SAFETY

Database

The database appeared to be adequate for analysis of the safety of nisoldipine, which includes cumulative experiences of nearly 4,200 hypertensive patients as of 10/29/93. Of 1,466 patients# (921 in US trials) who were treated with nisoldipine Coat-Care formulation, about 55% were exposed for at least 2 months (approx. 33% over 6 months) and a great majority were on doses of 20 to 60 mg.

The majority of comparative experience was based on the results of 6 randomized, double-blind, parallel group, placebo-controlled trials of 4-9 week duration (all U.S. double-blind controlled trials, see list in Efficacy Section)', which included 678 patients on nisoldipine and 280 patients on placebo.

Data from the first 120-day Safety Update were not incorporated into the following summary, however, the numbers added were small and did not change the safety profile of the drug (see Reviews of Safety Update by Dr. Dern).

Comparative Experiences

There were no surprising findings in the safety profile of nisoldipine CC used in hypertensive patients. Overall frequency and rates of some specific adverse clinical experiences and abnormal laboratory findings were more common in nisoldipine than placebo treated patients, but none were serious or unexpectedly frequent.

The percentage of nisoldipine-treated patients reporting an **adverse event** in controlled trials (68%, N=678) was higher than that in the placebo group (53%, N=280). Among the adverse experiences, the following were more common for nisoldipine than placebo with incidence of $\geq$ 3%:

ADE	Nisoldipine(%) N=678	Placebo(%) N=280
peripheral edema	22	10
headache	22	15
dizziness	5	4
asthenia	4	4
vasodilatation	4	2
palpitation	3	1

From draft SBA. Different numbers of trials and patients exposed were given in Integrated Summary and Draft SBA, the later is probably more updated. Some calculations shown below were based on data from Integrated Summary of Safety.

' Foreign data also included some placebo-controlled safety experiences (D90-006). However, a great majority of the non-U.S. studies were not controlled and thus were not considered in comparative experiences. Nisoldipine was used in all treatment groups in one U.S. study (D90-029), but results of that study were not excluded from the comparative analysis.

As expected, the most commonly reported adverse events were related to nisoldipine's vasodilating effects. Most were mild and infrequently leading to withdraw.

While the overall frequency of adverse experiences was not affected significantly by patient **age, sex, race,** or **body weight** in the controlled trials, some minor differences in the incidence of a few adverse events may change the tolerability of nisoldipine in demographic subgroups. Headache appeared to be less common in the elderly, which would be surprising if the adverse event is pharmacokinetics-related (see Clinical Pharmacology). Peripheral edema was more frequent in female (as suspected with other dihydropyridine agent) and heavier patients (>185 lbs), but the sex difference was only seen in foreign studies, not in the U.S. controlled trials. Compared to blacks, incidences of headache and edema were slightly higher in whites.

The percentage of nisoldipine-treated patients **withdrawn due to adverse clinical experiences** was higher than that of placebo (7.8 vs 3.2%, U.S. controlled trials only), and dose-related (up from 5.4% at 10 mg to 10.9% at 60mg). The reasons for withdrawal were mostly related to nisoldipine's pharmacologic activities and within the scope of common adverse experiences:

Reasons for withdrawal	Nisoldipine(%) N=678	Placebo(%) N=280
headache	3.8	0.4
peripheral edema	2.9	0.4
vasodilatation	1.5	0.0
nausea	0.9	0.0
palpitation	0.9	0.0
dizziness	0.7	0.4

There were 2 **deaths** (car accident and metastatic prostate cancer) in nisoldipine-treated patients in controlled trials (non-U.S. studies only), compared with two deaths in the placebo groups. None were considered drug-related. Other **serious events** occurred with similar frequencies in nisoldipine (2.0%) and placebo group (1.5%). However, they are dose-related (increased from 0.2% at 20 mg to 4.5% at 60 mg) and half of these serious events led to withdrawal.

Abnormal **laboratory** findings in controlled trials were both rare and no different between nisoldipine and placebo groups. In U.S. controlled trials, incidences of such reports were in the range of 0-4% for hematology, 0-2% for hepatic functions, 0-1% for creatinine/BUN and 0-6% for lipid profile. While there were more reports of increased fasting blood glucose in nisoldipine than in placebo group from non-U.S. controlled trial, the phenomenon was not dose-related, not seen in the U.S., and cases of increases to above 140 mg/dl were not more frequent (than placebo).

Overall Exposures

In general, the overall safety experiences in all patients treated with nisoldipine in all clinical trials were not unexpectedly different from those described above for controlled trials.

Approximately 62% of all patients reported one or more **adverse events**, while the incidence was lower in European trials (43% vs 75% in U.S.). Prominent complaints were similar to those in controlled trials (e.g., 18% headache, 15% edema in U.S. Trial).

In all clinical trials, about 9% were **withdrawn** due to adverse experiences (10% in U.S. studies), not too different from that of comparative experiences. There was no additional death other than those noted above in the comparative experiences. Accumulative experiences of abnormal **laboratory** findings in all U.S. studies were also similar to that in the controlled trials.

Class Specific Safety Issues

As noted above, adverse experiences relatively specific to calcium channel blockers were also reported in nisoldipine-treated patients. They were not more severe or frequent than in other members of the class; however, the database may not be large enough for detecting some of the rare events.

Clinically significant **hypotension** and other related adverse experiences in nisoldipine treated patients were not common and rarely resulted in withdrawal. As described earlier in time-effect relationship, at doses that produced adequate trough blood pressure reduction, average peak response was not excessive. In all U.S. and non-U.S. trials, symptomatic hypotension occurred in about 0.2% and syncope was reported in 0.1% of patients. **Orthostatic** hypotension and related symptoms were slightly more common, reported in approx. 0.4% of patients on nisoldipine monotherapy, but very few were considered serious and required intervention. Overall, hypotensive reactions to nisoldipine treatment did not appear to be more frequent or severe than those with other calcium channel blockers. Appropriate warning related to hypotensive reaction is included in the draft labeling.

Like other dihydropyridines, nisoldipine has no significant effects on electrophysiology or **cardiac rhythms**. Tachycardia was reported in about 1% of all nisoldipine-treated patients, with a small mean changes in heart rate (< 1bpm, placebo-adjusted). It is most likely due to hypotensive reflex, rarely led to withdrawal, and occurred equally frequently in placebo groups. Some minor changes in ECG (QRS) were noted more frequently than that in placebo group, especially in patients receiving concomitant atenolol, but the magnitudes were of no clinical meaning. While dose (plasma level) and magnitude of BP reduction related **T-wave flattening/inversions** were observed in a small phase II study (D90-022) with rapid dose escalation, such ECG finding was less clearly related to dose and not as frequent (similar to that in placebo groups) in a retrospective but blinded analysis of data from three efficacy trials. It is somewhat re-assuring that no angina or thallium test-documented ischemia were reported in any of the patients with T-wave changes in Study D90-022.

Limited experiences with concomitant use of nisoldipine and atenolol, lisinopril or HCTZ have not identified any unexpected safety or tolerability issue. Combination of nisoldipine with HCTZ or lisinopril may increase slightly the incidences of asymptomatic hypotension, tachycardia, palpitation and dizziness. Rebound hypertension after withdrawal has not been a problem with other dihydropyridines and was not significant in a small pharmacodynamic study for nisoldipine.

PEDIATRIC/GERIATRIC USE

There are no clinical trials assessing the efficacy or safety of nisoldipine in pediatric patients, either completed or in progress. The sponsor claimed that the drug has little potential for use in children and thus did not commit to any study in hypertensive children.

Efficacy and safety of nisoldipine as treatment for hypertension in the elderly (65 year and older) are not significantly different from that of general patient population.

DRAFT LABELING

The draft labeling submitted by the sponsor has been edited.

CONCLUSIONS

Nisoldipine appeared to be an effective and safe treatment for hypertension.

While there is little doubt that nisoldipine at 20-60 mg/day is an antihypertensive more effective than placebo, it is not certain if the entire useful dose range has been fully explored. Nisoldipine should be started at 10 mg once daily and titrated slowly (e.g. every few weeks) to 60 mg according to blood pressure response.

It is recommended that nisoldipine be approved with the edited draft labeling.

Shaw T. Chen, M.D.,Ph.D.

cc:
ORIG NDA- 20-356
HFD-110
HFD-110/CSO
HFD-110/SChen/10/26/94

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

Date: 03/11/94

From: Shaw T. Chen, M.D., Ph.D., Medical Group Leader, HFD-110
Through: Director, Division of Cardiorenal Drug Products, HFD-110
To: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: NDA 20-356, Nisoldipine Core-Coat for Hypertension and Angina
Summary of Efficacy Data

INTRODUCTION

This memorandum will only summarize results from major controlled efficacy trials intended to support the above application, so preliminary decisions regarding approvability of the two indications can be made and deficiencies in efficacy data delineated. For the approvable claim, dose-response, safety and other labeling related issues are not covered here and will be described in a more comprehensive secondary review later.

As of the date of this memo, all primary medical reviews have been completed for both indications. While the hypertension claim is clearly approvable, as described below, the support

addition, Pharmacology Review is pending CAC deliberation on some animal tumorigenicity findings, which may be a potential approvability/labeling issue. Most importantly, several serious deficiencies in chemistry section of the NDA have been identified in the Chemistry Review, which may be ground for non-approval since they have not been corrected despite repeated request to do so by the Division.

This package is being transmitted with a draft Summary Basis of Approval (SBA) prepared by the sponsor, which has not been edited by the Division but appears to be accurate in its contents to serve as one of the references for secondary/tertiary reviews of the application. In the draft SBA, any description or interpretation of the data different from that of this memo should be disregarded.

HYPERTENSION

Major Trials Supporting Approval

Nisoldipine has been evaluated as an antihypertensive treatment in 1,914 patients at dosages up to 80 mg day. The efficacy data supporting approval were derived from the results of 7 double blind, parallel placebo controlled studies in 1,360 patients with hypertension, 886 of whom received nisoldipine. Long-term efficacy was supported by five open-label, 6-12 month follow up of 554 patients (Studies X89-039, X90-019, X90-006, 675, 690).

The primary efficacy endpoint in each of these studies was the change in supine diastolic blood pressure (SDBP) from baseline at the end of dosing interval (trough effect) after 4-9 weeks of therapy. Data from five of the seven studies should be considered for major evidence of efficacy:

Study	Doses, mg day		Duration	Remarks
D88-054	10, 20, 30	QD	4 wks	fixed dose
D89-026	10-40	QD	9 wks	dose titrated per response
D89-029	5, 10	QD	6 wks	fixed dose (after week 1)
D89-039	2, 4, 8	QD	8 wks	fixed dose (after week 1)
D90-006	5, 20, 80	QD	6 wks	fixed dose (after week 1)

In the last three trials listed above, high doses were reached after one week of low dose administration. It should be noted that in Study D89-039, another group of 15 patients were randomized to receive nisoldipine CC 80 mg qd, but the arm was terminated due to safety concerns before collection of efficacy data. Nisoldipine was also compared with verapamil 240 mg (additional group of 78 patients) in this parallel placebo controlled study.

The remaining two controlled studies may provide instructions on how to use nisoldipine in a practical setting, but are not very useful as primary evidence for assessing efficacy of nisoldipine vs placebo in general population who are not treated with other concurrent antihypertensive agents): in one (Study D89-029) nisoldipine was evaluated in patients all receiving atenolol 50 mg qd as background therapy and the other (Study D90-029) compared lisinopril, hydrochlorothiazide (HCTZ) and placebo in the presence of nisoldipine in all groups. Results of these studies will be commented in the Section of "Comparison/Combination with other Antihypertensives" in the final secondary review.

Overall Treatment Effects vs Placebo

The **primary efficacy** data in the table on Page 4 demonstrate that nisoldipine, at 20-60 mg qd, is a consistently and significantly more effective antihypertensive agent than placebo with adequate duration of activity for once daily treatment. At this dose range,

the placebo subtracted net decreases in SDBP at trough ranged from 5.6 to 9.9 mmHg after 4-9 weeks of therapy. Treatment effects were less consistent for the 10 mg dose, but was superior to placebo in the larger trial (D90-089) with decent drop in SDBP. Similar results were obtained for supine systolic blood pressures (SSBP) (same Table) and standing blood pressures (excluding the smallest trial, D88-054, results not shown in this memo).

The **percentages of responders** (SDBP reduction of $\geq$ 10 mmHg at trough or to $\leq$ 90 mmHg) are also summarized in the table on next page. In the major trials, the response rates for 20-60 mg/day were in the range of 17-45% more than that of placebo. Again, less patients (around 17% over placebo) responded to 10 mg dose.

With respect to blood pressure changes and response rate, there were no significant differences between various statistical analyses, i.e. per-protocol or intent-to-treat (final visit).

[illegible] these are significantly different from placebo

Change in supine DBP from baseline at trough

Analysis	Treatment regimen: mg/day [titrated]	Last Visit Wk	N/arm	10	20	30	40	60	10-40 titrated
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	3.61	4.53			
[illegible]	[illegible]	[illegible]	[illegible]						8.35
	[illegible]	[illegible]	74			6.66		9.91	
	[illegible]	[illegible]	75		4.03		7.33		
	[illegible]	[illegible]	82	3.21	6.65	8.00			
	[illegible]			3.12	4.81	6.70	7.33	9.91	
[illegible]	[illegible]	[illegible]	[illegible]	5.31	8.43	7.65			
[illegible]	[illegible]	[illegible]	74						14.73
	[illegible]	[illegible]	73			9.86		14.90	
	[illegible]	[illegible]	[illegible]		7.33		13.96		
	[illegible]	[illegible]	82	8.84	17.78	15.88			
	[illegible]			[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	

Analysis	Treatment regimen: mg/day [titrated]	Last Visit Wk	N/arm	10	20	30	40	60	10-40 titrated
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]			35.5			
	[illegible]	[illegible]	74						40.9
	[illegible]	[illegible]	73			40.7		44.8	
	[illegible]	[illegible]	[illegible]		28.5		45.8		
	[illegible]	[illegible]	82		29.9	40.3			
	[illegible]			[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	

Pages 5-9

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CONFIDENTIAL

COMMERCIAL

INFORMATION

CONCLUSIONS

Based on the efficacy data, nisoldipine appeared to be approvable for treatment of hypertension.

Shaw T. Chen, M.D.,Ph.D.

cc:
ORIG: NDA 20-356
HFD-110
HFD-110/CSO
HFD-110/Dern/Duarte/Stockbridge
HFD-110/SChen/03-11-94

NDA REVIEW
Clinical Pharmacology

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
CDER/ODE-I/DIV CARDIO-RENAL DRUGS

FEB 16 1994

NDA	20-356
Name of Drug	Nisoldipine, Coat Core Tabs
Sponsor	Miles
Indications	Hypertension
Submitted	03/31/93
Received	04/05/93
Assigned	04/09/93
Reviewed	08/25/93
Review Completed	02/14/94
Reviewer	Shaw T. Chen, M.D.,Ph.D.

Overview of NDA (Clinical Pharmacology)

Nisoldipine is a calcium channel blocker of dihydropyridine derivative type being developed for the treatment of hypertension. In this initial application, approvals of a sustained release formulation (coat-core) for both indications are requested. As a part of new parallel, team approach, this medical review covers only the areas related to clinical pharmacology.

The sections on clinical pharmacology (Section 8.1 of NDA) contain data of 17 studies, involving 393 patients/subjects, on sustained released formulation (coat-core, referred to as CC in this memo). Of these, 183 participated in 6 U.S. studies. In addition, the submission also includes results of 47 studies on the immediate release preparation (IR), most of which were conducted in foreign countries. Except for three small studies (total 12 normal subjects, copies of publications only), full reports of all studies listed in Section 8.12 were submitted.

Additional pharmacokinetic and bioavailability data were presented in Section 6 of the NDA, which include 129 foreign studies on IR or other non-CC formulations not repeated in the clinical sections (Section 8, as noted above). These studies will be reviewed by the biopharmaceutical group of the Agency and not commented in this report.

TABLE of CONTENTS

PHARMACOKINETICS

Formulation Design

The new coat-core formulation, which has a slowly-dissolving coat and an immediate release core, was designed based on the observation that nisoldipine is readily absorbed in the upper gastrointestinal tract but cleared by a first pass rapidly and a marked decrease in the rate of absorption but lower first pass metabolism in the colon.

Absorption/Disposition

The absorption of radiolabeled oral nisoldipine solution was rapid (T_{max} 0.42 hr) and extensive (87%). Despite efficient absorption, absolute bioavailability of the parent drug was only 8.4%, due to a high first pass effect (Study 400). Measured by iv infusion, nisoldipine has a volume of distribution about 2.3 to 3.4 L/kg (Study 330).

In **single dose** studies (Studies 102-106), oral doses of nisoldipine 6-20 µg/kg administered as IR capsules resulted in C_{max} of 2.7-19.3 µg/L within 30 minutes[1] after dosing. Nisoldipine was detectable (>1 µg/L) 4 hrs later only in the high dose groups (12 µg/kg and above). Compared with the IR formulation, nisoldipine administered in the **controlled release** (CR) forms had reduced C_{max}, greater AUC, prolonged mean residence time (MRT), duration of plasma concentration above 0.3 ng/ml and T_{max} (6 subjects, Study 632):

Formulation	C_{max} ng/ml	AUC_{inf} g.hr/L	MRT hrs	$T_{>0.3}$ hrs	T_{max} hrs
CR (E 029)	0.86	57.2	21.2	23.9	12
IR	1.55	31.3	4.2	4.4	2
CR/IR (95% Confidence)	0.55 (0.34-0.90)	1.82 (1.30-2.56)	5.02 (3.48-7.23)	5.42 (3.48-8.45)	

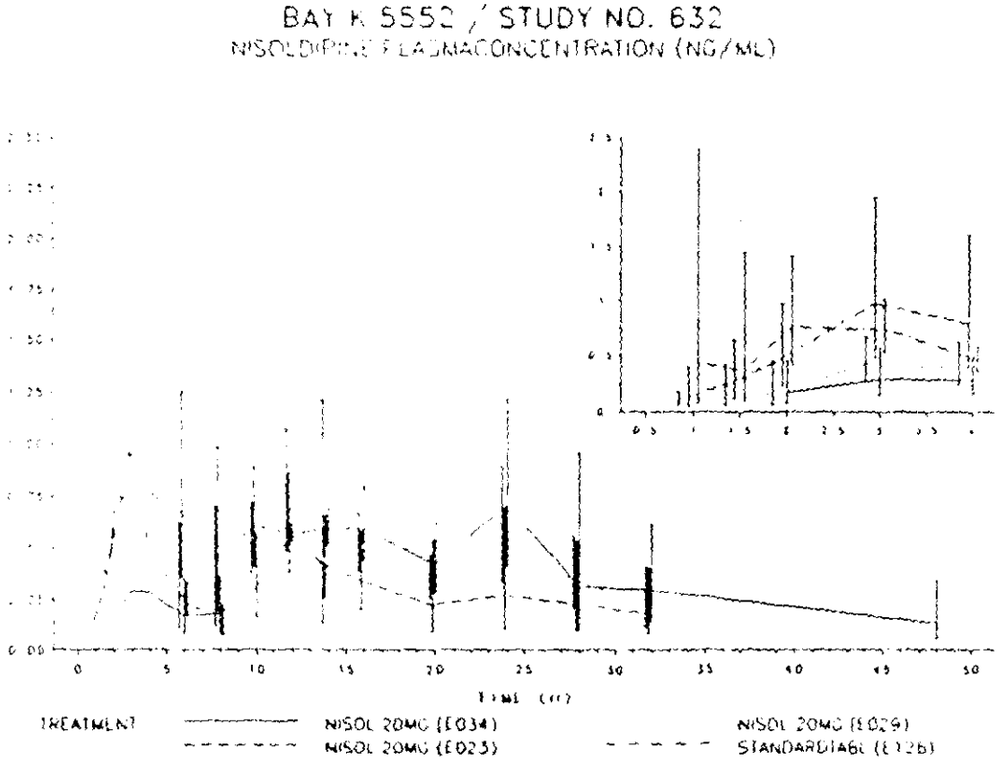
Time-courses of plasma concentrations after administration of the three CR formulations were compared with that of the IR dose in Figure 1. Based on these characteristics, the CR formulation E 029 was chosen from the three studied for further development. While the bioavailability was relatively higher than the IR form, absolute bioavailability of the CR formulation was still low (5.5%) in another study (Study 627).

Bioavailabilities (C_{max} and AUCs) of nisoldipine were dose-proportional for both the IR (at 2.5-20 mg, Studies 125, 339, D85-024-01) and CC (at 10-60 mg) formulation (Study D91-035)[2].

[1] There may be greater variability in T_{max}, which was longer (mean 2 hrs) for IR nisoldipine in another study (632).

[2] The 10 mg dose may not be as linear as other doses, but the deviation is non-significant

Figure 1


BAY K 5552 / STUDY NO. 632
NISOLDIPINE PLASMACONCENTRATION (NG/ML)
0.00
0
5
10
15
20
25
30
35
40
45
50
TIME (HR)
TREATMENT
NISOL 20MG (E034)
NISOL 20MG (E029)
NISOL 20MG (E023)
STANDARDTABL (E126)

Although there is no evidence of accumulation with **multiple doses** of IR formulation (10 mg bid), bioavailability of CC nisoldipine increased moderately after 7 days of daily 20 mg dosing (see Table below, Study 045). At these doses, fluctuations of plasma nisoldipine levels were lower for the CC formulation (113% vs 434% for IR).

Formulation		C_{max} ng/ml	$AUC_{norm.}$ g*h/L	$T_{c\&l3}$ hrs	T_{max} hrs
CC	Day 1	0.84	40.3	14.9	11.1
	Day 7	1.09	58.9	28.4	9.2
IR	Day 1	2.18	40.8	10.8	2.4
	Day 7	1.95	40.3	11.6	2.3
CC/IR	Day 7	0.56	1.46	2.45	
(95% Confidence)		(0.47-0.66)	(1.27-1.69)	(1.95-3.08)	

While the $AUC_{0-\infty}$ remain similar regardless of fed or fasted state, C_{max} was increased by 38-48% when 20 mg of CC nisoldipine was administered together with or 1 hour after breakfast (Study 666). **Dose-dumping by food** of the CC formulation (administered within 5 minutes after completion of a meal) was even more pronounced with 30 mg (65-236% increase in C_{max}, 11-42% decrease in AUC) and 40 mg (92-292% increases in C_{max}, 7-39% decrease in AUC) doses in Study D92-045-02 (range given are 90% CI's). This food effect was both dose and formulation dependent, since C_{max} and $AUC_{0-\infty}$ were increased only modestly (31 and 28% respectively) by food for the IR formulation (20mg, Study 323), and may have clinical implications in patients usually older than those in the food studies (see Comments on Individual Studies below).

At the concentrations 20 times or higher than that observed in kinetic studies, nisoldipine and its enantiomers are >99% **protein bound**. Partitioning between plasma and blood or erythrocytes were moderate (0.7 blood to plasma, 0.3 erythrocyte to plasma). (Study 339, Ref. 3 (PB19611))

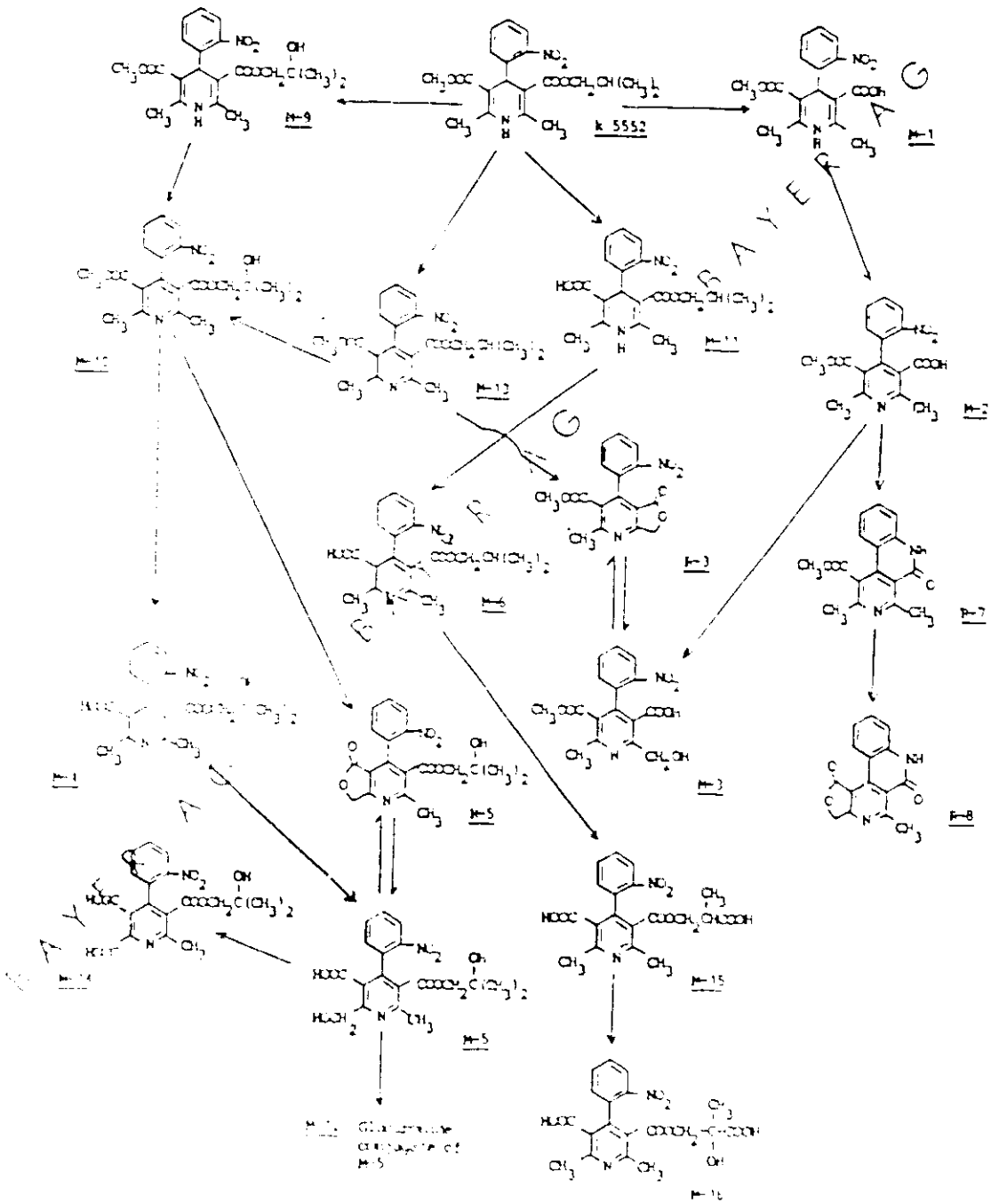
Metabolism

Following oral administration of nisoldipine solution, eleven metabolites, but no unchanged parent drug, was detected in the urine (Study 400, Reference 15 (PB 16676)). The proposed biotransformation pathways of nisoldipine in humans are described in Fig 2. In man, hydroxylation of the isobutyl ester appears to be the major product. Of the three metabolites detected in human plasma (M9, M10, M13), M-10 (Bay r 9590) is most abundant (approx 10-20 times of parent drug) and M-9 (Bay r 9425) is the only one with biological activity (about 1/10 of parent drug, Study P10108947). The latter is present at approximately the same[3], dose-proportional concentration as the parent drug (Studies 339, D85-024-01).

[3] In another study (No. 325), a metabolite was present at higher C_{max} and AUC (2-4 times of parent drug) which was later identified as sum of M-9 + M-10 due to nonspecific assay (12/20/93 Amendment).

Figure 2

PROPOSED METABOLIC PATHWAYS OF NISOLDIPINE



Excretion

Excretion of nisoldipine metabolites was predominantly renal (70% urine, 12% feces with oral dose), and varies little with route of administration (80% urine, 14% feces with iv dose) (Study 400). Terminal elimination half lives of iv nisoldipine, as measured in 4 healthy subjects in Study 330, were 11-12 hrs, with systemic clearance of 544-768 ml/hr*kg. Nisoldipine is probably not dialyzable (Study 311).

Pharmacokinetics of Enantiomers

The bioavailability of nisoldipine is dominated by the (+) enantiomer, when administered as racemic mixture with only the (+) enantiomer labeled with radioisotope (Ref 43), which is also the one with higher cardiovascular activity (in vitro studies). Since all clinical pharmacology studies and efficacy/safety trials were conducted using the racemic mixture, the kinetic parameters for the two enantiomers have no practical relevancy.

Pharmacokinetics in Disease States

Pharmacokinetics of nisoldipine CC formulation in **hypertensive patients** was examined in two double blind, parallel placebo controlled studies (D90-022 & D88-059). While the CC formulation was not compared with the IR form in any hypertensive groups, kinetics of nisoldipine in both normotensive and hypertensive elderly subjects were described in a third study (Study 712).

In study D90-022, 23 patients (5 placebo, 18 nisoldipine) were randomized and treated for 22 days with nisoldipine dosage increased every 4 days[3] from 30 to 60 mg and every 7 days from 90 to 120 mg. As summarized below, the kinetic parameters measured at the end of dosing periods were dose-proportional at 30-90 mg, non-linearity of 120 mg was dismissed for small number of patients (3). In this study, higher C_{max} of nisoldipine in hypertensive patients were reached at approximately the same T_{max} as that in normotensive subjects, but cross-study comparison is difficult to interpret.

Dose (mg qd)	C_{max} ng/ml	AUC_{0-24} ng.hr/ml	T_{max} hrs
30	4.79±0.68	74.28± 7.96	7.22±0.93
60	8.48±0.81	129.76±12.74	9.08±1.97
90	13.02±1.20	199.31±16.45	6.78±2.30
120	14.92±2.01	226.58±12.41	4.00±1.00

[3] [illegible] of CC nisoldipine increased moderately from Day 1 to Day 7 in a study on normotensive [illegible] (see above), 4 days may not be sufficient for reaching steady state for the two lower doses in this [illegible]. [illegible] were separated by washout period (see comments [illegible] study.

In the second study in hypertensive patients (D88-059), total of 69 patients were randomized in parallel to receive placebo, 5, 10, 20, or 30 mg CC nisoldipine for 7 days. Plasma nisoldipine concentrations were dose-proportional within the range of these doses both on Day 1 and Day 7 (with 40-70% increases from Day 1 to Day 7). The least square mean kinetic parameters at the end of 7 day dosing period are shown below:

Dose (mg qd)	C_{max} ng/ml	AUC_{0-24} ng.hr/ml	T_{max} hrs
5	0.65	8.39	9.21
10	1.02	16.17	4.79
20	2.13	28.24	3.65
30	2.79	40.34	3.73

At least in the elderly (>65), bioavailability of nisoldipine CC was not influenced by the elevated blood pressure in hypertensive patients (Study 712):

Day 7	Normotensive	Hypertensive
C_{max} (ng/ml)	2.61	2.59
AUC_{0-24} (ng*h/ml)	36.9	38.7

Bioavailability of CC nisoldipine in **angina patients** were also dose-proportional over the range of 20-60 mg (45 day treatment, long-term extension of Study D90-015). Data from 21 angina patients with mean age of 62 (range 43-77) resembled that of the elderly hypertensive patients (>65 year old, Study 712, see above).

Dose (mg qd)	No Patients	C_{max} ng/ml	AUC_{0-24} ng.hr/ml	T_{max} hrs
20	10	2.70	41.9	7.8
40	9	6.27	92.3	6.4
60	2	9.59	102.1	3.0

Despite the fact that metabolites of nisoldipine are eliminated predominantly by renal excretion, patients with various degrees of **renal impairment** (but not requiring dialysis) had similar pharmacokinetic parameters for the parent drug when given nisoldipine 20 mg in CC formulation for 7 day. (Study D92-001).

Cr clearance	>90	61-90	30-60	<30
Day 8, Mean (ml/min/1.73m^2)				
C_{max} (ng/ml)	3.33	3.21	2.54	2.97
AUC_{0-24} (ng*h/ml)	40.0	50.3	38.4	43.8

However, pharmacokinetic effects of renal function on nisoldipine were slightly greater (although not significantly) with initial doses (Day 1)

Cr clearance	>90	61-90	30-60	<30
Day 1, Mean (ml/min/1.73m^2)				
C_{max} (ng/ml)	1.77	2.37	2.71	2.57
$AUC_{(0,24}$ (ng*h/ml)	25.3	32.8	36.1	32.1

Thus the accumulations of nisoldipine with multiple doses appeared to be blunted somewhat by the decrease in renal function (Day 8 vs Day1). As expected, elimination of some metabolites was more affected by renal function than the parent drug, but like nisoldipine, the differences were noted mostly on the first day and diminished over multiple dosing (Study D92-001). While there is less pharmacodynamic concern because the only active metabolite (Bay r 9425) was the least influenced by renal impairment, it not clear whether substantial increases (with the initial doses) of other more abundant metabolites by renal impairment has any long-term toxic effect. In this study the group with moderate renal impairment (Cr Cl 30-60 ml/min/1.73m^2) had higher mean age (63 vs 52-54 for other groups), but there is no clear trend suggesting that the conclusion was affected by such difference in age. Administered in the IR form, nisoldipine bioavailability was increased by about 40% when creatinine clearance decreased from >80 to <25 ml/min (Study 364). While nisoldipine was not detectable in the dialysate, thus probably not removed by hemodialysis, bioavailability of nisoldipine IR in patients on dialysis resembled that in subjects of normal renal function in the same study.

Hepatic failure increases bioavailability of nisoldipine administered as CC tablets. Compared with normal subjects, cirrhotic patients who received 10 mg nisoldipine CC had higher C_{max} and $AUC_{0,24}$ (4-5 folds, Study D90-026). There appeared to be less effect of liver function on the bioavailability of nisoldipine in IR formulation, however, the studies were not controlled and the results were variable (Studies 294, 452).

Demographics Differences in Pharmacokinetics

While there is no significant difference with acute dosing (1 day), bioavailability of nisoldipine, administered as CC 20 mg daily for one week, was increased in the elderly normotensive subjects (65-84 years old), as compared with that in the younger subjects (Study 712):

Day 7	Young	Elderly
C_{max} (ng/ml)	1.41	2.61
$AUC_{0,24}$ (ng*h/ml)	14.7	36.9

Bioavailability of IR nisoldipine was also higher (2-3 folds) in the elderly, but little accumulation was observed after one week dosing (Study 503).

Drug Interactions

The **effects of other drugs on nisoldipine pharmacokinetics** were evaluated in the following studies

Immediate Release Formulation

Second Drug, Doses	Nisoldipine Doses	Interaction Observed	Study
Ranitidine, 300 mg qd X 3 days vs placebo	IR 20 mg one dose on Day 3	nisoldipine AUC increased 24%	385
Cimetidine, 400 mg one dose then 200 mg tid X 3 doses vs no treatment	oral & iv solution 10 mg po, 0.374 mg iv one dose each period	bioavailability of oral nisoldipine increased by 48%	399
Propranolol, 40 mg one dose vs placebo	IR 20 mg one dose	nisoldipine AUC, C_{max} increased by 30% & 57%	417

CC Formulation

Second Drug, Doses	Nisoldipine Doses	Interaction Observed	Study
Ranitidine, 150 mg bid X 6 days vs placebo	CC 20 mg one dose on Day 5	nisoldipine AUC, C_{max} decreased by 15-20%	738
Cimetidine 400 mg bid X 6 days vs placebo	CC 20 mg one dose on Day 5	nisoldipine AUC, C_{max} increased by 30-45% t_{max} decreased by 4 hrs	738
Propranolol, 40 mg tid X 5 days vs no treatment	CC 20 mg qd X 5 days	nisoldipine AUC, C_{max} unchanged, $t_{1/2}$ decreased by propranolol (by 20%)	704
Quinidine, 648 mg b.i.d x 2 doses vs no treatment	CC 20 mg qd x 1 dose	nisoldipine AUC reduced by 25%, C_{max} unchanged but at lower t_{max}	703

Bioequivalence of Various Formulations

Bioequivalence between clinical trial and market tablets and between various coat-core dosage forms have been determined in several studies. Details of the results are referred to the Biopharmaceutical Review

Comments on Individual Pharmacokinetic Studies

In general, pharmacokinetic studies on nisoldipine, either CC or IR formulation, were well designed and properly conducted. Compared with the translated foreign reports, the U.S. studies (study numbers beginning with D) were better documented and probably more reliable. Minor deficiencies for a few studies and interpretation of the data different from that of sponsor have been pointed out, mostly as footnotes, in previous sections. Other than that, there are no study defects collectively serious enough to invalidate the conclusion on kinetic behavior of CC nisoldipine. In addition to the following comments, which are arranged below in the order of Study numbers, further detailed reviews on individual kinetic studies are referred to the Biopharmaceutical Review.

Studies 102-106

Based on a summary report (no detailed protocol), there is nothing remarkable in these placebo controlled, dose-escalating, kinetic studies using IR formulation in 12 normal subjects.

Studies 125, 339

These were placebo controlled, double blind, randomized, 3-4 sequence crossover studies on dose proportionality of nisoldipine IR and metabolites in 12 normal subjects (6 actually treated in Study 125). While treatments were separated by at least one week in Study 339, they were given in three successive days in Study 125. Thus results of the latter study may be confounded by residual effect of preceding dose.

In vitro protein binding of nisoldipine at 20 ng/ml was also performed in Study 339, using each subject's pre-dose plasma.

Studies 294/452

These are two pharmacokinetic studies of IR nisoldipine and its metabolites in cirrhotic patients. The results were translated from foreign reports and no detailed protocols were submitted with the NDA. Subjects in Study 294 were hypertensive but the blood pressures were not described in Study 452. Neither was controlled with subjects of normal liver function.

Study 311

This is a kinetic and tolerability study in patients requiring regular hemodialysis. Seven patients were treated with nisoldipine 10 mg once daily, with dosage titrated up to 40 mg per day, for 3 months. The treatment effects on blood pressure and tolerability were not controlled.

Study 323

This is a food-pharmacokinetic study of IR nisoldipine 20 mg dose. Eight healthy, young male subjects were randomized to receive a single dose of the study drug either in a fasted (1.5 hrs pre meal) or fed (20 minutes after start of meal) state and crossed over to the opposite food state two weeks later. Small differences in heart rate response in fasted/fed states were noted (increased 10 bpm vs increase 15 bpm), but probably of no clinical significance.

Studies 330, 400

These were two uncontrolled bioavailability studies using oral/iv solution in small groups of healthy volunteers. A few subjects were excluded from data analysis due to radioisotope overdose in 2 of 12 subjects in Study 400 and leakage of infusion system in 2 of 6 subjects in Study 330. Washout interval was adequate for the crossover study (28 days, Study 400)

Study 364

This is also a kinetic study in renally impaired patients (Cr Clearance >80 ml/min, <25 ml/min or uremic on dialysis, 29 patients), but treated with single dose IR formulation only.
The sponsor concluded that a 40% increase in nisoldipine bioavailability in renal failure was not a significant effect (see discussion above).

Study 400 (PB 14514, Biotransformation)

This was part of study 400 (see above for comments on bioavailability study) which described metabolite profile in human urine after oral and iv administration of radioisotope labeled nisoldipine. Eleven metabolites were identified, but no test of biologic activity was performed and plasma metabolite profile was not investigated.

Study 563

This is a pharmacokinetic study of IR nisoldipine in 21 normotensive subjects, 9 were of 20-28 years of age and 12 were older than 65. All were treated with IR nisoldipine 10 mg for 8 days.

Study 632

Three controlled release formulations (CR) were evaluated in this non-blind, randomized, crossover, single dose study in six healthy volunteers. The kinetics of CR formulation was compared with that of IR nisoldipine administered in the fourth period to all subjects, the wash-out between treatment periods was 6 days. Treatments were given in fasted states.

Study 637

Bioavailability of a controlled release formulation (CR E 029) selected from Study 632 was evaluated further, relative to an iv solution of nisoldipine, in this non-blind, crossover study in 12 normal subjects. Washout out between treatments was adequate (6 days) and study drugs were administered in fasted states.

Study 645

Steady state pharmacokinetics of nisoldipine CC 20 mg was compared with that of IR formulation given as 10 mg bid in this non-blind crossover study. The study drugs were given in fasted state for one week and the treatments were separated by 7-day washout periods. Total of 18 male subjects were treated and included in the data analysis.

Study 666

This is an open-label study of food effect on pharmacokinetics of nisoldipine 20 mg CC tablets. Twelve young male subjects (24-33 years old) were randomized to receive the a single dose of the study drug in fasted (2 hrs pre-meal), together with (within 7 minutes after start of meal), or one hour after an American breakfast. All subjects also receive another dose together with a Continental dinner in a non-randomized fourth period. Treatment periods were separated by one week washouts. Moderate increase in C_{max} were observed when nisoldipine 20 mg CC was given in fed states, but no difference in clinical adverse events was noted.

Study 712

Bioavailabilities of nisoldipine CC in normotensive and hypertensive elderly (65 and older) patients were compared in this open-label, non-randomized study. In addition, influence of age on pharmacokinetic was also assessed in young and old normal subjects in the same study. Total of 58 subjects (46 young-elderly normal subjects, 12 hypertensive elderly) were treated with daily doses of 20 mg for 7 days.

Study D85-024-01

This is another dose-proportionality study using an ascending-dose, uncontrolled, non-crossover design. Single doses of nisoldipine IR 2.5-20 mg were administered at weekly intervals to twenty subjects. Kinetics of major metabolites in plasma were also described.

Study D88-059

This is a double blind, placebo-controlled, multiple-dose, kinetic and tolerability study of nisoldipine CC in 69 hypertensive patients. After a 3-week placebo run-in, patients with SDBP of 95-115 mmHg were randomized to receive placebo, 5, 10, 20 or 30 mg nisoldipine CC in five parallel groups and treated for 7 days. The study design was better than that of higher dose range (Day 0-42) above and dose proportionality in hypertensive patients was clear over the range of doses in this study. However, quite a few patients were excluded from the analyses for various reasons, which included 6 disqualified for low baseline SDBP (but included in the kinetic data), 10 without steady state blood samples and data of another 10 were considered invalid because these patients took nisoldipine instead of placebo on Day 0. One additional drop-out was due to blood drawing discomfort.

Study D90-015

Data from long term extension of this angina efficacy trial were cited for bioavailability of nisoldipine CC formulation in patients with angina. See Medical Review on angina efficacy by Dr. Stockbridge for description of trial design and execution.

Study D90-022

This is a double blind, placebo controlled multiple ascending dose, kinetic and tolerability study of nisoldipine CC in 23 hypertensive patients. Patients were randomized after a 3-week placebo run-in to [illegible] were increased every 4 days for the two low dose [illegible] (5, 10, 20, 60 mg)

and every 7 days for higher doses (90 & 120 mg) without washout intervals (see above). The concern was that 4 day may not be adequate for reaching steady state and the carry over effects from previous lower dose can not be excluded. Thus the results of this study should be accepted with reservation and dose proportionality is better supported by a parallel design (Study D88-059, but covered a lower dose range, see next).

Study D90-026

Pharmacokinetics of nisoldipine CC in patients with cirrhosis was evaluated and compared with that in normal subjects in this study. Sixteen, 8 in each group with well matched age, sex and weight, were treated with 10 mg daily for 7 days. Accumulation was observed in both groups.

Stucy D91-035

This was an open-label, four period crossover study on dose-proportionality of CC nisoldipine. Twenty-four healthy male subjects were randomized to one of four treatment sequences with single doses of 10-60 mgs. Treatments were separated by 7 day wash-outs and given in a fasted state.

Study D92-001

This is a unblinded pharmacokinetic study in patients with renal impairment, using the group with creatinine clearance of >90 ml min^{-1} 1.73m^{-2} as the control for renal function. In addition to the patent drug kinetics of three major metabolites were also described. Of the 46 patients were treated with nisoldipine CC 20 mg once daily for 7 doses, 42 had valid kinetic data. While the overall recruitment had encountered some difficulty, two subjects were excluded because of "over-enrollment" in the mild renal impairment group. Two additional patients had indeterminate renal function and were excluded from the kinetic analysis. As noted in the above, age was not well matched in the four treatment groups, but no trend was discernible.

Study D92-045-02

This is the most important pharmacokinetic study on food effects, which described a significant dose dumping phenomenon when nisoldipine CC was administered immediately (within 5 minutes) after a 20 minutes standard high-fat breakfast (see above for description of pharmacokinetic results). This was a randomized, open-label, two-way crossover study. Twenty-eight healthy, young (19-42 years), male subjects with near ideal weights were randomized to receive a single 30 or 40 mg CC tablet in a fasted (4 hour pre-meal) or fed state. After a one-week washout period, all subjects were crossed over at the same dose level to the opposite food state. Except for a higher heart rate in the fed state, the sponsor claimed that despite the marked change in kinetic behavior of nisoldipine CC, there were no pharmacodynamic consequences of dose dumping by food. In 5 subjects with fed/fasted C_{max} ratio of ≥ 5, more complaints of headache were seen in the fasted state (5 vs 1) and one subjects reported dizziness under fed state only.

Study PB 16626

This was a study on biotransformation of nisoldipine in several animal species and in human. Metabolites detected in urine, but not in plasma, were identified. There were no qualitative differences in biotransformation of nisoldipine in rats, dogs, monkeys or man. Biological activities of metabolites were not described.

Study PB 19611

This is an *in vitro* human plasma protein binding and erythrocyte-plasma partitioning study of nisoldipine over the concentration range of 0.1 to 10 µg/ml. Results of protein-binding in this ^{14}C study were consistent with that of Study 339.

Study P1010947

Results of this foreign study (possibly animal) was cited in the NDA as the basis of the biological activities of various nisoldipine metabolites. However, there is no synopsis of the study anywhere in the application and despite repeated request by the Agency, the sponsor has had difficulty locating and submitting a full report as of the date of this memo.

Study (Reference 43)

This is a manuscript published by Frost et al in *Dose-Response Relationships of Drugs*, no original data were submitted

Drug Interaction Studies

The results of studies on drug-interactions were separated into the following three groups.

Effect of other drugs on pharmacokinetics of nisoldipine
Effect of other drugs on pharmacodynamics of nisoldipine
Effect of nisoldipine on other drugs' pharmacokinetics/dynamics.

Only the first category was included in the Pharmacokinetic Sections, the remaining two will be discussed in the pharmacodynamic sections below. Limitations of individual studies, such as single/multiple doses, duration of treatment and use of control groups, have been described in tables summarizing the results

Summary of Pharmacokinetic Issues

In summary, pharmacokinetic properties of nisoldipine administered as IR or CC formulations have been studied adequately and well-described in the submission. Bioavailability of nisoldipine CC was low due to high first pass effect and appeared to be linearly proportional to doses of 5-90 mg. Accumulation after 7 days of administration was modest and products of extensive metabolism were excreted renally. Relative to the young subjects, nisoldipine was slightly more bioavailable in the elderly after one week dosing. Bioavailability of nisoldipine was not significantly different in patients with hypertension, angina or renal impairment (except for the initial doses for the latest), but were markedly increased in hepatic failure subjects. Pharmacokinetics of nisoldipine was affected by concomitant administration of cimetidine, ranitidine and quinidine, other drug interactions may be possible through high degree of protein binding (> 99%).

While the kinetic data of CC nisoldipine support a longer dosing interval than the IR form, clinical effectiveness of once-daily dosing depends on the correlation with pharmacodynamic and efficacy findings. It should also be noted that the problem of dose-dumping when nisoldipine CC is administered in a non-fasted state can not be ignored and must be addressed appropriately in the labeling. This phenomenon is more pronounced than with the IR formulation and not exactly unexpected since similar problem has been observed in another approved drug formulated identically and developed by the same sponsor.

The differences in food effects between 20 mg (Study 666) and 30, 40 mg doses (Study D92-045-02) of nisoldipine CC may be due to variations in relative timings of drug administration and meal ingestion both in fasted and fed states. Both the absolute C_{ss} and increase relative to fasted state were greatest when nisoldipine CC 30-40 mg was administered immediately after completion of a meal and compared to a prolonged fasted state (for additional 4 hrs post dose) (Study D92-045-02). While this sustained "fasted state" (4 hrs post dose) may not be a realistic simulation of large efficacy trials or practical settings, the dramatic increase in plasma nisoldipine concentration by food may result in excessive hypotension because nisoldipine plasma levels in efficacy trials resembled that of fasted state and there is a good kinetic-dynamic correlation (see below). Thus nisoldipine CC should not be administered concomitantly with meal, but instead after overnight fast and 1-2 hours before breakfast. Appropriate instruction to avoid dose administration in a fed state should be included in the labeling.

PHARMACODYNAMICS

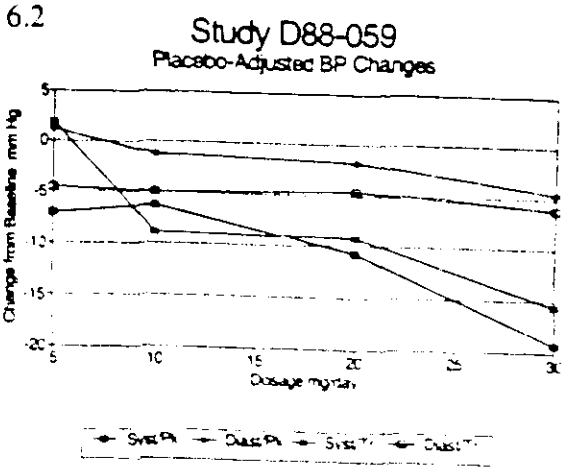
Pharmacodynamic data of nisoldipine are described in three groups in this review: **Principal cardiovascular effects**, **Non-cardiovascular pharmacological activities** and **Drug interactions**. Integrated summary of pharmacodynamic data as presented in the application and reviewed below were in general based on U.S. studies. Results of small scale foreign studies, mostly open and uncontrolled, were only commented briefly whenever appropriate. It should be noted that most dynamic data were obtained from studies using iv or IR oral form, only the effects on blood pressure and heart rate have been evaluated with the CC formulation. Issues related to **tolerability** of nisoldipine CC formulation in kinetic studies were also summarized at the end of this section.

Principal Cardiovascular Effects

In contrast with what the sponsor has stated in the NDA, **blood pressure and heart rate** changes were not small nor inconsistent in normal subjects treated with CC nisoldipine. Approximately 4-8 hours after a single dose of 10-60 mg, mean supine diastolic pressure decreased by a maximum of 5-7 mm Hg and mean heart rate increased by 6-11 bpm in a uncontrolled study (D91-035, Appendix 13.9.10). Similar but slightly greater effects (8-9 mm Hg) on SDBP were also seen with a single 60 mg dose in Study D90-020, before adjusted for a placebo response of about 4 mm Hg drop (Study Report Section 13.9.4). Mean Heart rate increases in the same study were 10-15 bpm (vs 2-4 bpm for placebo). The IR formulation produced more rapid but comparable degrees of heart rate and SDBP changes (Study 125, single dose). Relative to the normal subjects, the blood pressure responses of hypertensive patients were certainly more notable with multiple dosing at 5-40 mg/day (about 10 mm Hg drop in SDBP over placebo with IR form, Study 372). *Placebo-subtracted* blood pressure reductions in patients with mild to moderate hypertension were dose-related from 5 to 30 mg of CC nisoldipine (especially in systolic pressures, Study D88-059):

Dose	Changes in Systolic/Diastolic BPs, mm Hg, Day 7	
mg/d	8 hrs post dose	24 hrs post dose
5	- 7.1 / + 1.2	+ 1.9 / - 4.6
10	- 6.3 / - 1.2	- 8.8 / - 4.9
20	-10.7 / - 1.7	- 9.2 / - 4.6
30	-19.2 / - 4.6	-15.6 / - 6.2

On the right dose-response plot, 8 hrs data were used for peak effects. However, due to a large placebo response at 6-10 hours post dose in this study, the SDBP changes at 8 hrs did not appear to be the peak effects and greater antihypertensive activity was noted around 14 hours (-2.8 to -8.6 mm Hg over placebo, Study Report Section 13.9.4).



However, such dose-response relationship was not observed at higher dose range (30-90 mg/day) in another study of hypertensive patients (see Table on next page, D90-022)[5].

Study D90-022

Dose	Changes in Diastolic BPs, Placebo adjusted, mm Hg	
mg/d	8 hrs post dose	24 hrs post dose
30	- 9.4	- 6.4
60	- 9.2	- 6.7
90	- 9.1	- 6.6

Responses to 120 mg was distinctively higher (19.0 and 9.3 mm Hg at 8 and 24 hrs post dose) in the same study, but there were too few patients (3) received this maximum dose. As noted before, the dosages were forced-escalated in rapid sequence and not evaluated in parallel groups in this study, thus made the interpretation difficult.

Changes of heart rate in hypertensive patients due to nisoldipine appeared to be less consistent than that observed in normal subjects (Study 372, IR form). As noted in the NDA, heart rate increases were in the range of 5-10 bpm over baseline values for all nisoldipine groups in Study D90-022. However, it is not apparent whether the changes were caused by nisoldipine since the variation of heart rate in the placebo patients was in the same range and there is no clear trend in the difference between groups that suggests a treatment effect. Heart rate changes were not documented in Study D88-059.

With intravenous administration of nisoldipine at 3-13 µg/kg, changes in blood pressures (decreased by 11-16 mm Hg) and heart rates (increased by 10-30%) were dose-related and slightly more pronounced than that observed in the oral studies. Most of such studies were single-dose, uncontrolled, in small number of patients with underlying coronary artery disease (some were receiving concurrent beta blockers during measurements, Studies 344, 560, Ref 1-5)[6]. Directly compared with diltiazem in a published report (Ref 4)[6], nisoldipine (6 µg/kg) increased heart rate (by 14 bpm) but not diltiazem (500 µg/kg). Both drugs reduced peak systolic pressure by 24-28%.

[5] The sponsor claimed that blood pressure reductions not adjusted for placebo response were dose-related (see Summary of Clinical Pharmacology, Section 8.14), but admitted that the relationship did not exist when placebo responses were subtracted (see Study Report, Section 10.6).

[6] For reference cited, see List and Location of Publications, Section 8.14.3, NDA Vol 116. It should be noted that Ref 1-4 were reported by the same group of investigators.

Effects of nisoldipine on **other hemodynamic parameters** were summarized from 9 studies, three with full reports submitted with the NDA and 6 from published literatures (Ref 1-6, reprints only, no original data). Of these, hemodynamic effects were measured with intravenous administration of nisoldipine in 7 reports and with oral dosing in 2. In a double-blind, placebo controlled study (Study 372), nisoldipine IR titrated from 5 to 40 mg/day (bid) every 2 weeks were effective in blood pressure reduction for 36 hypertensive patients (see above) and reduced peripheral resistance as expected from a calcium channel blocker. Compared to placebo, cardiac index was increased either at rest or during exercise, but the effect of oral nisoldipine on LVEF was probably not meaningful. Changes in hemodynamic parameters as measured by radionuclide techniques are summarized below:

Changes/Baseline.	Resting		Exercise	
Parameters	Nisoldipine	Placebo	Nisoldipine	Placebo
Heart rate (bpm)	- 3.1/77	- 1.9/76	- 0.5/129	- 3.4/131
SBP (mm Hg)	- 24.1/172	- 8.5/171	- 20.4/219	- 13.3/217
DBP (mm Hg)	- 20.1/110	- 8.3/109	- 13.8/123	- 3.5/116
Total Peripheral Resistance (dynes/s/cm^5)	-302/1435	-132/1519	-165/953	- 32/940
Cardiac Index (L/min/m^2)	+ 0.31/4.1	+ 0.05/3.9	+ 0.37/7.4	- 0.01/7.4
Stroke Index (ml/m^2)	+ 5.6/53	+ 2.4/52	+ 2.7/58	+ 1.3/56
LVEF	+ 0.04/0.63	0.01/0.67	+ 0.02/0.75	+ 0.00/0.75
Double Product	- 23.9/134	- 9.2/130	- 27.4/283	- 24.7/288

A single oral dose of 10 or 20 mg IR nisoldipine decreased systemic vascular resistance significantly, but maintained same cardiac output, as measured invasively in 12 patients undergoing electrophysiology evaluations (Study 178, uncontrolled).

	Baseline	2 hrs post dose	
Heart rate	64	73	bpm
arterial pressures, Syst/Diast	130/76	120/69	mm Hg
Right Atrial Pressure	4	2	mm Hg
Pulmonary Arterial Pressures S/D	19/8	20/8	mm Hg
Pulm Cap Wedge Pressure	8	7	mm Hg
Cardiac Index	3.75	3.71	L/min/m^2
Systemic Resistance	1307	1038	dynes/s/cm^5
Stroke Index	50	53	ml/m^2

In another single oral dose study (Ref 6)[illegible], nisoldipine 10 mg attenuated both the drop in exercise LVEF in patients with coronary disease (-10% after vs -18% before treatment) and the decrease in LVEF during cold pressor test in patients with signs of ischemia but normal coronary artery .

Acute effects of intravenous nisoldipine on invasive hemodynamic factors are fairly consistent in Study 560 and several published reports (Ref 1-5)[illegible]. As noted above, most of these studies were single dose (3-13 µg/kg)[illegible], uncontrolled, in small number of patients with coronary artery disease (some were on concurrent beta blockers during measurements). Similar to that observed with oral administration, total systemic vascular resistance was decreased (by about 30-35%) with iv nisoldipine in all studies described (Ref 1-3)[illegible]. Nisoldipine iv also decreased intra-aortic and left ventricular systolic pressure (by 15-30%), but not LV end diastolic pressure in these studies. Nisoldipine did not appear to have negative inotropic activities. Ejection fraction and stroke volume were increased by 16 and 21-24%, respectively, and cardiac out was up 26-36%.

The hemodynamic effects of nisoldipine on **coronary blood flow** have been examined in several uncontrolled studies using intravenous nisoldipine. In patients with coronary artery disease, nisoldipine administered intravenously increased coronary blood flow, but only in normal vessels or in area with collateral supplies (by 38-52%, 6 patients on background therapy of atenolol, Study 324). It also dilated the coronary arteries for up to 15 minutes after a 0.5 or 1.0 mg dose infused over 4 minutes, which was not plasma level related, however (Study 560). In several published reports, nisoldipine increased coronary blood flow by 17-50% (Ref 1-4)[illegible], thus reduced the calculated coronary vascular resistance by 46-50%. Myocardial oxygen consumption changes (decreased by 4-8%) were not significant in these studies. Similar to nicardipine, nisoldipine reduced myocardial lactate production in patients evaluated for angina (Ref 5)[illegible].

In patients with stable angina, nisoldipine IR given as a single oral dose of 20 mg increase exercise tolerance by 2[illegible] watts-min as compared with 10 watts-min for placebo (Study 126). Nisoldipine also reduced ST segment change more than that by placebo at the maximum stress (0.8 mm vs. 1 mm) in the same study. Exercise duration was increased in a published report (Ref 6)[illegible], but the study was not controlled and no data on ST change were described. These **anti-ischemic effects**, however, were not observed in another single oral dose study of similar design (Study [illegible]).

In an **electrophysiology** study using oral nisoldipine (baseline controlled, Study 178), sinus cycle length and AH intervals were shortened by 12% and 9%, respectively, about 120 minutes after a 10 or 20 mg single dose. Other ECG changes (QTc, QRS, HV, corrected sinus recovery time, effective ventricular and atrial refractory periods) were not significant. In another similar study using the same IR oral doses (Study 135), there were no significant changes in intra-cardiac conduction times and the effects of nisoldipine on sinus node were also mild (except for sinus node recovery time and S-A conduction time, which were decreased by 11-15%, all other changes were less than 10%). However, electrophysiological measurements may not be performed at time of maximum effect (within 20-45 minutes of dosing) in the latter study.

[illegible] were [illegible] after a 4.5 µg/kg bolus followed by [illegible] infusion of

With intravenous administration at single dose of 1.5 µg/kg, similar shortening of sinus period were noted at 15-40 minutes post dose, without other changes in refractory periods (Study 144). The effect of iv nisoldipine on sinus cycle length was blunted some what (to 7%) if the patients were pre-treated with beta-blockers (Study 257).

Some ECG changes, usually seen as T-wave flattening or inversion, appeared to associated with nisoldipine in several tolerability studies. They occurred most frequently (65% of 17 treated patients vs none of placebo) in Study D90-022, a 3-week double blind, placebo-controlled, ascending dose study in hypertensive patients. The incidence is probably dose and plasma concentration related, since in this study, it increased from 22% to 80% with doses up from 30 mg to 120 mg, observed at peak but not trough drug level in some patients, and reversible when study drug was discontinued. Also, patients who reported the ECG changes had significantly higher C_{max} than those who did not (Study Report Section 10.8.4). These reactions were correlated with blood pressure drop and hemodynamic effects have been proposed as the mechanism in literatures for this and other calcium blockers. Higher rate of T-wave ECG changes in Study D90-022 was attributed to rapid and forced dose escalation. This issue has been discussed in more details by Dr. Dern in his Medical Review.

Based on one double-blind, crossover study comparing **neurohormonal effects** of nisoldipine IR 10 mg, nifedipine 20 mg, and placebo (Study 199), nisoldipine has no significant effects on the renin-angiotensin-aldosterone system or noradrenaline concentration in normal volunteers.

Nisoldipine IR at 20 mg daily increased **regional blood flows** in liver and kidney, but the effect was transient and no different from that of placebo by Day 4 (Ref 7)[redacted]. Similar time course of effects was noted for some renal function parameters (GFR and sodium excretion) in the same study. Nisoldipine, administered intravenously, increased blood flow in forearm more than nitrendipine or nifedipine (Ref 8)[redacted].

Non-cardiovascular Pharmacological Activities

Nisoldipine IR given at 10 mg bid for 4 weeks has no significant effect on **thyroid** function tests or **prolactin** level in young and healthy subjects (placebo controlled, Study 670). Similar to verapamil (160 mg), nisoldipine inhibit **platelet aggregation** after 4 days of treatment with 20 mg daily. However, nisoldipine plasma concentration at such dose did not have the same anti-platelet effect *in vitro* and unlike verapamil, nisoldipine did not displace yohimbine from specific platelet binding sites (Ref 9)[redacted]. The sponsor claimed that nisoldipine, at 5-20 mg daily for 4 weeks, induced favorable changes (increased HDL and apoprotein A) in plasma **lipoproteins** in a group of 15 hypertensive patients (Ref 10)[redacted]. The study, however, had no concurrent controls. In a double-blind, placebo-controlled 3-week trial in normal subjects (Study 479), there were no significant differences between placebo and nisoldipine (10 mg bid) in **psychomotor** performance tests.

[redacted] Publication, Section 8.15.3, NDA Vol 116, Ref [redacted]
[redacted] Publication, 3.4 of the [redacted], Page 08 15 0000010, Section 8.15.3

Drug Interactions

Pharmacodynamic interaction studies were conducted for nisoldipine and the following drugs:

a. Effect of Other Drugs on Nisoldipine Pharmacodynamics

Immediate Release Formulation

Second Drug, Doses	Nisoldipine Doses	Interaction Observed	Study
Ranitidine, 300 mg qd X 3 days vs placebo	IR 20 mg one dose Day 3	no differences in hemodynamics	385
Cimetidine, 400 mg one dose then 200 mg tid X 3 doses vs no treatment	oral & iv solution 10 mg po, 0.374 mg iv one dose each period	cimetidine had no additional hemodynamic effects*	399
Propranolol, 40 mg one dose vs placebo	IR 20 mg one dose	propranolol attenuates heart rate increase by nisoldipine	417

CC Formulation

Second Drug, Doses	Nisoldipine Doses	Interaction Observed	Study
Propranolol, 40 mg tid X 5 days vs no treatment	CC 20 mg qd X 5 days	no significant changes in hemodynamics	704

b. Effect of Nisoldipine on Other Drugs

Second Drug, Doses	Nisoldipine Doses	Interaction Observed	Study
Quinidine, 500 mg bid X 5 doses	IR 10 mg bid X 7 days vs placebo	quinidine AUC increased 17-26%, no ECG changes	384
Quinidine, 648 mg bid x 2 doses	CC 20 mg qd x 1 dose vs no treatment	no significant changes in quinidine kinetics	703
Warfarin, "steady state"	IR 10 mg bid X 21 days vs placebo	no change on warfarin level or anti-coagulation effect	349

* [illegible] dependent, see comments on individual studies

Propranolol 40 mg one dose	IR 20 mg one dose vs placebo	propranolol AUC, C_{max} increased by 43% & 68% no effect on beta blockade	417
Propranolol 80 mg bid X 7 days	IR 10 mg bid for 7 days vs placebo	propranolol AUC, C_{max} unchanged, higher heart rate w/ nisoldipine	Ref 57
Propranolol 160 mg qd X 2 weeks	IR 20 mg qd for 2nd week vs placebo	propranolol AUC, C_{max} increased by 30% & 50%, further BP reduction and higher heart rate by nisoldipine	382[30]
Propranolol 40 mg tid X 5 days	CC 20 mg qd X 5 days vs no treatment	propranolol AUC, C_{max} decreased by 14-15%, $t_{1/2}$ increased by 25%, no effects on hemodynamics	704
Atenolol 100 mg qd X 2 weeks	IR 20 mg qd for 2nd week vs placebo	atenolol C_{max} increased by 20% further BP reduction and higher heart rate by nisoldipine	382[29]
Digoxin 0.25 mg qd X 2 days then 0.5 mg qd X 21 days	IR 10 mg bid Days 9-22 vs controls	digoxin plasma level increased 7% (95%CI 3-20%)[55] no dynamic interaction	413
Digoxin 0.25 mg bid X 7 days (pretreated 14 days)	IR 10 mg bid X 7 days vs placebo	digoxin plasma level increased 15% ($p<0.05$) no dynamic interaction[55]	Ref 58[55]

[illegible] individual studies for design problems

[illegible] kinetic interaction in this study but the data are consistent with the results of Ref 58

[illegible] steady-state periods 139±21 ms with placebo vs 129±11 ms with nisoldipine

Tolerability Findings

Tolerability findings in pharmacologic studies are described as follows, which are included as part of dynamic characterization and not to be relied on heavily for safety assessment. For complete assessment of adverse experiences and safety profile of nisoldipine, reference is made to the reviews on efficacy/safety trials by Drs. Dern and Stockbridge.

As noted in the above sections on pharmacokinetics, studies referred to as "dose-tolerability" in the NDA were of short term (mostly 7 days) and conducted in small number of subjects. In general, nisoldipine administered in CC formulation was well-tolerated in healthy volunteers (Studies D91-035, D90-020), hypertensive patients (D88-059, D90-022) and in subjects with hepatic (D90-026) or renal impairment (D92-001). Adverse experiences reported frequently in these small studies were roughly dose-related and not unexpected from those observed for other calcium channel antagonists, which included dizziness, edema, flushing, headache, nausea, postural hypotension and tachycardia. Somnolence was a frequent complaint in patients with hepatic dysfunction and overall frequency of adverse events was higher in the renally impaired patients. Abnormal ECG with T-wave change (see above in Pharmacodynamics, Electrophysiology) was noted in at least three studies, one in normal subjects (D90-020), one in hypertensive patients (D90-022) and one in patients with renal impairment (D92-001). However, it was not clear whether the ECG changes were correlated with any clinically intolerable signs and symptoms. Similar ECG changes had not been as prominent in Phase III efficacy/Safety trials (see Dr Dern's Review)

Comments on Individual Pharmacodynamic Studies

While most pharmacodynamic studies of nisoldipine were of reasonable design and execution, some dynamic activities were not controlled and such results should be accepted with reservation. Again, the U.S. studies were better documented and probably more reliable than the translated foreign reports. Minor deficiencies and interpretation different from that of NDA have been noted in the discussion above. The following comments are arranged in the order of Study numbers for reference.

Studies 101-107, 109, 110, 115, and 116

These were all small tolerability studies in 4-6 normal subjects each. The adverse effects observed were similar to that of U.S. studies.

Study 125

In this single dose, placebo controlled crossover study, decreases in SDBP and increases in heart rate were dose-related in 6 normal subjects. However, there may not be adequate separation between doses to rule out carry over effect (see Comments on Individual Pharmacokinetic Studies for study design).

Study 126

Effect of a single oral dose (20 mg) nisoldipine on exercise tolerance was evaluated in this placebo-controlled, double blind study in 12 patients with stable angina. While nisoldipine improved exercise capacity and ST-segment changes, single dose data are basically of little use for these endpoints.

Study 135

This a electrophysiologic study of single oral dose of nisoldipine (10-20 mg). No significant changes in intra-cardiac conduction were found, but the study was not controlled and the measurements may have been performed too soon after dosing (see description of data above).

Studies 144, 257

These were uncontrolled electrophysiology studies of similar design and same dosage (1.5 µg/kg iv). In Study 257, patients were pretreated with atenolol 75 mg/day or pindolol 15 mg/day for 3 days.

Study 178

This is an uncontrolled hemodynamic and electrophysiologic study of nisoldipine in 12 patients undergoing arrhythmia evaluation. The patients were randomized to receive a single dose of nisoldipine IR 10 or 20 mgs orally. Invasive hemodynamic and electrophysiologic data were collected before and 120 minutes after dosing. Electrophysiology data were not cited by the sponsor in the pharmacodynamic summary (see description of results above).

Study 199

In this double blind, single dose, crossover study, nisoldipine IR 10 mg was compared with nifedipine 20 mg and placebo in 9 young and healthy subjects. Effects on blood pressure, heart rate and neurohormonal system were measured. Separation of treatment periods was adequate (one week).

Study 201

This is a small study in 12 Japanese healthy subjects. Nisoldipine was well-tolerated at single doses of 2.5-20 mg. No kinetic data were available.

Study 344

This is an open label, uncontrolled study of iv nisoldipine (3 µg/kg/3 min) on regional myocardial blood flow in 6 patients with coronary heart disease. Background therapy with atenolol 100 mg/day was continued for all patients.

Study 372

This is a double blind, placebo controlled, 8-week study in 72 hypertensive patients. After a 3 week washout, the dosage was forced-titrated from 5 mg qd to 20 mg bid every two weeks. Ergometric exercise was performed by every patient but hemodynamic measurements were done in only randomly selected half of the patients. Treatment groups were well-matched.

Study 382

This is a double blind, placebo controlled drug-interaction study to assess the effects of adding nisoldipine to established beta blocker (atenolol or propranolol) therapies in normotensive subjects. Eight young and healthy subjects were randomized to one of the following two treatment sequences (a or b) (reproduced from p. 11, NDA Vol 148, Page 08 17 00130290):

a	Weeks	0-----1-----2-----3-----4-----5
	beta blocker (b) or placebo (p)	bbbbbbbbbbbb-----pppppppbbbbbb
	nisoldipine (n) or placebo (q)	------nnnnnn-----------qqqqqq
b	Weeks	0-----1-----2-----3-----4-----5
	beta blocker (b) or placebo (p)	pppppppbbbbbb--------bbbbbbbbbbbb
	nisoldipine (n) or placebo (q)	--------qqqqqq----------nnnnnn

In the above scheme, the third week (between the end of Week 2 and beginning of Week 4) was a washout. With either sequence, nisoldipine (n) was administered after one week of beta blocker (b) therapy but the matching placebo (q) was given after a week of beta blocker matching placebo (p), although they were both concomitant with beta blockers during Week 2 or 5. Thus the nisoldipine treatment was not well controlled.

Study 399

This is another drug interaction study to evaluate the effects of pretreatment with cimetidine on the pharmacokinetics and pharmacodynamics of nisoldipine. Eight normal subjects were given a single dose of nisoldipine as a 10 mg oral solution or 0.374 mg iv infusion over 40 minutes (two crossover periods separated by 8 days), without (no placebo) and with cimetidine treatment (400 mg x 1 followed by 200 mg tid the next day of measurement). Using a sigmoidal E_{max} model, it was calculated that the hemodynamic changes due to cimetidine pre-treatment can be attributed to a 48% increase in nisoldipine bioavailability, and the sponsor concluded that cimetidine has no additional dynamic interaction with nisoldipine.

Study 479

This a double blind, parallel placebo controlled, 3 week study to evaluate the effect of nisoldipine (10 mg IR bid) on psychomotor functions in 30 normal healthy subjects.

Study 560

Changes in diameters of coronary arteries before and after iv nisoldipine (0.5 or 1.0 mg over 3 minutes) treatment were measured by angiography in 26 patients with coronary heart disease in this uncontrolled study. Plasma drug levels correlated with other hemodynamic activities but not vasodilating effects.

Study 648/649

This is another [illegible] study on the anti-ischemic effect of nisoldipine, but unlike Study 126 (see above) a QD dose (20 mg) was tested. Despite a rigorous design (double blind, parallel, placebo controlled, [illegible] nisoldipine had no effect on several angina endpoints investigated. But again, [illegible] as seen with a single dose study.

Study 670

The study was double blind, placebo controlled in young and healthy subjects. Effects of nisoldipine IR treatment (10 mg bid for 4 weeks) on thyroid function and prolactin were assessed.

Study D88-059

See Comments on Individual Pharmacokinetic Studies for study design and execution.

Study D90-029

This is a double blind, parallel placebo controlled, two single dose crossover study designed to demonstrate bioequivalence of 3x20mg and 2x30mg nisoldipine CC tablets. Blood pressure and heart rate effects, as well as tolerability data, were also collected.

Study D90-022

See Comments on Individual Pharmacokinetic Studies for study design and execution.

Study D91-035

See Comments on Individual Pharmacokinetic Studies for study design and execution

Ref 1-4

As noted above, these four studies were published by the same group of investigators, thus not to be considered as four independent reports. Invasive hemodynamics, both systemic and coronary, of nisoldipine were measured in patients with coronary heart disease. Except for Ref 4, which compared nisoldipine (6 µg/kg) with diltiazem (500 µg/kg), none of the other studies were controlled. For these studies, only publication reprints were submitted (no original data).

Ref 5

Effects of nisoldipine on myocardial metabolism were compared with that of nicardipine in this paper published by M F. Rousseau et al. Thirty-two patients with angina pectoris were treated with nisoldipine (0.06-0.12 µg/kg) infused intravenously over 10 minutes. Measurements were performed at basal state and during a cold pressor test.

Ref 6

Effects of nisoldipine on left ventricular function were assessed in patients with chronic stable angina and coronary disease and in patients with ischemic pain and abnormal exercise test but normal coronary arteries. The study was not controlled. Reprint of published report was presented in the NDA.

Ref 7, 8 (References 1, 2 of Section 8.15.3)

These are published reports of studies on regional blood flow (liver and kidney, Ref 7, [illegible] Ref 8). Nisoldipine was given orally (20 mg/day x 4 days) in the former and intravenously [illegible] in the latter. The investigation was conducted in young, normal subjects in both studies. Reprint only, no original data reviewed.

Ref 9 (References 3 of Section 8.15.3)

Effects of nisoldipine (20 mg/day for 4 days) on platelet aggregation was compared with that of verapamil (480 mg/day for 4 days) in this published report. Reprint only, no original data.

Ref 10 (References 4 of Section 8.15.3)

With no concurrent control, the claimed favorable effects of nisoldipine on lipoproteins [illegible] cannot be taken seriously and will not be described in the labeling. Also reprint of published report, no original data.

Drug Interaction Studies

As noted above in the kinetic sections, drug interactions were separated into the following three groups

Effect of other drugs on pharmacokinetics of nisoldipine
Effect of other drugs on pharmacodynamics of nisoldipine.
Effect of nisoldipine on other drugs' pharmacokinetics/dynamics.

The first category has been described in the Pharmacokinetic Sections. For the remaining two, limitations of individual studies, such as single/multiple doses, duration of treatment and use of control groups, have been described in tables summarizing the results. Studies 382 and 399 have been commented above in this section

Summary of Pharmacodynamic Issues

Antihypertensive activity of nisoldipine has been demonstrated for both IR and CC formulations. Dosages ranging from 5 to 120 mg have been studied in at least 6 studies, however, dose-response relationship in these short-term studies on CC formulation was not consistent. While the placebo-adjusted blood pressure reductions were proportional to doses of 5-30 mg/day, the response was flat over higher doses of 30-90 mg/day in a less well-designed study (see comments above). Systemic vascular resistance was consistently decreased by nisoldipine, but heart rate changes appeared to be mild in the pharmacodynamic studies.

Nisoldipine did not appear to have significant negative inotropic activities and except for a modest decrease in sinus cycle length, had no appreciable chronotropic effects either. However, the changes in T wave as observed in a few pharmacologic studies need to be re-examined in large efficacy/safety trials. Nisoldipine may increase coronary blood flow in patients with coronary artery disease but other measurements of anti-ischemic effect were not consistent.

[redacted] were no significant pharmacodynamic interactions between nisoldipine and ranitidine, [redacted] propranolol (with CC formulation of nisoldipine). Nisoldipine may increase bioavailability of quinidine, propranolol, atenolol and digoxin, but the extends were variable and of [redacted] consequences or clinical meaning.

[redacted] nisoldipine had no adverse pharmacologic effects [redacted] system, renal blood flow, thyroid and prolactin activity, lipoprotein profile or [redacted]. Nisoldipine may inhibit platelet aggregation and its clinical implication [redacted] in the safety data. Nisoldipine CC appeared to be well-tolerated in the small [redacted] studies, some of which were conducted in normotensive subjects. [redacted] seen in other calcium channel blockers.

PHARMACOKINETICS/DYNAMICS CORRELATIONS

As noted in the Summary of Pharmacodynamic Issues, placebo-adjusted blood pressure changes were dose-related for 5-30 mg (one week study on CC formulation, Study D88-059), correlation with plasma drug concentration and total bioavailability was also good:

Dose	C_{max}	AUC_{0-24}	Changes in Systolic/Diastolic BPs, mm Hg, Day 7	
mg/d	ng/ml	ng.hr/ml	8 hrs post dose	24 hrs post dose
5	0.65	8.39	- 7.1 / + 1.2	+ 1.9 / - 4.6
10	1.02	16.17	- 6.3 / - 1.2	- 8.8 / - 4.9
20	2.13	28.24	-10.7 / - 1.7	- 9.2 / - 4.6
30	2.79	40.34	-19.2 / - 4.6	-15.6 / - 6.2

Fit by linear regression was reasonable and suggested that plasma nisoldipine concentration of 2 ng/ml was required for a 5 mm Hg drop in diastolic BP over placebo. Such relationship was less clear at higher doses (30-90 mg/day, Study D90-022). When blood pressure responses and plasma drug concentrations were fitted with linear regression which included placebo data for plasma level of zero, estimated slopes were significant or nearly so for 30 mg and 60 mg. However, as noted before, placebo-corrected blood pressure reductions were not dose-related and the dosages were forced titrated rapidly in sequence in this study.

In four large hypertension efficacy trials (D88-054, D89-029, D89-039 and D90-019), which covered doses from 10 to 60 mgs/day, overall correlation between systolic/diastolic blood pressure reductions and plasma drug concentration was good for each study pooled over all dosages, and for 30-60 mg doses pooled over all four studies (Table on next page). The estimated slopes from linear regression analysis indicated that trough supine diastolic blood pressure decreased by 1.57 mm Hg per 1 ng/ml (overall pooled analysis), or 1.67-2.38 mm Hg per ng/ml for the three monotherapy studies.

In two Phase III angina trials (D88-060 and D90-015), placebo-subtracted changes in exercise durations from baseline were related to dose/plasma concentration as follows:

Dose	C_{max}	C_{min}	Changes in seconds, p for correlation with drug levels			
mg/d	ng/ml	ng/ml	Peak	p	Trough	p
10	-	0.79	-		1	0.037
20(D88-060)	-	1.25	-		20	0.905
20(D90-015)	2.10	1.57	29	0.012	34	0.184
30	-	2.16	-		32	0.054
40	3.70	2.56	6	0.108	7	0.017
60	5.70	4.07	34	0.577	37	0.777

Of these, the estimated slopes were significant or nearly so for 10, 30, 40 mgs at trough, and 20 mg at peak. Pooled over all dosages, the correlation was significant in Study D88-060 (10-30 mg, trough) only. Overall correlations between dose, plasma level and exercise tolerance in angina patients were poor.

Correlation of trough nisoldipine level and blood pressure responses:
(From NDA Section 8.13.8.2, Vol 116)

NISOLDIPINE/EFFICACY POOL
US CC HTN

TABLE
CORRELATION COEFFICIENTS OF TROUGH BLOOD LEVELS WITH TROUGH BLOOD PRESSURE
FOR ALL PATIENTS VALID FOR EFFICACY ANALYSIS

PROTOCOL	NIS CC 00 DRUG GROUP	N	SUPINE SYSTOLIC BP R	SUPINE SYSTOLIC BP P	SUPINE DIASTOLIC BP SLOPE	SUPINE DIASTOLIC BP R	SUPINE DIASTOLIC BP P	STANDING SYSTOLIC BP R	STANDING SYSTOLIC BP P	STANDING DIASTOLIC BP R	STANDING DIASTOLIC BP P
D88-054	ALL	87	-0.17853	0.0900	-2.11	-0.30895	0.0036	-0.11794	0.2766	-0.29230	0.0060
D89-029	ALL	158	-0.14792	0.0636	-1.03	-0.30298	0.0001	-0.22569	0.0044	-0.39461	0.0001
D89-039	ALL	114	-0.38121	0.0001	-2.38	-0.40175	0.0001	-0.31050	0.0001	-0.50104	0.0001
D90-019	ALL	119	-0.30830	0.0006	-1.67	-0.44582	0.0001	-0.29118	0.0013	-0.46786	0.0001
D88-054	10MG	30	0.01600	0.9331	---	-0.34391	0.0628	0.11973	0.5286	-0.23418	0.2129
D88-054	20MG	30	-0.47472	0.0080	---	-0.39234	0.0320	-0.39317	0.0316	-0.36920	0.0447
D88-054	30MG	27	-0.22195	0.2658	---	-0.16068	0.4233	-0.21617	0.2780	-0.34713	0.0761
D89-029	20MG	56	0.15089	0.2482	---	0.14361	-0.2910	0.12065	-0.3757	0.07007	0.6078
D89-029	40MG	49	-0.15621	0.2838	---	-0.32064	0.0247	-0.14882	0.3075	-0.53553	0.0001
D89-029	60MG	53	-0.09154	0.5145	---	-0.31901	0.0199	-0.17371	0.2135	-0.22792	0.1007
D89-039	20MG	59	-0.15072	0.2545	---	-0.26441	0.0430	-0.19165	0.1459	-0.28609	0.0280
D89-039	40MG	55	-0.36456	0.0062	---	-0.35004	0.0080	-0.41101	0.0018	-0.45768	0.0004
D90-019	30MG	66	-0.27969	0.0229	---	-0.32130	0.0085	-0.35486	0.0035	-0.42230	0.0004
D90-019	60MG	53	-0.27936	0.0420	---	-0.44821	0.0008	-0.22532	0.1048	-0.44383	0.0009
ALL	10MG	30	0.01600	0.9331	---	-0.34391	0.0628	0.11973	0.5286	-0.23418	0.2129
ALL	20MG	145	-0.05009	0.5496	---	-0.09741	0.2438	-0.15034	0.0604	-0.20408	0.0138
ALL	30MG	93	-0.26392	0.0106	---	-0.28517	0.0056	-0.31687	0.0020	-0.39962	0.0001
ALL	40MG	104	-0.22874	0.0195	---	-0.32601	0.0007	-0.25083	0.0102	-0.47381	0.0001
ALL	60MG	106	-0.17762	0.0685	---	-0.39735	0.0001	-0.19492	0.0453	-0.35785	0.0002
ALL	ALL	478	-0.26588	0.0001	-1.57	-0.39170	0.0001	-0.28519	0.0001	-0.45456	0.0001

CONCLUSIONS

It appears that pharmacokinetic properties of nisoldipine has been well-described for both the immediate release (IR) and the control release (CC) formulations. Variations in bioavailability of nisoldipine in patients of different concurrent diseases and demographic characteristics have been examined, which should be addressed in relevant sections of labeling, especially the issues of dose-dumping by food. Once daily use of nisoldipine CC tablets for hypertension is supported by the kinetic data.

While the pharmacodynamic profile of nisoldipine was studied mostly using intravenous and the IR formulations, it has been demonstrated that nisoldipine is a vasodilating antihypertensive with minor electrophysiologic effects and insignificant inotropic activities. There is no reason to expect significantly different behavior for the CC tablets. For major cardiovascular effects of nisoldipine, there is good correlation with dose (5-30 mg/day) and plasma drug concentration.

It is concluded that clinical pharmacology of nisoldipine CC has been adequately characterized for the patients to be treated that instructions on its clinical use for hypertension can be written for the labeling.

Shaw T. Chen, M.D.,Ph.D.

cc:
ORIG: NDA- 20-356
HFD-110
HFD-110/CSO 4
HFD-426/PMarroum
HFD-110/PDem, CDuarte, NStockbridge
HFD-110/SChen/02/15/94

D (R) Duarte

DIVISION OF CARDIO-RENAL DRUG PRODUCTS

MEDICAL OFFICER'S REVIEW OF NDA AUG 4 1993

NDA Number : 20-356

Name of Drug : Nisoldipine (NIS CC)

Drug Category : Calcium Channel Blocker

Indication : Hypertension

Sponsor : Miles Inc Pharmaceutical Division

Date of Submission : March 31, 1993

Date Received : April 1, 1993

Date Review Completed : July 30, 1993

Reviewer : Cristobal G. Duarte, MD

Background. NIS CC is an extended release tablet dosage form of the dihydropyridine calcium channel blocker Nisoldipine The sponsor has submitted a NDA for approval of Nisoldipine for the treatments of hypertension This review will be concerned only with the efficacy in the treatment of hypertension.

As pivotal protocols in support of the effectiveness of Nisoldipine in the control of hypertension the sponsor is submitting the following studies : D90-006, D90-019, D89-026, D89-029, and D89-039.

Protocol D89-026

Title of Study : " A Pilot Dose-Titration Study of the Safety and Efficacy of Nisoldipine Coat-Core 10 mg, 20 mg, 30 mg and 40 mg versus Placebo in Patients with Mild to Moderate Hypertension ".

Investigators : Ginsberg D, Flamenbaum W, Canzanello V, Townsend R, Winer N,Schnaper H.

Places of Study. Harleysville, Englewood Cliffs, Winston-Salem, Galveston, Kansas City, Birmingham/USA

Objectives. The objectives of this study were :

1. To determine whether Nisoldipine given once daily lowers the blood pressure significantly more than placebo.

2. To determine the efficacy and safety of Nisoldipine when titrated from 10 mg to 40 mg qd.

Inclusion Criteria. Ambulatory patients, male and female, 21 years of age or older, with a history of essential hypertension were eligible for the study. Hypertension was defined as mean supine diastolic blood pressure of 95-115 mmHg.

Exclusion Criteria. Patients with the following conditions were excluded from the study :

1. Labile hypertension.
2. Recent myocardial infarction
3. Patients with cerebrovascular accident or signs suggesting impending MI or CVA, heart failure, angina pectoris, intermittent claudication, major arrhythmia or cardiac conduction disturbances.
4. Insulin-dependent diabetes mellitus, failure of a major organ system, impaired renal function (serum creatinine >2 mg/dl), severe infection, malignancy or psychosis.
5. Patients likely to have impaired drug absorption such as with chronic diarrhea, ulcerative colitis, regional enteritis, diverticulitis, partial or complete gastrectomy or small bowel resection.
6. Women of childbearing potential, alcohol or drug abusers, history of allergy to dihydropyridines.
7. Excluded concomitant medications were : antihypertensive drugs, cimetidine, monoamino oxidase inhibitors, sedatives, tranquilizers, tricyclic antidepressants, neuroleptic drugs, anorectics and decongestants.

Qualification for Randomization. Patients with mild or moderate hypertension discontinued previous antihypertensive treatment and were given a single-blind placebo once daily (regimen A) during a three to four-week qualifying run-in period. There was an optional extension of one week if the blood pressure was not in the qualifying range. Those patients with mean supine diastolic pressure ≥ 95 mmHg to ≤ 114 mmHg after three to four weeks of placebo were randomized and were given regimen B (Nisoldipine or placebo).

Drug-Regimen Protocol. At week 0 qualified subjects were given either Nisoldipine 10 mg qd or placebo qd (regimen B) for 2 weeks. On subsequent visits 5 through 7 (scheduled every two weeks) the once daily dose of Nisoldipine was titrated in a stepwise fashion to 20 mg (regimen C), 30 mg (regimen D), or 40 mg (2X20 mg) (regimen E) if mean trough supine diastolic pressure for that visit was ≥ 85 mmHg. Patients randomized to placebo underwent corresponding dummy titration. Two patients were randomized to Nisoldipine for each patient that was randomized to placebo.

Patients took two tablets before 11 am through the study but did not take the medication on the morning of clinical visits until trough blood pressure has been measured. Patients took the medication fasting or with food.

Patients were seen either weekly or biweekly in the morning throughout the study. At each visit supine and standing blood pressures were measured 24 hours ± 30 minutes after the last dose.

The duration of the double-blind phase was 9 weeks.

Expulsion. A subject was to be dropped from the study if the mean supine diastolic blood pressure was greater than 114 mmHg at any visit or if they had significant physical or laboratory abnormalities or a significant concurrent illness.

They also were to be withdrawn for blatant non-compliance, for missing visits or significant adverse experiences.

Assessment. Patients were seen in the morning at weekly or biweekly intervals. A history was taken at the first visit. Complete physical examination and 12-lead electrocardiogram were done at the first visit, at baseline (after 3 to 4 weeks on placebo) and at the last visit (after 9 weeks of double blind drug). Brief physical examinations were done at all other visits. A chest X-ray was done at the first visit unless a report was available within the previous 6 months.

Blood was drawn for the following laboratory tests at the first visit, after 3 to 4 weeks of single-blind placebo, and after 9 weeks of double-blind drug : CBC, differential, and platelet count, serum glucose, uric acid, calcium, phosphate, sodium, potassium, chloride, bicarbonate, creatinine, BUN, total protein, albumin, cholesterol, triglycerides, CPK, SGOT, LDH, alkaline phosphatase and total bilirubin, Complete urinalysis including microscopic and casts.

The primary endpoint of the study was a change in trough diastolic blood pressure (measured 24 hours after dosing) from baseline (mean of diastolic blood pressure after 3 or 4 weeks of single-blind placebo) to endpoint (the last valid visit on double-blind drug for each valid patient) in the Nisoldipine group compared to the placebo group.

Secondary endpoints were supine systolic blood pressure at trough and standing blood pressure at trough.

Statistical Analysis. The primary efficacy analysis was based on change from baseline in trough supine diastolic blood pressure at endpoint. No analysis based on level of titration achieved was done. Responders were considered those who achieved efficacy results according to the following criteria : blood pressure 90 mmHg or less, at least a 10 mmHg fall in blood pressure from baseline, either of the above and both of the above.

All tests were two-sided and based on the least square means estimated by the model.

Data from previous hypertension studies had suggested that the standard deviation of change from baseline in trough supine diastolic blood pressure at endpoint would be 7.5 mmHg. In order to detect a 5 mmHg difference from placebo in an alpha = 0.05, two tailed tests of

significance, and in order to obtain as much data as possible on the Nisoldipine 40 mg qd, it was decided to randomized 72 patients to Nisoldipine and 36 to placebo. Based on this information, the study, as designed, had 80 % power to detect a significant difference of at least 5 mmHg.

Subjects Studied. Of 166 patients enrolled, 43 were disqualified for randomization. The reasons for which patients did not qualify for randomization id given in the following table :

Mean Diastolic blood pressure at visit 4 did not qualify for randomization (95 mmHg to 114 mmHg)	26
Supine diastolic blood pressure >114 mmHg-	
At any time	4
Non compliance	1
Illness not due to medication	3
Other	9
Total	43

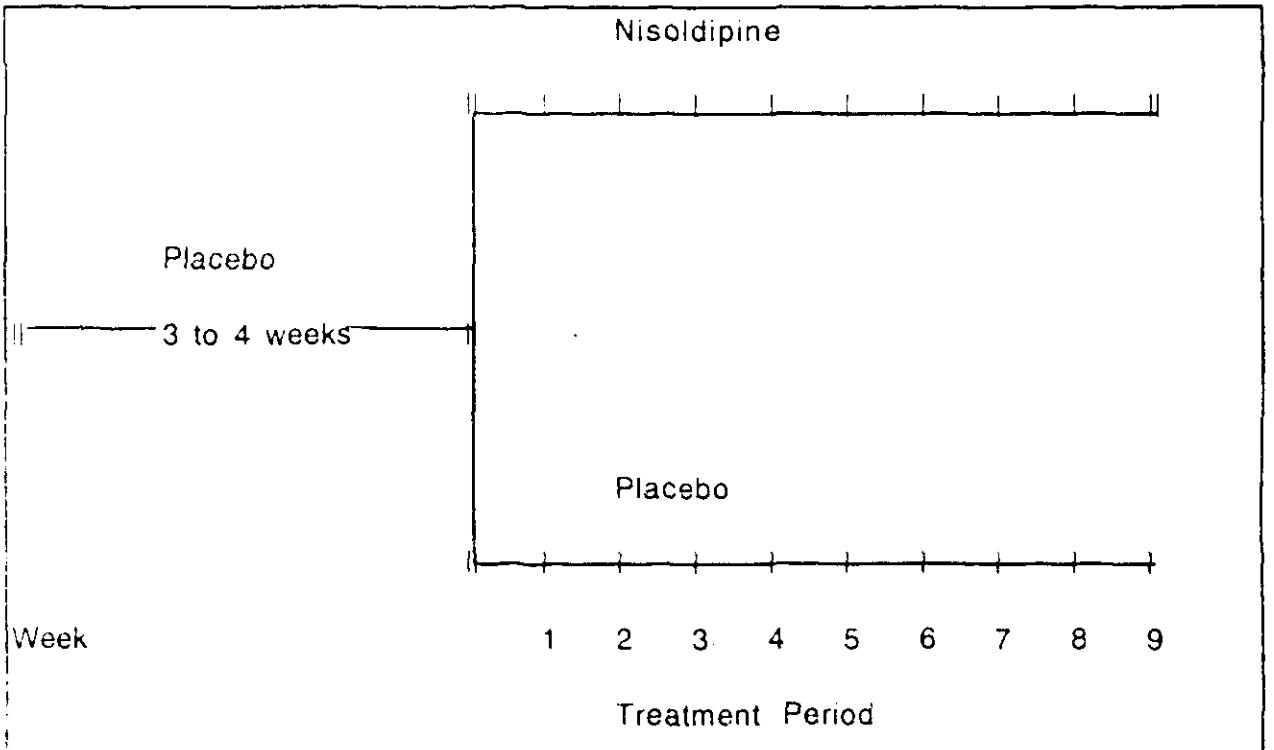
The demography and baseline characteristics of the patients valid for analysis of efficacy is given in the following table :

		Nisoldipine (n=79)	Placebo (n=38)
Sex	Male	46 (58 %)	22 (58 %)
	Female	33	16
Race	Caucasian	53 (67 %)	21 (55 %)
	Black	25	16
	Other	1	1
Age (years)	Mean	53	57
Years of hypertension	Mean	10	14
Baseline blood pressure	Supine	153/100	160/101
	Standing	149/100	156/102

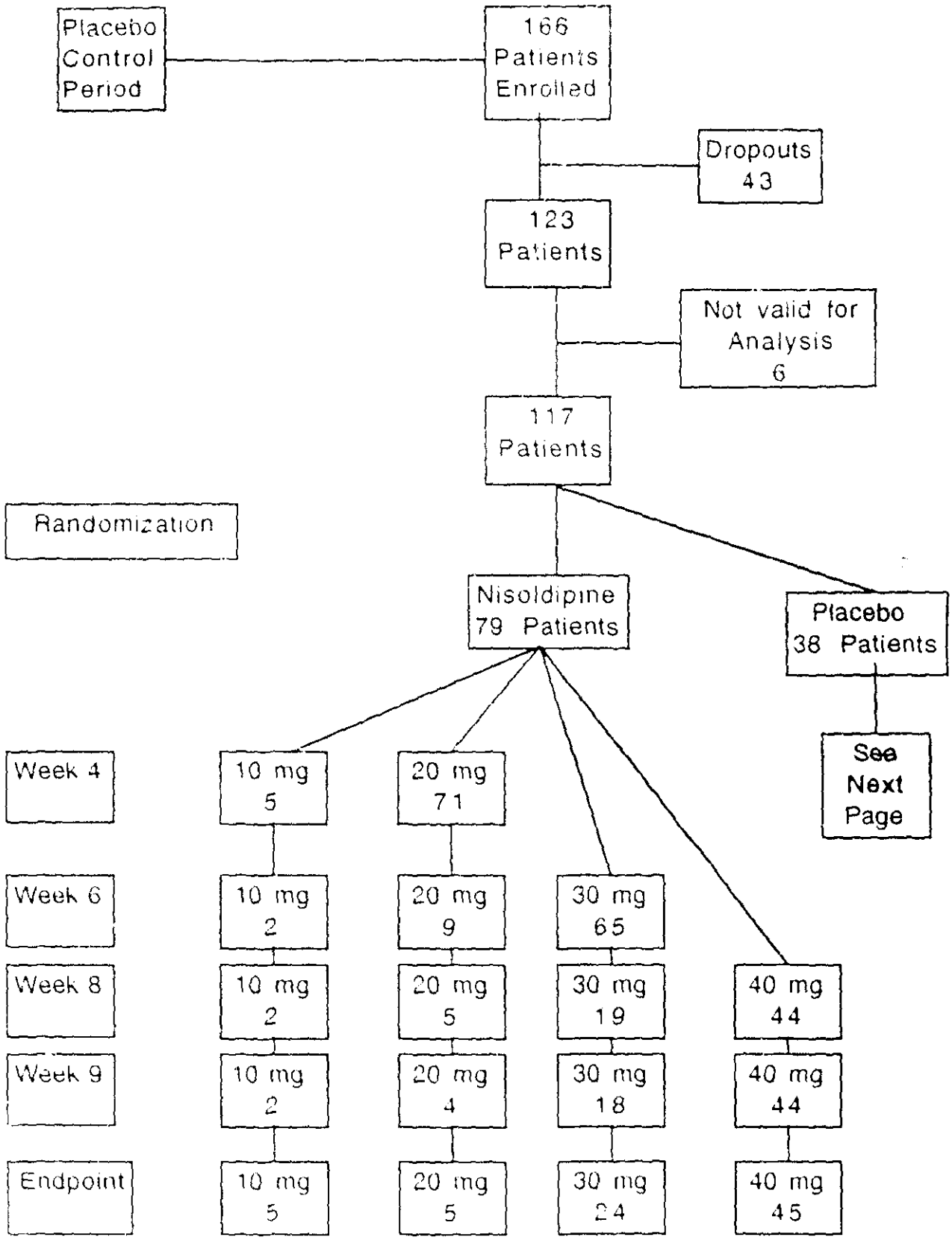
The reasons for discontinuation of double-blind therapy are given in the following table :

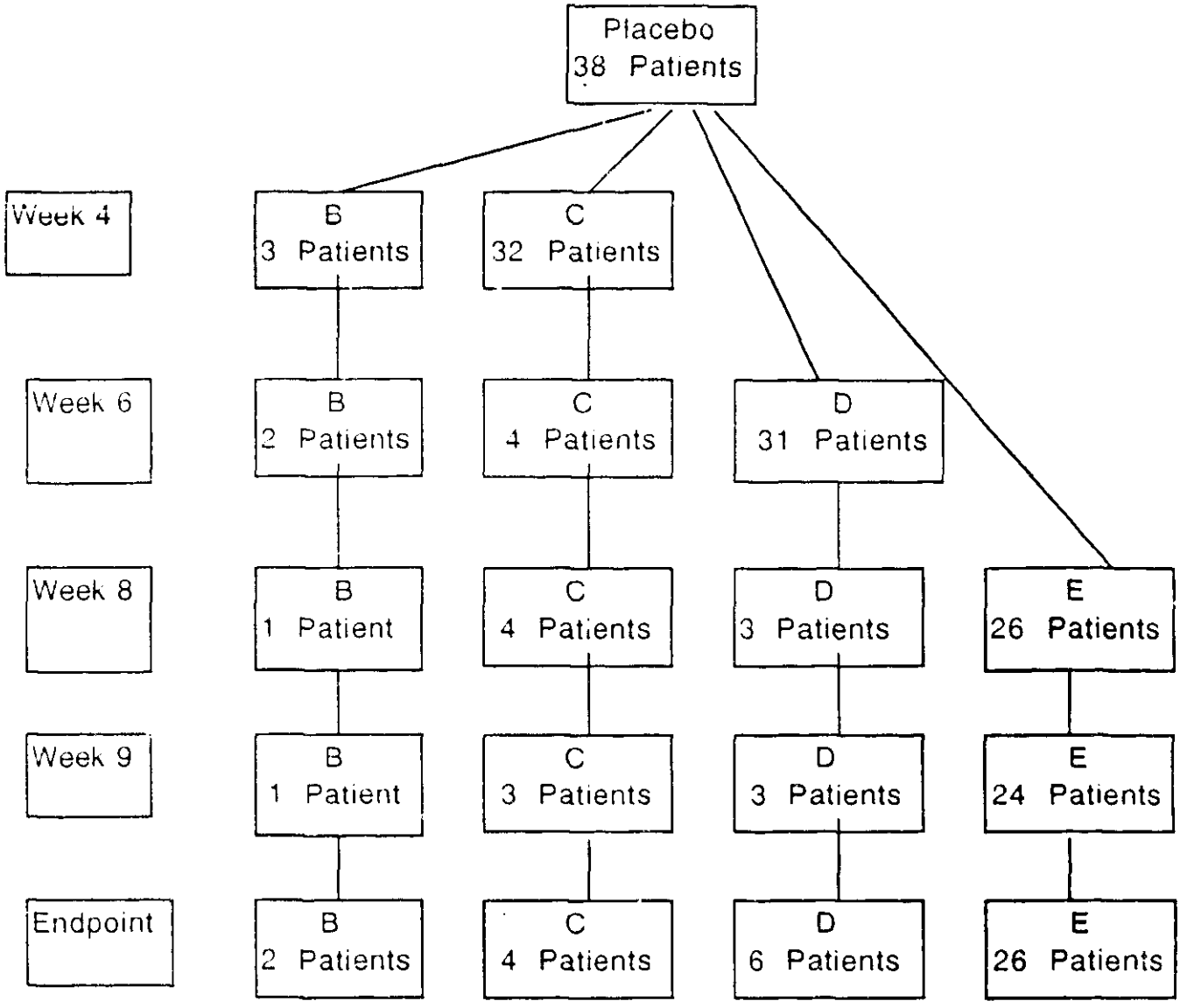
Reason	Nisoldipine n=83	Placebo n=40
Lack of Efficay	0	5
Adverse Event	6	0
Abnormal Laboratory Value	0	1
Lost to Follow-up	3	0
Other	2	9

The protocol that was followed is represented schematically in the following graph



The randomization of patients is given in the following two pages


Placebo Control Period
166 Patients Enrolled
Dropouts 43
123 Patients
Not valid for Analysis 6
117 Patients
Randomization
Nisoldipine 79 Patients
Placebo 38 Patients
See Next Page
Week 4
Week 6
Week 8
Week 9
Endpoint
10 mg 5
20 mg 71
10 mg 2
20 mg 9
30 mg 65
10 mg 2
20 mg 5
30 mg 19
40 mg 44
10 mg 2
20 mg 4
30 mg 18
40 mg 44
10 mg 5
20 mg 5
30 mg 24
40 mg 45


Placebo
38 Patients
Week 4
B
3 Patients
C
32 Patients
Week 6
B
2 Patients
C
4 Patients
D
31 Patients
Week 8
B
1 Patient
C
4 Patients
D
3 Patients
E
26 Patients
Week 9
B
1 Patient
C
3 Patients
D
3 Patients
E
24 Patients
Endpoint
B
2 Patients
C
4 Patients
D
6 Patients
E
26 Patients

Efficacy. Doses of Nisoldipine were titrated from regimen B (10 mg QD) to regimen E (40 mg QD) in 10 mg steps. The following table shows the actual number of patients that were given each dose level and had valid visits at each week for the population of patients valid for efficacy :

			Week of Therapy					
			2	4	6	8	9	End-point
Group	Reg.	Dose	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)
NIS	B	10 mg QD	79 (100)	5(7)	2 (3)	2 (3)	2 (3)	5 (6)
	C	20 mg QD		71 (93)	9 (12)	5 (7)	4 (6)	5 (6)
	D	30 mg QD			65 (86)	19 (27)	18 (27)	24 (30)
	E	40 mg QD				44 (63)	44 (65)	45 (57)
PLA	B		38 (100)	3 (9)	2 (5)	1 (3)	1 (3)	2 (5)
	C			32 (91)	4 (11)	4 (12)	3 (10)	4 (11)
	D				31 (84)	3 (9)	3 (10)	6 (16)
	E					26 (77)	24 (77)	26 (68)

The following table shows trough supine diastolic blood pressure response at different weeks of treatment and at endpoint for both groups for the set of all valid patients

Trough Supine Diastolic Blood pressure
Mean Change (mmHg) by visit

	Week 2	Week 4	Week 6	Week 8	Week 9	End-point
Nisoldipine						
(n)	(79)	(76)	(76)	(70)	(68)	(79)
Mean Change	-5.7*	-6.5	-10.1*	-10.6*	-10.0*	-9.5*
Placebo						
(n)	38	35	37	34	31	
Mean Change	-3.0	-4.9	-3.4	-4.4	-3.4	-1.2

* Significantly different from placebo

In the following table, changes from baseline at endpoint by treatment regimen at endpoint for all valid patients is demonstrated :

	Nisoldipine			
	Reg B (n=5)	Reg C (n=5)	Reg D (n=24)	Reg E (n=45)
Supine				
Systolic	-8.3	-23.1	-10.9	-16.7
Diastolic	-5.9	-12.4	-9.4	-9.8
Standing				
Systolic	-5.3	-13.5	-10.3	-15.3
Diastolic	-7.2	-9.9	-6.6	-8.6

	Placebo			
	Reg B (n=2)	Reg C (n=4)	Reg D (n=6)	Reg E (n=26)
Supine				
Systolic	-19.7	-8.0	+20.4	-2.2
Diastolic	+ 1.7	-10.0	+6.9	-1.4
Standing				
Systolic	-9.7	-1.3	+13.9	-0.1
Diastolic	-0.7	-7.5	+5.0	-1.6

The responder rates are given in the following table :

Responder Rates
Based on Trough Supine Diastolic Blood Pressure at Endpoint

	Nisoldipine	Placebo
BP<90 mmHg at endpoint	49 %	18 %
At least a 10 mmHg fall at Endpoint	54 %	13 %
Either of the Above	62 %	21 %
Both of the Above	42 %	11 %

The change from baseline in trough blood pressure by treatment for all patients valid for analysis of efficacy is given in the following table :

Supine Diastolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg.B	Reg. B or C	Reg. BC or D	Reg.BCD or E	Reg BCDorE	
Nisoldipine n	79	76	76	70	68	79
Baseline Mean LS	100.36	100.36	100.36	100.14	100.36	100.36
LS Mean Change	-5.65*p	-6.52*	-10.06*p	-10.58*p	-9.98*p	-9.51*p
SE of Change	0.68	0.75	0.88	0.84	0.80	0.89

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group 300 ppm, sex ♂

Animal No. 273	Testes	Leydig cell tumour (b), unilateral
No. 274	Mesentery	Leiomyosarcoma which has infiltrated the pancreas and the skeletal muscle (diaphragm)
No. 276	RHS	Histiocytary sarcoma with foci in the heart, spleen, liver, lung, pancreas, bones, bone marrow, stomach, orbit, oesophagus, trachea, thyroid, epididymis and adrenals
No. 285	Testes	Leydig cell tumour (b), unilateral
No. 288	Adrenals	Phaeochromocytoma (b), unilateral
No. 289	RHS	Malignant lymphomas in the spleen, lymph nodes, liver, lung, pancreas, kidneys, mesentery, mediastinum and heart
No. 290	Skin (lower abdomen)	Cornifying squamous cell carcinoma
No. 291	Testes	Leydig cell tumour (b), unilateral
No. 296	Pituitary Lung	Adenoma Adenoma
No. 297	Testes	Leydig cell tumour (b), unilateral
No. 299	Subcutis (penis)	Haemangiosarcoma

Dose group 1800 ppm, sex ♂

Animal No. 361	Testes Adrenals	Leydig cell tumour (b), unilateral Phaeochromocytoma (b), unilateral
No. 363	Heart Pituitary	Endocardial fibromatosis (b) Adenoma
No. 368	Testes Pituitary	Leydig cell tumour (b), unilateral Adenoma
No. 370	Adrenals	Phaeochromocytoma (b), bilateral
No. 372	Subcutis (lower leg)	Haemangiosarcoma

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group 1800 ppm, sex ♂

Animal No. 374	Adrenals	Phaeochromocytoma (b), unilateral
No. 375	Subcutis (head)	Sarcoma. The soft-tissue tumour has infiltrated the periorbital and retro-orbital tissue and the pituitary.
No. 376	Adrenals	Phaeochromocytoma (b), unilateral
No. 377	Thyroid	C cell carcinoma
	Brain	Meningioma (b)
No. 378	Testes	Leydig cell tumour (b), unilateral
No. 379	Pituitary	Adenoma
No. 380	Pituitary	Adenoma
No. 382	Pituitary	Adenoma
	Adrenals	Phaeochromocytoma (b), unilateral
No. 383	Testes	Leydig cell tumour (b), unilateral
No. 384	Pituitary	Adenoma
	Adrenals	Phaeochromocytoma (b), unilateral
No. 385	Subcutis (Femur, ear)	Sarcoma with infiltrates or metastases in the skeletal muscle, bone, bone marrow, and lung
No. 390	Testes	Leydig cell tumour (b), bilateral
No. 391	Pituitary	Adenoma
No. 392	RHS	Malignant lymphomas in the thymus, spleen and lymph nodes
	Pituitary	Adenoma
	Adrenals	Phaeochromocytoma (b), unilateral
No. 393	Epididymides	Sarcoma
No. 398	Pituitary	Adenoma
	Adrenals	Phaeochromocytoma (b), unilateral
No. 400	Testes	Leydig cell tumour (b), unilateral
	Thyroid	Follicular carcinoma
	Brain	Meningioma (b)
	Subcutis (base of tail)	Sarcoma
No. 404	Testes	Leydig cell tumour (b), unilateral
No. 410	Testes	Leydig cell tumour (b), unilateral
No. 415	Pituitary	Adenoma
No. 416	Pituitary	Adenoma
	Skin (head)	Cornifying squamous cell carcinoma

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group 0 ppm, sex ♀

Animal No. 61	Pituitary	Adenoma
No. 66	Thyroid	C cell adenoma
No. 68	Uterus	Endometrial stromal tumour (polyp, b)
	Thyroid	C cell adenoma
No. 69	Uterus	Endometrial stromal tumour (polyp, b)
	Pituitary	Adenoma
No. 72	Pituitary	Adenoma
No. 74	Pituitary	Adenoma
No. 75	Uterus	Adenocarcinoma with infiltrates in the mesentery
No. 76	Uterus	Endometrial stromal tumour (polyp, b)
No. 77	Pituitary	Adenoma
	Mammary gland	Adenocarcinoma
No. 82	Uterus	Endometrial stromal tumour (polyp, b)
No. 84	Urinary bladder	Adenoma
No. 85	Pituitary	Adenoma
No. 89	Kidney	Sarcoma
	Thyroid	C cell adenoma
No. 90	Uterus	Endometrial stromal tumour (polyp, b)
	Thyroid	C cell adenoma
No. 92	Pituitary	Adenoma
No. 93	Uterus	Endometrial stromal tumour (polyp, b)
	Thyroid	C cell adenoma
No. 94	Pituitary	Adenoma
No. 97	Uterus	Endometrial stromal tumour (polyp, b)
No. 98	Thyroid	Follicular carcinoma
	Subcutis (head)	Haemangiosarcoma
No. 99	Thyroid	C cell carcinoma
No.100	Pituitary	Adenoma
	Adrenals	Cortical adenoma, unilateral
No.102	Uterus	Endometrial stromal sarcoma infiltrating the urinary bladder
No.104	Pituitary	Adenoma

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group 0 ppm, sex ♀

Animal No.106	Pituitary	Adenoma
No.107	Pituitary	Adenoma
	Subcutis (inguinal region)	Sarcoma
No.110	Kidney	Adenoma
	Pituitary	Adenoma
No.111	Pituitary	Carcinoma with infiltrates in the brain
No.113	Pituitary	Adenoma
No.114	Thyroid	C cell adenoma
No.117	Uterus	Endometrial stromal sarcoma with infiltration into the mesentery
No.118	Uterus	Endometrial stromal tumour (polyp, b)
No.119	Pituitary	Adenoma
No.120	Pituitary	Adenoma

Dose group 50 ppm, sex ♀

Animal No. 181	Uterus	Endometrial stromal tumour (b)
No. 187	Uterus	Endometrial stromal tumour (polyp, b) Infiltrating adenocarcinoma with metastases in the liver, pancreas and mesentery
No. 188	Uterus	Endometrial stromal tumour (polyp, b)
No. 192	Adrenals	Cortical adenoma, unilateral
No. 196	Uterus	Adenocarcinoma
No. 199	Thyroid	C cell adenoma. Tentative diagnosis because of the severe autolysis.
No. 212	Mammary gland	Adenoma
No. 213	Mammary gland	(Fibro)adenoma with pericanalicular and intracanalicular growth
No. 219	Uterus	Endometrial stromal tumour (polyp, b)
No. 220	Pituitary	Adenoma

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group 300 ppm, sex ♀

Animal No.333	Pituitary	Carcinoma infiltrating the brain
	Thyroid	C cell adenoma
No.334	Uterus	Endometrial stromal tumour (polyp, b)
	Pituitary	Adenome
No.335	Uterus	Endometrial stromal tumour (polyp, b)
	Mammary gland	Adenoma
No.336	Uterus	Infiltrating adenocarcinoma with squamous metaplasia and sarcomatous tissue areas
	Pituitary	Adenoma
No.340	Thyroid	C cell adenoma
No.341	Uterus	Endometrial stromal tumour (polyp, b)
No.345	Uterus	Endometrial stromal sarcoma infiltrating the mesentery and pancreas
No.347	Uterus	Endometrial stromal tumour (polyp, b)
No.348	Uterus	Endometrial stromal tumour (polyp, b)
No.350	Ovaries	Granulosa-theca cell tumour (b), unilateral
	Pituitary	Adenoma

Dose group 1800 ppm, sex ♀

Animal No.425	Thyroid	C cell adenoma
No.426	Ovaries	Granulosa-theca cell tumour (m)
No.427	Uterus	Endometrial stromal tumour (polyp, b
	Pituitary	Adenoma
No.429	Skin (head)	Papilloma (b)
No.431	Ovaries	Granulosa-theca cell tumour (b)
	Pituitary	Adenoma
	Adrenals	Phaeochromocytoma, unilateral. The tumour has penetrated the organ capsule,m
No.435	Uterus	Adenocarcinoma with infiltrates in the mesentery
No.445	Uterus	Endometrial stromal tumour (polyp, b)
	Pituitary	Adenoma
No.446	Uterus	Endometrial stromal tumour (polyp, b)
	Pituitary	Adenoma

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group 50 ppm, sex ♀

Animal No.221	Uterus	Endometrial stromal tumour (polyp, b) Adenocarcinoma
No.222	Uterus	Endometrial stromal tumour (polyp, b)
No.223	Uterus	Endometrial stromal tumour with squamous metaplasia (polyp, b)
	Pituitary	Carcinoma infiltrating the brain
No.224	Uterus	Adenocarcinoma with metastases in the liver, pancreas, stomach, intesti and mesentery
No.230	Uterus	Endometrial stromal tumour (polyp, b)
No.231	Pituitary	Adenoma
No.233	Uterus	Endometrial stromal tumour (polyp, b)
	Pituitary	Adenoma
No.234	Mammary gland	Adenocarcinoma
No.236	Uterus	Endometrial stromal tumour (polyp, b)
No.237	Uterus	Endometrial stroma, tumour (polyp, b)
No.240	Uterus	Endometrial stromal tumour (polyp, b)

Dose group 300 ppm, sex ♀

Animal No.301	Uterus	Endometrial stromal sarcoma
No.305	Pituitary	Adenoma
No.308	Uterus	Adenocarcinoma
No.310	Uterus	Endometrial stromal tumour (polyp, b)
No.312	Pituitary	Adenoma
No.313	Uterus	Endometrial stromal tumour (polyp, b)
No.314	Uterus	Endometrial stromal tumour (polyp, b)
No.318	Mesentery	Malignant mesothelioma of the serous lining of the adrenals, uterus, ovarie spleen, stomach, intestines and urinar bladder
No.325	Uterus	Endometrial stromal tumour (polyp, b)
No.326	Uterus	Endometrial stromal tumour (polyp, b)
	Mammary gland	Adenocarcinoma
No.330	Uterus	Endometrial stromal tumour (polyp, b)

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group 1800 ppm, sex ♀

Animal No.447	Uterus	Endometrial stromal tumour (polyp, b)
No.450	Uterus	Endometrial stromal tumour (polyp, b)
No.453	Pituitary	Adenoma
No.457	Uterus Adrenals	Endometrial stromal tumour (polyp, b) Phaeochromocytoma (b), unilateral
No.459	Intestines Uterus	Fibroma Endometrial stromal sarcoma
No.460	Subcutis (flank)	Sarcoma infiltrating through the muscles of the flank and extending into the mesentery.
No.463	Thyroid	Follicular adenoma
No.464	Uterus Subcutis (ear)	Endometrial stromal tumour (polyp, b) Sarcoma
No.465	Uterus Mammary gland	Endometrial stromal tumour (polyp, b) Adenoma
No.466	RHS	Histiocytary sarcoma in the mesentery liver, pancreas, skeletal muscle and subcutis
No.467	RHS	Malignant lymphoma in the spleen, lymph nodes, liver, lung, kidneys, urinary bladder, uterus, thyroid, skeletal muscle, bone marrow, mediastinum and mesentery
No.469	Uterus	Endometrial stromal tumour (polyp) with squamous metaplasia (b) Adenocarcinoma with metastases in the lung, liver, pancreas, kidneys, ovaries and mesentery
No.471	Uterus	Endometrial stromal sarcoma with metastases in the liver, urinary bladder, ovaries and mesentery
No.472	Pituitary	Adenoma
No.475	Mammary gland	(Fibro)adenoma
No.476	Uterus	Endometrial stromal tumour (polyp, b)
No.477	Uterus	Endometrial stromal tumour (polyp, b)
No.478	Uterus	Endometrial stromal tumour (polyp, b)
No.479	Uterus	Endometrial stromal tumour (polyp, b)
No.480	Pituitary	Adenoma

Histopathological findings on the lower jaw in the region of the gnawing teeth

Dose group 0 ppm, sex ♂

Animal No. 33 Nothing abnormal detected (N.A.D.)

No. 36 N.A.D.

No. 41 Small focal necrosis in the hard substance of the crown of the teeth (caries)

No. 47 Slight to moderate round-cell infiltration in the connective tissue of the gums and muscles

No. 51 N.A.D.

Dose group 1800 ppm, sex ♂

Animal No.390 N.A.D.

No.393 N.A.D.

No.407 Moderate acute, focal gingivitis in the crown-root region

No.411 Moderate acute, focal gingivitis in the crown-root region

No.413 N.A.D.

Dose group 0 ppm, sex ♀

Animal No. 90 Very slight acute, focal gingivitis in the crown-root region

No. 94 N.A.D.

No.114 N.A.D.

No.118 Very slight acute, focal gingivitis in the crown-root region
Small focus of necrosis in the hard substance of the teeth and the pulp.

No.119 N.A.D.

Dose group 1800 ppm, sex ♀

Animal No.341 Moderate subchronic myositis. The epithelium over this is hyperkeratotic. It is assumed that the changes are the result of an external injury.

No.443 Very slight acute, focal gingivitis in the crown-root region

No.457 Slight acute, necrotic focal gingivitis in the crown-root region.
Small focus of necrosis in the hard substance of the crown of the teeth (caries).

No.461 Slight necrotic focal gingivitis in the crown-root region.
Focal oedema in the connective tissue of the gums.

No.466 Slight acute, focal gingivitis in the crown-root region.

ATTACHMENT

Histopathology Incidence

Twenty-One Month Mouse Study

A Brief Coversheet for Carcinogenesis Study Review

NDA 20-356
Drug: Nisoldipine
Sponsor: Miles Inc. Pharmaceutical Division

1. Species & Strain	Mouse, Bor:NMRI (SPF HAN)
2. Name of Laboratory	
3. No./sex/group	50 (additional 20 mice/sex/group were included for interim sacrifice at 12 months)
4. Doses (C, L, M & H)	0, 100, 300 and 900 ppm in diet
5. Basis for dose selection stated: yes (x) no ( )	28-day dietary dose rangefinding study in mice
6. Interim sacrifice	yes
7. Total duration	21 months
8. No. alive at termination (C, L, M & H)	Males: 36, 35, 31 & 10 Females: 22, 16, 16 & 18
9. Statistical methods used	Two-tailed U test, generalized Wilcoxon test & Peto analysis

10. Tumor and non-tumor data for each tissue attached.

MOUSE STUDY

The following findings were observed in control and dose animals: no test substance effect (group specificity) was detectable (see Tables in Appendix, p. 341 to 381).

1. Non-blastomatous changes: (interim kill)

Liver : Slight to moderate lipid storage in hepatocytes, isolated round cell infiltrates and slight intracytoplasmic vacuoles were seen in several animals.

Kidneys : Tubular dilations (partially with epithelial desquamation and/or plasma basophilia), PAS-positive casts, cysts in the papillary area and round cell infiltrates in the area of the venae stellatae and the pelvis were found in most animals.

Bladder : Round cell infiltrates in the submucosa were found in several animals.

Testes : Several animals showed spermiogranuloma, atrophy, some convoluted seminiferous tubules and round cell infiltrates.

Uterus : Several animals showed endometrial cysts.

Salivary glands: Slight round cell infiltrates were found in some animals.

Stomach : In several animals, the glandular mucosa showed retention cysts in the fundal area, to some extent with squamous metaplasia and epithelial proliferation. Some animals had round cell infiltrates in the area of the submucosa.

Intestine : Animals 215, 216 (both died) and 503 (all from the 900 ppm dose group) showed a tightly filled, dilated colon.

Eyes : Some animals showed slight round cell infiltrates in the episcleral area.

Thyroid : Several animals showed small cysts (remains of the thyroglossal duct).

Individual findings

No. 14 (0 ppm; ♂), Epididymis: aspermia
18 (0 ppm; ♂), Pancreas: islet cell hyperplasia
Spleen: increased haematopoiesis
Prostate: purulent prostatitis and urethritis
Bladder: purulent cystitis
225 (900 ppm; ♂), Nasopharynx: cellular or inflammatory cellular infiltration, slight
226 (900 ppm; ♂), Subcutis: abscess
284 (0 ppm; ♀), Large intestine: section containing helminthes
286 (0 ppm; ♀), Adrenals: intracytoplasmic vacuoles in adrenal cortex cells
289 (0 ppm; ♀), Kidneys: glomerular atrophy
Ovaries: follicular cysts
291 (0 ppm; ♀), Stomach: erosion with round cell infiltrates in the area of the keratinising squamous epithelium
437 (300 ppm; ♀), Lung: multifocal bronchopneumonia

Heart, brain, seminal vesicle, oesophagus, lymph nodes, pituitary, trachea, skin, bones, bone marrow, skeletal muscles and spinal cord were histopathologically normal.

2. Blastomas

A synoptic comparative review of the tumours is shown on Page 340. All tumours are regarded as having occurred spontaneously and are not attributed to the treatment with the test substance BAY k 5552, as no dose-dependency is recognisable from the number, type or location.

Tumour carriers (interim kill)

Group 0 ppm, ♂

Animal No.	14	Kidney	Malignant lymphoma

Group 900 ppm, ♂

Animal No.	211	Lung	Alveologenic carcinoma (Kimura Type A)+
	212	Kidney	Malignant lymphoma
	214	Liver	Malignant hepatoma
	224	Lung	Alveologenic carcinoma (Kimura Type AP)

Group 0 ppm, ♀

Animal No.	281	Lung	Alveologenic carcinoma (Kimura Type A)
+	289	Kidney, salivary gland, pancreas, bladder	Malignant lymphoma
	290	Stomach	Keratoacanthoma
	291	Kidney, salivary gland	Malignant lymphoma

Group 900 ppm, ♀

Animal No.	493	Thymus, kidney, serosa	Malignant lymphoma
	499	Kidney	Malignant lymphoma
	501	Lung	Alveologenic carcinoma (Kimura Type A)
		Kidney	Malignant lymphoma
	502	Kidney, bladder	Malignant lymphoma
	503	Lung	Alveologenic carcinoma (Kimura Type A)

Group 300 ppm, ♀

Animal No.	430	Vagina	Keratoacanthoma
	438	Lung	Alveologenic carcinoma (Kimura Type AP)
	439	Thymus	Thymic lymphosarcoma

+ Kimura, J.: Progression of pulmonary tumor in mice. Acta path. jap. 21, 13 ff (1971)

8.5 Autopsy findings

G	died
ZS	sacrificed at interim kill
VE	sacrificed at end of study
MG	killed in extremis

8.6 List of abbreviations for Tables with individual histopathological findings

A	= autolysis
Abd	= abdominal cavity
Abz	= abscess
An	= inflammatory congestion of secretions in the anal gland
Ap	= angiopathy
Asp	= aspermia
At	= atrophy
b	= bilateral
Cy	= cyst(s)
De	= degeneration (for pituitary: cystic degeneration)
Dil	= dilation
e	= unilateral
EPI	= epididymis
Er	= erosion
F	= hepatocellular fatty infiltration/fat marrow
Fca	= foci of cellular alteration
Gg	= biliary proliferation
H	= pituitary
Hä	= haemorrhage
Hb	= bladder
HD	= Harder's gland
He	= helminthes
Hn	= hydronephrosis
Hp	= increased haematopoiesis
Ht	= skin
Hy	= hyperplasia for the pituitary: diffuse and/or focal hyperplasia for the stomach: adenomatous hyperplasia of the glandular stomach for the uterus: cystic/hyperplastic endometrium for the adrenals: proliferated subcapsular cortical cells for the pancreas: multifocal hyperplasia of the islets of Langerhans for the testes: hyperplasia of the Leydig cells
Iz	= inflammatory infiltrates
Iz Ne	= inflammatory necroses
Kv	= lipid-free, cytoplasmatic vacuoles, lying close to the nucleus. In male mice, the vacuoles are often so paranuclear, that the nucleus is deformed into a crescent shape.

Kz = diffusely proliferated Kupffer's cells
LE = liver
LN = lymph nodes
LU = lung
Ma = mammary gland
Mh = arterial medial hyperplasia
Med = mediastinum
Mes = mesentery
Mi = increased occurrence of mitoses
Min = mineralisation foci in the gastric mucosa
Mp = epithelial metaplasia
Mz = in the liver: megalohepatocytes
in the brain: malacia
Ne = necrosis
Nez = increased occurrence of unicellular necroses
NN = adrenals
NNH = paranasal sinuses
Np = progressive senile nephropathy
O = without histopathological findings: findings within normal variability which correspond particularly to species age of study animals and conventional keeping condition. Normal findings in all animal groups include, amongst others: effects from the kill, preparation artefacts (e.g. different degrees of exsanguination, varying degrees of lung collapse, artificial emphysema, slight autolysis), small round-cell infiltrates e.g. of the head salivary glands, liver, kidneys, bladder; dysontogenetic cysts in the kidneys; individual small endometrium cysts; slight to moderate brown degeneration of the adrenal x-zone (ceroid deposit in the area around the corticomedullary junction, almost only in female animals) and slight subcapsular cell reaction; intranuclear inclusions in the hepatic cells; distended excretory ducts of the mammary glands filled with secretion; slight to moderate progressive senile nephropathy
Oph = ophthalmopathy
Ov = ovary
e = not investigated, no histological sample available
Ö = oedema
P = pelvic serosa
PA = pancreas
PD = hyperplasia of the preputial gland
PDS = secretion congestion in the preputial gland
Pel = peliosis hepatis
Pi = increased occurrence of pigment
PIT = pituitary gland
PRO = prostate gland
PT = organ investigated histopathologically, no parenchyma tumour found
RHS = reticulohistiocytary system
Rz = cellular infiltrates consisting mainly of round cells
Sc = subcutis
SLGL = salivary gland
Spgr = spermiogranuloma
SV = seminal vesicle

T = one tumour
TM = tumor metastasis
bT = benign tumour
mT = malignant tumour
mTL = systemic, malignant tumour, e.g. malignant lymphoma
Ta = telangiectasis
Th = thrombus
Thy = thymus
THYR = thyroid gland
UBL = urinary bladder
Ul = ulcer
Ut = uterus
V = vacuoles

Investigations after 12 months

A = postmortal autolysis
Asp = aspermia (epididymis)
At = atrophy
Cy = cystic changes (e.g. retention cysts in the glandular stomach)
Dil = colon dilated and engorged
emH = extramedullary haematopoiesis
Er = erosion in area of forestomach mucosa
F = Oil Red-O positive substances in hepatocytes
Glo = glomerula atrophy
Ihyp = islet cell hyperplasia (pancreas)
Iz = cellular and/or inflammatory cellular infiltration
Met = squamous epithelium metaplasia in gastric retention cysts
Ne = hepatic cell necrosis
O = findings within normal variability which correspond particularly to species, age of study animals and conventional keeping conditions.
ø = not investigated (not available)
P = cut surface of parasites (intestine)
Pro = proliferation
Sg = spermiogranuloma
T = tumour (blastoma)
Tub = tubular renal changes (dilation, desquamation, epithelial basophilia)
V = intracytoplasmatic vacuoles (liver: lipid-free vacuoles)
Zy = PAS-positive cylinder in renal tubule

Degrees of intensity

\+ = very slight
1 = slight
2 = moderate
3 = severe

FINDINGS	INCIDENCES OF NON-NEOPLASTIC LESIONS *)							
DOSE (ppm)	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
heart								
n	50	48			50	50		
A2	3	0			5	1		
Ap1	0	1			0	0		
Ap2	1	0			0	2		
De1	1	0			4	0		
De2	1	0			0	0		
Fi1	0	2			1	0		
Fi2	1	0			0	0		
Iz1	1	0			0	1		
Iz3	1	0			0	0		
Rz1	1	0			2	1		
Th1	0	2			0	0		
Th2	0	1			0	0		
trachea								
n	50	44			50	50		
A2	3	0			4	1		
Mp1	0	0			1	0		
D3	0	1			0	0		
Rz2	0	0			1	0		
lung								
n	50	48			50	50		
A2	2	0			5	2		
Iz2	1	0			0	0		
Iz3	1	0			1	0		
D2	2	1			1	0		
D3	0	0			0	1		
Rz2	1	1			0	0		
Th1	0	0			0	1		
Th2	1	0			0	0		
head salivary glands								
n	49	48			50	50		
A2	5	0			3	0		
A3	0	0			3	0		
Ap1	0	3			0	0		
Ap2	0	1			0	0		
At2	0	0			1	0		
D2	0	1			0	1		
Rz2	2	8			1	1		

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FINDINGS — INCIDENCES OF NON-NEOPLASTIC LESIONS *)

DOSE (ppm)	0		100 *)		300		900 **)	
Sex	M	F	M	F	M	F	M	F
liver								
n	50	47			50	50		
A2	3	0	2	4	2	1	3	3
A3	0	0	0	1	0	0	0	0
Ap2	1	0	0	0	0	0	0	0
clear cell foci	0	0	0	0	0	0	0	1
F2	9	16	11	0	7	6	18	4
F3	0	0	0	0	1	1	2	0
Fca	0	0	0	0	2	0	2	0
Fca1	0	0	0	0	0	1	0	0
Fi1	1	0	0	0	0	1	0	1
Gg3	0	0	0	0	0	1	0	0
Ha2	0	0	0	0	0	1	0	0
Hp1	1	1	3	0	1	1	0	0
Hp2	1	2	0	0	1	1	0	1
Hp3	0	1	0	0	0	0	0	0
Kv1	4	6	7	9	9	6	6	4
Kv2	1	2	1	7	3	8	4	10
Kv3	0	3	1	4	4	8	6	16
Kz2	1	0	1	0	0	0	0	0
Mi	2	1	0	0	0	0	0	0
Mi1	1	0	2	0	0	0	1	0
Mh1	2	0	2	0	1	0	0	0
Mz1	1	0	0	0	0	0	0	0
Mz3	0	0	0	0	0	1	0	0
Ne1	2	1	1	2	4	1	2	0
Ne2	1	1	0	2	1	1	0	0
Ne3	1	0	1	0	0	1	0	0
Nez	0	0	0	0	0	0	0	1
Nez1	3	1	0	0	1	0	0	0
Pi1	0	0	0	0	1	0	0	0
Pi2	1	0	0	0	0	0	0	1
Rz1	6	1	3	0	2	0	1	0
Rz2	0	1	0	0	0	1	0	0
Ta2	0	0	0	0	0	1	0	0
Th2	0	1	0	0	0	0	0	0
Vi	1	0	0	0	0	0	0	0
pancreas								
n	49	45			50	49		
A2	2	0			5	0		
A3	0	0			0	1		
Ap1	1	0			0	0		
Ap2	0	1			1	0		
Ap3	0	1			0	0		
At1	0	2			0	0		
At2	0	1			0	1		
He	0	0			0	1		
Hy3	1	0			0	0		
Di	0	0			0	2		
Di2	0	2			0	3		
Rz2	0	1			0	2		
Rz3	0	1			0	0		

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FINDINGS — INCIDENCES OF NON-NEOPLASTIC LESIONS *)

DOSE (ppm)	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
oesophagus								
n	50	45			50	50		
A2	1	0			0	0		
Iz Ne3	0	0			1	0		
stomach								
n	49	48	49	49	50	50	49	49
A2	2	0	4	6	6	6	14	6
A3	1	1	0	3	0	1	2	0
Ap1	1	1	1	0	1	0	0	0
Ap2	0	0	1	0	0	0	0	0
Er1	0	0	0	0	1	0	0	0
Hy	0	0	0	0	1	0	0	0
Hy1	5	1	5	4	7	1	3	6
Hy2	3	2	6	3	5	3	6	1
Hy3	1	0	4	1	6	1	3	1
Iz2	0	0	0	0	0	0	1	0
Min2	0	0	0	0	0	1	0	0
D2	0	2	1	0	0	0	0	2
Ul1	0	0	0	0	0	2	3	0
Ul2	0	0	0	0	0	1	0	0
small intestine								
n	49	48			50	50	49	49
A2	4	1			5	10	15	6
A3	0	2			1	3	2	1
Ap1	0	2			0	0	0	0
D1	0	1			0	1	0	0
large intestine								
n	49	48			50	50	49	49
A2	5	1			5	11	15	7
A3	1	2			1	3	2	0
Ap1	1	2			0	0	0	0
Ap2	0	0			0	1	0	0
Ap3	0	1			0	0	0	0
Ne	4	0			5	1	10	1
D2	0	3			0	2	1	4
Ul2	0	0			0	0	0	1

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FINDINGS — INCIDENCES OF NON-NEOPLASTIC LESIONS *)

DOSE (ppm)	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
spleen								
n	49	48			50	49		
A2	3	0			6	1		
At2	0	0			1	0		
At3	0	1			0	0		
Hp1	5	1			4	0		
Hp2	2	6			4	3		
Hp3	0	4			0	3		
Hy2	1	5			3	9		
Hy3	0	0			2	2		
lymph nodes								
n	42	47			48	36		
A2	1	0			5	0		
Ap1	0	1			0	0		
Ap3	0	2			0	0		
Hp2	0	1			0	0		
Hp3	0	1			0	0		
Hy2	0	2			0	5		
Hy3	0	1			0	1		
kidneys								
n	96	96			100	100		
A2	5	0			6	4		
Ap1	2	3			1	2		
Ap2	5	3			3	0		
Ap3	0	0			0	0		
Hn	0	0			1	0		
Hp3	0	6			0	6		
Rz2	10	11			6	11		
Rz3	0	1			0	0		
Th1	0	1			0	1		
testes								
n	98	-			100	-		
A2	2	-			5	-		
Ap1	7	-			9	-		
Ap2	3	-			5	-		
At1b	2	-			0	-		
At1e	6	-			1	-		
At2b	7	-			4	-		
At2e	7	-			8	-		
At3e	0	-			1	-		
Hy1	0	-			2	-		
Hy2	0	-			1	-		

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FINDINGS	INCIDENCES OF NON-NEOPLASTIC LESIONS *)							
DOSE (ppm)	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
epididymis								
n	95	-			95	-		
A2	2	-			5	-		
Asp	18	-			14	-		
Spgr	1	-			0	-		
prostate								
n	45	-			48	-		
A2	2	-			5	-		
Ap1	1	-			0	-		
Iz2	0	-			2	-		
Iz3	2	-			0	-		
seminal vesicle								
n	49	-			50	-		
A2	1	-			6	-		
Ap1	2	-			1	-		
Ap2	0	-			2	-		
Ma2	0	-			1	-		
Iz1	9	-			8	-		
Iz2	20	-			8	-		
urinary bladder								
n	48	47			50	48		
A2	2	2			5	6		
A3	1	1			1	3		
Ma3	0	0			1	0		
Iz1	1	0			0	0		
Rz2	0	7			0	2		
ovaries								
n	-	92			-	96		
Ap1	-	1			-	2		
Ap2	-	2			-	3		
Ap3	-	8			-	3		
Cy3	-	4			-	2		
Th3	-	0			-	1		
Amyloid 1	-	1			-	0		

INCIDENCES OF NON-NEOPLASTIC LESIONS *)

DOSE (ppm)	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
uterus								
n	-	46	-	47	-	49	-	46
A3	-	1	-	0	-	0	-	0
Ap1	-	0	-	0	-	2	-	0
Ap2	-	1	-	0	-	0	-	0
Ap3	-	1	-	0	-	2	-	0
Dil1e	-	0	-	0	-	0	-	5
Dil2	-	1	-	0	-	0	-	0
Dil2e	-	0	-	1	-	0	-	0
Dil2b	-	0	-	0	-	0	-	1
Dil3	-	0	-	0	-	2	-	0
focal pellosis	-	0	-	0	-	0	-	1
granular cell focus	-	0	-	1	-	0	-	0
Hy2	-	3	-	11	-	12	-	8
Hy3	-	3	-	5	-	4	-	5
He2	-	0	-	0	-	1	-	0
Th3	-	0	-	1	-	1	-	0
mamma								
n	-	40			-	33		
D3	-	0			-	1		
Rz2	-	0			-	1		
skin								
n	49	47			50	50		
Abz	0	0			1	0		
Iz1	0	0			0	2		
Iz2	0	0			1	2		
Iz3	1	3			0	3		
Iz He3	0	0			4	0		
He	0	1			0	4		
D3	0	1			0	1		
pituitary gland								
n	40	35	-	38	46	41	-	38
A1	0	0	-	4	0	0	-	0
A2	1	0	-	0	5	0	-	0
De1	0	1	-	0	0	0	-	0
Dil1	0	1	-	2	0	0	-	0
Dil2	0	1	-	4	0	0	-	0
Hy1	0	2	-	1	0	2	-	8
Hy2	0	2	-	3	0	3	-	5
Hy3	0	2	-	1	0	4	-	3
thyroid gland								
n	45	40			48	47		
A2	1	0			3	1		
Ap1	0	1			0	0		

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FINDINGS — INCIDENCES OF NON-NEOPLASTIC LESIONS *)

DOSE (ppm)	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
adrenals								
n	95	88			99	99		
A2	3	0			6	1		
Ap2	2	1			0	0		
Cy3	0	0			1	0		
Ma1	0	1			0	0		
My2	12	2			12	0		
My3	5	0			1	0		
Iz1	0	0			0	1		
Pi3	0	23			0	10		
Rz3	0	0			1	0		
Th1	0	0			0	1		
Th3	0	0			1	0		
V2	0	1			0	1		
V3	0	0			0	1		
brain								
n	49	48			50	50		
A2	5	1			6	1		
A3	0	0			0	1		
Cy2	0	0			1	0		
Ma3	0	1			0	0		
Mz1	0	0			1	0		
Rz1	0	0			0	2		
Rz2	0	4			0	1		
spinal cord								
n	49	48			50	50		
A2	3	0			6	1		
A3	1	0			0	0		
Cy1	0	0			1	0		
Rz1	1	2			0	1		
Rz2	0	2			0	1		
eye								
n	98	96			99	98		
A2	5	3			2	9		
A3	2	1			4	3		
Hz1	0	0			4	0		
Iz2	0	0			1	1		
Iz Me2	0	0			2	0		
Iz Me3	0	0			1	0		
Oph2e	1	0			1	0		

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INCIDENCES OF NON-NEOPLASTIC LESIONS *)

	0		100 *)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
muscle								
n	50	48			50	50		
A2	5	0			6	1		
Ap1	1	1			0	0		
Ap2	0	1			0	0		
Ap3	0	1			0	0		
D3	0	0			0	1		
Rz1	0	0			1	0		
Rz2	0	2			0	2		
bone, bone marrow								
n	50	48			50	50		
A2	3	0			6	0		
F2	1	0			0	0		
Fi1	2	3			0	11		
Fi2	0	20			0	21		
My1	1	0			0	0		
others								
MESENTERIUM (Mes:)								
n #)								
Ap1	0	0			0	1		
Ap2	2	0			1	0		
Iz3	0	0			1	0		
Melz3	0	0			0	1		
Rz2	0	4			3	7		
(PO:)								
(preputial gland)								
n #)								
PDS2	1	0			0	0		
PD3A2	0	0			1	0		
PD3	4	0			1	0		
SUBCUTIS (Sc:)								
n #)								
DZ	0	1			0	1		
D3	0	1			0	2		
Iz Me2	1	0			0	0		
Iz3	1	0			0	0		
Abz	1	0			0	0		
An2	0	1			0	2		
Ap3	0	1			0	0		

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FINDINGS	INCIDENCES OF NON-NEOPLASTIC LESIONS *)							
DOSE (ppm)	0		100 **)		300		900 **)	
SEX	M	F	M	F	M	F	M	F
paranasal sinuses (# #)								
1z2	1	0			0	0		
Harder's gland		47				49		
A3		2				1		

*) /Animals sacrificed at interim kill were not considered.

**) In this group, only stomach, uterus, pituitary and liver were examined.

***) In this group, stomach, uterus, pituitary, liver, large and small intestine were examined completely. Organs (extent see group 0 ppm and 300 ppm) were examined in respect to tumors.

#) Only macroscopically changed organs were examined.

Table 17: Summary of number of male and female mice with benign and/or malignant tumours, as well as frequency of benign and malignant tumours encountered

Sex	♂			♀		
Dose ppm	0	300	900	0	300	900
No. of animals investigated	50	50	49	48	50	50
No. of animals with tumours	27	28	24	34	31	30
No. of animals with only benign tumours	7	6	8	6	6	6
No. of animals with only malignant tumours	12	15	14	21	18	17
No. of animals with benign and malignant tumours	8	7	2	7	7	7
No. of animals with more than one primary tumour	15	11	5	13	9	8

Table 18: Comparative summary of tumours occurring according to location, type, number and dignity $ (animals scheduled for terminal kill)

Sex	♂				♀			
Dose ppm	0	100	300	900	0	100	300	900
Lung:		-				-		
bronchiolo-alveolar adenoma	2		3	4	2		1	3
bronchiolo-alveolar carcinoma (malig.)	13		12	5	8		6	5
Stomach:								
papilloma	0	0	0	2	0	0	0	0
sarcoma (malig.)	0	0	2	1	0	0	0	0
Liver:								
hepatocellular adenoma	2	2	2	3	0	0	0	1
hepatocellular carcinoma (malig.)	3	4	5	8	0	0	0	1
haemangiosarcoma (malignant)	1	0	0	0	0	0	0	0
Kidneys:		-				-		
tubular carcinoma (malignant)	0		1	0	0		0	0
haemangiosarcoma (malignant)	0		1	0	0		0	0
Bladder:		-				-		
stromal tumour (benign)	1		1	2	0		0	0
stromal tumour (malignant)	0		1	1	0		0	0
Ovary:	-	-	-	-		-		
granulosa-theca cell tumour (ben.)					5		5	3
granulosa-theca cell tumour (malig.)					1		0	0
luteoma (benign)					2		2	0
tubular adeno-carcinoma (malig.)					1		0	0
Sertoli cell tumour (benign)					0		0	1
Uterus:	-	-	-	-				
adenoma					0	0	1	0
carcinoma (malig.)					1	0	0	0
fibroma					0	0	1	0
myoma					0	0	1	2
myosarcoma (malig.)					0	0	0	1
stromal tumour (benign)					3	0	2	2
stromal sarcoma (malignant)					1	3	2	2

Table 18 (continued):

Sex	♂				♀			
Dose ppm	0	100	300	900	0	100	300	900
Testes:		-				-	-	-
Leydig cell tumour (benign)	2		2	0				
adenoma of rete testis	1		0	0				
Pituitary:		-						
adenoma	2		0	0	0	3	3	1
Thyroid:		-				-		
follicle cell adenoma	0		1	0	0		0	0
papillary cyst-adenoma	1		0	0	0		0	0
Adrenals:		-				-		
cortical adenoma	3		3	1	2		0	0
phaeochromocytoma (benign)	1		0	0	1		0	2
phaeochromocytoma (malignant)	0		0	1	0		0	0
RH system:		-				-		
lymphoma (malig.)	7		3	1	18		12	14
lymph node sarcoma (malignant)	1		0	0	1		0	0
Skin/subcutis:		-				-		
epithelioma (malignant)	0		0	1	0		0	0
sarcoma (malig.)	1		0	0	0		3	0
Mammary gland:	-	-	-	-		-		
carcinoma (malig.)					3		0	0
adeno-ancanthoma (malignant)					0		1	1
Harder's gland:		-				-		
papillary adenoma	3		2	0	1		1	1
Spinal marrow:		-				-		
schwannoma (malig.)	0		0	0	0		1	0
Bones:		-				-		
osteosarcoma (malignant)	0		0	0	0		1	0
Abdomen:		-				-		
haemangiosarcoma (malignant)	0		0	0	0		1	0
Pelvic serosa:		-	-	-	-	-	-	-
sarcoma (malig.)	1							

- Organ not investigated $ Bilateral tumours counted twice

List of blastomas occurring in the histopathologically investigated mice from the terminal kill

Dose group: 0 ppm, male

Animal No.	21	Lung	Bronchiolo-alveolar carcinoma
	22	Liver	Hepatocellular carcinoma
		Adrenal	Phaeochromocytoma, benign
	23	Lung	Bronchiolo-alveolar carcinoma
	28	Testes	Leydig cell tumour, benign
	30	Lung	Bronchiolo-alveolar carcinoma
	32	Adrenal	Cortical adenoma
	36	Lung	Bronchiolo-alveolar carcinoma
	42	Lung	Bronchiolo-alveolar adenoma
	44	Lung	Bronchiolo-alveolar carcinoma
		Liver	Haemangiosarcoma
	46	Harder's gland	Papillary adenoma
	47	Lung	Bronchiolo-alveolar carcinoma
		RHS	Malignant lymphoma of spleen, lymph nodes, liver, kidney, mesentery
	48	Lung	Bronchiolo-alveolar carcinoma
		RHS	Malignant lymphoma of spleen, lymph nodes, heart, lung, head salivary glands, liver, pancreas, stomach, intestine, kidney, bladder, prostate, skin, skeletal muscles, bone marrow, mesentery, urethra
	50	Lung	Bronchiolo-alveolar carcinoma
		RHS	Malignant lymphoma of spleen, lymph nodes
	51	Pituitary	Adenoma
		Subcutis (ear)	Sarcoma
	52	Harder's gland	Papillary cystadenoma
	54	Lymph nodes	Sarcoma with metastases in the pelvic serosa

Animal No. 55	Testes	Leydig cell tumour, benign
58	Testes	Papillary adenoma of the rete testis
	Adrenals	Cortical adenoma
59	RHS	Malignant lymphoma of spleen, heart, lung, liver, pancreas, prostate, skin, skeletal muscles
63	Lung	Bronchiolo-alveolar carcinoma
	Liver*	Hepatocellular adenoma
64	Lung	Bronchiolo-alveolar carcinoma
	Thyroid gland	Papillary cystadenoma
65	Liver	Hepatocellular carcinoma
	RHS	Malignant lymphoma of mesentery
66	Liver*	Hepatocellular adenoma
	RHS	Malignant lymphoma of spleen, lymph nodes, heart, lung, pancreas, oesophagus, stomach, kidneys, bladder, prostate, seminal vesicle, skin, adrenals, skeletal muscles, preputial gland, mesentery
67	Lung	Bronchiolo-alveolar carcinoma
	RHS	Malignant lymphoma of lymph nodes, mesentery
	Pituitary	Adenoma
68	Lung	Bronchiolo-alveolar carcinoma
	Bladder	Polypous stromal tumour with adenomatous parts. The tumour contains foci of polymorphous, partially epithelioid cells with central necrosis and localised argentophilic fibrous structures (benign)
	Adrenals	Cortical adenoma
	Harder's gland	Papillary adenoma
69	Lung	Bronchiolo-alveolar carcinoma
	Pelvic serosa	Sarcoma, infiltrates the neighbouring skeletal muscles

* = The tumour was found in one of the additionally prepared Paraplast blocks

Animal No. 70	Lung	Bronchiolo-alveolar adenoma
	Liver	Hepatocellular carcinoma

Dose group 100 ppm, male

Only the hepatocellular neoplasias found are reported here.

Animal No. 98 Liver: Hepatocellular adenoma
No. 101 Liver: Hepatocellular adenoma
No. 110 Liver: Hepatocellular carcinoma
No. 115 Liver: Hepatocellular carcinoma
No. 125 Liver: Hepatocellular carcinoma
No. 129* Liver: Hepatocellular carcinoma

Dose group 300 ppm, male

Animal No.	161	Liver	Hepatocellular carcinoma
	163	Lung	Bronchiolo-alveolar carcinoma
	164	Lung	Bronchiolo-alveolar carcinoma
		Testes	Leydig cell tumour, benign
	167	Lung	Bronchiolo-alveolar carcinoma
		Kidneys	Haemangiosarcoma
	168	Liver	Hepatocellular carcinoma
		Harder's gland	Papillary adenoma
	172	Testes	Leydig cell tumour, benign
	173	Stomach	Sarcoma
		Kidney	Tubular renal epithelial carcinoma
	175	Lung	Bronchiolo-alveolar carcinoma
	176	Liver*	Hepatocellular adenoma
		RHS	Malignant lymphoma of spleen, lymph nodes, kidneys, mesentery
	177	Lung	Bronchiolo-alveolar adenoma
	180	Lung	Bronchiolo-alveolar carcinoma
		Liver*	Hepatocellular carcinoma
	181	Lung	Bronchiolo-alveolar carcinoma
	182	Lung	Bronchiolo-alveolar carcinoma
		Thyroid gland	Follicle cell adenoma
	183	Lung	Bronchiolo-alveolar adenoma
	184	Lung	Bronchiolo-alveolar carcinoma
		Harder's gland	Papillary adenoma
	188	RHS	Malignant lymphoma of lymph nodes and kidneys
	189	Liver	Hepatocellular carcinoma
		Bladder	Small, polypous, benign stromal tumour covered with hyperplastic urothelium, in the centre of which lies a nest of large, epithelioid, polymorphous cells

Animal No. 190 RHS		Malignant lymphoma of spleen, lymph nodes, head salivary glands, liver, pancreas, stomach, kidneys, bladder, epididymis, seminal vesicle
191	Adrenals	Cortical adenoma
195	Stomach	Sarcoma
	Adrenals	Cortical adenoma
196	Liver	Hepatocellular carcinoma
199	Lung	Bronchiolo-alveolar carcinoma
201	Liver*	Hepatocellular adenoma
204	Lung	Bronchiolo-alveolar carcinoma
205	Lung	Bronchiolo-alveolar carcinoma
206	Adrenals	Cortical adenoma
	Lung	Bronchiolo-alveolar adenoma
207	Bladder	Malignant stromal tumour in the subepithelium, consisting of spindle-shaped to epithelioid cells and interspersed with fine argentophilic fibres. The tumour infiltrates the muscular layer of the urinary bladder
210	Lung	Bronchiolo-alveolar carcinoma

Dose group 900 ppm, male

Animal No. 234	Bladder	Stromal tumour (benign)
	Lung	Bronchiolo-alveolar adenoma
238	Lung	Bronchiolo-alveolar adenoma
239	Liver	Hepatocellular carcinoma
240	Liver	Hepatocellular carcinoma
242	RHS	Malignant lymphoma
	Bladder	Benign stromal tumour: a small node in the propria, infiltrated by leucocytes, which consists of spindle-shaped to epithelioid cells. On the lumen side, the propria is oedematous. Overpigmented macrophages are present at the periphery of the node. The urothelium above the tumour is hyperplastic.
	Stomach	Inverted papilloma of pars cutanea
244	Liver	Hepatocellular carcinoma
248	Liver	Hepatocellular carcinoma
	Lung	Bronchiolo-alveolar carcinoma
244	Liver	Hepatocellular carcinoma
250	Lung	Bronchiolo-alveolar carcinoma
251	Liver*	Hepatocellular adenoma
252	Lung	Bronchiolo-alveolar carcinoma
255	Lung	Bronchiolo-alveolar carcinoma
257	Liver	Hepatocellular adenoma (frozen section)
258	Liver	Hepatocellular adenoma
260	Skin (ear)	Epithelioma

Animal No. 264	Lung	Bronchiolo-alveolar carcinoma
	Liver	Hepatocellular carcinoma
268	Adrenals	Malignant phaeochromocytoma; the voluminous tumour has numerous mitoses and multi-focal necroses
270	Stomach	Inverted papilloma of pars cutanea
271	Lung	Bronchiolo-alveolar adenoma
273	Adrenals	Cortical adenoma
275	Lung	Bronchiolo-alveolar adenoma
	Liver	Hepatocellular carcinoma
277	Stomach	Subserous sarcoma
279	Stomach	Metastases of an osteosarcoma
280	Liver	Hepatocellular carcinoma

Dose group 0 ppm, female

Animal No. 302	RHS	Malignant lymphoma of spleen, lymph nodes, heart, lung, head salivary glands, liver, stomach, kidneys, bladder, ovaries, uterus, thyroid, adrenals, brain, spinal cord, skeletal muscles, bone marrow, subcutis
303	Mammary gland	Carcinoma with focal necroses
	Lymph nodes	Sarcoma
305	Lung	Bronchiolo-alveolar carcinoma
306	RHS	Malignant lymphoma of mesentery
307	Mammary gland	Carcinoma with focal necroses
308	RHS	Malignant lymphoma of spleen, lymph nodes, head salivary glands, pancreas, stomach, kidneys, bladder, ovaries, skeletal muscles, mesentery
309	Adrenals	Cortical adenoma
310	Lung	Bronchiolo-alveolar carcinoma
313	RHS	Malignant lymphoma of lymph nodes, pancreas, kidneys, thymus, mesentery
314	Adrenals	Cortical adenoma
315	Lung	Bronchiolo-alveolar carcinoma
	RHS	Malignant lymphoma of spleen, lymph nodes, lung, liver, kidneys, brain, bone marrow, skeletal muscles, mammary gland, thyroid

Animal No. 315	Uterus	Stromal tumour (benign)
316	Lung	Bronchiolo-alveolar adenoma
317	RHS	Malignant lymphoma of lymph nodes, liver, kidneys, bladder, skeletal muscles
	Ovary	Granulosa theca cell tumour (benign)
319	Lung	Bronchiolo-alveolar carcinoma
	Ovary	Granulosa theca cell tumour, which infiltrates neighbouring fatty tissue (malignant)
320	RHS	Malignant lymphoma of lymph nodes, heart, lung, pancreas, stomach, kidneys, uterus, mammary gland, pituitary, skeletal muscles, mesentery
	Ovary	Luteoma (benign)
321	Ovary	Granulosa theca cell tumour (benign)
322	RHS	Malignant lymphoma of lymph nodes, lung, head salivary glands, pancreas, kidneys, mammary gland, skin, eye, skeletal muscles, mesentery
323	Uterus	Stromal sarcoma, sarcomatous metastases of lymph nodes, lung, liver, (mes)ovary
324	RHS	Malignant lymphoma of lymph nodes, pancreas, kidneys, mesentery
325	Lung	Bronchiolo-alveolar adenoma
	Uterus	Stromal tumour (benign)

Animal No. 329	RHS	Malignant lymphoma of spleen, lymph nodes, heart, lung, head salivary glands, liver, stomach, kidneys, ovaries, mammary gland, skeletal muscles, bone marrow, mesentery
332	Lung	Bronchiolo-alveolar carcinoma
	RHS	Malignant lymphoma of spleen, lymph nodes, heart, lung, liver, pancreas, stomach, brain, skeletal muscles, mesentery
334	RHS	Malignant lymphoma of lymph nodes, heart, pancreas, kidneys, bladder, ovaries, uterus, mesentery
	Uterus	Stromal tumour (benign)
335	RHS	Malignant lymphoma of lymph nodes, lung, skeletal muscles, mesentery
337	RHS	Malignant lymphoma of lymph nodes, spleen, heart, trachea, lung, head salivary glands, liver, pancreas, stomach, kidneys, bladder, ovaries, uterus, mammary gland, skin, adrenals, brain, spinal cord, eyes, skeletal muscles, bone marrow, mesentery
338	Ovary	Luteoma
	RHS	Malignant lymphoma of thymus
339	Ovary	Granulosa theca cell tumour (benign)
	Adrenals	Phaeochromocytoma (benign)
340	RHS	Malignant lymphoma of spleen, lymph nodes, heart, trachea, lung, head salivary glands, pancreas, kidneys, ovaries, brain, spinal cord, skeletal muscles

Animal No. 341	RHS	Malignant lymphoma of heart, lung, liver, ovaries, skeletal muscles
	Uterus	Carcinoma
342	Ovary	Tubular adenocarcinoma
344	Lung	Bronchiolo-alveolar carcinoma
	Mammary gland	(Adeno)carcinoma
	Harder's gland	Papillary cystadenoma
345	Lung	Bronchiolo-alveolar carcinoma
346	Lung	Bronchiolo-alveolar carcinoma
	Ovaries	Granulosa theca cell tumour, bilateral (benign)
350	RHS	Malignant lymphoma of lymph nodes, pancreas, bladder, brain, skeletal muscles, subcutis, mesentery

Dose group 100 ppm, female

Animal No.	402	Pituitary	Adenoma
	404	Pituitary	Adenoma
	414	Pituitary	Adenoma

Dose group 300 ppm, female

Animal No. 442	RHS	Malignant lymphoma of thymus
444	RHS	Malignant lymphoma of lymph nodes, lung, liver, kidneys, mesentery, skeletal muscles
445	Bone system	Osteosarcomatous metastases of lung and liver
446	Ovary	Granulosa theca cell tumour (benign)
448	Uterus	Polypous stromal sarcoma
449	Spinal marrow	Schwannoma (malignant)
450	Lung	Bronchiolo-alveolar carcinoma
	RHS	Malignant lymphoma of spleen, lymph nodes, lung, mesentery, skeletal muscles
	Ovary	Granulosa theca cell tumour (benign)
451	Abdomen	Haemangiosarcoma, which infiltrates the cortex of one kidney
452	RHS	Malignant lymphoma of lymph nodes, kidney, mesentery, skeletal muscles
	Ovary	Granulosa theca cell tumour (benign)
	Pituitary	Adenoma of the pars intermedia
453	Lung	Bronchiolo-alveolar carcinoma
	Ovary	Luteoma (benign)
	Uterus	Stromal tumour (benign)

Animal No. 454	RHS	Malignant lymphoma of lymph nodes, heart, lung, liver, kidneys, ovaries, uterus, mesentery
455	Uterus	Polypous stromal tumour (benign)
	Subcutis (ear)	Sarcoma (schwannoma)
457	Ovary	Bilateral granulosa theca cell tumour (benign)
458	Lung	Bronchiolo-alveolar carcinoma
	Uterus	Myoma
459	RHS	Malignant lymphoma of pancreas, mesentery, mediastinum, skeletal muscles
460	Lung	Bronchiolo-alveolar carcinoma
	Subcutis (ear)	Sarcoma (schwannoma)
461	Uterus	Fibroma (benign)
464	Subcutis (ear)	Sarcoma (schwannoma) with necroses
465	Uterus	Adenoma
466	RHS	Malignant lymphoma of lymph nodes, heart, lung, pancreas, stomach, kidneys, skeletal muscles, mesentery, mediastinum
471	Lung	Bronchiolo-alveolar carcinoma
	Pituitary	Adenoma
472	Uterus	Stromal sarcoma

Animal No. 473	Lung	Bronchiolo-alveolar carcinoma
475	Ovary	Luteoma
478	RHS	Malignant lymphoma of spleen, lymph nodes, pancreas, stomach, large intestine, bladder, skin, thyroid, skeletal muscles, mesentery
479	Pituitary	Adenoma
	Harder's gland	Papillary adenoma
482	RHS	Malignant lymphoma of spleen, lymph nodes, head salivary glands, kidneys, bladder
483	RHS	Malignant lymphoma of pancreas, skeletal muscles, mesentery
484	Mammary gland	Adenoacanthoma (malignant)
485	Lung	Bronchiolo-alveolar adenoma
	RHS	Malignant lymphoma of spleen, lymph nodes, heart, lung, head salivary glands, liver, pancreas, bladder, ovaries, mammary gland, skin, skeletal muscles, bone marrow, mesentery
486	RHS	Malignant lymphoma of pancreas, skeletal muscles, mesentery

Dose group 900 ppm, female

Animal No. 511	RHS	Malignant lymphoma
512	RHS	Malignant lymphoma
515	RHS	Malignant lymphoma, stomach
517	Uterus	Stromal sarcomatous metastases of liver and mesentery
519	Adrenals	Phaeochromocytoma (benign)
	Uterus	Myosarcoma
520	RHS	Malignant lymphoma
521	Adrenals	Phaeochromocytoma (benign)
523	Ovary	Granulosa theca cell tumour (benign)
524	Ovary	Granulosa theca cell tumour (benign)
	Lung	Bronchiolo-alveolar carcinoma
525	RHS	Malignant lymphoma
526	Mammary gland	Adenoacanthoma (malignant)
527	RHS	Malignant lymphoma
528	Liver	Hepatocellular carcinoma
	Uterus	Myoma (benign)
530	Lung	Bronchiolo-alveolar carcinoma

Animal No.	531	Lung	Bronchiolo-alveolar adenoma
	535	Lung	Bronchiolo-alveolar carcinoma
	537	RHS	Malignant lymphoma
	538	RHS	Malignant lymphoma
	540	RHS	Malignant lymphoma
	541	Lung	Bronchiolo-alveolar carcinoma
		Harder's gland	Papillary cystadenoma
	542	RHS	Malignant lymphoma
	545	Lung	Bronchiolo-alveolar carcinoma
	546	RHS	Malignant lymphoma
		Lung	Bronchiolo-alveolar adenoma
	547	Uterus	Angiomatous stromal sarcoma
		Pituitary	Adenoma
	551	RHS	Malignant lymphoma
	553	Ovary	Granulosa theca cell tumour (benign)
		Lung	Bronchiolo-alveolar adenoma
	556	RHS	Malignant lymphoma
	557	Uterus	Polypous stromal tumour (benign)
	559	Uterus	Stromal tumour (benign)
	560	RHS	Malignant lymphoma
		Uterus	Myoma (benign)
		Liver	Hepatocellular adenoma

STATISTICAL REVIEW AND EVALUATION

NDA #: 20-356 Drug Class: Date: JAN 4 1994

Applicant: Miles Pharmaceutical Division

Name of Drug: Nisoldipine (Bay k 5552) Coat-core Tablets, 10, 20, 30, and 40 mg, q.d.

Indication: 1) Hypertension, alone or in combination with other antihypertensive agents; and 2) chronic stable angina (classical effort-associated angina). This review addresses the Hypertension claim.

Documents Reviewed: Volumes 1-5, 379, 388, and 400 of the NDA submission dated March 31, 1993. Also the data for the primary efficacy variable, supine diastolic blood pressure, was submitted on diskette for the double-blind portions of Studies D90-019, D90-029, and D90-039.

Medical Officer: The medical officer for this review is Dr. Cristobal Duarte.

I. INTRODUCTION

Nisoldipine is a dihydropyridine calcium channel blocker derived from nifedipine. The product is currently approved in 23 countries, although clinical development was discontinued in the United States because the brief duration of effect required multiple daily dosing. This application is for an extended-release formulation of nisoldipine, and studies once daily dosing for both hypertension and for angina. The coat-core tablet consists of an inner core containing 20% of the nisoldipine dose in an immediate-release form, surrounded by an outer coat containing 80% of the nisoldipine dose in a slow-release form.

This submission consisted of the results of two Phase II and three Phase III trials for hypertension which were carried out in the United States, and one South African hypertension study. The submission also contained two Phase III trials for angina which were carried out in the United States, and two foreign angina trials, one multi-national and one carried out in Israel. The angina claims will be addressed in another review.

II. CONTROLLED CLINICAL TRIALS

II.A. PROTOCOL NO. D90-019

II.A.1 Study Description

Study D89-019 was a sixteen-center dose-response study designed to compare three fixed doses of diltiazem with placebo in patients with mild to moderate hypertension.

The plan called for patients to be randomized to one of four parallel groups, receiving either placebo, nisoldipine 30 mg qd, nisoldipine 60 mg qd, or nisoldipine 90 mg qd, over an six week double-blind period. The 90 mg nisoldipine arm was deleted from the protocol by amendment before any patients were randomized.

After a four week washout period, patients who had a supine diastolic blood pressure (DBP) between 100 and 114 mmHg on each of the last two pre-randomization visits were eligible for randomization to double-blind treatment. The two supine DBP readings were required to be within 7 mmHg of each other. Blood pressure measurements were taken at trough (24 hr ± 30 minutes post-dose).

A total of 309 patients were enrolled in the placebo run-in period, and 221 were randomized to treatment group, with 72 assigned to placebo, 76 to nisoldipine 30 mg qd, and 73 to nisoldipine 60 mg qd. Nine placebo patients dropped out of the study, as did three low dose and 12 higher dose nisoldipine patients, while 197 patients completed the study A total of 213 were considered valid for efficacy analyses.

Patients were instructed to take three tablets each morning prior to 11 a.m. All patients randomized to nisoldipine began at 30 mg once daily, and those randomized to the 60 mg dose were titrated after one week. The relationship of drug administration to meals was not specified. The double-blind treatment phase lasted a total of six weeks, with patients evaluated weekly for the first four weeks and then again at the end of the study (week 6).

The primary efficacy variable was change from baseline (mean of weeks 3 and 4 of the placebo run-in) to endpoint in supine diastolic blood pressure at trough, 24 hours post-dose. Secondary efficacy variables included change from baseline in trough standing DBP and supine and standing systolic blood pressure (SBP). Response rates at trough were also analyzed, with response define in four different ways, (1) supine DBP no more than 90 mmHg; (2) a fall in supine DBP of at least 10 mmHg, (3) supine DBP≤ 90 mmHg **or** a fall in supine DBP ≥ 10 mmHg; and (4) supine DBP≤ 90 mmHg **and** a fall in supine DBP ≥ 10 mmHg.

At eight of the 16 centers 24-hour ambulatory blood pressure monitoring (ABPM) was performed at week 3 of the placebo run-in and at week 5 of the double-blind treatment period. The sponsor used the ABPM data to analyze the peak/trough ratio. A total of 117 patients had 24-hour ABPM monitoring. In-clinic blood pressure measurements were also taken at for 12 hours post-dose at seven centers after 4 weeks of placebo run-in and after six weeks of double-blind treatment.

II.A.2 Sponsor's Analysis

The sponsor performed both an evaluable patient analysis, including those patients with at least two post-baseline evaluations who were not protocol violators, and an intent-to-treat analysis including all patients at least one post-baseline evaluation. A last-observation-carried-forward approach was used to include the available data from patients who dropped out of the study. The initial analysis of variance model for the

primary and secondary change from baseline endpoints included treatment group, investigator, and treatment by investigator interaction terms. The interaction term was dropped if it was not significant at the .05 level. The initial comparison was the average of the nisoldipine groups versus placebo. If this was significant then pairwise comparisons were done to identify which nisoldipine doses were favored over placebo.

The sample size for this study was selected to give 90% power to detect a difference of at least 5 mmHg between the nisoldipine groups and the placebo group. This was calculated using an estimated standard deviation of 8 mmHg. The sponsor compared the demographics of the four groups at baseline using a Mantel-Haenszel test on sex, race, smoking status, and use of previous antihypertensive medications, and found no significant differences. The groups were also similar with respect to age, weight, height, and years of hypertension, which were compared using an analysis of variance model.

Change from Baseline Blood Pressure Measurements at Endpoint (LOCF) (Intent-to-Treat Data Set)

	Supine DBP	Supine SBP	Standing DBP	Standing SBP
Placebo (N=71)				
Baseline mean	103.64	155.31	102.91	151.22
Change from baseline	-4.68	-1.88	-2.60	-1.94
Nisoldipine 30 mg (N=76)				
Baseline mean	104.41	157.29	103.82	153.57
Change from baseline	-11.58	-12.10	-9.59	-13.36
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Nisoldipine 60 mg (N=73)				
Baseline mean	104.76	158.43	103.74	154.12
Change from baseline	-14.46	-16.67	-12.41	-15.94
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Interaction p-value	0.4675	0.1074	0.2651	0.4209

The results of the sponsor's analysis of their evaluable patient data set were similar, with all of the active drug groups demonstrating a highly statistically significant difference from the placebo group. The same was true of the analysis at each of the various time points after at least two weeks of double-blind therapy. Each active treatment group tended to reach a plateau in blood pressure response after two or three weeks of double-blind therapy, and the results for the group were very consistent beyond that point. The treatment by center interaction term was not significant at the .05 level.

The response variables also demonstrated results similar to the change from baseline blood pressure values, as can be seen below.

Response Rates

	Placebo N=71	Nisoldipine 30 mg qd N=76	Nisoldipine 60 mg qd N=66
A) DBP ≤ 90 mmHg	16 (23%)	26 (34%)	37 (56%)
B) DBP decrease ≥ 10 mmHg	22 (31%)	37 (49%)	50 (76%)
C) A) or B)	22 (31%)	37 (49%)	50 (76%)
D) A) and B)	16 (23%)	26 (34%)	37 (56%)

The ambulatory blood pressure data was analyzed by smoothing the hourly means from the eight centers using a Fourier transform. The mean trough and peak values and trough to peak ratios were obtained from the smoothed data. Peak effect for each nisoldipine group was defined as the greatest difference between the nisoldipine group and placebo in mean change from baseline. Trough effect was defined as the 24-hour post-dose difference from placebo.

The peak diastolic blood pressure response occurred at 13 hours post-dose for the nisoldipine 30 mg group and at 4 hours post-dose for the nisoldipine 60 mg group. The peak/trough ratios for the placebo subtracted ambulatory measurements were -12.1/-9.5 = 78% and -15.2/-14.2 = 93%, respectively. The corresponding systolic peak/trough ratios were 83% and 76%.

The number of adverse events experienced in the placebo group was statistically significantly different from both of the nisoldipine groups. The most frequently reported adverse events included headache and peripheral edema in the placebo group and both nisoldipine groups. Adverse events were most likely to occur early in the first two weeks of double-blind therapy, although they continued to occur at a reduced level throughout the study. One patient experienced a cardiac arrest and died while receiving Placebo during the double-blind portion of the study. No other patients died during the study.

Adverse Events

	Placebo N=72	Nisoldipine 30 mg qd N=76	Nisoldipine 60 mg qd N=73
Patients with ≥ one event	32 (44%)	47 (62%)	54 (74%)
Possibly drug related	19 (26%)	23 (30%)	33 (45%)
Serious adverse events	2 (3%)	4 (5%)	10 (14%)
Withdrew due to a. e.'s	3 (4%)	1 (1%)	11 (15%)

II.A.3. Reviewer's Comments

This study gives substantial evidence that nisoldipine, in doses of 30 and 60 mg qd, reduces blood pressure when compared to placebo. After establishing a drug effect by comparing the mean of the combined nisoldipine groups with placebo and reaching statistical significance, the sponsor compared each dose group directly with placebo, and both were highly statistically significant. The protocol and the study report do not mention any adjustments for multiple comparisons. However, the results of this study were so significant that even using the conservative Bonforonni adjustment for the multiple comparisons, they remain highly statistically significant for the primary and secondary blood pressure endpoints.

This reviewer performed several additional analyses on the data including analysis of covariance using baseline as a covariate for both change from baseline and for endpoint supine DBP. The results of these additional analyses did not differ substantially from the results submitted by the sponsor, and demonstrated the robustness of the efficacy results.

The results of this study demonstrate a dose-response relationship for the 30 mg qd and 60 mg qd doses of nisoldipine in both efficacy and safety. The higher dose group consistently showed a greater reduction in all four blood pressure measurements. This group also had more adverse events and more serious adverse events. This study involved a forced titration, and many of the patients in the high dose group possibly had an adequate response at the lower dose, and did not need the additional risk of adverse events which came with the additional blood pressure reduction. This reviewer feels that doses of nisoldipine lower than those studied here might be adequate for many patients.

The protocol stated that the original model for the analysis of the blood pressure variables would include an interaction term, but that the interaction term would be dropped if it was not significant at the .05 level. The test for interaction is a test with very low power and therefore interaction is usually tested at the .15 level. Using this level, one of the secondary endpoints, supine SBP, demonstrated a significant

treatment by center interaction in the endpoint analysis (p= 0.1074). The primary endpoint also had interaction terms with p-values which were greater than .05 but less than .15 at several time points, but not in the endpoint analysis. The inclusion of the interaction term in the analysis of variance model did not change the statistical significance of any of the primary or secondary endpoints. This reviewer calculated the results by center and found that while the response at the various centers was different in magnitude, they trended in the same direction in almost all centers. The interaction appears to be quantitative in nature, and probably a result of the variability of the blood pressure responses.

II.B. PROTOCOL NO. D89-029

II.B.1. Study Description

D89-029 was a sixteen-center dose-response parallel study comparing placebo with three once daily doses of nisoldipine on a background of atenolol 50 mg qd in patients with mild to moderate hypertension. Patients were randomized to one of four parallel groups, receiving either placebo or nisoldipine 20 mg qd, nisoldipine 40 mg qd, or nisoldipine 60 mg qd, over an six week double-blind period.

Following a two-week placebo run-in period, patients with supine diastolic blood pressure between 100 and 119 mmHg entered a single-blind four week run-in period where all received atenolol 50 mg qd, but no other hypertensive therapy. Patients who had a supine diastolic blood pressure between 95 and 114 mmHg after the four weeks of atenolol therapy were eligible for randomization to one of the four double-blind treatments, while continuing their atenolol therapy.

A total of 418 patients were enrolled in the placebo run-in, and 313 continued in the atenolol run-in period. Most of the patients who did not continue were not eligible because their blood pressure had dropped too much while receiving atenolol. A total of 251 patients were randomized to double-blind treatment group; 62 to placebo, 62 to nisoldipine 20 mg qd, 63 to nisoldipine 40 mg qd, and 64 to nisoldipine 60 mg qd. Three placebo patients dropped out of the study, as did one low dose, five mid-dose, and seven higher dose nisoldipine patients. A total of 238 patients were considered valid for the efficacy analyses.

Patients were instructed to take two tablets and one capsule each morning prior to 11 a.m. All patients randomized to nisoldipine began at 20 mg once daily, and those randomized to the higher dose groups were titrated weekly. The relationship of drug administration to meals was not specified. The double-blind treatment phase lasted a total of six weeks, with patients evaluated at weeks 1, 2, 4, and 6. The primary efficacy variable was change from baseline (mean supine DBP at week 4 of the single-blind atenolol phase) to endpoint (the last double-blind visit) supine diastolic blood pressure (DBP) at trough, 24 hours post-dose. Secondary efficacy variables included change from baseline in standing DBP and supine and standing SBP. The change

from baseline during the atenolol phase was also evaluated. Response rates at trough were also analyzed, with response define in four different ways, (1) supine DBP no more than 90 mmHg; (2) a fall in supine DBP of at least 10 mmHg, (3) supine DBP≤ 90 mmHg or a fall in supine DBP ≥ 10 mmHg; and (4) supine DBP≤ 90 mmHg and a fall in supine DBP ≥ 10 mmHg.

At eight of the 16 centers 24-hour ambulatory blood pressure monitoring (ABPM) was performed at week 3 of the atenolol run-in and at week 5 of the double-blind treatment period. The sponsor used the ABPM data to analyze the peak/trough ratio. A total of 141 patients (centers 01 through 08) had 24-hour ABPM monitoring, including 33 randomized to placebo, 34 to nisoldipine 20 mg, 35 to nisoldipine 40 mg, and 34 to nisoldipine 60 mg.

II.B.2. Sponsor's Analysis

The sample size for this study was selected to give 90% power to detect a difference of at least 5 mmHg between the nisoldipine 40 mg group and the placebo group. This was calculated using an estimated standard deviation of 8 mmHg. The sponsor compared the demographics of the four groups at baseline using the Cochran-Mantel-Haenszel test (adjusting for center) on sex, race, smoking status, and several other factors. The analysis of the previous use of antihypertensive medications was marginally significant with p=0.065. Only three patients had previously been treated, and two of those three were randomized to the placebo group. The groups were also similar with respect to age, weight, height, and years of hypertension, which were compared using an analysis of variance model.

The sponsor performed both an evaluable patient analysis, including patients with at least 19 days of double-blind therapy, and an intent-to-treat analysis including all patients who had at least one post-baseline evaluation. A last-observation-carried-forward approach was used to include the available data from patients who dropped out of the study. The initial analysis of variance model for the primary and secondary change from baseline endpoints included treatment group, investigator, and treatment by investigator interaction terms. The interaction term was dropped if it was not significant at the .05 level. The initial comparison was the average of the nisoldipine groups versus placebo. If this was significant then pairwise comparisons were done to identify which nisoldipine doses were favored over placebo.

Change from Baseline Blood Pressure Measurements at Endpoint (LOCF) Intent-to-Treat Data Set

	Supine DBP	Supine SBP	Standing DBP	Standing SBP
Placebo + Atenolol (N=62)				
Baseline mean	100.74	158.30	102.10	154.20
Change from baseline	-3.90	-0.12	-1.66	+2.30
Nisoldipine 20 mg + Atenolol (N= 62)				
Baseline mean	100.58	158.19	102.13	153.81
Change from baseline	-10.00	-12.51	-8.85	-10.14
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Nisoldipine 40 mg + Atenolol (N= 62)				
Baseline mean	100.97	159.32	103.46	157.22
Change from baseline	-12.01	-19.03	-12.39	-21.75
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Nisoldipine 60 mg + Atenolol (N= 64)				
Baseline mean	100.81	160.90	102.32	155.72
Change from baseline	-13.73	-22.38	-14.30	-21.89
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Nisoldipine versus placebo	0.0001	0.0001	0.0001	0.0001
Interaction p-value	0.1107	0.3041	0.5361	0.4636

The results of the sponsor's analysis of their evaluable patient data set were similar, with all of the active drug groups demonstrating a highly statistically significant difference from the placebo group. The same was true of the analysis at each of the various time points after the first week of double-blind therapy. Each active treatment group tended to reach a plateau in blood pressure response after two or three weeks of double-blind therapy, and the results were very consistent beyond that point. The treatment by center interaction term was significant at the .05 level for the analysis of supine DBP at the first double-blind visit, but not for the secondary blood pressure variables at that visit, nor for any blood pressure variables at later visits or at endpoint.

The response variables also demonstrated results similar to the change from baseline blood pressure values, as can be seen below.

Response Rates

	Placebo N=59	Nisoldipine 20 mg qd N=61	Nisoldipine 40 mg qd N= 59	Nisoldipine 60 mg qd N= 59
A) DBP ≤ 90 mmHg	19 (32%)	34 (56%)	40 (68%)	39 (66%)
B) DBP decrease ≥ 10 mmHg	14 (24%)	31 (51%)	40 (68%)	44 (75%)

The ambulatory blood pressure data was analyzed by smoothing the hourly means from the eight centers using a Fourier transform. The mean trough and peak values and trough to peak ratios were obtained from the smoothed data. Peak effect for each nisoldipine group was defined as the greatest difference between the nisoldipine group and placebo in mean change from baseline. Trough effect was defined as the 24-hour post-dose difference from placebo.

The peak diastolic blood pressure response occurred at 3 hours post-dose for the nisoldipine 20 mg group, at 23 hours post-dose for the nisoldipine 40 mg group, and at one hour post-dose for the nisoldipine 60 mg group. The peak/trough ratios were -5.0/-9.4 = 53%, -12.8/-13.1= 97%, and -12.9/-13.0 = 99%, respectively. The corresponding systolic peak/trough ratios were 86%, 100%, and 94%.

Adverse Events

	Placebo N=62	Nisoldipine 20 mg qd N= 62	Nisoldipine 40 mg qd N= 63	Nisoldipine 60 mg qd N= 64
Patients with ≥ one event	28 (62%)	37 (60%)	44 (70%)	42 (66%)
Possibly drug related	14 (23%)	16 (26%)	28 (44%)	28 (44%)
Serious adverse events	2 (3%)	5 (8%)	2 (3%)	5 (8%)
Withdrew due to a. e.'s	1 (2%)	1 (2%)	4 (6%)	4 (6%)

The most frequently reported adverse events included headache and peripheral edema in the nisoldipine groups. The most frequently reported adverse events in the placebo group included rhinitis, peripheral edema, and headache. Adverse events were most likely to occur early in the study (prior to week 4), although they continued

to occur at a reduced level throughout the study. There were no deaths reported during this study.

II.B.3. Reviewer's Comments

This study demonstrated that nisoldipine treatment resulted in additional blood pressure reduction when used in the presence of atenolol. The primary and secondary endpoints all demonstrated statistically significant reductions in blood pressure during the six week study. This reviewer again performed several alternative analyses and found that the results were robust.

The results also demonstrate a dose-response trend for the 20 mg qd through 60 mg qd doses of nisoldipine in both efficacy and safety. The higher dose groups consistently showed a greater reduction in each of the blood pressure measurements, and also an increasing number of adverse events. This study again involved a forced titration, and many of the patients in the higher dose groups possibly had an adequate blood pressure response at lower doses and did not need the additional risk of adverse events which came with the additional blood pressure reduction. This reviewer feels that doses of nisoldipine lower than those studied here might be adequate for many patients.

The protocol stated that the original model for the analysis of the blood pressure variables would include an interaction term, but that the interaction term would be dropped if it was not significant at the .05 level. The test for interaction is a test with very low power and therefore interaction is usually tested at the .15 level. Using this level, the primary endpoint, supine DBP, demonstrated a significant treatment by center interaction in the endpoint analysis for both the evaluable patient data set ($p=0.1478$) and for the intent-to-treat data set ($p=0.1107$). This reviewer calculated the results by center and compared them. The centers vary substantially in the response of the various nisoldipine groups, but in no case did the placebo group have a greater response to therapy than did the treated groups.

II.C. PROTOCOL NO. D89-039

II.C.1. Study Description

D89-039 was a four-arm parallel study comparing placebo, two once daily doses of nisoldipine, 20 mg. and 40 mg, and verapamil 240 mg bid, in patients with mild to moderate hypertension. The protocol for the sixteen center study included a fifth group randomized to nisoldipine. 80 mg qd, but this group was dropped shortly after the beginning of the study (not prior to randomization) after the sponsor received information from another study that nisoldipine doses in excess of 60 mg qd were not well-tolerated..

After a four week washout period, patients who had an average supine DBP between 95 and 114 mmHg on each of the last two pre-randomization visits were eligible for

randomization to double-blind treatment. Blood pressure measurements were taken at trough (24 hr ± 30 minutes post-dose). The double-blind portion of the study lasted 12 weeks, but patients randomized to placebo were switched to verapamil 240 mg qd for the final four weeks of the study.

A total of 413 patients were enrolled in the placebo run-in period, and 320 were randomized to treatment group; 75 to placebo, 78 to verapamil, 76 to nisoldipine 20 mg qd, 76 to nisoldipine 40 mg qd, and 15 to nisoldipine 60 mg qd. Eleven patients randomized to the placebo group dropped out before the end of the study, as did five verapamil patients, 12 low dose nisoldipine patients, and 12 medium dose nisoldipine patients. The 15 patients who had been randomized to high dose nisoldipine were dropped from the study when that arm was deleted, so 265 patients completed the study A total of 290 patients were considered valid for efficacy analyses.

Patients were instructed to take two tablets and one capsule each morning prior to 11 a.m. and another capsule 12 hours later. All patients randomized to nisoldipine began at 20 mg once daily, and those randomized to the 40 mg dose were titrated after one week. The relationship of drug administration to meals was not specified. The double-blind treatment phase lasted a total of eight weeks at the original randomized dose groups, followed by four weeks where the placebo group received verapamil 240 mg qd and the other three groups remained on their randomized therapy. Patients were evaluated weekly for the first four weeks, and then biweekly for the rest of the study.

The primary efficacy variable was change from baseline (defined as the mean of six readings, three taken at week 3 and three at week 4 of the placebo run-in period) to endpoint (defined as week 8 of the double-blind portion of the study) in supine diastolic blood pressure (DBP) at trough, 24 hours post-dose. The primary efficacy comparison was between the 40 mg qd nisoldipine group and the placebo group. The comparison of the 20 mg qd nisoldipine group and the placebo group was of secondary importance. Secondary efficacy variables included trough standing DBP and supine and standing systolic blood pressure (SBP). Response rates at trough were also analyzed, with response define in four different ways, (1) supine DBP no more than 90 mmHg; (2) a fall in supine DBP of at least 10 mmHg, (3) supine DBP≤ 90 mmHg or a fall in supine DBP ≥ 10 mmHg; and (4) supine DBP≤ 90 mmHg and a fall in supine DBP ≥ 10 mmHg.

At eight of the 16 centers 24-hour ambulatory blood pressure monitoring (ABPM) was performed at week 3 of the placebo run-in and at week 7 of the double-blind treatment period. The sponsor used the ABPM data to analyze the peak/trough ratio.
A total of 141 patients (centers 01 through 08) had 24-hour ABPM monitoring, including 34 randomized to placebo, 36 to verapamil, 35 to nisoldipine 20 mg, and 36 to nisoldipine 40 mg. A total of 163 patients (centers 06 through 13) had 12-hour post-dose in-house blood pressure readings (every two hours) at week 4 of the placebo run-in and at week 8 of the double-blind treatment period.

II.C.2. Sponsor's Analysis

The sample size for this study was selected to give 90% power to detect a difference of at least 5 mmHg between the nisoldipine 40 mg group and the placebo group. This was calculated using an estimated standard deviation of 8 mmHg. The sponsor compared the demographics of the four groups at baseline using the Cochran-Mantel-Haenszel test (adjusting for center) on sex, race, smoking status, and use of previous antihypertensive medications, and no significant differences were found. The groups were also similar with respect to age, weight, height, and years of hypertension, which were compared using an analysis of variance model.

Change from Baseline Blood Pressure Measurements at Endpoint (LOCF) Intent-to-Treat Data Set

	Supine DBP	Supine SBP	Standing DBP	Standing SBP
Placebo (N=75)				
Baseline mean	99.76	154.69	100.58	151.18
Change from baseline	-4.30	-1.93	-2.09	-2.40
Nisoldipine 20 mg qd (N=75)				
Baseline mean	99.99	152.67	100.80	[illegible]
Change from baseline	-7.97	-10.17	-6.99	-11.42
p-value vs placebo	0.0004	0.0001	0.0001	0.0001
Nisoldipine 40 mg qd (N=76)				
Baseline mean	100.35	154.22	101.27	150.20
Change from baseline	-11.22	-15.50	-11.34	-14.90
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Verapamil 240 mg bid (N=78)				
Baseline mean	99.95	151.67	100.66	148.62
Change from baseline	-14.48	-14.79	-13.21	-14.95
p-value vs placebo	0.0001	0.0001	0.0001	0.0001
Interaction p-value	0.6287	0.1693	0.5060	0.6371

The sponsor performed both an evaluable patient analysis, including those patients with at least two post-baseline evaluations who were not protocol violators, and an intent-to-treat analysis including all patients who had at least one post-baseline evaluation. A last-observation-carried-forward approach was used to include the available data from patients who dropped out of the study. The initial analysis of variance model for the primary and secondary change from baseline endpoints included treatment group, investigator, and treatment by investigator interaction terms. The interaction term was dropped if it was not significant at the .05 level.

The results of the sponsor's analysis of their evaluable patient data set were similar, with all of the active drug groups demonstrating a highly statistically significant difference from the placebo group. The same was true of the analysis at each of the various time points after at least two weeks of double-blind therapy. Each active treatment group tended to reach a plateau in blood pressure response after two or three weeks of double-blind therapy, and the results were very consistent beyond that point. The treatment by center interaction term was significant for some visits, but not for the final two visits or for the endpoint values for either data set.

The response variables also demonstrated results similar to the change from baseline blood pressure values, as can be seen below.

Response Rates

	Placebo	Nisoldipine 20 mg qd	Nisoldipine 40 mg qd	Verapamil 240 mg bid
	N=70	N=72	N=76	N=72
A) DBP ≤ 90 mmHg	19 (26%)	35 (50%)	50 (69%)	62 (82%)
B) DBP decrease ≥ 10 mmHg	10 (14%)	28 (40%)	47 (65%)	59 (78%)
C) A) or B)	20 (28%)	38 (54%)	53 (74%)	67 (88%)
D) A) and B)	9 (13%)	25 (36%)	44 (61%)	54 (71%)

The ambulatory blood pressure data was analyzed by smoothing the hourly means from the eight centers using a Fourier transform. The mean trough and peak values and trough to peak ratios were obtained from the smoothed data. Peak effect for each nisoldipine group was defined as the greatest difference between the nisoldipine group and placebo in mean change from baseline. Trough effect was defined as the 24-hour post-dose difference from placebo.

The peak diastolic blood pressure response occurred at 4 hours post-dose for the nisoldipine 20 mg group, at 24 hours post-dose for the nisoldipine 40 mg group, and at 4 hours after the morning dose for the verapamil 240 mg bid group. The peak/trough ratios were -6.7/-9.7 = 66%, -11.7/-11.7 = 100%, and -11.1/-12.9 = 86%, respectively. The corresponding systolic peak/trough ratios were 66%, 100%, and 78%.

Adverse Events

	Placebo N=75	Nisoldipine 20 mg qd N=76	Nisoldipine 40 mg qd N=76	Verapamil 240 mg bid N=78
Patients with ≥ one event	48 (64%)	49 (64%)	57 (75%)	55 (71%)
Possibly drug related	34 (45%)	33 (43%)	42 (55%)	39 (50%)
Serious adverse events	7 (9%)	9 (12%)	12 (16%)	3 (4%)
Withdrew due to a. e.'s	3 (4%)	10 (13%)	11 (14%)	4 (5%)

The overall incidence of adverse events was not statistically significantly different for the four treatment groups. The most frequently reported adverse events included headache and peripheral edema in the placebo group and both nisoldipine groups. The most frequently reported adverse events in the verapamil group included constipation and headache. Adverse events were most likely to occur early in the study (prior to week 4), although they continued to occur at a reduced level throughout the study. There were no deaths reported during this study.

II.C.3. Reviewer's Comments

This study clearly demonstrates the efficacy of nisoldipine when compared to placebo in the treatment of mild-to-moderate hypertension. A dose-response exists for both diastolic and systolic blood pressure, and for adverse events. The differential between the groups appeared within a few weeks of treatment, and was consistent for the rest of the study. This reviewer again analyzed the data using several other models, and found the results to be consistent.

The group randomized to verapamil consistently demonstrated results which were at least as good as the higher nisoldipine dose, and were often superior (although not often statistically significantly better). The response rates for the verapamil group were also higher than those of the nisoldipine groups. The sponsor stated that verapamil is often used as once-a-day therapy, and the twice-daily dosing used in this study could have given that group an advantage over the once-daily dosing of nisoldipine. Although the verapamil group did not usually achieve statistical significance when compared to the nisoldipine groups, the study was not powered as an equivalence trial and the results should not be interpreted as showing the treatments are the same.

II.D. OTHER HYPERTENSION STUDIES

II.D.1. Study Descriptions

The sponsor submitted the results of three additional placebo-controlled clinical trials, two which were carried out in the United States. These studies lend supportive

evidence of the efficacy and safety of nisoldipine in the treatment of mild to moderate hypertension.

Study D88-054 was a pilot parallel dose-ranging eight-center study comparing placebo with three once daily doses of nisoldipine, 10 mg. 20 mg. and 30 mg over a four week period. This was the first exploratory clinical trial carried out in the target population and approximately 30 patients were randomized to each dose. The 20 mg and 30 mg dose groups both achieved statistically significantly greater reductions in supine DBP, supine SBP, and standing SBP than did the placebo group.

Study D89-026 was a pilot parallel dose titration study comparing placebo with nisoldipine, 10 - 40 mg once daily over a nine week period. Patients randomized to nisoldipine received 10 mg qd for the first week and were titrated upward an additional 10 mg on a bi-weekly basis if their supine DBP remained ≥ 85 mmHg at trough. A total of 72 patients were randomized to nisoldipine and 34 to placebo, which was also titrated based on blood pressure response. At the end of the nine weeks of treatment 6% of the nisoldipine patients remained at 10 mg qd, 6% were receiving 20 mg qd, 30% were receiving 30 mg qd, and 57% had been titrated to 40 mg qd. The nisoldipine group achieved statistically significantly greater reductions in supine and standing DBP and SBP.

Study D90-006 was a parallel multicenter study comparing placebo with three once daily doses of nisoldipine, 10 mg. 20 mg. and 30 mg over a six week period. This study was carried out in South Africa. All patients randomized to nisoldipine began at 10 mg qd and were titrated to their assigned dose after one week. Approximately 50 patients were randomized to each group. In the endpoint analysis all three nisoldipine groups achieved statistically significantly greater reductions in supine and standing DBP and SBP than did the placebo group.

IV. OVERALL SUMMARY AND CONCLUSIONS

The sponsor submitted the results of six trial involving patients with mild-to-moderate hypertension, including three Phase III studies performed in the United States. Study D90-019 was a dose-response study comparing two doses of nisoldipine (30 mg and 60 mg) with placebo in once-daily dosing. Both nisoldipine dose groups had statistically significantly better reduction in blood pressure than did the placebo group, and the groups demonstrated a dose-response relationship for both efficacy and safety. Study D90-029 compared placebo with three once daily doses of nisoldipine (20 mg, 40 mg, and 60 mg) on a background of atenolol 50 mg qd. All three nisoldipine/atenolol groups had statistically significantly better reduction in blood pressure than did the placebo/atenolol group. The responses trended in a dose-response order for both blood pressure reduction and for adverse experiences. Study D90-039 was a four-arm placebo and active-controlled study comparing two doses of nisoldipine (20 mg and 40 mg) with verapamil 240 mg bid and placebo. The nisoldipine groups had statistically significantly better reduction in blood pressure than the placebo group. The verapamil group had a somewhat better response than

did the nisoldipine groups, although the results were not often statistically significant. However, this trial was not designed as an equivalence trial, and was under-powered to detect the differences seen in the study. The study could not detect a difference, but that does not imply that the treatments are the same.

Clearly the nisoldipine doses studied in this trial (20 mg to 60 mg, qd) are effective at lowering blood pressure. What is not clear is that the dose range has been adequately examined, especially at the lower end. The maximal dose appears to be limited by adverse reactions. This reviewer feels that doses of nisoldipine lower than those studied here might be adequate for many patients and should be examined.

There were two potential problems in the design of these studies. The original model for the primary analysis of variance included a treatment by center interaction term which was dropped if the p-value for interaction was less than .05. The test for interaction is a very low powered test, and interaction is usually tested at the .15 level. The results of these studies were robust whether or not an interaction term was included in the model, and the interactions which were statistically significant appeared to be qualitative rather than quantitative. These studies each involved multiple doses of nisoldipine, and none of the analysis plans included adjustments for multiple comparisons. The p-values that resulted from the analyses, however, were all less than 0.001, and thus could stand up to a Bonforonni adjustment.

The overall summary and conclusions section may be conveyed to the sponsor.

Nancy D Smith

Nancy D. Smith, Ph.D.
Mathematical Statistician

Concur: Dr. Chi Chi 1/4/94

for Dr. Dubey SDn 1-4-94

BIO REVIEWS

FOI

DEC - 1 1994

NDA No: 20-356
Date of Document: November 14, 1994
Generic Name: Nisoldipine
Brand Name: NISOCOR
Formulation: Nisoldipine CC (Extended Release Tablets)
Sponsor: Miles Incorporated
Type of Submission: NDA Amendment
Reviewers: Olof Borga, Ph.D. and Alfreda Burnett, Ph.D.

BACKGROUND

Nisoldipine is a dihydropyridine calcium antagonist, similar to felodipine. NISOCOR tablet contains either 10, 20, 30, or 40 mg of nisoldipine as a Coat-Core (CC) formulation: the coat is a slow release formulation while the core is a fast release formulation. Previous studies with felodipine (1), nifedipine (1) and nitrendipine (2), all dihydropyridines (DHPs), have shown that simultaneous intake of grapefruit juice increases the bioavailability of the DHP drug. These drugs are all subject to first-pass metabolism, and the mechanism of interaction is believed to be inhibition of cytochrome P450 3A4 responsible for this step. Grapefruit juice is known to contain high amounts of flavonoids, mainly present as glycosides, which probably undergo hydrolysis in the intestine to the corresponding aglycones and sugars (3). In vitro studies in rat and human liver microsomes have shown that the aglycones, mainly narengenin, quercitin, and kaempferol all have inhibitory potency with regard to the metabolism of DHPs (4).

The sponsor has conducted a clinical study to investigate the possibility of an interaction between grapefruit juice and NISOCOR (nisoldipine cc) with respect to the pharmacokinetics and pharmacodynamics of nisoldipine and its major metabolites in plasma. An account of the study has also been published (5).

References:

1 Bailey, D.G, et al. Interaction of citrus juices with felodipine and nifedipine. Lancet 1991, Feb 2; 337(8736): 268-269.
2. Soons, P.A., et al. Grapefruit juice and cimetidine inhibit stereoselective metabolism of nitrendipine in humans. Clin Pharmacol Ther 1992; 50(4): 394-403.
3. Buening, M. K., et al. Activation and inhibition of benzo(a)pyrene and aflatoxin B1 metabolism in human liver microsomes by naturally occuring flavonoid. Cancer Res 1981; 41: 67-72.
4. Miniscalco, A., et al. Inhibition of dihydropyridine metabolism in rat and human liver microsomes by flavonoids found in grapefruit juice. J Exp Pharmacol Ther, 1992; 261(3): 1195-99.
5. Bailey, D.G., et al. Effect of grapefruit juice and naringin on nisoldipine pharmacokinetics. Clin Pharmacol Ther 1993; 54(6): 589-94.

SYNOPSIS

The sponsor has submitted a food-drug interaction study. The primary objective was to investigate a possible interaction between grapefruit juice and nisoldipine cc with respect to the pharmacokinetics of nisoldipine (BAY k 5552) and its major plasma metabolites (the hydroxylated metabolite BAY r 9425 and the pyridine metabolite BAY 0 3199). The secondary objective was to investigate the hypothesis that one bioflavonoid, naringin, in grapefruit juice is responsible for the proposed interaction. The study was conducted in 12 normotensive male subjects. Subjects randomly received each of 3 treatments on 3 separate study days, separated by a washout period of at least 7 days. Blood

pressure and heart rate assessments and determination of plasma concentrations of nisoldipine and its two major plasma metabolites were carried out for 48 hours after each treatment. Also, included in this submission is the proposed annotated package insert for NISCOR (nisoldipine).

STUDY SUMMARY

See Appendix.

CONCLUSION:

The sponsor has demonstrated that
a) grapefruit juice alters the pharmacokinetics of nisoldipine cc in healthy subjects
b) naringin does not appear to be the bioflavonoid in grapefruit juice responsible for the interaction
c) all treatments produced minor effects on supine blood pressure and heart rate, probably because subjects were normotensive

LABELING COMMENTS: The following wording is suggested by the firm:"In a study of twelve healthy male subjects, the bioavailability of nisoldipine was increased by as much as 3-fold when taken with grapefruit juice, compared to when taken with water. A similar finding has been seen with some other dihydropyridine calcium antagonists, but to a somewhat lesser extent than seen with nisoldipine." We suggest the following wording:**"In a study in twelve healthy male subjects, the AUC of nisoldipine was increased up to 4-fold (mean 75%) and C_{max} increased up to 7-fold (mean 350%) when taken with grapefruit juice, compared to when taken with water. This is probably caused by inhibition of first-pass elimination of the drug. A similar interaction has been seen with other dihydropyridine calcium antagonists, but to a somewhat lesser extent."**

RECOMMENDATION:

The Division of Biopharmaceutics recommends that the label be amended to reflect that after intake of grapefruit juice:
a) In 12 healthy males the C_{max} of nisoldipine increased up to 7-fold. The mean C_{max} increased 350%.
b) The AUC_{0-48} increased up to 4-fold. The mean AUC_{0-48} increased approximately 75%.

Olof Borga, Ph.D. — Alfreda Barnett, Ph.D. — Date
Pharmacokinetics Review Branch

FT Initialed by Ameeta Parekh,Ph.D. 12/21/94

cc: NDA 20-350, HFD-110, HFD-420 (Fleischer, Parekh), Drug, FOI (HFD-19), Chron, HFD-340 (Vishwanathan), F

NDA: 20-356
Nisoldipine Coat-Core tablets
10, 20, 30 and 40 mg
Sustained release tablets
Miles Pharmaceutical Division
Priority: 1S

Submission Date: [illegible]
August 2, 1993
September 24,1993.
October 14,1993.
November 04,1993.
November 23,1993.
December 29,1993.
March 14, 1994.
March 18, 1994.
June 27, 1994.
July 18, 1994.
July 29, 1994.

Type of submission: new molecular entity. Reviewer: Patrick J. Marroum.

Synopsis:

-The sponsor has adequately studied the pharmacokinetics (single and multiple dose) of nisoldipine coat-core tablet.
-Dosage form proportionality has been established between the 20 and 30 mg tablets only but not between the 20 and 40 mg strengths.
-Dose proportionality was established for AUC and CMAX in the dosing range of 20 to 60 mg. The 10 mg tablet strength gave more than dose proportional plasma levels when compared to the higher strengths.
-The effect of food on the rate and extent of nisoldipine absorption was investigated in 3 separate studies. Food increased CMAX by up to 300 % while decreasing AUC by up to 26 %.
-Adequate studies have been performed in elderly normal and hypertensive subjects.
-The effect of liver and renal disease has been adequately characterized in this NDA.
-Drug interaction studies between nisoldipine and warfarin, cimetidine, ranitidine, digoxin, quinidine, propranolol and atenolol have been performed.
-The sponsor failed to characterize the effect of gender on the pharmacokinetics of nisoldipine.
-The sponsor has adequately validated the gas chromatographic assay used in these studies.
-The sponsor attempted to correlate the in vitro dissolution with the in vivo performance of 3 different formulations of nisoldipine but failed to establish any relationship due to the fact that nisoldipine undergoes site specific gut wall metabolism.
-The dissolution method proposed by the sponsor for nisoldipine C.C. seems to be acceptable with the specifications recommended by the Division of Biopharmaceutics.

RECOMMENDATION:

The sponsor's NDA 20-356 appears to be acceptable for meeting the biopharmaceutics requirements provided that the comments on Pages 13 to 15 are adequately addressed by the sponsor.

TABLE OF CONTENTS:

The following studies were not reviewed because they either pertain to the immediate release formulation which is not subject for approval under this NDA or were not deemed pertinent for the approval of nisoldipine C.C.

Study 339	Investigation of the relation between the systemic bioavailability and the dose of BAY K5552 in healthy subjects.
Study 115	Plasma concentrations after oral administration of capsules and tablets (micronized substance and coprecipitate) to healthy volunteers.
Study 297	Investigation into the bioequivalence of various oral formulations of nisoldipine in healthy volunteers by a crossover trial.

Study D85-038	Bioequivalence study of formulations of nisoldipine 2.5 mg, 5 mg and 10 mg tablets in normal subjects.
Study D85-037	Bioequivalence study of formulations of nisoldipine 20 mg tablets in normal subjects.
Study 116	Plasma concentrations after the oral administration of 5 and 10 mg tablets. Comparison of micronized and ground active substance.
Study 330	Steady-state pharmacokinetics of nisoldipine in healthy male volunteers.
Study 102-106	Plasma concentrations after oral administration of different doses (6 to 20 μg/kg) of BAY K5552 to healthy test subjects.
Study 125	Plasma levels in volunteers after oral administration of 10 and 20 mg of BAY K5552 in the form of tablets.
Study 294	Plasma concentrations during treatment with BAY K5552 of hypertensive patients with impaired liver function.
Study 452	Pharmacokinetics and hemodynamic effects of nisoldipine in patients with liver cirrhosis after PO and IV administration.
Study 364	Nisoldipine pK in renal dysfunction.
Study 399	Pharmacokinetics and hemodynamic effects of nisoldipine and its interaction with cimetidine in healthy volunteers.
Study D88-054	Comparative double blind pilot study of the safety and efficacy of once daily doses of nisoldipine 10, 20, 30 mg CC tablets vs placebo in hypertensive patients.
Study D89-029	Double blind randomized study of safety and efficacy of once daily doses of nisoldipine 20, 40 and 60 mg (2x30 mg) CC tablets vs placebo in combination with atenolol 50 mg in hypertensive patients.
Study D89-039	Comparative double blind study of safety and efficacy of once daily doses of nisoldipine 20, 40 and 80 mg CC tablets vs a twice daily doses of verapamil SR 240 mg caplets vs placebo in hypertensive patients.
Study	A double blind randomized study of the safety and efficacy

D90-019 [redacted] of once daily doses of nisoldipine CC tablets 10, 20 (2x10), 30 (3x10) mg vs placebo in hypertensive patients.

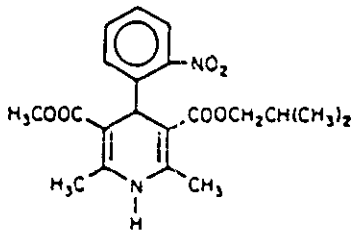
Study D88-060 Efficacy and safety of CC nisoldipine 10, 20 and 30 mg qd vs placebo in patients with stable exertional angina pectoris.

Study 0417 Acute pK/pD interaction of nisoldipine and propranolol.

Appendix II:

Background:

Nisoldipine is a dihydropyridine calcium channel blocker. It is 3,5-pyridinedicarboxylic acid,1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-methyl 2-methylpropyl ester.It has the following structural formula:



Nisoldipine is a yellow crystalline substance, practically insoluble in water but soluble in ethanol. No information about the octanol/water partition coefficient was submitted in this NDA. It has a molecular weight of 388.4. Nisoldipine coat core tablets consist of an external coat and internal core. Both contain nisoldipine, the coat in a slow-release formulation and the core in a fast-release formulation. Nisoldipine CC tablets contain either 10, 20, 30 or 40 mg of nisoldipine for once a day oral administration.

Nisoldipine C.C. is indicated for the treatment of hypertension and may be used alone or in combination with other antihypertensive agents. In general, therapy should be initiated with 10 mg orally once daily. The usual maintenance dosage is 20 or 30 mg once daily. Doses beyond 40 mg are not recommended.

It is to be noted that this NDA was first submitted on March 31, 1993. An approvable letter was issued on March 25, 1994 based on deficiencies in both the Chemistry and the Clinical sections of the application. It was resubmitted to the Agency on August 3, 1994. The user fee goal for this application is February 3, 1995.

SUMMARY OF BIOAVAILABILITY/PHARMACOKINETICS/PHARMACODYNAMICS:

I-BIOAVAILABILITY/BIOEQUIVALENCE:

A-Absolute Bioavailability:

The absolute bioavailability of 20 mg nisoldipine coat core compared to the dose corrected IV dose infused over 1 hour was 5.5 % with a 95 % CI ranging from 4.8 % to 6.4 %. (Study 0637).

B-Food Effects:

The effect of food on the pharmacokinetics of nisoldipine C.C. was investigated in 2 separate studies. Study 666 where a 20 mg nisoldipine C.C. tablet was given fasted, together with a high fat breakfast, 1 hour after a high fat breakfast and together with dinner showed that the coadministration with meals remarkably increased CMAX from 26 %(with dinner) up to 48% (together with breakfast) with a corresponding shortening of TMAX by about 2 to 3 hours. However food did not seem to have any effect on the extent of bioavailability of nisoldipine since there was no difference between the AUCs in fed and fasted states.

Study D92-045-02 showed that the effect of food was even more pronounced on the 30 and 40 mg C.C. tablets. Food increased the CMAX for nisoldipine on the average by 250 to 300 % (CMAX increased from 1.9 to 4.5 ng/ml for the 30 mg and from 2.7 to 7.5 ng/ml for the 40 mg strength) while decreasing the AUC by 26 % in the fed state as compared to the fasted state (AUC decreased from 49.2 to 35.4 ng*hr/ml for the 30 mg and from 70.4 to 53 ng*hr/ml for the 40 mg strength).

Similar results as far as CMAX is concerned were observed with the 20 mg IR tablet of nisoldipine. Food increased the AUC by 28 % and CMAX by 31 % compared to the fasting state (Study 323).

C-Bioequivalence:

Study 5678 showed that 1x40 mg was bioequivalent to 2x20 mg C.C. tablets as far as AUC was concerned since the 90 % CI of the log transformed AUC was 85.26 to 112.24 %. The same could not be said for CMAX because the 90 % CI of the log transformed CMAX was 94.62 to 140.03 %. Thus, 1x40 mg C.C. tablet is considered not bioequivalent to 2x20 mg tablets.

[redacted]

On the other hand Study D90 020 (a study in which 5 mg C.C. tablets were bioequivalent to 3x20 mg tablets), the AUC ratio (5 mg/3x20) was [redacted] with a log transformed 90 % CI of 95 to 115 while the CMAX ratio was [redacted] with a corresponding log transformed 90 % CI of 83.64 to 109.87 %.

Study KF 715 established the bioequivalence between the old 20 mg and the new 20 mg C.C. formulation as far as AUC was concerned. As for CMAX, none of the three strengths of the new formulation were bioequivalent to the old 20 mg formulation. The 5, 10 and 20 mg strengths were higher on the upper limit of the 90 % CI compared to the old 20 mg strength.

II.PHARMACOKINETICS:

The terminal half-life of nisoldipine was estimated to be about 7 hours after an infusion of 0.08 mg/kg for 20 hours. This corresponded to a systemic clearance of 544 to 768 ml/hr/kg. The volume of distribution was estimated to be between 2.3 and 3.4 l/kg (Study 330).
Following the administration of nisoldipine C.C. 20 mg once a day for 7 days, the CMAX was 0.84 ng/ml on day 1 and 1.09 ng/ml on day 7. The AUCnorm was 40.3 g*hr/l on day 1 and 58.9 g*hr/l for day 7 giving an accumulation ratio based on AUC of 1.46. CMAX following administration of an immediate release 10 mg tablet CMAX was 2.18 ng/ml on day 1 and 1.95 ng/ml on day 7 indicating that there is no accumulation of nisoldipine with the immediate release formulation. The fluctuation index for the IR tablet given bid was 439 % as compared to 113 % following the controlled release formulation (Study 645).
Study 606/618 showed that after IV administration similar plasma concentrations were obtained for both enantiomers. However, after oral administration the concentration of the (+) nisoldipine (which is pharmacodynamically more active than the (-)) was about 6 times higher than the (-) nisoldipine. The CMAX for the (+) hydroxylated dihydropyridine (M9) was 7.6 times higher than the (-) enantiomer.

III-METABOLISM:

In man, hydroxylation of the isobutyl ester appears to be the major biotransformation pathway. 70 to 80 % of the urinary metabolites in the first 12 hours after administration are the metabolites M4, M5 and M12 (Study 400),(see metabolic scheme in Appendix II). Metabolites M1 and M2 represent about 10 % of the urine metabolites in man (Study 16626). Only metabolite M12 was hydrolyzable with beta glucuronidase yielding M5 as the aglycon. The only metabolite with known activity is BAY r9425 (M9) with 10 % of the activity exhibited by nisoldipine and is present in approximately equal concentrations in the plasma as nisoldipine. Study 600 provided some evidence that nisoldipine undergoes some degree of gut wall metabolism which is decreasing from the proximal to the distal parts of the intestine with no metabolism occurring in the colon.
It is to be noted that even though nisoldipine seems to be extensively metabolized, the sponsor did not identify the enzymes responsible for its biotransformation.

IV-DOSE PROPORTIONALITY:

[redacted]

The dose proportionality of immediate release nisoldipine was established between the doses of [redacted] 10, 20 and 40 mg since both AUC and CMAX increased in a dose proportional manner (Study D85-024-01).

As for the coat core formulation, the dose proportionality was established for doses in the range of 20 to 60 mg (using the 20 mg tablet except for the 10 mg dose where the 10 mg tablet was used). However, the dose normalized values for CMAX and AUC for the 10 mg dose were somewhat higher and statistically different as compared to the values for the 20, 40 and 60 mg doses (Study D91-035). These differences in results were not explained by the sponsor.

V-SPECIAL POPULATIONS:

A.Renal Impairment:

In patients with severe renal impairment (creatinine clearance less than 30 ml/min), nisoldipine plasma concentrations on day 1 were higher by as much as 2 fold compared to subjects with normal renal function. However, this difference seemed to have subsided by day 7. Even though renal impairment does not seem to alter significantly the pharmacokinetics of nisoldipine C.C. and its metabolites. Caution should be exercised in dosing and titrating these patients. (Study D92-001).

B.Hepatic Impairment:

Study D90-026-01 shows that liver impairment has a pronounced effect on the pharmacokinetics of nisoldipine C.C. since both AUC and CMAX were increased four fold as compared to normal volunteers. Therefore extra care should be exercised when giving this drug to patients with impaired liver function.

C.Elderly:

Study 563 shows that the plasma concentrations of nisoldipine following the administration of 10 mg IR for 8 days tended to be higher in the elderly as compared to the young. CMAX was 1.76 (+/-0.84)ng/ml compared to 4.96 (+/-3.22) ng/ml in the elderly. AUC in the young was 7 (+/-3.12) ng/hr/ml compared to 15.04 (+/-9.33) ng/hr/ml in the elderly. However, there was no difference in the single dose and multiple dose of nisoldipine in both the young and the elderly and there was no accumulation upon multiple dose administration.
On the other hand, study 712 where 20 mg nisoldipine C.C. was administered for 7 days showed that after single dose the elderly normal and hypertensive patients tended to have about 50 % higher AUCs than young healthy subjects. CMAX in the elderly hypertensives was also about 50 % higher than either the healthy young or elderly volunteers. Moreover, upon multiple administration, there was a greater tendency for increase in AUC and CMAX for both the elderly healthy and hypertensive subjects compared to the young. It is to note that there was essentially no accumulation in the young in this study but in the elderly healthy and hypertensives, the accumulation ratio was around 2.

D-Gender:

The effect of gender on the pharmacokinetics of nisoldipine [illegible] investigated by the sponsor. An attempt by this reviewer to correlate gender with [illegible] and CMAX from data obtained from Study 712 was inconclusive due to insufficient data.

V-DISEASE STATES:

A-Hypertension:

Studies D90-022 and D88-059 showed that hypertension does not have any effects on the pharmacokinetics of nisoldipine C.C. since the plasma levels obtained from these studies were similar to those obtained in healthy subjects.

VI-DRUG INTERACTIONS:

A-Ranitidine:

Coadministration of ranitidine with nisoldipine C.C. did not have any effect on the pharmacokinetics of nisoldipine (Study 738).

B-Cimetidine:

Cimetidine seems to have a pronounced effect on nisoldipine C.C. pharmacokinetic parameters since there was more than 50 % increase in some parameters of interest. Multiple dose administration of 400 mg of cimetidine increase nisoldipine CMAX from 1.05 to 1.74 ng/ml and its AUC from 14.97 to 23.2 mcg*hr/l. Therefore, great caution should be exercised when both these drugs are administered concomitantly, the patients should be monitored and dose adjustments made as necessary (Study 738).

C-Warfarin:

Study 349 showed that coadministration of steady state doses between 3 and 10 mg of warfarin with 10 mg IR nisoldipine did not have any effect on the pharmacokinetics of nisoldipine. Moreover, nisoldipine did not affect the prothrombin times of patients that were on warfarin.

D-Quinidine:

Coadministration of twice a day of 10 mg of nisoldipine IR with 600 mg of quinidine bid increased quinidine's AUC by 25 % (Study 854). The effect of quinidine on nisoldipine pharmacokinetics could not be assessed since no nisoldipine plasma concentrations were measured in this study.

E-Propranolol:

Coadministration of 20 mg nisoldipine capsules either acutely or chronically caused a significant increase in both AUC and CMAX for propranolol. The propranolol AUC increased from 1556 (+/-) 1135) to 2098 (+/- 1501) with single dose nisoldipine and up to 2482 (+/- 2099) ng.hr/ml with multiple dosing of nisoldipine. CMAX increased from 143 (+/- 44) ng/ml to 222 (+/- 67) ng/ml with either single dose or multiple dose administration of the calcium channel blocker (Study 3982).
However Study 704 showed that coadministration of 20 mg nisoldipine C.C. with 40 mg propranolol tid did not have any significant effects on the plasma concentrations of either drugs.

F-Atenolol:

Coadministration of 20 mg nisoldipine capsules either acutely or chronically caused a significant increase in both AUC and CMAX of atenolol. The CMAX for atenolol increased from 455 (+/- 135) ng/ml to 540 (+/- 146) ng/ml while the AUC for the beta blocker increased from 5854 (+/- 2291) ng*hr/ml to 6987 (+/- 2269) ng*hr/ml. These results are very similar to what was seen when nisoldipine was coadministered with propranolol (Study 3982).

G-Beta Acetyl Digoxin:

Coadministration of 10 mg of nisoldipine IR tablets bid with 0.6 mg/day of beta acetyl digoxin did not seem to have any effect on the pharmacokinetics of beta acetyl digoxin (Study 413).
(Note: In the labelling, the results of this study appear as lack of interaction with Digoxin and not acetyldigoxin. This issue was discussed with Dr Chen supervisory Medical Officer HFD 110 who thought that labelling the results of this study as no interaction with digoxin was appropriate.)

VII-PHARMACOKINETIC/PHARMACODYNAMIC RELATIONSHIPS:

The sponsor attempted to establish a pharmacokinetic/pharmacodynamic model in hypertensive patients using the results of study D90-022. This pharmacodynamic model was established using the program Attract which is based on linear system analysis and methods utilizing hysterisis minimization. The sponsor reported that the pharmacodynamic responses (mainly drop in blood pressure) follow a sigmoid EMAX model.
The modelling method used by the sponsor is not valid due to the fact that it is very difficult to see hysterisis with this formulation of this drug (see also the comments following Study D90-022).
The Division of Biopharmaceutics attempted to model the pharmacodynamics with the

pharmacokinetics of Nisoldipine C.C. using a population approach. The data from study D90-022 and study D88-059 were used in this attempt. However due to the fact that both studies for the same 30 mg dose gave totally different plasma concentrations (study D90-022 gave almost double the plasma concentrations of study D88-059), these studies could not be combined and the final model was established using data from the same study that the sponsor model. The best model that describes this set of data was found to be an EMAX model with a maximal reduction of diastolic blood pressure of -23.9 mm of Hg. The EC50 was estimated to be 3.94 ng/ml.

VIII-FORMULATION:

The coat-core tablet consists of a slowly dissolving coat surrounding a more rapidly dissolving core. Within the coat the active ingredient is finely distributed in a matrix of a hydrophilic gel-forming polymer. On contact with water a swelling process begins at the tablet surface and the soft material formed is continuously eroded. The active ingredient contained in the eroded material can then be dissolved and absorbed. Initially, the diameter of the tablet and thus its surface area change very little resulting in a constant release of active ingredient over a period of about 6 to 8 hours (i.e. zero order dissolution kinetics). When the erosion of the coat has advanced, the dissolution of the fast-release core causes an increase in the release rate over a period of about 2 hours. Thus, the decreasing release rate of drug from the tablet coat (due to diminishing tablet surface area) is countered by the rapid dissolution of the core.
The composition of the different strengths tablets of nisoldipine Coat Core is summarized in Appendix II.
It is to be noted that all the pivotal clinical trials were done using the to be marketed formulation.

IX-PROTEIN BINDING:

The plasma protein binding of nisoldipine is very high since less than 1 % is unbound at a concentration range between 100 ng/ml and 10 mcg/ml. The binding is primarily to albumin. There was no stereoselectivity in binding since both enantiomers had similar degree of binding as observed with the racemate. (study 19611)

X-RED BLOOD CELL PARTITIONING:

The erythrocyte/plasma partition coefficients are about 0.3 mostly independent of the concentration in the range studied 0.1 to 10 mcg/ml. However, the partition between erythrocytes and plasma water is very high in the order of 56 indicating a high affinity of the blood cells and other tissues to nisoldipine. There was no difference in partitioning between the racemate and its enantiomers. (Study 19611).

XI-DISSOLUTION:

The proposed dissolution method for the coat-core tablet formulation was the USP method II (paddle method) at a paddle speed of 50 rpm. The medium was 900 ml of phosphate buffer with

1% sodium lauryl sulfate. The proposed dissolution specifications were:
-3 hours
-6 hours
-12 hours: Not less than
*For the evaluation after stage 2 of the USP acceptance table, single tablets and mean value are specified by the limits % respectively.
Based on the performance of the bio lots submitted in this NDA, the following dissolution specifications are recommended:
-3 hours:
-6 hours:
-12 hours: not less than .

XII-IN VIVO IN VITRO CORRELATION:

An attempt was made by the sponsor to correlate the in vitro and in vivo performance of the nisoldipine coat-core tablets. However, the sponsor could not establish any level of correlation (A,B or C) due to the fact that nisoldipine undergoes variable gut wall metabolism which is dependent on the site in the gastro-intestinal tract.

XIII-ASSAY:

Concentrations of nisoldipine and its metabolites from biological fluids were determined using capillary gas chromatography with electron capture as a detection mode. Overall, the assay methodology as well as its validation were satisfactory.

Comments to be Sent to the Firm:

1-Nisoldipine appears to exhibit stereospecific first pass metabolism. After P.O. administration the (+) nisoldipine plasma levels are 6 times higher. Since the (+) enantiomer of nisoldipine is responsible for most if not all the activity of this drug, the sponsor should have used a stereospecific assay for all pk studies.

2-The sponsor did not determine the ratio of the two enantiomers in special populations. The sponsor is asked to submit any available data on the ratio of the enantiomers of the parent compound as well as any relevant metabolites in dose proportionality, drug interaction studies and special populations (such as liver impairment patients, elderly etc...) as compared to healthy volunteers.

3-The sponsor failed to identify the specific enzymes that are responsible for the metabolism of nisoldipine. The sponsor is requested to identify the enzyme systems responsible for the metabolism of nisoldipine even though the sponsor believes that there is no need to conduct such studies because it is believed that the same enzyme that is responsible for the metabolism of nifedipine (i.e. 3A4) is also responsible for the metabolism of nisoldipine. Nevertheless, it is necessary to confirm this by conducting an in vitro metabolic study.

4-The dissolution data submitted in this NDA showed a great deal of variability specially around the 9 hour time point. Some of the higher strengths lots i.e. the 20 and 60 mg tablet strength, seem to give different results when the same lot studied under the same conditions were tested within the same day and also on different days. The firm is requested to give an explanation for this variability.

5-Based on the performance of the bio lots submitted in this NDA, the following dissolution test and specifications are recommended:
USP paddle at a speed of 50 rpm in 900 ml of phosphate buffer pH 6.8 with 1 % sodium lauryl sulfate
-3 hours:
-6 hours:
-12 hours: not less than

6-Studies D90-022 and D88-059 do not give the same plasma levels for the 30 mg dose. In the first study (D90-022) the plasma levels obtained with the 30 mg dose are almost double than what was obtained with the same dose in Study D88-059. AUC was 74.28 +/- 7.96 compared to 33.097 +/- 6.077 ng*hr/ml while CMAX was 4.79 +/- 0.68 compared to 2.473 +/- 0.458 ng/ml. The sponsor is asked to explain the observed discrepancy between the 2 studies.

7-In several of the studies, the sponsor did not include any assay validation. It is the Division of Biopharmaceutics policy to recommend that a full description of the analytical assay be included in the study reports. Data on the linearity, specificity, sensitivity and on the accuracy and precision of the analytical methodology should be included in each study report.

8-The modelling approach taken by the sponsor to analyze the relationship between the pK of nisoldipine and its pd was found to be inadequate by the reviewer due to the reasons outlined in the Comments following Study D90-022 on page 189.

9-In Studies D90-022 and D88-059, CMAX and AUC (only in Study D90-022) increased in a less than dose proportional manner with dose. Yet, the results of Study D91-035 indicate that the pharmacokinetics of nisoldipine from the coat-core formulation should be linear between 20 and 60 mg. The sponsor is asked to explain the discrepency in these results.

10-Quinidine is a known inhibitor of the cytochrome P450 isoenzyme DII6. In the drug interaction study between quinidine and IR nisoldipine, the sponsor only measured the plasma concentrations of quinidine. The sponsor should have also measured the nisoldipine plasma concentrations to see whether quinidine had any effect on the metabolic pathways responsible for the metabolism of this dihydropyridine compound.

11-In the renal impairment study, the sponsor should have determined the protein binding of nisoldipine. It is not uncommon to see significant protein binding changes which will affect the plasma levels of the drug under study.

12-The sponsor did not investigate either the effect of gender [illegible] on the pharmacokinetics of nisoldipine. The sponsor is asked to submit any additional data that might be available that would address this issue.

13-The Pharmacokinetics and metabolism section of the package insert should be rewritten as follows:

Pharmacokinetics:

Nisoldipine activity is primarily due to the (+) enantiomer. Studies with radiolabelled drug have demonstrated that administered nisoldipine is relatively well absorbed into the systemic circulation with 87 % of the radiolabel recovered in urine and faeces. Elimination of nisoldipine is exclusively by metabolism with no unchanged nisoldipine recovered in the urine. Nisoldipine pharmacokinetics are independent of dose in the range of 20 to 60 mg. Upon multiple dosing, nisoldipine accumulation is predictable from a single dose. The bioequivalency between 2x30 mg and 3x20 mg nisoldipine has been established. However 1x20 vs 2x10 and 2x20 mg vs 1x40 mg tablets were inequivalent with respect to CMAX.

Absorption: The absolute bioavailability of nisoldipine was found to be 5.5 %. Nisoldipine's low bioavailability is due to presystemic metabolism with evidence of gut wall metabolism which decreases from the proximal to the distal parts of the intestine with no metabolism occurring in the colon.

Food has a pronounced effect on the release of nisoldipine from the coat-core formulation. CMAX increased by up to 300 % and AUC decreased by up to 26 %. However, the food effect was not as pronounced on the immediate release capsule since AUC increased by 28% and CMAX by 31 %. Concomitant intake of food with nisoldipine Coat-Core is contraindicated.

The volume of distribution of nisoldipine after IV administration was estimated to be between 2.3 and 3.4 l/kg. The plasma protein binding is very high since less than 1 % is unbound over the plasma concentrations of 100 ng/ml to 10 mcg/ml. Nisoldipine poorly penetrates into red blood cells with a blood/plasma ratio of 0.3 mostly independent of concentration over the range of 0.1 to 10 mcg/ml.

Maximal plasma concentrations of nisoldipine are reached 6 to 12 hours after dosing. The terminal elimination half-life ranges from 7 to 12 hours. With a 40 mg tablet of nisoldipine Coat-Core, CMAX was 3.1 ng/ml and the AUC_{0-48} was 54.3 ng*hr/ml. After oral administration, the concentration of the (+) nisoldipine was about 6 times higher than the (-) isomer.

Metabolism: 11 metabolites have been identified in the urine. In man the major biotransformation pathway appears to be the hydroxylation of the isobutyl ester. Metabolite M9, which is the hydroxylated derivative of the side chain of nisoldipine, is the only one that appears to have any activity (10 % of the parent compound) and is present in equal amounts as nisoldipine in plasma. Cytochrome P450 is believed to play a major role in the metabolism of nisoldipine, however, the particular isoenzyme system that is responsible for its metabolism has not been identified.

Excretion: No unchanged nisoldipine is eliminated in the urine.

Special Populations:

Geriatric: Elderly patients have been found to have 2 to 3 fold higher plasma concentrations than young subjects.

Renal dysfunction: Because renal elimination is not a significant pathway, dosing adjustments in patients with mild to moderate renal impairment is not necessary.

Hepatic Insufficiency: In patients with liver cirrhosis given 10 mg nisoldipine C.C., plasma concentrations of the parent compound were found to be 4 to 5 times higher than healthy young subjects. Thus lower maintenance doses may be required in both cirrhotic patients and in the elderly.

Gender and Race: The effect of gender or race on the pharmacokinetics of nisoldipine has not been investigated.

Disease States: Neither hypertension nor stable exertional angina pectoris alter the pharmacokinetics of nisoldipine.

Drug-Drug Interactions: No significant interactions were found between nisoldipine immediate release (IR) and warfarin or beta acetyl-digoxin. However, IR nisoldipine increased plasma quinidine concentrations by about 20 %.
A 30 to 40 % increase in AUC and CMAX of nisoldipine was observed with concomitant administration of 400 mg cimetidine twice daily. There was no interaction with ranitidine 150 mg twice daily..
Coadministration of 20 mg nisoldipine IR with 160 mg propranolol once daily caused a 35 % increase in propranolol AUC and 55 % increase in CMAX. The interaction with nisoldipine C.C. was negligible. Atenolol's AUC and CMAX were increased by 20 % when coadministered either acutely or chronically with 20 mg IR nisoldipine capsules.

9-In the Dosage and Administration section of the package insert, a statement should be included that nisoldipine coat-core should be taken on an empty stomach.

P Marroum 11/18/1994
Patrick J. Marroum Ph.D.

Biopharm Day October 4 1994 (Collins, Ludden, Malinowski, Fleischer, Chen, Gillespie, Parekh, Marroum).
RD/ FT initialed by A Parekh Ameeta Parekh 11/21/94

cc: NDA 20-356, HFD 110, HFD 426 (Marroum, Fleischer), Chron, Drug, FOI (HFD 19), HFD 340 (Vishwanathan), F, CR, A, DI, Pk/PD, RI, A, CD.

END

Effects on the Offspring: There were no significant intergroup differences in number of pups per litter at birth or after 1 and 2 weeks. However, there was a higher incidence of stillbirths and a higher rate of neonatal mortality throughout the 2 week postpartum study period for offspring of nisoldipine treated dams. The body weights of pups in the nisoldipine treated group were lower than control at the time of birth, and after 1 and 2 weeks. There was no effect on sex ratio at birth or at PPD 14 (See table which follows).

DOSE (MG/KG)	TOTAL NO. OF YOUNG (ALIVE + DEAD)	TOT. NO. OF DEAD YOUNG AT BIRTH	UP TO TIME OF 1ST WEIGHING	AFTER 1 WEEK	2 WEEKS
CONTROL	208	2	2	7	11
30	227	22*	30**	42**	50**

* significant difference from the controls, $p < 0.01$
**significant difference from the controls, $p < 0.001$

Comment: The increase in mean duration of gestation, followed by a higher incidence of stillbirths and a higher rate of neonatal mortality throughout the 2 week postpartum study period, and the lower body weight at birth for offspring of nisoldipine treated dams, were observed in both the main study and in the present study. These observations are suggestive of dystocia. Dystocia is defined as abnormal labor, which is usually accompanied by increased duration of labor and an increaed incidence of stillbirths and neonatal mortality.

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(MEAN VALUES AND STANDARD DEVIATIONS FOR THE GR

DATA FOR ANIMALS ALLOWED TO REAR THEIR OWN YOU

INVESTIGATION	CONTROLS	.G/KG
DURATION OF GESTATION IN DAYS	21.6 0.5	22.1+ 0.5
WEIGHT GAIN DAY 0 to DAY 20 (G)	93	78.9*** 13.3
WEIGHT GAIN DAY 0 to DAY 16 (G)	.5 8.9	57.7 6.7
WEIGHT GAIN DAY 16 to DAY 20 (G)	37.5 8.9	21.2++ 10.2
BODY WEIGHT IN G DAY 0 P.C.	195.6 7.2	196.6 8.6
DAY 16 P.C.	251.1 13.3	254.4 10.8
DAY 20 P.C.	288.6 20.2	275.5** 17.2
DAY 1 [illegible]	222.8 13.0	210.2*** 16.6
1 P.P.	240.3 10.5	231.1* 13.6
WEEKS P.P.	246.3 14.6	245.9 14.3
NUMBER OF IMPLANTATIONS	10.0 3.3	10.4 2.8

* Significant difference from the controls, $p < 0.01$
** Significant difference from the controls, $p < 0.025$
***Significant difference from the controls, $p < 0.005$
\+ Significant difference from the controls, $p < 0.001$
++ Significant difference from the controls, $p < 0.0005$

BAY k 5552 T 3008898

BAY K 5552/T3008898

(MEAN VALUES AND STANDARD DEVIATIONS FOR THE GR

DATA ON THE YOUNG

INVESTIGATION	CONTROLS	MG/KG
NO. OF YOUNG AT BIRTH	9.4 3.3	8.5 3.1
AFTER 1 WEEK	[illegible]	8.4 3.1
AFTER 2 WEEKS	[illegible].0 3.1	8.0 3.1
AFTER 3 WEEKS	0.0 0.0	0.0 0.0
WEIGHTS OF THE YOUNG AT BIRTH (G)	5.9 0.7	5.3** 0.6
AFTER 1 WEEK	11.7 1.9	10.2* 2.1
AFTER 2 WEE[illegible]	20.3 4.2	18.3 3.9
T[illegible] DAYS P.P.) OF: [illegible]LDING OF PINNAE	3.5 0.7	3.5 0.6
[illegible]EVELOPMENT OF COAT	10.4 0.6	10.7 1.0

* Significant difference from the controls, $p < 0.01$
**Significant difference from the controls, $p < 0.005$

MUTAGENICITY STUDIES (S. Stolzenberg)

1. Salmonella/Microsome Test

Study No.: Not provided. Pharma Report No. 9634

Performing Laboratory:

Date Performed: August, 1980 to September, 1980

Quality Assurance: A signed statement of compliance with GLP is not included.

Background: This widely used mutagenicity assay detects point mutations (base pair by TA 1535 and TA 100 and frame shift by TA1537 and TA 98), caused by chemical agents, *in vitro*. The reversion rates to prototrophy of histidine requiring (his-) mutants to the wild type histidine independent strain (his+), are evaluated in a medium with a low content of histidine. In the presence of test compound, an increase in reversion rate, measured by an increase in colony growth on the agar plate, is an indication of mutagenicity.

Procedure: The evaluation was performed with and without metabolic activation (provided by S-9 fraction of livers of Aroclor pretreated rats). *S. typhimurium* strains TA1535, TA100, TA1537 and TA98 were used. Two studies with TA 1535 but only one with the remaining 3 strains were carried out with 4 plates per concentration of test substance, DMSO control or postive control substances. Concentrations tested were 0, 20, 100, 500, 2500 and 12500 ug/plate.

Positive Controls:

a). without S-9 activation
Endoxan (cyclphosphamide) for TA1535 and TA100
Trypaflavin for TA1537 and TA98

b). with S-9 activation
2- aminoanthracene for all 4 tester strains

Results: 2500 ug/plate was toxic to bacterial growth for all 4 strains, whereas 12500 was toxic and caused precipitation, making it not possible to evaluate colony growth. 500 ug/plate was toxic for TA1535. In the first test, the positive control for TA1535 without S-9 showed no response, and the negative controls, both and without S-9, were unusually low. Therefore, the test with TA1535 was repeated. In the second test with TA1535, no indication of mutagenicity was seen with or without S-9. Similarly, Bay k 5552 showed no mutagenic effects on TA100, TA1537 and TA98. Positive controls gave adequate responses; i.e. well over double those of the negative controls.

In this test system, Bay k 5552 was considered not mutagenic at non-toxic, soluble concentrations.

Salmonella/Microsome Test with BAY k 5552 on Salmonella typhimurium TA 1535.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment / M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
12500			Could not be evaluated		
2500	0	0.8	64.4**	-	0.80
500	8.5	6.5	126.9**	8.50 a)	6.50 a)
100	9.0	6.3	158.4	9.00 a)	6.30 a)
20	14.5	5.0	146.0	14.50 a)	5.00 a)
Negative Control 0	1.0	1.0	152.4	1.00	1.00
Positive Control Endoxan 435	0.5	1.0	163.7	0.50 a)	1.00 a)
Positive Control 2-Aminoanthracene 10	32.8	19.0	5.2**	32.80 a)	19.00 a)

** Bacteriotoxic effect
a) See the "Results" section

Salmonella/Microsome Test with BAY k 5552 on Salmonella typhimurium TA 100.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment / M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
12500			Could not be evaluated		
2500	4.3	19.5	2.0**	0.06	0.04
500	80.5	34.5	4.3	1.18	0.71
100	72.3	62.0	4.7	1.06	1.27
20	53.5	49.3	6.2	0.78	1.01
Negative Control 0	68.5	48.8	4.7	1.00	1.00
Positive Control Endoxan 435	273.8	82.0	3.9	4.00*	1.68
Positive Control 2-Aminoanthracene 10	1136.0	81.8	0.6**	16.58*	1.68

* Mutagenic effect
** Bacteriotoxic effect

Salmonella/Microsome Test with BAY k 5552 on Salmonella typhimurium TA 1537.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
12500			Could not be evaluated		
2500	0	0.5	45.9**	-	0.13
500	3.3	1.5	49.1	0.66	0.38
100	7.3	2.0	51.3	1.46	0.50
20	5.0	1.5	51.5	1.00	0.38
Negative Control 0	5.0	4.0	50.9	1.00	1.00
Positive Control Trypaflavine 200	162.8	114.8	44.7**	32.56*	28.70*
Positive Control 2-Aminoanthracene 10	37.3	15.3	0.3**	7.46*	3.83*

* Mutagenic effect

** Bacteriotoxic effect

Salmonella/Microsome Test with BAY k 5552 on Salmonella typhimurium TA 98.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
12500			Could not be evaluated		
2500	3.0	1.3	100.0**	0,18	0,27
500	22.0	3,0	127.3	1.31	0,69
100	19.3	4,8	151.70	1.15	1,00
20	14.0	5,5	-	0,83	1.14
Negative Control 0	16,8	4,8	121.5	1.00	1.00
Positive Control Trypaflavine 200	460,0	4.3	144,3	27.38*	0.90
Positive Control 2-Aminoanthracene 10	845.3	8,0	70,7**	50.32*	1.67

* Mutagenic effect
** Bacteriotoxic effect

Salmonella/Microsome Test with BAY k 5552 on Salmonella typhimurium TA 1535. Repeat.

Dose in µg per Plate	Mutants / Plate (M/P)		Total Organism Count per ml	M/P Treatment M/P Negative Control	
	+ S-9 Mix	- S-9 Mix	x 10^8	+ S-9 Mix	- S-9 Mix
2500	0	0	toxic	-	-
500	0	0	toxic	-	-
100	6.5	6.0	non-toxic	1.08	1.33
20	6.8	7.0	non-toxic	1.13	1.56
Negative Control 0	6.0	6.5	non-toxic	1.00	1.00
Positive Control Endoxan 435	427.3	24.3	non-toxic	71.22*	5.40*
Positive Control 2-Aminoanthracene 10	266.0	8.5	non-toxic	44.33*	1.89

* Mutagenic effect

2. Salmonella/Microsome Test

Study No.: T 5027709

Performing Laboratory:

Date Performed: 1/29/88 to 3/11/88

Quality Assurance: A signed statement of compliance with GLP is included.

Procedure: Tester strains used were *S. typhimurium* TA1535, TA100, TA1537 and TA98. Two different forms of S-9 were used; from livers of Aroclor 1254 pretreated male NMRI mice, and from livers of NMRI male mice that had been pre-treated for 28 days with 2000 ppm Bay k 5552 in the diet. All the studies were carried out with 4 plates per concentration of test substance, control or postive control substances. Initially, concentrations tested both without and with metabolic activation were 0, 20, 100, 500, 2500 and 12500 ug/plate. Subsequently, concentrations tested were 0, 75, 150, 300, 600, 1200 and 2400 ug per plate. There is no explanation as to why two methods of enzyme activation were employed.

Positive Controls:

a). without S-9 activation
Sodium azide for TA1535
Nitrofurantoin for TA100
4-nitro-1,2-phenylene diamine for TA 1537 and TA98

b). with S-9 activation
2- aminoanthracene for all 4 tester strains

Results: No indication of mutagenicity was observed at any concentration tested. However, 20 ug/plate, the lowest concentration tested, was the only concentration at which bacteriotoxic problems were not encountered. Starting at 150 ug/plate, precipitation problems were also encountered. Positive controls gave adequate responses; i.e. well over double the colony count of the negative controls.

Bay k 5552 was considered not mutagenic in this test system, but this study is valid only at 20 ug/plate because it was the only concentration at which toxicity and/or precipitation problems were not encountered with all 4 bacterial strains.

BAY k 5552
Salmonella/microsome test
Study No. T 5027709

Tabulated summary of data

Summary of means from Tables 1-8 without S-9 mix

Tables +group mcg/pl.	Strain TA 1535	TA 100	TA 1537	TA 98
1-4				
0	17	126	11	17
20	20	128	10	15
100	17	94	10	14
500	16	71	8	14
2500	--	9	--	9
12500	--	---	--	--
Na-Azid	926			
NF		405		
4-NPDA			126	187
5-8				
0	15	80	7	18
75	13	72	5	20
150	10	72	7	18
300	11	56	8	15
600	9	59	6	16
1200	--	38	8	12
2400	--	--	-	13
Na-Azid	1089			
NF		370		
4-NPDA			118	96

BAY k 5552
Salmonella/microsome test
Study No. T 5027709

Summary of means from Tables 1-12 with S-9 mix
of Aroclor induced male NMRI mice

Tables +group mcg/pl.	Strain TA 1535	TA 100	TA 1537	TA 98
1-4 30% S-9				
0	19	146	11	35
20	17	128	12	30
100	18	125	9	32
500	13	83	10	32
2500	--	46	7	18
12500	--	---	--	--
2-AA	81	812	138	1018
5-8 30% S-9				
0	13	118	10	24
75	16	105	7	24
150	14	86	10	16
300	10	85	8	17
600	14	82	4	20
1200	10	62	6	17
2400	--	23	--	8
2-AA	89	1505	102	1290
9-12 10% S-9				
0	15	99	10	33
75	12	76	10	24
150	9	74	8	27
300	11	70	9	22
600	7	64	10	28
1200	--	63	8	21
2400	--	4	--	9
2-AA	264	2510	505	2402

BAY k 5552
Salmonella/microsome test
Study No. T 5027709

Summary of means from Tables 13-20 with S-9 mix
of BAY k 5552 treated male NMRI mice

Tables +group mcg/pl.	Strain TA 1535	TA 100	TA 1537	TA 98
13-16 30% S-9				
0	12	105	11	24
75	11	100	8	15
150	14	102	7	13
300	12	76	5	18
600	11	45	8	9
1200	4	---	--	--
2400	--	---	--	--
2-AA	78	893	44	748
17-20 10% S-9				
0	15	95	7	26
75	13	93	6	19
150	13	88	7	25
300	8	56	3	20
600	8	77	7	25
1200	--	32	-	--
2400	--	--	-	--
2-AA	349	1628	274	1066

3. CHO HGPRT Forward Mutation Assay

Study No.: T 1023114 (sponsor's number)

Performing Laboratory

Sponsor:

Date Performed: 7/11/86 to 10/29/86

Quality Assurance: A signed statement of compliance with GLP is included.

Background: "The objective of this study was to evaluate the test article for its ability to induce forward mutation at the HGPRT locus in the CHO-K1-BH Chinese hamster cell line, as assessed by colony growth in the presence of 6-thioguanine (TG). Hypoxanthine guanine phosphoribosyl transferase (HGPRT) is a cellular enzyme that allows cells to salvage hypoxanthine and guanine from the surrounding medium for use in DNA synthesis. If a purine analog such as TG is included in the growth medium, the analog will be phosphorylated via the HGPRT pathway and incorporated into nucleic acids, eventually resulting in cellular death. The HGPRT locus is located on the X chromosome. Since only one of the two X chromosomes is functional in the female CHO cells, a single-step forward mutation from HGPRT+ to HGPRT- in the functional X chromosome will render the cell unable to utilize hypoxanthine, guanine, or TG supplied in the culture medium. Such mutants are viable as wild-type cells in normal medium because DNA synthesis may still proceed by de novo synthetic pathways that do not involve hypoxanthine or guanine as intermediates. The basis for the selection of HGPRT- mutants is the loss of their ability to utilize toxic purine analogs (e.g., TG), which enables only the HGPRT- mutants to grow in the presence of TG. Cells which grow to form colonies in the presence of TG are therefore assumed to have mutated, either spontaneously or by the action of the test article, to the HGPRT- genotype."

Procedure: In preliminary, range finding tests, concentrations of 50 and 100 ug/ml Bay w 5552 (batch #828305) without metabolic activation were found to be 100% cytotoxic to the cell culture, but in the presence of metabolic activation (provided by S-9 fraction of livers of Aroclor 1254 pretreated rats), 100 ug/ml caused only a 39% decrease in survival index.

Three tests without S-9 included duplicate cultures with concentrations between 10 to 40 ug/ml, and two tests with S-9 included duplicate cultures with concentrations of 5 to 85 ug/ml. Positive controls were 5-bromodeoxyuridine without S-9 and 3-methylcholanthrene in the presence of S-9.

Results: Decreases in relative cell survival were seen at 30 ug/ml or higher concentrations without S-9, and at 50 ug/ml and higher with S-9. There were sporadic, small but statistically significant increases in mutant frequencies in each of the 3 tests without S-9 and in one of the two tests with S-9. In every case the increase occurred in only one of the two duplicate cultures. In spite of a suggestion of a concentration relationship at 40 and 60 ug/ml without S-9 (see first 2 tables which follow), it was claimed that a concentration relationship did not exist. It should, however, be noted that even among the duplicate controls, there were wide variations in mutant frequencies in most of the tests.

Bay k 5552 was considered not mutagenic in this test system.

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ _____ TEST ARTICLE: Bay k 5552 mikron. ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: August 20, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants
Expression Time: 7 days

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES/DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
NONACTIVATION:																		
Vehicle Control	258.0 ± 13.5	100.0	100.0	0	1	0	0	0	0	0	0	0	0	0	0	1	118.5 ± 8.7	0.4
	275.0 ± 1.0			2	2	0	1	0	1	2	1	2	3	0	4	18	108.8 ± 13.9	6.9*
Positive Control	145.7 ± 1.5	54.7	67.4	26	20	22	17	26	17	26	24	18	20	22	23	261	88.5 ± 1.3	122.9**
(50 µg/ml BrdU)[b]	149.3 ± 9.1	56.0	68.4	23	22	25	18	35	27	18	23	31	31	18	25	296	86.8 ± 7.3	142.1**
TEST ARTICLE																		
10.0 µg/ml	242.7 ± 6.5	91.1	169.6					NOT CLONED										
10.0 µg/ml	251.7 ± 12.7	94.4	159.6					NOT CLONED										
12.5 µg/ml	237.3 ± 7.6	89.1	131.4					NOT CLONED										
12.5 µg/ml	263.7 ± 32.0	98.9	139.6					NOT CLONED										
15.0 µg/ml	243.3 ± 7.5	91.3	127.3	0	2	0	0	0	0	0	0	1	C	0	0	3	83.5 ± 3.6	1.6
15.0 µg/ml	245.0 ± 22.6	91.9	164.6	0	0	1	0	0	0	0	0	0	0	0	0	1	80.5 ± 6.1	0.5
20.0 µg/ml	243.0 ± 21.7	91.2	143.7					NOT CLONED										
20.0 µg/ml	232.7 ± 11.6	87.3	136.2					NOT CLONED										
25.0 µg/ml	215.7 ± 10.2	80.9	124.1	1	1	1	1	3	1	2	0	2	0	1	0	13	84.0 ± 4.0	6.4
25.0 µg/ml	206.7 ± 28.6	77.5	115.2	2	0	1	1	0	0	1	1	1	1	2	0	10	100.3 ± 2.3	4.2
30.0 µg/ml	249.0 ± 6.6	93.4	99.7	C	C	C	C	C	C	C	C	C	C	C	C	-	100.0 ± 0.0[f]	-
30.0 µg/ml	227.3 ± 10.0	85.3	66.1	0	0	0	0	4	0	1	1	2	0	1	1	10	113.3 ± 4.6[x]	3.7
35.0 µg/ml	237.7 ± 21.5	89.2	59.8	C	C	C	C	C	C	C	C	C	C	C	C	-	122.5 ± 6.4[x]	-
35.0 µg/ml	227.7 ± 11.7	85.4	55.1	0	0	0	0	2	0	0	0	0	1	0	1	4	133.7 ± 4.4	1.2
40.0 µg/ml	193.3 ± 9.3	72.5	39.6	4	2	1	1	4	3	3	0	0	3	0	0	21	105.2 ± 5.5	8.3**
40.0 µg/ml	193.0 ± 13.0	72.4	42.3	0	0	1	0	0	0	0	0	0	0	0	1	2	109.8 ± 3.3	0.8

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: Bay k 5552 mikron ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: September 19, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants
Expression Time: 7 days

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL. POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E. ± S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
NONACTIVATION:																		
Vehicle Control	193.7 ± 6.0	100.0	100.0	0	3	1	1	0	2	2	0	1	0	0	0	10	102.3 ± 4.9	4.1
	187.0 ± 15.4			0	2	1	1	2	2	0	1	0	1	0	0	10	111.3 ± 13.7	3.7
Positive Control (50 µg/ml BrdU)[b]	148.3 ± 15.8	77.9	37.6	12	14	16	10	13	13	15	14	13	16	17	15	168	103.3 ± 4.0	67.8**
	139.7 ± 7.8	73.4	32.9	20	12	18	16	20	15	22	14	16	13	14	10	190	105.0 ± 6.6	75.4**
TEST ARTICLE																		
10.0 µg/ml	147.3 ± 12.1	77.4	43.6	0	4	0	1	0	1	0	0	0	1	0	0	7	129.0 ± 5.3	2.3
10.0 µg/ml	156.3 ± 11.8	82.1	59.5	3	0	2	2	1	3	3	0	3	3	1	0	21	125.8 ± 6.4	7.0
15.0 µg/ml	152.0 ± 17.3	79.9	44.0	1	1	1	1	1	1	1	0	2	0	1	2	12	127.0 ± 14.1	3.9
15.0 µg/ml	151.3 ± 11.7	79.5	39.8	1	0	1	0	0	4	1	0	3	1	3	0	14	142.7 ± 14.7	4.1
20.0 µg/ml	137.0 ± 23.1	72.0	44.8	0	0	2	0	1	0	0	0	1	0	2	0	6	112.3 ± 7.4	2.2
20.0 µg/ml	151.3 ± 11.2	79.5	31.8	1	2	4	5	2	2	1	2	1	0	1	1	22	141.3 ± 2.8	6.5
30.0 µg/ml	125.3 ± 3.2	65.8	42.9	0	2	1	1	0	0	0	1	2	0	2	0	9	110.2 ± 1.2	3.4
30.0 µg/ml	135.7 ± 8.6	71.3	49.9	3	1	0	1	1	2	2	2	3	5	2	0	22	115.5 ± 4.4	7.9*
40.0 µg/ml	42.7 ± 9.0	22.4	7.0	0	0	1	0	0	0	1	0	0	1	2	1	6	129.3 ± 15.7	1.9
40.0 µg/ml	42.3 ± 8.1	22.2	7.4	0	3	2	4	3	0	4	3	4	3	7	3	44	96.3 ± 1.5	19.0**
60.0 µg/ml	0.0 ± 0.0	0.0	< 0.1	NOT CLONED														
60.0 µg/ml	0.0 ± 0.0	0.0	< 0.1	NOT CLONED														

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: Bay k 5552 mikron ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: October 10, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants
Expression Time: 7 days

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES: DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
NONACTIVATION:																		
Vehicle Control	152.3 ± 11.2 172.0 ± 8.5	100.0	100.0	1 1	2 2	0 0	0 0	1 2	2 3	0 3	0 3	0 2	0 1	0 3	3 1	9 21	112.8 ± 6.8 126.5 ± 11.0	3.3 6.9
Positive Control (50 µg/ml BrdU)[b]	84.7 ± 1.2 84.7 ± 14.5	52.2 52.2	42.9 41.1	24 23	25 22	27 21	15 21	40 24	25 26	20 17	21 13	27 28	24 20	21 30	28 20	297 265	101.3 ± 11.5 90.7 ± 5.5	122.2** 121.7**
TEST ARTICLE																		
10.0 µg/ml	140.0 ± 6.1	86.3	76.0	2	2	1	3	5	2	2	3	1	2	2	0	25	108.7 ± 1.3	9.6*
10.0 µg/ml	145.7 ± 4.0	89.8	95.6	0	3	0	1	3	1	2	0	2	1	2	5	20	101.8 ± 11.3	8.2
15.0 µg/ml	146.7 ± 13.1	90.4	85.1	0	C	1	0	0	1	0	2	0	2	3	1	10	120.5 ± 1.7	3.5
15.0 µg/ml	136.7 ± 12.0	84.3	72.4	2	2	3	3	2	1	3	0	3	0	1	1	21	115.8 ± 13.8	7.6
20.0 µg/ml	138.7 ± 11.5	85.5	67.7	0	2	1	0	1	0	0	2	0	1	0	1	8	124.2 ± 6.6	2.7
20.0 µg/ml	128.0 ± 20.9	78.9	73.9	0	2	3	3	3	4	2	3	1	2	1	3	27	100.3 ± 9.4	11.2**
30.0 µg/ml	102.7 ± 7.4	63.3	65.0	1	2	1	1	1	3	2	1	1	1	3	3	20	104.2 ± 3.2	8.0
30.0 µg/ml	108.3 ± 9.0	66.8	57.7	T	T	T	2	2	1	1	5	2	1	4	0	18	122.3 ± 11.0	8.2
40.0 µg/ml	45.7 ± 12.1	28.2	8.2	6	0	0	1	2	4	4	0	1	0	3	1	22	116.8 ± 3.3	7.8
40.0 µg/ml	47.0 ± 5.3	29.0	9.1	1	0	1	0	1	1	3	0	1	1	2	0	11	113.5 ± 3.9	4.0
60.0 µg/ml	0.3 ± 0.6	0.2	< 0.1	NOT CLONED														
60.0 µg/ml	0.3 ± 0.6	0.2	< 0.1	NOT CLONED														
80.0 µg/ml	0.0 ± 0.0			NOT CLONED														
80.0 µg/ml	0.0 ± 0.0			NOT CLONED														
100.0 µg/ml	0.0 ± 0.0			NOT CLONED														
100.0 µg/ml	0.0 ± 0.0			NOT CLONED														

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: Bay k 5552 mikron. ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: September 10, 1986

Selective Agent: 15 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants
Expression Time: 7 days S9 batch: 1-186

TEST CONDITION	SURVIVAL TO TREATMENT MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
S9 ACTIVATION:																		
Vehicle Control	294.3 ± 12.9	100.0	100.0	0	2	2	2	1	0	4	1	0	2	0	0	14	110.3 ± 5.1	5.3
	396.3 ± 122.1			0	0	0	2	1	1	0	0	0	0	0	0	4	110.2 ± 5.8	1.5
Positive Control (5 µg/ml 3-MCA)[d]	276.0 ± 20.2	79.9	55.4	69	55	53	08	53	70	69	60	61	63	61	52	754	97.2 ± 9.0	323.2**
	256.7 ± 12.0	74.3	37.8	86	81	65	64	80	64	54	62	63	77	68	83	841	117.3 ± 5.1	298.7**
TEST ARTICLE																		
10.0 µg/ml	272.7 ± 16.3	79.0	194.8	NOT CLONED														
10.0 µg/ml	267.3 ± 4.5	77.4	167.8	NOT CLONED														
20.0 µg/ml	295.3 ± 11.7	85.5	111.4	NOT CLONED														
20.0 µg/ml	324.7 ± 8.4	94.0	145.5	NOT CLONED														
35.0 µg/ml	305.0 ± 26.6	88.3	140.3	1	0	1	0	1	0	0	0	0	1	0	0	4	96.2 ± 10.2	1.7
35.0 µg/ml	293.0 ± 9.5	84.9	153.9	0	1	0	0	0	0	0	0	1	0	0	0	2	94.5 ± 5.9	0.9
50.0 µg/ml	680.3 ± 43.7	197.0	108.1	0	0	3	0	1	2	2	3	0	3	1	1	16	94.7 ± 3.8	7.0*
50.0 µg/ml	250.0 ± 11.5	72.4	94.4	1	0	2	0	0	1	0	0	0	0	0	0	4	87.8 ± 5.0	1.9
60.0 µg/ml	215.3 ± 13.6	62.4	77.2	0	0	0	0	0	0	0	0	0	0	0	0	0	91.7 ± 6.0	0.0
60.0 µg/ml	250.0 ± 13.0	72.4	37.5	0	0	0	0	1	0	1	0	3	2	0	0	7	95.7 ± 4.5	3.0
70.0 µg/ml	124.7 ± 2.1	36.1	17.3	0	2	0	0	0	0	0	0	0	0	1	0	3	81.3 ± 5.9	1.5
70.0 µg/ml	119.7 ± 12.7	34.7	17.8	0	0	0	0	1	0	1	2	0	1	0	0	5	79.8 ± 1.3	2.6
85.0 µg/ml	22.0 ± 1.7	6.4	2.9	0	0	0	0	0	0	0	0	0	0	0	0	0	102.2 ± 5.5	0.0
85.0 µg/ml	24.0 ± 3.6	7.0	3.0	0	0	0	0	0	0	0	0	0	0	0	0	0	98.3 ± 4.8	0.0
100.0 µg/ml	6.3 ± 1.5	1.8	0.3	NOT CLONED														
100.0 µg/ml	5.0 ± 2.6	1.4	0.3	NOT CLONED														

CHO/HGPRT MUTAGENESIS ASSAY RESULTS

CLIENT: _____ TEST ARTICLE: Bay k 5552 mikron. ASSAY NO: E-9510

VEHICLE: DMSO TEST DATE: October 1, 1986

Selective Agent: 10 µg/ml thioguanine Cells seeded for analysis: 200/dish for C.E.; $2x10^5$/dish for mutants
Expression Time: 7 days S9 batch: 1-186

TEST CONDITION	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER ± S.D.	SURVIVAL TO TREATMENT: PERCENT NEG.CONTROL	REL.POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES: DISH NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ. IN 10^{-6} UNITS[a]
S9 ACTIVATION:																		
Vehicle Control	287.3 ± 12.7	100.0	100.0	0	C	0	0	0	C	C	1	2	1	0	1	5	101.8 ± 3.5	2.7
	347.3 ± 31.5			1	0	0	1	1	1	2	1	3	2	2	1	15	125.8 ± 9.5	5.0
Positive Control (5 µg/ml 3-HCA)[d]	259.7 ± 1.5	81.8	49.4	C	71	C	94	C	80	69	93	71	72	73	73	696	80.2 ± 7.2	482.1**
	266.3 ± 24.0	83.9	46.4	80	84	87	89	79	82	70	70	73	62	65	74	915	85.8 ± 7.7	444.3**
TEST ARTICLE																		
20.0 µg/ml	332.0 ± 26.5	104.6	90.3	0	0	1	0	1	0	2	2	1	0	1	0	8	119.7 ± 4.0	2.8
20.0 µg/ml	283.0 ± 43.8	89.2	80.6	2	2	0	1	1	1	1	0	1	0	1	3	13	127.3 ± 2.1	4.3
35.0 µg/ml	341.7 ± 13.1	107.7	99.2	0	1	0	0	2	0	1	0	0	1	0	0	5	122.3 ± 6.8	1.7
35.0 µg/ml	266.0 ± 16.6	83.8	132.5	0	0	0	0	0	0	0	1	0	0	1	0	2	129.2 ± 10.2	0.6
50.0 µg/ml	268.7 ± 7.6	84.7	C	NOT CLONED														
50.0 µg/ml	272.0 ± 7.8	85.7	C	NOT CLONED														
60.0 µg/ml	278.7 ± 39.4	87.8	75.4	2	0	0	1	3	2	3	0	1	1	1	C	14	117.3 ± 3.8	5.4
60.0 µg/ml	280.7 ± 4.6	88.5	82.3	1	1	>0	0	1	0	0	0	1	0	1	1	6	141.5 ± 4.8	1.8
70.0 µg/ml	169.3 ± 14.5	53.4	22.9	1	1	0	1	0	1	0	0	0	1	0	0	5	116.8 ± 9.4	1.0
70.0 µg/ml	142.7 ± 10.1	45.0	31.6	0	0	1	0	2	0	0	1	0	0	0	0	4	99.5 ± 4.8	1.7
85.0 µg/ml	46.0 ± 5.6	14.5	3.3	3	1	0	2	1	2	0	2	1	0	1	0	13	109.5 ± 2.8	4.9
85.0 µg/ml	34.0 ± 3.6	10.7	2.3	2	0	1	0	0	0	0	0	0	0	0	0	3	125.7 ± 0.6	1.0
100.0 µg/ml	5.0 ± 0.0	1.6	0.2	NOT CLONED														
100.0 µg/ml	3.3 ± 0.6	1.0	0.2	NOT CLONED														

EXPLANATION OF TABLES, ASSAY NO. E-9510

a = Mutant Frequency = Total mutant clones/ (No. of dishes x 2 x 10^5 x abs. C.E.)

b = BrdU = 5-Bromo-2'-deoxyuridine

c = 3-MCA = 3-Methylcholanthrene

** = Significant increase, p≤0.01

* = Significant increase, p≤0.05

C = Contaminated

T = Lost dishes due to a technical error

= = Two dishes lost due to contamination

x = One dish lost due to contamination

4. Mouse Hepatocyte Primary Culture DNA Repair Assay

Study No.: T2 023 386 (sponsor's number)

Performing Laborator

Sponsor

Date Performed: 8/14/86 to 2/25/87

Quality Assurance: A signed statement of compliance with GLP is included.

Background: Freshly obtained rodent, metabolically active hepatocytes are capable of limited biotransformation activity. Chemically induced damage to nucleic acid of the mammalian cells results in an effort by the enzyme systems to repair the defect(s), resulting in unscheduled DNA synthesis.

Procedure: Freshly prepared hepatocyte primary cell cultures from adult $B6C3F_1$ males were used, according to the method of Williams et al, Cancer Letters 6: 119-306, 1982. Eight concentrations between 1 mg/ml and 5×10^{-4} mg/ml (listed in the table which follows) were tested in triplicate against $5X10^5$ hepatic cells. DNA repair was determined by ^3H-thymidine uptake, to determine the net increase in nuclear grains induced by the test compound. Benzo(a)pyrene served as positive control and DMSO and pyrene served as negative controls. A test compound was considered positive when the mean net nuclear grain count exceeded that of the DMSO control by more than 2 standard deviations.

Results: Cytotoxicity was observed at concentrations of 1 and $5X10^{-1}$ mg/ml. No net increase in nuclear grain count was observed at concentrations between $5X10^{-4}$ and 10^{-1} mg/ml or with pyrene and DMSO (negative controls); the positive control, benzo(a)pyrene, was highly genotoxic, indicating that the hepatocytes were capable of metabolic transformation and DNA repair.

Under the conditions of this test system, Nisoldipine was considered to be not genotoxic at concentrations up to 10^{-1}M

NDI/IN VITRO Systems Facility
Report on: UPC/DNA Repair Assay

Date:	November 21, 1986
Expt #	NII2186
Chemical	Nisoldipine
Molecular wt	
Source/Purity	
Lot #	
Solvent/vehicle	DMSO
Volatility	
Precautions	
Positive control	Benzo(a)pyrene (BaP)
Negative control	Pyrene

Assay method	Autoradiography
Species/strain/sex/age/wt	House/B6C3F$_1$/Male/Adult/28-32g
Organ or tissue/condition	Liver
Cells/primary or line	Primary
Medium used:	Williams Medium E
Duration of chemical exposure	18 hrs.
Chemical dose range	1 to 5 x 10^{-4} mg/ml
Exposure method	In Situ
Label/uCi/ml or Ci/mM, final	^{3}H thymidine, 10uCi/ml
Interval between exp. and label	Simultaneous
Duration of label	18 hrs.

CONTROLS			TEST RESULTS				COMMENTS:
Positive	Conc.	Autoradiog. grains/nucleus (NET)*	Conc. mg/ml	Autoradiog. grains/nucleus (NET)*	Cytotoxicity	Evaluation	* Mean ± standard deviation of triplicate coverslips.
BaP	10^{-5}M	43.7 ± 5.0	1		Toxic		**Mean of duplicate coverslips.
			5 x 10^{-1}		Toxic		
			10^{-1}	-11.6 ± 1.7	Subtoxic	Negative	
			5 x 10^{-2}	-15.2**	Subtoxic	Negative	
			10^{-2}	-16.5 ± 1.8	Nontoxic	Negative	
			5 x 10^{-3}	-15.3 ± 2.4	Nontoxic	Negative	
Negative	conc.	grains/nucleus (NET)	10^{-3}	-16.5 ± 2.1	Nontoxic	Negative	
Pyrene	10^{-5}M	-11.1 ± 4.2	5 x 10^{-4}	-12.8 ± 0.9	Nontoxic	Negative	
DMSO	1%	-13.6 ± 1.3					
Cell Control		-13.9 ± 3.7					

5. Micronucleus Test in Mice

Study No.: T 1010 875

Performing Laboratory:

Date Performed: 11/6/81 to 12/1/81

Quality Assurance: A signed statement of compliance with GLP is not included.

Background: The micronucleus test permits the recognition of a mutagenic action on a somatic tissue, the femoral bone marrow, of an intact animal. Erythrocytes are normally anuclear. The increased occurrence of micronuclei (chromosome fragments) in polychromatic erythrocytes, compared to negative controls, indicates that the test substance brings about chromosome breaks, or has effects on the spindles in the erythroblasts.

Procedure: Bor: NMRI (SPF Han) mice, 8 to 12 weeks of age, weighing 23 to 32 g, 5 of each sex per group, received 0, 100 and 200 mg Bay k 5552/kg/day, p.o., on two consecutive days; positive controls received cyclophosphamide (Endoxan^{R}) orally. All animals were sacrificed 6 hours after the second dose and femoral bone marrow samples were removed for the purpose of determining the frequency of micronuclei observed, by examination of 1000 polychromatic erythrocytes per animal.

Results: Since there was no difference between males and females, the results obtained for both sexes were pooled. No deaths or clinical signs were observed. The significant decrease in normochromatic erythrocytes in the 100 mg/kg group was considered "not biologically significant" (see table on page which follows). It is therefore claimed that Bay k 5552 produced no inhibition of erythropoiesis.

There was no increase in polychromatic erythrocytes with micronuclei. The positive control caused a high level of response.

It was concluded that there was no indication of a mutagenic effect by this test.

Survey of the experimental evaluation he micronucleus test

with BAY k 5552

Experimental group	Number of investigated polychromatic erythrocytes	Number of normochromatic erythrocytes per 1,000 polychromatic erythrocytes	Number of cells with micronuclei	
			per 1,000 normochromatic erythrocytes	per 1,000 polychromatic erythrocytes
I Negative control	10000	682.6	1.0	1.6
II BAY k 5552 2 x 100 mg/kg	10000	465.6*	1.9	1.8
III BAY k 5552 2 x 200 mg/kg	10000	543.1	1.2	1.8
IV Positive control Endoxan 2 x 87 mg/kg	10000	724.0	1.2	31.4

* $P \leq 0.05$ in the distribution-free test of ranks according to NEMENIY related to the negative control (I)

6. Dominant-Lethal Test in Mice

Study No.: T 50 10 239

Performing Laboratory:

Date Performed: 5/11/81 to 11/10/81

Quality Assurance: A signed statement of compliance with GLP is not included.

Background: This test permits detection of mutations in germ cells, particularly stage-specific effects, during meiotic maturation of sperm cells, and is usually performed in mice or rats. A mutagenic test substance causes severe chromosomal damage or lethal germ cell mutations, resulting in embryo or fetal mortality. This is determined by increased occurrence of pre- or post-implantation loss in females (untreated) that were mated to test substance treated males.

Procedure: Bor: NMRI (SPF Han) mice, 8 to 12 weeks of age, body weights at initiation of study of 30-44 g for males and 25-30 g for females, were used. Males in the treated group (50/group) received 200 mg/kg Bay w 5552/kg in aqueous hydroxyethylmethyl-cellulose, single oral dose (Batch No. 576923); control males received vehicle. Starting on the day of drug administation, a new, untreated female was placed in the cage of each treated or control male at the start of each of 12 mating periods, lasting 4 days each (48 days total). About 14 days after the middle of the relevant mating period, the uterus of each female was examined for live and dead embryos, resorption sites and corpora lutea.

Criteria for Dose Selection: Claimed to be based on a preliminary study with female mice, 5/group, which received 200, 500 or 1000 mg/kg, p.o., and in which "200 mg/kg was tolerated with only slight symptoms".

Results: There were no effects of treatment on impregnation rate, fertility, pre- or post-implantation loss. Because there was no indication of early death of embryos at any stage of mating due to compound treatment, there was no indication of a dominant lethal effect (see tables on next 2 pages).

In conclusion, Bay K 5552 was considered to be not mutagenic in this test system.

DOMINANT-LETHAL-TEST

SINGLE TREATMENT OF MALE MICE WEEK DAY F 5002

STUDY .: 15010239

DOSE GROUP 200 MG/KG (P.O.)

PREIMPLANTATION LOSS

MATING PERIOD	CORPORA LUTEA				IMPLANTATIONS*				PREIMPLANTATIONS LOSS			
	TOTAL		PER IMPREGNATED FEMALE		TOTAL		PER IMPREGNATED FEMALE		TOTAL		PER IMPREGNATED FEMALE	
	CONTROL	DOSE GROUP	CONTROL	DOSE GROUP	CONTROL	DOSE GROUP	CONTROL	DOSE GROUP	CONTROL	DOSE GROUP	CONTROL	DOSE GROUP
1	621	504	12.9	13.3	577	453	12.0	11.9	44	51	0.92	1.34
2	472	535	12.8	12.4	442	502	11.9	11.7	30	33	0.81	0.77
3	543	596	12.3	13.8	518	566	11.8	12.3	25	30	0.57	0.65
4	572	425	13.6	12.1	532	380	12.7	10.9	40	45	0.95	1.29
5	419	433	13.1	12.4	408	400	12.8	11.4	11	33	0.34	0.94
6	518	558	12.6	13.0	474	505	11.6	11.7	44	55	1.07	1.28
7	531	544	14.8	13.6	504	503	13.3	12.6	27	41	0.71	1.02
8	591	503	13.7	13.2	536	469	12.5	12.3	55	36	1.28	0.89
9	541	512	12.9	13.1	507	463	12.1	11.9	34	49	0.81	1.26
10	624	514	13.8	13.5	581	462	12.1	12.2	43	52	0.90	1.37
11	568	561	13.5	13.7	543	527	12.9	12.9	25	34	0.60	0.83
12	604	525	14.4	12.5	568	491	13.5	11.7	36	34	0.86	0.81
1 -12	6404	6210	13.2	13.0	6190	5719	12.4	12.0	414	491	0.83	1.03

* Since it can occur that a placenta is found with two embryos on one implantation site, the number of implantations can be smaller than the total of living and dead implants.

DOMINANT - LETHAL - TEST
SINGLE TREATMENT OF MALE MICE WITH BAY K 5552
STUDY : 15010219

DOSE GROUP 200 MG/KG (P.O.)

POSTIMPLANTATION LOSS

MATING PERIOD	LIVING IMPLANTS		PER IMPREGNATED FEMALE		DEAD IMPLANTS		PER IMPREGNATED FEMALE	
	CONTROL GROUP	DOSE GROUP	CONTROL GROUP	DOSE GROUP	CONTROL GROUP	DOSE GROUP	CONTROL GROUP	DOSE GROUP
1	547	427	11.4	11.2	31	26	0.65	0.68
2	414	481	11.2	11.2	31	24	0.84	0.56
3	492	531	11.2	11.5	27	36	0.61	0.78
4	480	346	11.4	9.9	52	38	1.24	1.09
5	338	361	12.1	10.3	22	40	0.69	1.14
6	446	461	10.9	10.7	29	46	0.71	1.07
7	472	479	12.4	12.0	33	25	0.87	0.63
8	497	440	11.6	11.6	42	29	0.98	0.76
9	485	429	11.5	11.0	24	34	0.57	0.87
10	546	434	11.4	11.4	38	31	0.79	0.82
11	501	481	11.9	11.7	43	46	1.02	1.12
12	544	463	13.0	11.0	27	30	0.64	0.71
1 -12	5812	5333	11.6	11.2	399	405	0.80	0.85

7. In Vitro Chinese Hamster Ovary Cell (CHO) Test for Clastogenic Potency

Study No.: 2528 MIC

Performing Labora

Sponsor:

Date Performed: Not indicated. The report is dated 1/19/88

Quality Assurance: A signed statement of compliance with GLP is included.

Procedure: "CHO described by Puck" (Genetics 55:513-518, 1967), were used. After preliminary cytotoxicity tests, concentrations of Bay K 5552 tested without metabolic activation were 10, 20 and 30 uM, and with metabolic activation (provided by S-9 fraction of livers from Aroclor induced rats) they were 500, 600 and 800 uM. The highest dose levels were selected to give about a 50% inhibition of mitotic index, but allowed sufficient numbers of cells at the metaphase stage for analysis of chromosome and chromotid aberrations (breaks, gaps and exchanges); generally, a requirement for dose selection with this test. Positive control compounds were methyl-methane-sulfonate (MMS) without S-9 and cyclophosphamide (CP) with S-9. An 18 hour incubation period, which corresponds to one cell cycle, was selected for the main study. Aberrations were analyzed in 100 cells arrested at the metaphase stage of cell division for each concentration level. The assays with and without S-9 were performed twice.

Results: There were no increases in chromosome or chromatid aberrations at any concentration (see tables which follow). The positive controls (MMS or CP exposed cells) showed statistically significant, large increases in rates of aberration.

In conclusion, Bay K 5552 was considered to be not clastogenic in this test system.

ASSAYS WITHOUT S-9 MIX
Mean of both assays

A. Number of aberrations per 100 analysed metaphases

Chemical	Dose (µM)	Gap	Break	Chromatid Exchanges	Chromo-some Exchanges	Multiple aberra-tions	Total number of aberrations	
							Including gaps	Excluding gaps
-	-	8.5	2.0		1.0		11.5	3.0
DMSO	-	5.5	0.5		1.0		7.0	1.5
BAY K 5552	10	4.5	2.0			0.5	6.5	2.0
	20	2.5	5.5	1.0	2.5		11.5	9.0
	30	5.5	2.0				7.5	2.0
MMS	605	15.5	29.5	36.0	7.0	28.5	88.0	72.5

B. Percentage of cells containing aberrations

Chemical	Dose (µM)	Gap	Break	Chromatid Exchanges	Chromo-some Exchanges	Multiple aberra-tions	Percentage of cells containing aberrations	
							Including gaps	Excluding gaps
-	-	7.0	2.0		1.0		9.5	3.0
DMSO	-	5.5	0.5		1.0		6.5	1.5
BAY K 5552	10	4.5	2.0			0.5	7.0	2.5
	20	2.5	4.0	1.0	1.5		7.0	5.0
	30	4.5	1.5				6.0	1.5
MMS	605	13.0	18.0	26.5	6.5	28.5	73.0	65.0***

Break : chromatic and chromosome
Chromatic exchanges : inter and intrachange
Chromosome exchanges : inter and intrachange
Multiple aberrations : complex rearrangement

Statistical test used : X^2 *** $p \leq 0.001$

ASSAYS WITH S-9 MIX
Mean of both assays

A. Number of aberrations per 100 analysed metaphases

Chemical	Dose (µM)	Gap	Break	Chromatid Exchanges	Chromo-some Exchanges	Multiple aberra-tions	Total number of aberrations	
							Including gaps	Excluding gaps
-	-	8.0	1.0	1.0	1.5	2.0	11.5	3.5
DMSO	-	6.0	1.0	1.0	1.5		9.5	3.5
BAY K 5552	500	3.5	2.0		0.5		6.0	2.5
	600	2.0	0.5		1.0		3.5	1.5
	800	3.5	2.5	1.5	1.0	0.5	8.5	5.0
CPA	130	17.0	29.5	24.5	6.5	3.0	77.5	60.5

B. Percentage of cells containing aberrations

Chemical	Dose (µM)	Gap	Break	Chromatid Exchanges	Chromo-some Exchanges	Multiple aberra-tions	Percentage of cells containing aberrations	
							Including gaps	Excluding gaps
-	-	8.0	1.0	1.0	1.5	2.0	13.0	5.0
DMSO	-	6.0	0.5	1.0	1.5		8.0	2.5
BAY K 5552	500	3.5	2.0		0.5		6.0	2.5
	600	1.5	0.5		1.0		3.0	1.5
	800	3.5	2.0	1.0	1.0	0.5	7.5	4.0
CPA	130	15.0	25.5	20.0	5.5	3.0	53.5	44.0***

Break : chromatid and chromosome
Chromatid exchanges : inter and intrachange
Chromosome exchanges : inter and intrachange
Multiple aberrations : complex rearrangement

Statistical test used : χ^2 *** $p \leq 0.001$

8. Test for Inhibition of Liver Cell Culture Intercellular Communication

Study No.: T2 023 386 (Sponsor's number)

Performing Laboratory:

Sponsor:

Date Performed: 5/18/87 to 5/27/88

Quality Assurance: A signed statement of compliance with GLP is included.

Background Information: In this assay, the transfer of the toxic metabolite, 6-thioguanine (TG), from metabolically competant freshly isolated rat hepatocytes ($HGPRT^+$) to a mutant rat liver cell culture, ARL14-TG^R, which is purine analog resistant ($HGPRT^-$), is measured. When exposed to TG, the presence of the primary hepatocytes results in a reduction of TG^R colonies. The TG kills primary hepatocytes and ARL-TG^R cells to which the metabolite is transferred. If a test chemical inhibits membrane contact or intercellular communication (also referred to as metabolic cooperation), the reduction of TG^R colonies in the flasks containing primary hepatocytes will be diminished, leading to an increased survival and formation of colonies. It is claimed that "many but not all tumor promotors inhibit metabolic cooperation".

Procedure: Nisoldipine was tested at concentrations which ranged between $5X10^{-5}$ mg/ml and $5X10^{-4}$ mg/ml, to determine if it inhibited metabolic cooperation between rat primary hepatocytes (wild type cells) and ARL14-TG^R cells. It is claimed that toxicity had been previously observed at $5X10^{-3}$ mg/ml, but the data were not shown. Positive control used was DDT.

Results: A concentration dependent inhibition of metabolic cooperation was not observed with nisoldipine. DDT caused an inhibition of metabolic cooperation. (See table which follows).

It was concluded that Bay k 5552 did not inhibit metabolic cooperation in this test system.

Chemical	Concentration	Number of Colonies[a] No Hepatocytes	Number of Colonies[a] $1.25x10^6$ Hepatocytes
None	-	233 ± 38 (226,276,185,245)	97 ± 19 (98,122,93,75)
DMSO	0.1%	221 ± 22 (252,222,206,205)	118 ± 11 (103,117,123,129)
DDT	$5x10^{-5}$M	157 ± 10 (145,163,153,167)	151 ± 14 (132,156,151,164)
Nisoldipine	$5x10^{-4}$ mg/ml	187 ± 54 (235,230,160,124)	118 ± 7 (111,118,125)
	10^{-4} mg/ml	181 ± 9 (177,171,192,183)	118 ± 9 (114,112,129)
	$5x10^{-5}$ mg/ml	202 ± 17 (227,197,198,187)	89 ± 28 (114,69,112,60)

[a] Mean ± standard deviation of three to four flasks.

LABELING

Under PRECAUTIONS, the first sentence of the subsection "Carcinogenesis, Mutagenesis and Impairment of Fertility" presently reads:

Nisoldipine was administered orally to mice and rats for 21 and 24 months respectively, and was not shown to be carcinogenic.

We recommend the following revision :

Dietary administration of nisoldipine at doses up to 111 mg/kg/day for 24 months to rats or at doses up to 217 mg/kg/day for 21 months to mice (125 to 250 times the maximum recommended human dose of 40 mg/kg, based on a mg/kg comparison assuming a patient weight of 60 kg) revealed no evidence of a tumorigenic effect.

Under the same subsection "Carcinogenesis, Mutagenesis and Impairment of Fertility", the statement, "_____nisoldipine did not interfere with fertility at a dose more than 30 times the maximum recommended human dose" is meaningless. This should be changed to indicate the dosage in terms of mg/kg, then converted to dosage based on surface area, i.e. in terms of mg/m^2, which may then be compared to human dosage.

Based on the outcome of all the *in vitro* and *in vivo* tests, the statement in labeling, "The results of *in vitro* and *in vivo* mutagenic studies were negative" is reasonable.

Under *Pregnancy Category C*, we agree with the Category C classification, even though animal studies are only suggestive of potential fetal risk. The labeling should be modified to indicate that the fetal toxicity observed in the studies with animals are suggestive, not conclusive. It should specify that the monkey study was confounded by 1) the use of feral monkeys which are particularly susceptible to the stress of handling, and 2) the high rate of abortion and mortality, in both treated and control groups, resulting in only one surviving fetus in the 100 mg/kg group (which presented with anomalies) and only 3 surviving control fetuses. It should be stated that although it cannot be concluded that nisoldipine was teratogenic in the monkey study, such a possibility cannot be ruled out because the teratogenic effects observed (multiple left forelimb, finger and tail abnormalities observed externally, and related forelimb and vertebral abnormalities observed with skeletal examination) had not been previously observed in untreated animals of this species.

The proposed labeling does not specify the form of maternal toxicity that was observed in either the rat or rabbit. The phrase which states that nisoldipine caused a slightly increased malformation rate in rabbits should be omitted from the labeling. Of the two rabbit studies, slightly increased malformation rate was attributed to the stress of diarrhea, which occurred in one

of these two studies. There was no increase in any specific form of malformation.

The statement on fetotoxicity in rats and rabbits should be revised to read as follows:

Fetotoxicity in rats and rabbits was usually observed only at doses which caused a decrease in body weight gain of dams compared to control. In rats, an increase in post-implantation loss was observed at 100 mg/kg, and a decrease in fetal weight was observed at 30 and 100 mg/kg. In rabbits, decreases in fetal and placental weights were observed at 30 mg/kg.

For the rat and rabbit Segment II studies, the dosages cited as "*30 (or 100) times the maximum human dose*", should be expressed in terms of both mg/kg and mg/m^2.

Under a new section, *Labor and Delivery*, the labeling should state that the drug caused a slight prolongation of pregnancy in rats. The prolongation of pregnancy is presently noted under the *Pregnancy Category* section but should be moved here.

Under *Nursing Mothers*, it should be pointed out that "nisoldipine was found in the milk of lactating rats at concentrations which were lower than levels found in the plasma".

OVERALL SUMMARY AND EVALUATION

Nisoldipine coat core (Nis CC) tablet, proposed for the treatment of hypertension (alone or in combination with other antihypertensive agents) on a once-a-day dosage regimen, is an extended release formulation of the dihydropyridine calcium channel blocker nisoldipine. Nis CC tablet has an external coat of slow release formulation and an internal core of fast release formulation of nisoldipine.

Calcium channel blockers have recently emerged as a promising new class of antihypertensive agents. By blocking the channels which mediate calcium entry into smooth muscle cells, these agents decrease intracellular calcium levels, thereby inhibiting vascular smooth muscle contractions, resulting in a decrease in peripheral vascular resistance and reduction in blood pressure. Because calcium channel blockers inhibit coronary vasoconstriction and reduce vascular resistance and increase coronary blood flow, thereby protecting the heart against ischemia and reperfusion damage, they are also effective in the treatment

Nisoldipine was developed with the aim of improving the pharmacologic properties of nifedipine. Despite its chemical similarity to nifedipine, nisoldipine is a more potent dilator of coronary as well as peripheral blood vessels. Nisoldipine is 3 to 10 times more potent than nifedipine in increasing coronary blood flow and coronary venous oxygen saturation in dogs. In isolated vascular preparations, nisoldipine inhibits calcium- and potassium-induced contractions at concentrations 4-10 times lower than that of nifedipine. A negative inotropic effect, an adverse effect usually observed with other calcium channel blockers, has not been shown with nisoldipine in its therapeutic dose range.

An oral dose of 10-40 mg once daily is proposed for the treatment of hypertension and a dose of once daily is proposed for the treatment

This new drug application is supported by fairly extensive preclinical studies.

Nisoldipine was shown to produce dose-dependent reductions in blood pressure and total peripheral resistance in rats, dogs, cats and pigs. In SH rats, single oral doses of 0.315, 1.0 and 3.15 mg nisoldipine/kg produced 3, 12 and 18% reductions in blood pressure, respectively. The maximum effect was seen at 1 hr after drug administration and the blood pressure returned to the pretreatment level at 6 hr postdose. The antihypertensive effect of nisoldipine was much more pronounced in renal hypertensive rats than in SH rats; a dose of 3.15 mg/kg po produced a 39% reduction in blood pressure in renal hypertensive rats compared to an 18% reduction in SH rats. Long term treatment with nisoldipine (50-100 mg/kg/day in the diet for 60 weeks)

prevented the development of hypertension in SH rats (mean systolic blood pressure of 141 mm Hg in the drug treated group vs 214 mm Hg in the control group at the termination of the study) and other rat models. Chronic treatment with the test compound also significantly reduced the atrial natriuretic peptide and aldosterone concentrations in plasma and attenuated cardiac hypertrophy in SH rats.

The (+) enantiomer was found to be only slightly more potent than the racemic compound in its antihypertensive activity in SH rats, but it was about 20 times more potent than the (-) enantiomer.

In terms of antihypertensive effect, nisoldipine was about equipotent to nifedipine, nicardipine and hydralazine (ED20 = 4 mg/kg po) in SH rats; however, it was less potent than the other drugs in other hypertensive rat models.

Single oral doses of nisoldipine (0.03-1.0 mg/kg) produced dose-dependent decreases in mean arterial blood pressure in conscious renal hypertensive dogs. At 0.3 mg/kg, a 24% reduction in blood pressure was produced within 2 hr after drug administration and the hypotensive action lasted for about 12 hours. A tachycardia, lasting for about 3 hr, also occurred at the above dose level. Nifedipine produced about the same degree of hypotension (lasting about 6 hr) as that produced by an oral dose of 0.3 mg nisoldipine/kg at about a 10 fold higher dose level (ED20 - 0.14 mg/kg for nisoldipine vs 1.68 mg/kg for nifedipine). The antihypertensive activity of orally administered nisoldipine in renal hypertensive dogs significantly correlated with plasma concentrations of the drug.

Low doses of nisoldipine increased coronary blood flow and protected the heart against ischemia and reperfusion damage in various experimental models. In isolated rat hearts subjected to ischemia and reperfusion, nisoldipine (3 nM) doubled coronary blood flow. At 1 nM, nisoldipine increased coronary blood flow 31% and improved the recovery of contractile function and tissue ATP levels. Radioactive microsphere studies in conscious rats showed that oral administration of nisoldipine (0.3 mg/kg) produced a marked increase in coronary blood flow as well as a decrease in coronary vascular resistance. In anesthetized dogs, nisoldipine (5 µg/kg iv) increased coronary sinus blood flow by 111% and coronary sinus oxygen content by 50%. In dogs with acute myocardial infarction, nisoldipine (5 µg/kg iv 15 min, 2 and 4 hr after coronary artery occlusion) reduced myocardial infarct size by 31%. In pigs with gradual coronary occlusion, the test drug (30 µg/kg po, every 6 hr for 1 month) significantly reduced infarct size and increased endocardial and transmural blood flow by enhancing endocardial collateral circulation.

Nisoldipine did not depress cardiac function at doses needed to increase coronary blood flow or lower blood pressure in hypertensive animals. The drug reduced or abolished ventricular fibrillation and reperfusion arrhythmias, improved cardiac

output, reduced mortality, and improved ventricular function in several animal models. The beneficial effects are attributed to increases in perfusion of the ischemic zone, and a reduction in afterload through a decrease in peripheral resistance.

In vitro studies have shown nisoldipine to be twice as potent as nifedipine in inhibiting contractions of isolated pig coronary artery; however, it was only 1/3 as potent as nifedipine in inhibiting femoral artery contractions, indicating its selectivity for coronary vasculature.

Nisoldipine produced diuretic and natriuretic effects in rats, the effects being more pronounced in hypertensive than in normotensive rats. The natriuretic effect was attributed to the suppression of distal tubular sodium reabsorption.

Nisoldipine binds with very high affinity (Kd=<0.1 nM) to L-type calcium channels of various cell types. Compared to nifedipine, nisoldipine is found to have 2 to 30 times higher binding affinity depending on the cell type and experimental conditions. Several studies have shown that there is good agreement among binding affinity and the IC50 values both for inhibiting ^{45}Ca influx and aortic contraction. Moreover, the degree of nisoldipine inhibition of calcium channel current was found to increase with membrane depolarization.

Isolated membrane studies showed that the (+) isomer had a binding affinity 100 times higher than that of the (-) isomer.

General pharmacological studies in rodents showed analgesic and anticonvulsant effects, prolongation of anesthesia, attenuation of aggressive behavior, elevation of blood glucose and reduction of intestinal motility at dose levels 15 to 150 times the maximum recommended human dose on a body weight basis. (It is noted that the above effects were seen at dose levels 33 to 333 times the dose that produced the desired pharmacological effects in rats.)

Bay R 9425, an active metabolite with a dihydropyridine structure, exhibited pharmacological effects similar to the parent compound but was 1/3 to 1/10 less potent. The other metabolites had no significant effects.

Combined administration with propranolol prolonged the anti-hypertensive action of nisoldipine and reduced the reflex tachycardia in dogs.

Based on the ratio of percent of administered radioactivity excreted in urine for the i.v./oral doses, nisoldipine has been shown to be virtually completely absorbed in rats, dogs and humans, following oral doses of 5 mg/kg in the animals or 10 to 40 mg Nisoldipine CC tablets, in man. Despite the high rates of oral absorption, bio-availabilities (F) of parent compound were low, averaging 11.7% or less in the 3 species, which was attributed to an extensive first pass effect (shown in the dog to

be due to metabolism in both the gut and liver). In man, the F value for immediate release compound was 8.4%, but for the core-coated tablet, it was 5.5%. When administered by the oral route, $CEQ_{max,norm}$'s (C_{max} normalized to 1 mg/kg dose, based on radioactivity equivalence) for parent compound were similar in rat, dog, monkey and man, whereas $CEQ_{max,norm}$'s for total radioactivity (which includes parent compound plus all metabolites) were 4.7 to 7.5-times higher in man than in the 3 animal species. AUC_{norm}'s for parent compound were also similar in all 4 species whereas AUC_{norm}'s for total radioactivity (parent compound plus metabolites) were 6 to 10 times higher in man than in the three animal species. The plasma half-lives for parent compound following oral administration were only 0.7, 2.3, 3.8 and 4.0 hours, respectively, for the 4 species, whereas the plasma half lives for total radioactivity (parent compound plus metabolites) were 14.9, 54.4, 41.8 and 80.3 hours for the rat, dog, monkey, and man, respectively.

At steady state in humans, dose proportionality was seen for both immediate release and coat core tablets, based either on AUC or C_{max}. Correspondingly, decreases in systolic and diastolic blood pressures showed a general dose related decline from baseline.

Tissue distribution of total radioactivity following a single oral dose of 5 mg/kg in rats, determined between 0.5 and 72 hours post-dosing, reached maximum values at 1 hour, with liver, fat, kidney and adrenal glands generally containing the highest levels, brain and skeletal muscle the lowest. There was no indication of a change in organ pattern distribution with time. In dogs, tissue distribution (measured only 72 hours) after oral dosing was similar to that observed in rats. Placental transfer in pregnant rats, and secretion into milk of lactating rats, were observed. Whole-body autoradiography in rats indicated rapid tissue distribution and penetration of the blood-brain barrier within 5 minutes after i.v. dosing.

Protein binding, determined by equilibrium dialysis, was greater than 97.5% in rats and dogs and around 99.4% in humans. Nisoldipine was rapidly and extensively metabolized in the rat, dog, monkey and man; only a small percentage of unchanged labelled test substance could be found in the circulation within 30 or 60 minutes after an oral dose. Partial enterohepatic recirculation of metabolites was detected in rats. Hepatic enzyme levels of cytochrome P_{450}, aminopyrine N-demethylase and aniline hydroxylase were decreased following oral administration for 2 weeks, but these decreases were found to be reversible within one week following treatment termination.

At least 18 biotransformation products have been identified in urine and serum of rat, dog, monkey or man, 6 of which account for 80% of radioactivity in urine of all 4 species. After oral administration, there were no important differences in plasma or urinary metabolic profile between the 4 species. The investigators describe the biotransformation steps in all 4

species as follows:

-hydroxylation of the isobutyl ester
-dehydrogenation to the pyridine derivative
-cleavage of the ester to form the carboxylic acid
-reduction of the nitro group to the amino group
-glucuronidation (phase II enzymatic reaction)

In acute toxicity studies, oral LD50 values of nisoldipine were greater than 10,000 mg/kg for mice and rats and greater than 5000 mg/kg for rabbits and dogs. Acute iv LD50 values for the above four species ranged from 1.9 to 2.5 mg/kg. Propranolol pretreatment had no effect on the iv LD50 in the rat.

In the rat three month oral (gavage) toxicity study (0, 10, 30 and 100 mg nisoldipine/kg/day), elevation of plasma urea levels was seen in the high dose female group. Although absolute and/or relative weights of heart and liver (mid and high dose rats of both sexes) and thymus (mid and high dose males) were significantly higher than control, no significant histopathological findings were observed. The "no effect level" for liver and heart weight effects in the above study was found to be 10 mg/kg/day.

In reply to a request for justification of dose selection for the three dog studies, we were informed by the sponsor that doses were selected for the initial 4 week study on the basis of previous experience with the pharmacologically similar dihydropyridines, nifedipine and nitrendipine. The rationale for selection of the highest dose in the 52-week study was the avoidance of papillary muscle scars that were observed with the highest doses employed in the 4 and 13 week experiments. Such lesions were considered life threatening by the sponsor. Myocardial scars in one or both left ventricular papillary muscles, observed at 10 mg/kg in the 4 week study, and 6.25 mg/kg in the 13-week study, are generally attributed to hypoxic damage associated with vasodilator induced heart rate increase, a known damage mechanism in dogs. In the 4-week study, the associated ST drops and tachycardia were most pronounced in the dog with the most severe lesions. The pharmacologic effects (decreases in blood pressure and increases in heart rate) were usually reversible within 24 hours after treatment. No other treatment related effects were noted in the 4 and 13 week studies. The doses selected for the 52-week study were 0.3, 1.0 and 3 mg/kg, which caused dose-related decreases in blood pressure and increases in heart rate. Also noted were slight ST segment depressions, T-wave inversions and QT segment depressions, but no gross or histologically observable heart muscle damage or other indications of toxicity. In humans, nisoldipine is known to cause ST segment depression along with a decrease in peripheral vascular resistance, and side effects include palpitation and tachycardia.

The two year oral (dietary) carcinogen bioassay in the rat at doses of 0, 50, 300 and 1800 ppm nisoldipine (2.15, 13.13 and 82.40 mg/kg/day, respectively, in males and 2.78, 18.04 and 110.68 mg/kg/day, respectively, in females) did not show any evidence of a treatment-related increased incidence of tumors according to sponsor's analysis. However, a statistically significant (at 0.05 level) linear trend was reported by FDA statisticians for brain granular cell tumor ($p=0.0411$) in male rats; occurrence of the above tumor was limited to 3 (of 50) high dose males. Pairwise comparison showed no significant difference between high dose and control groups ($p=0.1594$). According to the sponsor, the incidence rate for the brain granular cell tumor is within the spontaneous range for male rats. It is noted that the above tumor incidence was observed at a dose level which is about 125 times the maximum recommended human dose of 40 mg/day, based on a mg/kg comparison assuming a patient weight of 60 kg. Hypertrophy of the zona glomerulosa cells of the adrenal cortex (high dose males and females) and increased incidence of progressive nephropathy (high dose females) were the major nonneoplastic findings of the above study. Although mean body weights for the high dose group (both sexes), for the duration of the study, were significantly lower than control values except on few occasions, the body weight decrement in high dose males was less than 10% throughout the study. In high dose females, beginning week 45 of treatment, the weight decrement was 10% or more, compared to control, till the end of the study. (The terminal weight decrements for high dose males and females were 5.6% and 22%, respectively.) There were no statistically significant differences in the survival of drug treated and control rats (male or female) in the above study.

In the mouse, dietary administration of nisoldipine at 0, 100, 300 and 900 ppm (19.37, 58.06 and 162.93 mg/kg/day, respectively, in males and 24.99, 74.36 and 217.28 mg/kg/day, respectively, in females) for 21 months showed no evidence of a drug related carcinogenic effect except for significant positive linear trends (at 0.05 level) for hepatocellular carcinoma and hepatocellular tumors (all) in male mice (sponsor's analysis). Analysis of the tumor data by FDA statisticians failed to confirm these trends. However, FDA analysis showed a significant positive linear trend ($p=0.0072$) for stomach papilloma in male mice [occurrence limited to 2 (of 50) high dose males]. Pairwise comparison showed the difference between high dose and control groups to also be significant ($p=0.0435$). The incidence rate for the stomach papilloma is reported to be within the historical control range for this tumor in NMRI mice. It is noted that the above tumor incidence occurred at dose level that is about 250 times the maximum recommended human dose on a body weight basis. Relevant non-neoplastic findings observed in this study included increased incidences of gastric mucosal hyperplasia (treated males and females - all groups) and pituitary hyperplasia (treated females - all groups). Chronic drug treatment had no significant effect on body weight in this study. The mortality rate of high dose males was significantly higher ($p<0.001$) than control (80%

in the high dose male group vs 28% in control). Though the mortality rate in high dose females (64% vs 56% in control) was also higher than control, the difference was not statistically significant.

Since the incidences of brain granular cell tumor in male rats and stomach papilloma in male mice are within the historical control range, and because nisoldipine has been shown not to be genotoxic, the drug-tumor association is considered to be not biologically relevant.

Only two of the 7 reproductive toxicology studies submitted were performed in accordance with GLPs; the Sprague-Dawley rat and the cynomolgus monkey teratology studies. All of the studies appear to be scientifically valid, but in the non-GLP studies, even with the amended tables, it was frequently not possible to determine the times of death.

In spite of the fact that the test substance had very low acute toxicity (LD_{50}> 10 g/kg) in Wistar rats, and could be administered chronically to Wistar rats in the carcinogenicity study at a dose as high as 220 mg/kg, doses given in the modified Segment I and III studies were only 3, 10 and 30 mg/kg. The only justification given for selection of these low doses was the undocumented statement, "The doses were chosen on the basis of toxicological results of other studies". Both studies included C-section of a proportion of the dams on day 20 of gestation.

In the Segment I study, nisoldipine produced no observable effects in the males (treatment started 70 days prior to mating) or females (treatment started 21 days prior to mating) in terms of clinical signs, body weight gain, mating, fertility and pregnancy rate. There were small but significant and dose related increases in mean fetal weight at 10 and 30 mg/kg but weights were said to have remained within normal limits for this strain. There were no differences from control in fetuses with external, soft tissue or skeletal malformations. In dams allowed to give birth, pregnancy duration was slightly, but significantly increased at all 3 dose levels.

In the Segment III study, nisoldipine administration was associated with a slight but statistically significant decrease in body weights (compared to control) of the high dose (30 mg/kg) dams of both the C-sectioned and rearing groups, after only the 4th day of treatment (day 20 of gestation). The only indication of toxicity to the offspring of the C-sectioned dams was a statistically significant decrease in mean fetal weight at the high dose. In the dams allowed to give birth, there was an increase in number of stillborn pups, and an apparent dose related increase in mortality of the newborn pups during the first week postpartum in the mid and high dose groups, but no statistical analysis was performed. The birth weight and the body weight gain of pups during lactation was reduced in the 30 mg/kg group compared to control.

In the supplemental Segment III study, where only the 30 mg/kg dose was tested, reduced maternal body weight gain was evident by day 20 of gestation (after only 4 days of treatment), with weights remaining below control weights through the first week of lactation. Gestation length was significantly prolonged, a finding that was interpreted by the sponsor as a "pharmacologically-induced tocolytic effect" (inhibition of uterine contractions), and there was a large increase in number of pup deaths at birth and during the first 2 weeks of lactation. Also, a decrease in pup weight was noted at birth and during the first week of lactation. These findings of maternal and fetal toxicity at 30 mg/kg confirm the observations noted for the 30 mg/kg group of the main Segment III study. Prolongation of gestation, which was seen in the supplemental Segment III study, had not been observed in the primary Segment III study but was observed in the Segment I study as well as in a Segment II study in another strain of rat (see table which follows and table on page 156).

DOSAGE THRESHOLDS FOR FERTILITY-REPRODUCTION STUDY AND PERINATAL-POSTNATAL STUDIES IN RATS

Report No.	T0002152	T1002153	T3008898
Study Type	Fertil-Reproduction	Peri- post-natal	Peri- post-natal
Strain	Wistar	Wistar	Wistar
Dose (mg/kg)	0, 3, 10, 30	0, 3, 10, 30	0, 30
Vehicle	glycerol-water-PEG	Glycerol-water-Lutrol	Glycerol-water-Lutrol
Days Administered	From 10 wks (males) or 3 wks (females) prior to mating to GD 7	GD 16 to PPD 21	GD 16 to PPD 14
C-Section Day (GD)	20	20	Not done
No. Fem/gp. C-Sectioned	23-27	25	N/A
No. Fem/gp. Littered	22-27	20-23	25
Maternal toxicity			
1. Decr. weight gain	>30 mg/kg	>10 $\leq$30 mg/kg#	$\leq$30 mg/kg#
2. Decr. food intake	>30	>30	>30
3. Prolonged Gestation	>30	>30	$\leq$30
Fetal (C-sect) toxicity			
1. Decr. survival	>30 mg/kg	>30 mg/kg	N/A
2. Decr. fetal weight	>30	>30	N/A
3. Decr. placental wt	>30	>30	N/A
4. Incr. malformation	>30	>30	N/A
Neonatal Toxicity			
1. Incr. stillborn	>30 mg/kg	>3 $\leq$10 mg/kg	$\leq$30 mg/kg
2. Decr. survival	>30	>3 $\leq$10 (1st week)	$\leq$30 (1st wk)
3. Decr. birth weight	>30	>10$\leq$30	$\leq$30
4. Decr. wt gain	>30	>10$\leq$30	$\leq$30

Limited to GD 16-20; effect was slight and barely significant in the first study, highly significant in the second.

Two Segment II studies were performed with rats, the first one with Long-Evans rats which received the drug in a polyethylene glycol-glycerol-water vehicle, and the second one with Sprague-Dawley rats which received the drug in an aqueous-Tylose vehicle. In both tests, the doses administered were 10, 30 and 100 mg/kg. In the second test with Sprague-Dawley rats (but not in the first one with Long-Evans rats), half the pregnant dams on test were allowed to litter and raise their young until 25 days postpartum; then selected males and females in each litter were monitored to sexual maturity. In both studies, a dose related decrease in body weight gain was noted for dams at the 2 highest doses. In the Long-Evans rat, there was no effect of nisoldipine on fetal weight, but in the Sprague-Dawley study, a dose related decrease was noted (significant at the 2 highest doses). There was an increase in postimplantation loss in the high dose group of the Sprague-Dawley study. There were indications of fetal immaturity in the 100 mg/kg group (more clearly noted with the Sprague-Dawley rat), as indicated by increased incidence of incomplete ossification of various bones and of stunted fetuses. Also in the Sprague-Dawley study (not shown in the following table), an increased number of fetuses with slightly increased (relative to normal control) dilatation of lateral ventricles and/or space between the body walls and organs occurred mainly in 2 litters of the 100 mg/kg group and was associated with low body weights of the fetuses in these two litters. Prolongation of gestation length was noted for the high dose group. Curiously, the birth weights of pups from the mid and high dose dams were slightly higher than control (the same observation was made for the 10 and 30 mg/kg groups of the Segment I study, but the opposite observation, i.e. a decrease in pup weight at birth, was made in the Segment III main and supplemental studies), and there was no increase in stillbirths and neonatal deaths, as had been observed in the Segment III studies. It is also pertinent to point out that in contrast to the Segment III studies where treatment was continued through the time of expected delivery, in the Segment II study with Sprague-Dawley rats, treatment was limited to days 7 to 17 of gestation. Thus, prolongation of pregnancy occurred a few days after treatment with nisoldipine had been discontinued.

DOSAGE THRESHOLDS FOR ADVERSE EFFECTS IN RAT DEVELOPMENTAL STUDIES

Report No.	7596	87 BAGO520/938
Strain	Long-Evans	Sprague-Dawley
Dose (mg/kg)	0, 10, 30, 100	0, 10, 30, 100
Vehicle	Glycerol-water-PEG	Aqueous tylose
Days Administered (GD)	6-15	7-17
Day of C-Section (GD)	20	20
No./Gp. C-Sectioned	20-21	21
No./Cp. Littered	N/A	11
Maternal Toxicity 1. Mortality 2. Decr. weight gain 3. Decr. food intake 4. Prolonged gestat	 >100 mg/kg >10 ≤30 Not measured N/A	 >100 mg/kg >10 ≤30 >10 ≤30 >30 ≤100
Fetal Toxicity 1. Incr. post-implant loss 2. Decr. fetal wt 3. Decr. placental wt	 >100 mg/kg >100 >30 ≤100	 >30 ≤100 mg/kg >10 ≤30 >100
Neonatal & postnatal toxicity (to time of breeding)	N/A	>100 mg/kg

In both Segment II rabbit studies, there was a compound related decrease in body weight gain of the pregnant does. In the first study, where the doses administered were 3, 10 and 30 mg/kg and the vehicle used was glycerol-water-PEG, a decrease in mean number of live male fetuses per doe ($P< 0.05$) resulted in a decrease in ratio of live male:female fetuses in the 30 mg/kg group. There was also a small increase in incidence of fetuses with anomalies (e.g. forelimb abnormalities and cleft palate) in the 30 mg/kg (high dose) group, but this was not associated with an increased incidence of any specific malformation. In the second study, where only the 30 mg/kg dose was tested and the vehicle used was aqueous tylose, mean fetal and placental weights were decreased and "underdeveloped forms" (defined as fetuses weighing $\leq$ 2.5 g), were increased vs control. The investigators attributed the increase in malformations in the first rabbit study to the increase in maternal stress. They suggested that the combination of the glycerol-water-PEG vehicle and the drug resulted in an increased incidence of diarrhea in the high dose dams, and that the diarrhea caused an increased incidence of does which aborted their entire litters (4 at high dose had diarrhea; 2 of these aborted and 1 died). However, diarrhea and abortion, also observed in 1 low dose dam and 1 mid dose dam, were considered "normal" for this strain of rabbit.

In both the rat and rabbit studies, increased incidence of malformations, increased fetal lethalities and/or depressed fetal weights were observed only with maternally toxic doses.

DOSAGE THRESHOLDS FOR ADVERSE EFFECTS IN RABBIT DEVELOPMENTAL STUDIES

Report No.	7595	7595 (Suppl)
Strain	Himalayan	Himalayan
Doses (mg/kg)	0, 3, 10, 30	0, 30
Vehicle	Glycerol-water-PEG	Aqueous tylose
Days Administered (GD)	6-18	6-18
Day of C-section (GD)	29	29
No./Group C-Sectioned	10-13	11
Maternal Toxicity		
1. Decr. body wt gain	>10 ≤30 mg/kg	≤30 mg/kg
2. Lethality	>10 ≤30*	>30
3. Diarrhea	>10 ≤30*	>30
4. Spontan. abortion	>10 ≤30*	>30
Fetal Toxicity		
1. Decr. survival	>10 ≤30 mg/kg#	>30 mg/kg
2. Decr. fetal wt.	>30	>10 ≤30
3. Decr. placental wt	>30	>10 ≤30
4. Incr. malformation	>10 ≤30**	>30

* In the first study with glycerol-water-polyethylene glycol vehicle, there were 4 high dose does with diarrhea, 2 of which aborted and 1 of which died. There was also 1 at low dose with diarrhea which aborted and one at mid dose which aborted but did not have diarrhea; none of the controls aborted. In the second test with aqueous tylose vehicle, there were no deaths and none of the treated animals aborted or had diarrhea. The high dose deaths and abortions in the first study were attributed by the sponsor to diarrhea, caused by an interaction of vehicle and compound, although the 2 vehicles were not directly compared in the same experiment.

The mean number of male live fetuses was significantly reduced in the 30 mg/kg group, resulting in a reduction in ratio of males:females in the first study. The sponsor considered the reduction in ratio of males:females a spontaneous occurrence and the overall decrease in number of live fetuses was attributed to the stress of diarrhea.

** Malformations in fetuses of the high dose group occurred in 3 dams. There was an increase in total number of fetuses with malformations and total number of dams that had fetuses with malformations (no statistical analysis), but no increase in any specific malformation. The investigators attribute the increase in malformation rate to increased stress due to diarrhea.

In the study with cynomolgus monkeys, clinical signs, which included heavy bleeding (followed by abortion), reduced food intake, diarrhea and vomiting, occurred during the treatment period in all the groups, including vehicle control. Although the symptoms were generally more frequent and of longer duration in the 100 mg/kg treated monkeys, they were still considered by the investigators to be due to treatment with the vehicle (polyethylene glycol-glycerin-water). In the opinion of this reviewer, the stress of handling these feral monkeys (rather than, or in addition to, the vehicle), contributed to the observed symptoms. Of the 10 or 11 pregnant monkeys on test in each group, 6 to 8 of them aborted in every group, and deaths occurred in 3 control, 1 mid-dose (30 mg/kg) and 5 high dose (100 mg/kg) monkeys. The single death at the mid dose, and 4 of the 5 deaths at the high dose, were associated with a volvulus (twisting or knotting of the intestine), which was not seen in control animals which died. Malformations were observed in the only surviving fetus of the 100 mg/kg group (left forelimb and tail anomalies, observed externally and by skeletal examination). In spite of the very few surviving fetuses that could be examined for malformations (3 controls, 2 low dose and 1 high dose) it is claimed, "The skeletal abnormalities in this one fetus are considered to be related to treatment with BAY k 5552 because similar defects were never observed in control fetuses of previous studies of the same type in Macaca fascicularis". No historical control data or details on how many previous studies or the number of control monkeys that were examined for teratogenicity are provided to support this statement. It should be pointed out that malformations occurred only at a dose level that was highly maternally toxic.

Bay k 5552 (nisoldipine) was tested for mutagenicity by five *in vitro* test systems (Salmonella/microsome, CHO HGPRT forward mutation assay, mouse hepatocyte primary culture DNA repair, a CHO test for clastogenicity, and by a test for inhibition of intercellular communication between two types of liver cells, 1) a primary rat culture ("wild type cells") and 2) the ARL14-TG^R cell line. It was further tested for mutagenicity in two *in vivo* systems (mouse micronucleus and mouse dominant-lethal). All the tests for mutagenicity appeared to be adequately performed, and positive controls in all of them confirmed the acceptability of the studies. Based on the outcome of these *in vitro* and *in vivo* studies, nisoldipine was not found to be mutagenic.

In conclusion, the preclinical studies summarized above have demonstrated the efficacy of the test drug as an antihypertensive and Adverse effects are seen only at high multiples of the maximum recommended human dose.

RECOMMENDATION

The NDA is approvable with suggested changes in labeling.

Sidney Stolzenberg, Ph.D

Xavier Joseph, DVM
August 29, 1994

ATTACHMENTS (3)

cc:
Orig.NDA
HFD-502
HFD-345/GJames
HFD-110
HFD-110/CSO
HFD-110/SStolzenberg
HFD-110/XJoseph
CAR 8/31/94

ATTACHMENT

CDER Statistical Review of

Carcinogenicity Studies

STATISTICAL REVIEWS

Statistical Review and Evaluation

IND #: Date: DEC 3 1992

Applicant: Miles Inc.

Name of Drug: Nisoldipine (Bay K 5552)

Documents Reviewed:

1. IND Submission Volume 19.1 & 19.2, Information Amendment Serial No. 031, "Carcinogenicity study on NMRI mice (feeding study over 21 months)", Pharma. Report no. 16329 (E), Date: Dec. 1987, Date of Document, August 11, 1988.

2. IND Submission Volume 9.1, Additional Bay K 5552 (Nisoldipine) Reports, "Chronic Toxicological investigations on rats (Feeding Study over 24 months)", Pharma Report No.: 13016(E), Date: May 6, 1985, Date of Document, Dec. 1985.

3. IND Special Submission, Data diskettes and print-outs for two animal tumorigenicity studies, Date of Document, April 1, 1992.

I. Background

Two animal carcinogenicity studies (one in mice and one in rats) were included in this IND submission. The purpose of this study was to evaluate the tumorigenic potential of Nisoldipine (Bay K 5552), when administered in the diet to mice and rats for two years, respectively. Dr. Xavier Joseph, HFD-110, who is the reviewing pharmacologist of this IND has requested the Division of Biometrics to perform the statistical review and evaluation of these two studies. The data submitted on computer floppy diskettes were used in the reviewer's independent analyses.

II. The Mouse Study

II. a. Design

In this study, four groups of 50 male and 50 female SPF-bred NMRI mice (strain Bor:NMRI(SPF HAN), breeder Winkelmann, Borchen) were admixed with 0, 100, 300, or 900 ppm Nisoldipine (Bay K 5552) (converted to 0, 19.37, 58.06, 162.93 mg/kg/day for males or 0, 24.99, 74.36, 217.28 mg/kg/day for females, respectively) in their diet for up to 21 months. In each dose and sex group, 20 additional mice were treated for up to 12 months and then sacrificed for interim investigations. The dosages were selected on the basis of the results of a previous subacute study with the administration of the substance in the food. Food consumption was monitored on a weekly basis up to 23rd week and every two weeks thereafter. Autopsies were done on all animals which died during the course of the study or that

were killed in extremis, and also on those that were sacrificed at 12 months and at the termination of the study. At the end of the study, some tissues from 0, 300, and 900 ppm groups were examined histopathologically. In addition, non-blastomatous lesions in the alimentary canal, the pituitary and the liver of animals in the 900 ppm group were recorded. Of the animals in the 100 ppm group (low dose group), the stomach, pituitary, and liver were examined histopathologically.

II. b. Sponsor's Analyses

The sponsor indicated that "no neoplasms were entered for the female animals in the 100 ppm group; therefore, the data for this group were not recorded." The following four animals (animal no. 333 and 343 in female control group, animal no. 550 in female high dose group, and animal no. 131 in male low dose group) were not included in the analysis due to autolysis. The pituitary was not examined in all animals. The stomach and liver were examined only in male animals from the 100 ppm group, hence, the low dose group is taken into consideration only in the evaluation of the mortality data and of tumors with the relevant location.

Mortality rates of female and male mice at 6, 12, 18, and 21 months reported by the sponsor are presented in Table 1. The survival curves for female and male mice are graphed in Figures 1-2, respectively. For male animals, the survival curves are significantly different at 0.05 level, regardless of whether all the groups are taken into consideration or the 100 ppm group is excluded. The significant difference in survival curve is due to the high mortality in the high dose group. For female animals, the survival curves of control, medium, and high dose groups are not significantly different ($p = 0.8192$).

The above survival data were analyzed by applying the BMDP statistical software package which uses the generalized Wilcoxon test in the life table and survival functions programs.

The sponsor indicated that the methods described in Peto et al. ("Guidelines for Simple, Sensitive Significance Tests for Carcinogenic Effects in Long-Term Animal Experiments", In Long-Term and Short-Term Screening Assays for Carcinogens: A Critical Appraisal, International Agency for Research on Cancer Monographs, Annex to Supplement 2, World Health Organization, 311-426, 1980) were used to test the linear trend in the tumor data. Since the data do not have information relating the tumors with the death of an animal, hence, malignant tumors are in general evaluated with the help of the death-rate method and benign tumors with the help of the prevalence method. The ordinal dose levels 0, 1, 2, 3 are used as the weighing factors for the control, low, medium, and high dose groups, respectively.

Table 2 summarizes the tumor bearing animals according to location

and tumor type. Table 3 lists the number of male and female mice with benign and/or malignant tumors for control, medium, and high dose groups. Table 4 lists the statistical analyses of selected tumor data. The results of the above analyses showed that there were significant (at 0.05 level) positive linear trends in hepatocellular carcinoma (death rate: $p = 0.0015$, prevalence: $p = 0.0267$), hepatocellular tumors (death rate: $p = 0.0004$, prevalence: $p = 0.0334$), and malignant tumors (death rate: $p = 0.0035$) in male mice.

Based on the above results, the sponsor concluded that "hepatocellular tumors were found in male mice from all four groups. If the incidences determined in these groups are compared with the historical control values which stem from six long-term studies with animals of this strain between 1980 and 1984 (see Table 5), the occurrence of 7 hepatocellular tumors in male mice from the 300 ppm group (medium dose) lies within the upper range of the norm. The number of hepatocellular tumors in male animals from the highest dose group is markedly higher than the values of the 0 ppm group and historical control values. This increased incidence is a result of a higher rate of hepatic tumors in mice which died, while animals from the terminal kill were not affected more frequently than the other groups. .... The increased incidence of hepatocellular tumors in males of the 900 ppm group is interpreted as a secondary effect of chronic liver overloading. A primary carcinogenic effect was not determined."

II.c. Reviewer's analyses and Comments

The sponsor did not include data of female low dose group in the computer diskettes. Hence, female low dose group was not included in the following survival and tumor data analyses. The Cox test and the generalized Wilcoxon test described in the paper of Thomas, Breslow, and Gart ("Trend and Homogeneity Analyses of Proportions and Life Table Data", Computers and Biomedical Research, 10, 373-381, 1977) were used to test for heterogeneity in survival distributions. The p-values of the Cox test were <0.00001 and 0.6081 for males and females, respectively. Hence, there was a statistically significant difference (at 0.05 level) in the survival distribution in male mice. No significant difference in the survival distribution was detected in female mice. A similar conclusion was obtained in the generalized Wilcoxon test. The p-values of the test were <0.00001 and 0.8213 for males and females, respectively.

The intercurrent mortality rates for both male and female mice (see Table 6) were tested for linear trend according to the death rate method described in the paper of Peto et al. (1980) using the time intervals 0-50, 51-80, 81-103 weeks. The actual dose levels 0, 100, 300, and 900 ppm were the scores assigned to the control, low, medium, and high dose groups, respectively. The results of the analyses showed that there was a significant (at 0.05 level) linear trend in the intercurrent mortality rate in male mice ($p < 0.00001$), but not in female mice ($p = 0.2716$).

The methods described in Peto et al. (1980) and the methods of age-adjusted exact permutation trend test were used to test the linear trend in the tumor data. The results of the above analyses showed that there were significant (at 0.05 level) positive linear trends in reticulo-histiocytary system malignant lymphoma ($p \leq 0.00001$) in female mice, and urinary bladder stromal tumor ($p = 0.0337$), lung bronchiolo-alveolar adenoma ($p = 0.0358$), and stomach inverted papilloma of pars cutanea ($p = 0.0072$) in male mice.

However, the prevalence rates of lung bronchiolo-alveolar adenoma in male mice in the concurrent control group is greater than one percent. They are considered as a common tumor in this strain of mice. For a common tumor, we consider a positive linear trend not to occur by chance of variation only if the p-value is smaller than 0.01. Therefore, we do not regard the positive linear trend in lung bronchiolo-alveolar adenoma in male mice as statistically significant. The incidence rates of reticulo-histiocytary system malignant lymphoma in female mice, and urinary bladder stromal tumor and stomach inverted papilloma of pars cutanea in male mice are given in Tables 7 to 9.

If all of the malignant lymphoma in different organ of female mice were combined, then there is no significant linear trend in malignant lymphoma of combined organ in female mice.

The sponsor's analyses showed that there were significant (at 0.05 level) positive linear trends in hepatocellular carcinoma and hepatocellular tumors in male mice. However, the reviewer found that there were not significant positive linear trends in hepatocellular adenoma ($p = 0.2641$), hepatocellular carcinoma ($p = 0.0762$) and hepatocellular tumors ($p = 0.0514$) in male mice. Tables 10 and 11 listed the incidence rates of hepatocellular adenoma and carcinoma in male mice. The different results of p-values are due to (1) the sponsor did not apply the survival-adjusted method and (2) the ordinal dose levels 0, 1, 2, and 3 were used in sponsor's analyses.

III. The Rat Study

III. a. Design

In this study, three groups of 50 male and 50 female SPF-bred rats (Wistar strain TNO/W 74, Winkelmann (Breeder), Borchen) received Nisoldipine (Bay K 5552) in doses of 50, 300, and 1800 ppm administered in their feed for up to 2 years. The dose levels can be converted to 2.78, 18.04, and 110.68 mg/kg/day for female, and 2.15, 13.13, and 82.4 mg/kg/day for male rats, respectively. An additional 50 male and 50 female rats received untreated diet and were designated as controls. The study lasted for 105 weeks (Nov. 1980 to Nov. 1982). The subsequent autopsy on the surviving animals extended over approximately further 2 weeks. The body weights of the experimental animals were recorded at the start of the study, then

each week up to the 27th week of the study, and thereafter at intervals of 2 weeks. A histopathological evaluation was carried out on selected organs or tissues from all the animals which died spontaneously, and those sacrificed in a moribund condition or at the end of the study from the control and the highest dose group (1800 ppm). For low and medium dose groups, the adrenals, the genital organs (testes, epididymis or ovaries, uterus) and skin changes suspected of being tumorous on gross inspection, as well as the kidneys from the females, were processed for microscopic assessment.

III. b. Sponsor's Analyses

The following are summaries of results of sponsor's analyses included in Volume 9.1 submitted on Dec. 9, 1985. It seems that second volume of rats study was missing. The reviewer has discussed this with the reviewing CSO, Mr. David Roeder, HFD-110.

Table 12 lists the mortality rates for each dose/sex group after 12, 18, and 24 months (at the end of the 52nd, 79th, and 105th weeks of the study). Figures 3 and 4 plot the cumulative mortality rates for each sex/dose group over the entire study period.

Based on the above mortality tables and graphs, the sponsor stated that "there was no significant statistical or biological effect on the mortality at any time".

The number of the blastoma carriers in the individual dose group was summarized in Table 13. The sponsor indicated that the numbers of blastoma carriers in the female rats from the control and the highest dose groups which had malignant or both malignant and benign tumors were approximately the same. In contrast, more male rats from the highest dose group had malignant blastomas compared with control rats. This is a finding which, considering the marked variability of the occurrence of spontaneous blastomas, is considered to be unrelated to the treatment. Table 14 lists all blastomas according to location, type, and status. The sponsor also indicated that no oncogenic activity of the test substance can be deduced from the table, which shows rather the variability of spontaneously arising blastomas.

Based on the above analyses, the sponsor concluded that "the type, localization, time of appearance and frequency of the benign and malignant tumors found provided no evidence of an oncogenic action of Bay K 5552. Therefore, under the conditions described, the 50 and 300 ppm doses are regarded as being tolerated without ill effects."

III.c. Reviewer's analyses and Comments

The Cox test and the generalized Wilcoxon test described in the paper of Thomas et al. (1977) were used to test for heterogeneity in

survival distribution. The p-values of the Cox test were 0.66 and 0.2525 for males and females, respectively. Hence, there was no statistically significant difference (at 0.05 level) in the survival distribution in either male or female rats. A similar conclusion was obtained in the generalized Wilcoxon test. The p-values were 0.7856 and 0.4237 for males and females, respectively.

The intercurrent mortality rates for both male and female rats (see Table 15) were tested for linear trend according to the death rate method described in the paper of Peto et al. (1980) using the time intervals 0-50, 51-80, and 81-105 (female)/81-109(male) weeks. The actual dose levels 0, 50, 300, and 1800 ppm were the scores assigned to the control, low, medium and high dose groups, respectively. The results of the analyses showed that there was no significant (at 0.05 level) linear trend in the intercurrent mortality rate in male or female rats.

The methods described in Peto et al. (1980) and the methods of age-adjusted exact permutation trend test were used to test the linear trend in the tumor data. The tumor intervals 0-50, 51-80, 81-105(female)/81-109(male) and terminal sacrifice were used in those methods. The results of the above analyses showed that there was a statistically significant (at 0.05 level) linear trend in brain granular cell tumor ($p = 0.0411$) in male rats. The incidence rates of this tumor are given in Table 16.

IV. Summary

IV. a. The Mouse study

The oncogenic potential of nisoldipine was evaluated in this mouse study when administered in the diet continuously to the animals at dosage levels of 0, 100, 300, or 900 ppm for up to 21 months.

Noted that the sponsor did not include data of female low dose group in the computer diskettes. Hence, female low dose group was not included in the following survival and tumor data analyses.

The Cox and the generalized Wilcoxon methods were used to test the heterogeneity in survival distribution. The test results revealed that there was no statistically significant difference (at 0.05 level) in the survival distribution in female mice. However, there was a statistically significant difference (at 0.05 level) in the survival distribution in male mice.

The statistical methods given in the paper of Peto et al. (1980) and an exact permutation trend test were used to test the positive linear trend in intercurrent mortality and incidental tumor rates. Applying the above methods to the data on sponsor's computer diskettes, the results of the analyses showed that there was a significant (at 0.05 level) linear trend in the intercurrent mortality rate in male mice

($p < 0.00001$), but not in female mice ($p = 0.2716$). Results of tumor data analyses showed that there was a significant (at 0.05 level) positive linear trend in urinary bladder stromal tumor ($p = 0.0337$), and stomach inverted papilloma of pars cutanea ($p = 0.0072$) in male mice.

The sponsor's analyses showed that there were significant (at 0.05 level) positive linear trends in hepatocellular carcinoma and hepatocellular tumors in male mice. However, the reviewer found that there were not significant positive linear trends in hepatocellular adenoma ($p = 0.2641$), hepatocellular carcinoma ($p = 0.0762$) and hepatocellular tumors ($p = 0.0514$) in male mice. The different results of p-values are due to (1) the sponsor did not apply the survival-adjusted method and (2) the ordinal dose levels 0, 1, 2, and 3 were used in sponsor's analyses.

IV. b. The Rat Study

The oncogenic potential of nisoldipine was evaluated in this rat study when administered orally and continuously to the animals at dosage levels of 50, 300, or 1800 ppm for 105 weeks.

The Cox and the generalized Wilcoxon methods were used to test the heterogeneity in survival distribution. The test results revealed that there was no statistically significant difference (at 0.05 level) in the survival distribution in female or male rats.

The statistical methods given in the paper of Peto et al. (1980) and an exact permutation trend test were used to test the positive linear trend in intercurrent mortality and incidental tumor rates. Applying the above methods to the data on sponsor's computer diskettes, the results of the analyses showed that there was not significant (at 0.05 level) linear trend in the intercurrent mortality rate in male or female rats.

Results of tumor data analyses showed that there was a significant positive linear trend in brain granular cell tumor ($p = 0.0411$) in male rats.

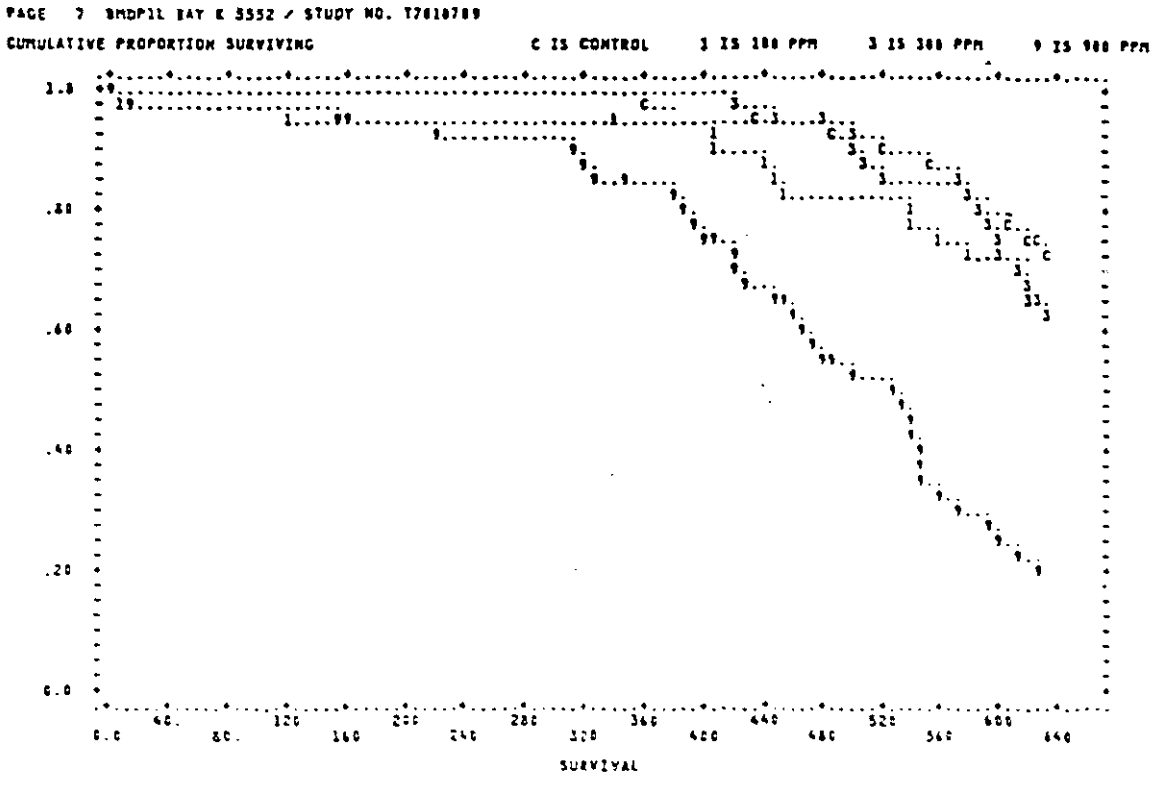
Daphne Lin

Daphne Lin, Ph.D.
Mathematical Statistician

Concur: Karl K. Lin 11/27/92

Karl K. Lin, Ph.D., Group Leader, SARB

Figure 1


PAGE 7 BMDP1L BAY E 3552 / STUDY NO. T7610789
CUMULATIVE PROPORTION SURVIVING
C IS CONTROL
1 IS 100 PPM
3 IS 300 PPM
9 IS 900 PPM
1.0
.80
.60
.40
.20
0.0
0.0
40.
80.
120
160
200
240
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320
360
400
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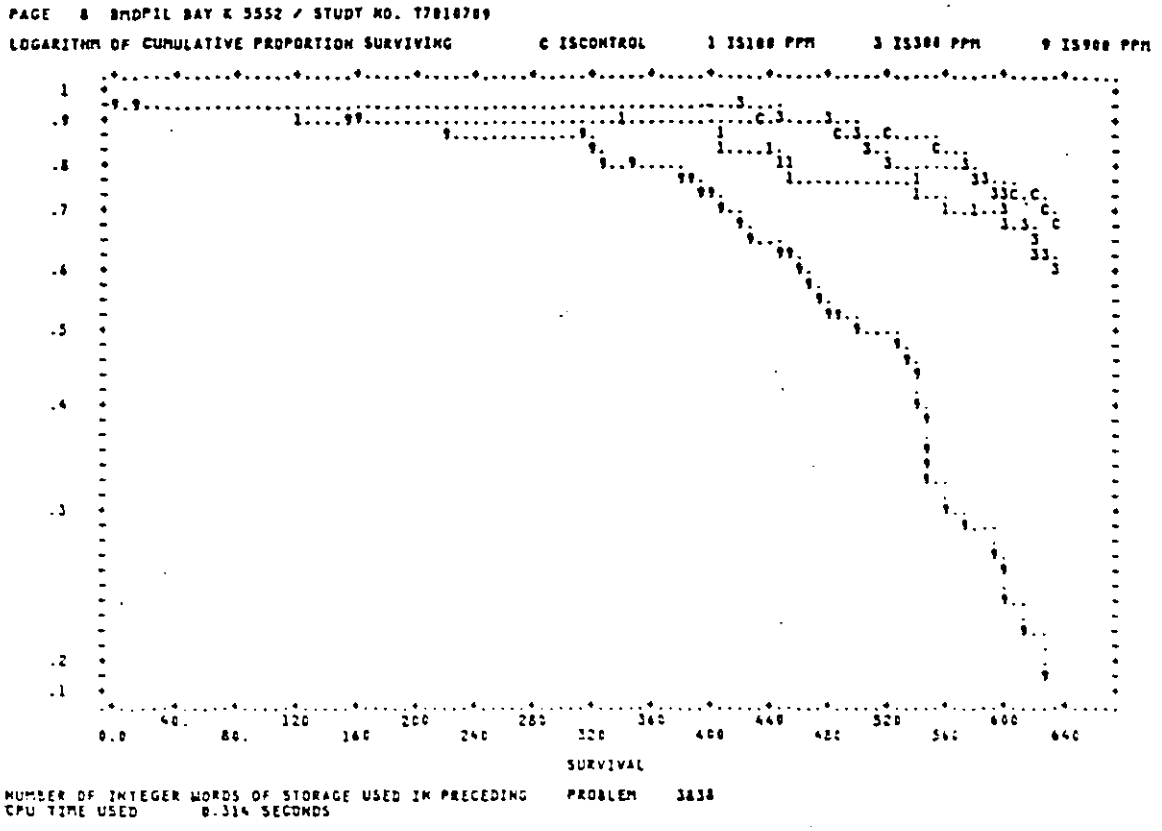
480
520
560
600
640
SURVIVAL

Figure 2



NUMBER OF INTEGER WORDS OF STORAGE USED IN PRECEDING PROBLEM 3838
CPU TIME USED 0.314 SECONDS

Figure 3

001

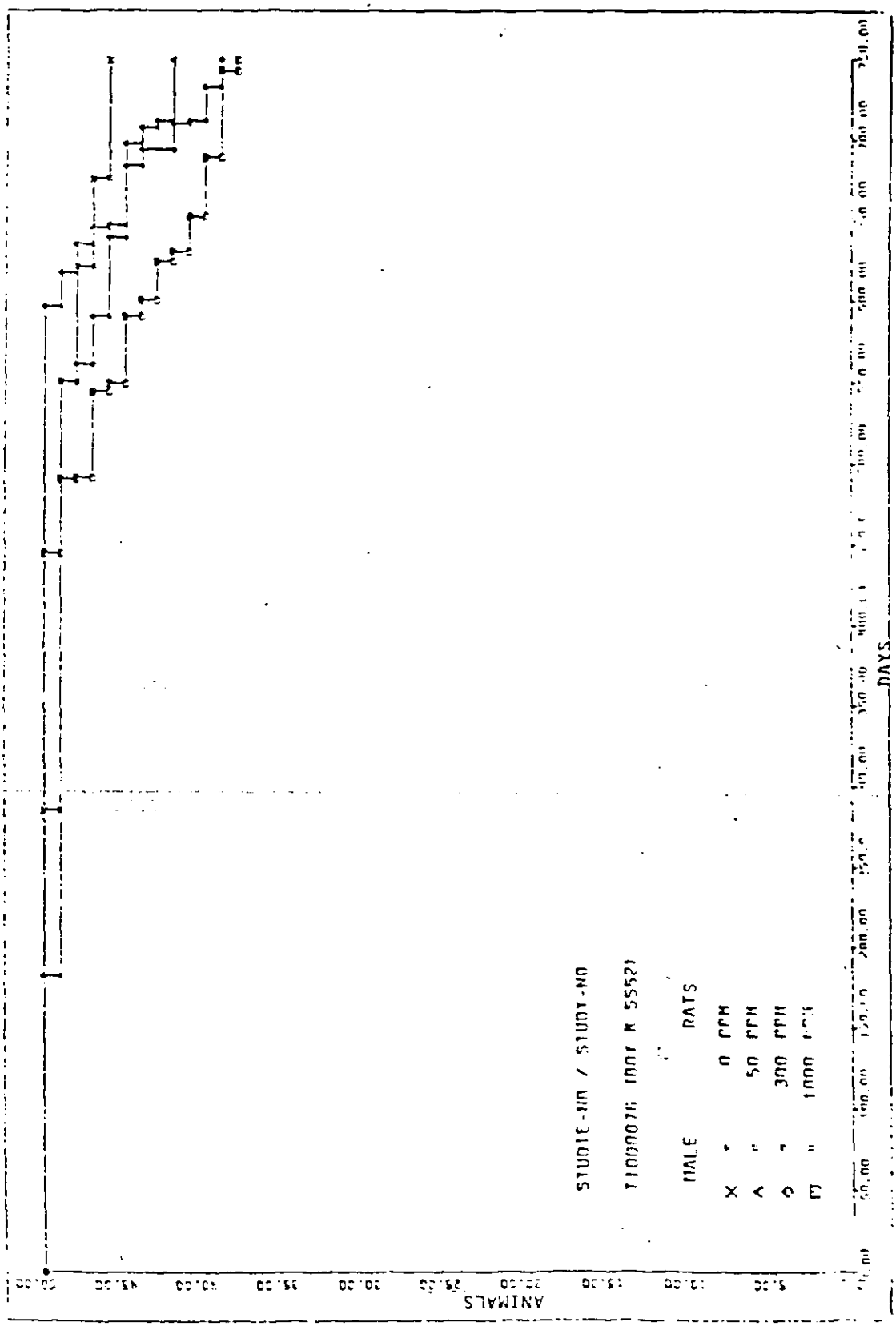


fig. 2: Mortality curves of male rats which received BAY k 5552 with the food for 24 months.

Figure 4

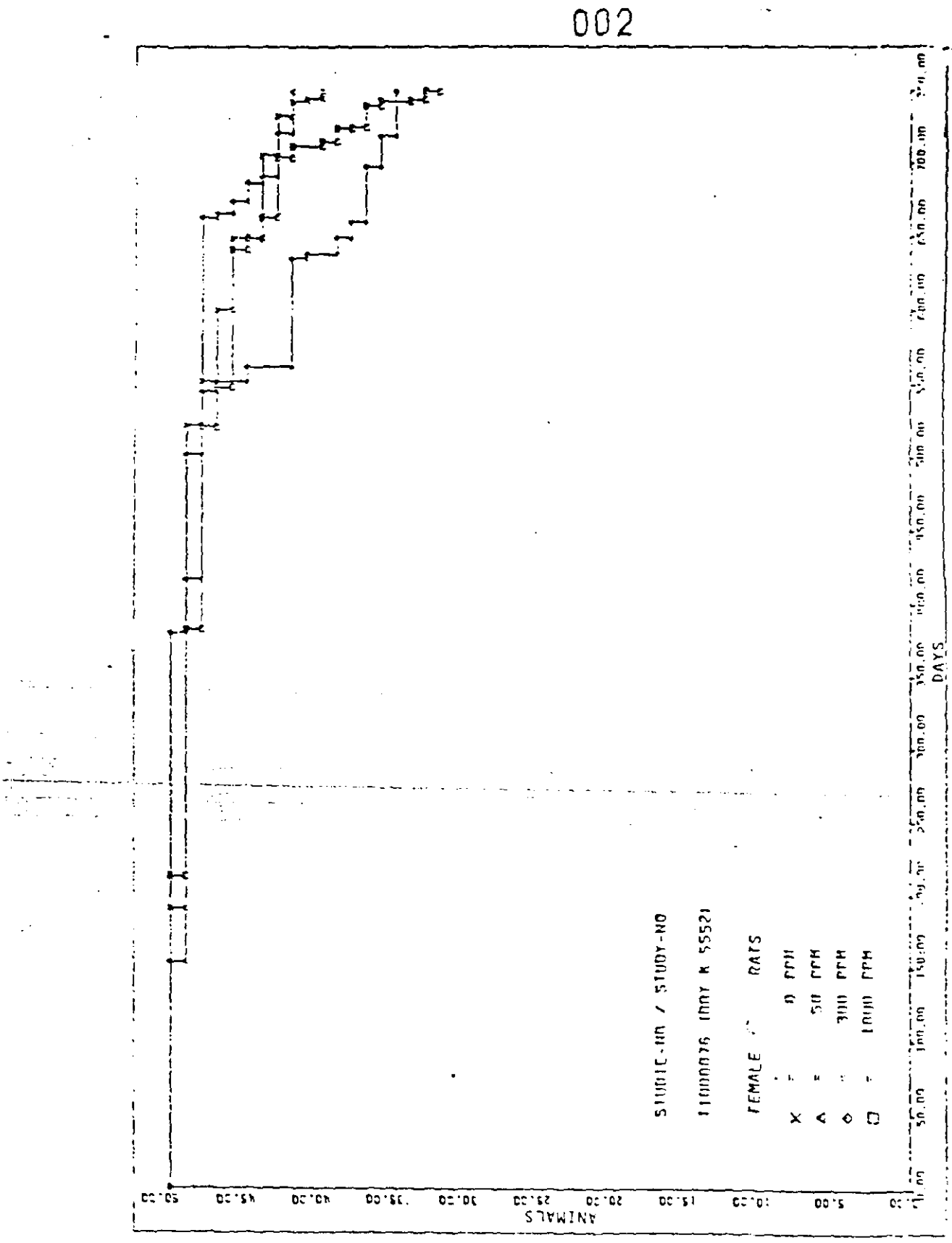


Fig. 3: Mortality curves of female rats which received BAY k 5552 with food for 24 months

Table 1 Appendix 1

Table 2

MORTALITY

DOSE ppm	NUMBER USED *	NUMBER DIED	MORTALITY %
	6 MONTHS		
	MALE		
0	50	0	0.0
100	50	2	4.0
300	50	0	0.0
900	50	3	6.0
	FEMALE		
0	50	0	0.0
100	50	0	0.0
300	50	0	0.0
900	50	1	2.0
	12 MONTHS		
	MALE		
0	50	0	0.0
100	50	3	6.0
300	50	0	0.0
900	50	8	16.0
	FEMALE		
0	50	2	4.0
100	50	5	10.0
300	50	3	6.0
900	50	5	10.0
	18 MONTHS		
	MALE		
0	50	5	10.0
100	50	11	22.0
300	50	7	14.0
900	50	29	58.0
	FEMALE		
0	50	21	42.0
100	50	19	38.0
300	50	20	40.0
900	50	21	42.0
	21 MONTHS		
	MALE		
0	50	14	28.0
100	50	15	30.0
300	50	19	38.0
900	50	40	80.0
	FEMALE		
0	50	28	56.0
100	50	34	68.0
300	50	34	68.0
900	50	32	64.0

* Animals scheduled for terminal kill

Table 18: Comparative summary of tumours occurring according to location, type, number and dignity $ (animals scheduled for terminal kill)

Sex	♂ (m)				♀ (f)			
Dose ppm	0	100	300	900	0	100	300	900
Lung:		-				-		
bronchiolo-alveolar adenoma	2		3	4	2		1	3
bronchiolo-alveolar carcinoma (malig.)	13		12	5	8		6	5
Stomach:								
papilloma	0	0	0	2	0	0	0	0
sarcoma (malig.)	0	0	2	1	0	0	0	0
Liver:								
hepatocellular adenoma	2	2	2	3	0	0	0	1
hepatocellular carcinoma (malig.)	3	4	5	8	0	0	0	1
haemangiosarcoma (malignant)	1	0	0	0	0	0	0	0
Kidneys:		-				-		
tubular carcinoma (malignant)	0		1	0	0		0	0
haemangiosarcoma (malignant)	0		1	0	0		0	0
Bladder:		-				-		
stromal tumour (benign)	1		1	2	0		0	0
stromal tumour (malignant)	0		1	1	0		0	0
Ovary:	-	-	-	-		-		
granulosa-theca cell tumour (ben.)					5		5	3
granulosa-theca cell tumour (malig.)					1		0	0
luteoma (benign)					2		2	0
tubular adeno-carcinoma (malig.)					1		0	0
Sertoli cell tumour (benign)					0		0	1
Uterus:	-	-	-	-				
adenoma					0	0	1	0
carcinoma (malig.)					1	0	0	0
fibroma					0	0	1	0
myoma					0	0	1	2
myosarcoma (malig.)					0	0	0	1
stromal tumour (benign)					3	0	2	2
stromal sarcoma (malignant)					1	3	2	2

Table 18 (continued):

Sex	♂				♀			
Dose ppm	0	100	300	900	0	100	300	900
Testes:	-				-	-	-	-
Leydig cell tumour (benign)	2		2	0				
adenoma of rete testis	1		0	0				
Pituitary:		-						
adenoma	2		0	0	0	3	3	1
Thyroid:		-				-		
follicle cell adenoma	0		1	0	0		0	0
papillary cyst-adenoma	1		0	0	0		0	0
Adrenals:		-				-		
cortical adenoma	3		3	1	2		0	0
phaeochromocytoma (benign)	1		0	0	1		0	2
phaeochromocytoma (malignant)	0		0	1	0		0	0
RH system:		-				-		
lymphoma (malig.)	7		3	1	18		12	14
lymph node sarcoma (malignant)	1		0	0	1		0	0
Skin/subcutis:		-				-		
epithelioma (malignant)	0		0	1	0		0	0
sarcoma (malig.)	1		0	0	0		3	0
Mammary gland:	-	-	-	-		-		
carcinoma (malig.)					3		0	0
adeno-ancanthoma (malignant)					0		1	1
Harder's gland:		-				-		
papillary adenoma	3		2	0	1		1	1
Spinal marrow:		-				-		
schwannoma (malig.)	0		0	0	0		1	0
Bones:		-				-		
osteosarcoma (malignant)	0		0	0	0		1	0
Abdomen:		-				-		
haemangiosarcoma (malignant)	0		0	0	0		1	0
Pelvic serosa:		-	-	-	-	-	-	-
sarcoma (malig.)	1							

- Organ not investigated § Bilateral tumours counted twice

Table 17: Summary of number of male and female mice with benign and/or malignant tumours, as well as frequency of benign and malignant tumours encountered

Sex	♂			♀		
Dose ppm	0	300	900	0	300	900
No. of animals investigated	50	50	49	48	50	50
No. of animals with tumours	27	28	24	34	31	30
No. of animals with only benign tumours	7	6	8	6	6	6
No. of animals with only malignant tumours	12	15	14	21	18	17
No. of animals with benign and malignant tumours	8	7	2	7	7	7
No. of animals with more than one primary tumour	15	11	5	13	9	8

Table 15: Statistical Analysis of Tumour Data

sex	target character	groups (ppm)	trend test	incidence	$\hat{z}$	p
male	hepatocellular tumours	0/100/300/900	death-rate	5/ 6/ 7/11	3.321	0.0004
male	hepatocellular tumours	0/100/300/900	prevalence	5/ 6/ 7/11	1.833	0.0334
male	hepatocellular tumours	0/100/300	death-rate	5/ 6/ 7	0.821	0.2059
male	hepatocellular tumours	0/900	death-rate	5/11	3.430	0.0003
male	hepatocellular tumours	0/300	death-rate	5/ 7	0.855	0.1964
male	hepatocellular tumours	0/100	death-rate	5/ 6	0.374	0.3541
male	tumour, benign	0/300/900	prevalence	15/13/10	0.286	0.3876
male	tumour, malignant	0/300/900	death-rate	20/22/16	2.692	0.0035
male	hepatocellular adenoma	0/100/300/900	death-rate	2/ 2/ 2/ 3	1.450	0.0735
male	hepatocellular carcinoma	0/100/300/900	death-rate	3/ 4/ 5/ 8	2.970	0.0015
male	hepatocellular carcinoma	0/100/300/900	prevalence	3/ 4/ 5/ 8	1.931	0.0267
male	hepatocellular carcinoma	0/100/300	death-rate	3/ 4/ 5	0.876	0.1905
male	hepatocellular carcinoma	0/900	death-rate	3/ 8	3.067	0.0011
male	hepatocellular carcinoma	0/300	death-rate	3/ 5	0.919	0.1790
male	hepatocellular carcinoma	0/100	death-rate	3/ 4	0.434	0.3321
female	tumour, benign	0/300/900	prevalence	13/13/13	0.040	0.4842
female	tumour, malignant	0/300/900	death-rate	28/25/24	-0.257	0.6015

Table 5

Historic control values: NMRI mouse 1980 to 1984

	Number					
Test No.	1	2	3	4	5	6
Adenomatous gastric mucosal hyperplasias in males	9*	20	10	32	5	27
n	50	50	50	44	47	48
%	18	40	20	73	11	56
in females	1*	8	11	14	10	13
n	50	48	49	46	47	48
%	2	17	22	30	21	27
*classified as adenoma						
Liver tumour in males	7	3	9	5	1	6
n	50	50	50	45	46	48
%	14	6	18	11	2	12
Uterine hyperplasias	0	19	21	23	0	33
n	50	46	49	45	45	46
%	0	41	43	51	0	71

Glucose concentration in the plasma: 4.32 - 9.36 mmol/l (male)
4.51 - 7.75 mmol/l (female)
Urea concentration in the plasma: 5.96 - 15.12 mmol/l (male)
4.05 - 14.99 mmol/l (female)

n = Number of organs evaluated

Table 6
Intercurrent Mortality Rates
Male Mice

Weeks	Control			Low			Medium			High		
	S	D	%	S	D	%	S	D	%	S	D	%
0-50	50	0	0	49	2	4.0	50	0	0	50	4	8
51-80	50	3	6.0	47	7	14.8	50	2	4	46	16	34
81-103	47	11	23.4	40	5	12.5	48	17	35.4	30	20	66
Term.	36			35			31			10		

Female Mice

Weeks	Control			Medium			High		
	S	D	%	S	D	%	S	D	%
0-50	48	2	4.1	50	1	2	49	3	6.1
51-80	46	10	21.7	49	9	18.3	46	9	19.5
81-103	36	14	38.8	40	24	60	37	19	51.3
Term.	22			16			18		

Notes: S: Number of animals starting during the period
D: Deaths
%: Percent of death during the period

Table 7
Tumor Incidence Rates
Female Mice, Reticulo-histiocytary System Malignant Lymphoma

Weeks	Control		Medium		High	
	T	N	T	N	T	N
0-50	0	2	0	1	0	3
51-80	0	10	0	9	3	9
81-103	0	14	0	24	8	19
Terminal	0	22	0	16	2	18
Total	0	48	0	50	13	49

Table 8
Tumor Incidence Rates
Male Mice, Urinary Bladder Stromal Tumor

Weeks	Control		LOW		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	0	7	0	2	0	16
81-103	0	11	0	5	0	17	1	20
Terminal	0	36	0	35	0	31	1	10
Total	0	50	0	49	0	50	2	50

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 9
Tumor Incidence Rates
Male Mice, Stomach inverted Papilloma of Pars Cutanea

Weeks	Control		LOW		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	0	7	0	2	0	16
81-103	0	11	0	5	0	17	0	20
Terminal	0	36	0	35	0	31	2	10
Total	0	50	0	49	0	50	2	50

Table 10
Tumor Incidence Rates
Male Mice, Hepatocellular Adenoma

Weeks	Control		LOW		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	0	7	0	2	1	16
81-103	1	11	0	5	0	17	2	20
Terminal	1	36	2	35	2	31	0	10
Total	2	50	2	49	2	50	3	50

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 11
Tumor Incidence Rates
Male Mice, Hepatocellular Carcinoma

Weeks	Control		LOW		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	2	7	0	2	2	16
81-103	0	11	0	5	2	17	5	20
Terminal	3	36	2	35	3	31	1	10
Total	3	50	4	49	5	50	8	50

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 3

STERBLICHKEIT / MORTALITY *			
DOSE DOSIS PPM	NUMBER USED EINGESETZTE TIERE	NUMBER DIED VERENDETE TIERE	MORTALITY STERBLICH KEIT %
12 MONATE / 12 MONTHS			
MAENNLICH/MALE			
0	50	1	2.0
50	50	0	0.0
300	50	1	2.0
1800	50	0	0.0
WEIBLICH/FEMALE			
0	50	1	2.0
50	50	1	2.0
300	50	0	0.0
1800	50	2	4.0
18 MONATE / 18 MONTHS			
MAENNLICH/MALE			
0	50	2	4.0
50	50	0	0.0
300	50	2	4.0
1800	50	5	10.0
WEIBLICH/FEMALE			
0	50	3	6.0
50	50	2	4.0
300	50	5	10.0
1800	50	4	8.0
24 MONATE / 24 MONTHS			
MAENNLICH/MALE			
0	50	4	8.0
50	50	8	16.0
300	50	11	22.0
1800	50	11	22.0
WEIBLICH/FEMALE			
0	50	8	16.0
50	50	8	16.0
300	50	15	30.0
1800	50	13	26.0

* The animals selected for the interim autopsy are not considered in this Table.

Table 31: Number of the blastoma carriers in the individual dose groups

Sex	♂				♀			
Dose (ppm)	0	50	300	1000	0	50	300	1000
Total number of rats investigated	49	50	50	48	48	48	48	48
Number of the blastoma carriers	31	16	21	26	33	21	21	28
Number of rats with exclusively benign blastomas	28	10	12	18	24	15	14	18
Number of rats with exclusively malignant blastomas	2	3	8	4	6	3	4	6
Number of rats with benign and malignant blastomas	1	3	1	4	3	3	3	4
Number of blastoma carriers as % all rats investigated	63	32	42	54	69	44	44	58
Number of blastoma carriers with exclusively benign blastomas as % all rats investigated	57	20	24	38	50	31	29	38
Number of blastoma carriers with exclusively malignant blastomas as % all rats investigated	4	6	16	8	11	6	8	13
Number of blastoma carriers with benign and malignant blastomas as % all rats investigated	2	6	2	8	6	6	6	8

Table 32: List of all blastomas according to number, localisation, type and status (male rats)

(Dose ppm)	0	50	300	1800
Adenohypophysis*	14	0	3	11
Adenoma	0	2	0	0
Carcinoma				
Thyroids*	6	0	0	0
C cell adenoma	1	0	0	1
C cell carcinoma	0	0	0	1
Follicular carcinoma				
Adrenal cortex	1	1	0	0
Adenoma unilateral				
Adrenal medulla	7	5	3	7
Phaeochromocytoma (b) unilateral	0	1	0	1
Phaeochromocytoma (b) bilateral	0	1	1	0
Phaeochromocytoma (m) unilateral				
Parathyroids*	3	0	0	0
Adenoma				
Testes	4	5	6	7
Leydig cell tumour (b) unilateral	0	1	2	1
Leydig cell tumour (b) bilateral				
Pancreas* endocrine	2	0	0	0
Adenoma				
Pancreas* exocrine	3	0	0	0
Adenoma				
Heart*	2	0	0	1
Endocardial fibromatosis (b)				
Lung*	0	0	1	0
Adenoma				
Epididymis	0	0	0	1
Sarcoma				
Brain*	0	0	0	2
Meningioma (b)				
RHS*	0	1	3	1
Malignant lymphoma	0	0	1	0
Histiocytary sarcoma				
Skin*	0	1	1	1
Cornified squamous cell carcinoma				
Subcutis*	1	1	1	3
Sarcoma	0	0	1	1
Haemangiosarcoma				
Mesentery*	0	0	1	0
Leiomyosarcoma				
Abdomen*	1	0	0	0
Fibrosarcoma	0	1	0	0
Sarcoma				

b = benign

m = malignant

* This organ was not routinely histologically investigated in all the rats from the 50 and 300 ppm groups.

Table 32: List of all blastomas according to number, localisation, type (continuation) and status (female rats)

(Dose ppm)	0	50	300	1800
Adenohypophysis				
Adenoma	16	3	5	7
Carcinoma	1	1	1	0
Thyroics*				
C cell adenoma	6	1	2	1
C cell carcinoma	1	0	0	0
Follicular adenoma	0	0	0	1
Follicular carcinoma	1	0	0	0
Adrenal cortex				
Adenoma unilateral	1	1	0	0
Adrenal medulla				
Phaeochromocytoma (b) unilateral	0	0	0	1
Phaeochromocytoma (m) unilateral	0	0	0	1
Ovary				
Granulosa-theca cell tumour (b)	0	0	1	1
Granulosa-theca cell tumour (m)	0	0	0	1
Uterus				
Endometrial stromal tumour (polyp) (b)	9	12	11	13
Endometrial stromal sarcoma	2	0	2	2
Adenocarcinoma	1	4	2	2
Mammary gland				
Adenoma	0	2	1	2
Adenocarcinoma	1	1	1	0
Kidneys				
Adenoma	1	0	0	0
Sarcoma	1	0	0	0
Urinary bladder*				
Adenoma	1	0	0	0
RHS*				
Malignant lymphoma	0	0	0	1
Histiocytary sarcoma	0	0	0	1
Intestine*				
Fibroma	0	0	0	1
Mesentery*				
Malignant mesothelioma	0	0	1	0
Skin				
Papilloma	0	0	0	1
Subcutis				
Haemangiosarcoma	1	0	0	0
Sarcoma	1	0	0	2

b = benign
m = malignant
* This organ was not routinely histologically investigated in all the rats from the 50 and 300 ppm groups.

Table 15
Intercurrent Mortality Rates
Male Rats

Weeks	Control			Low			Medium			High		
	S	D	%	S	D	%	S	D	%	S	D	%
0-50	50	1	2	50	0	0	50	1	2	50	0	0
51-80	49	1	2.0	50	0	0	49	0	0	50	3	6
81-109	48	3	6.2	50	8	16	49	10	20.4	47	8	17.
Term.	45			42			39			39		

Female Rats

Weeks	Control			Low			Medium			High		
	S	D	%	S	D	%	S	D	%	S	D	%
0-50	50	1	2	50	1	2	50	0	0	50	2	4
51-80	49	2	4.0	49	1	2.0	50	8	16	48	2	4.1
81-105	47	5	10.6	48	6	12.5	42	7	16.6	46	9	19.5
Term.	42			42			35			37		

Notes: S: Number of animals starting during the period
D: Deaths
%: Percent of death during the period

Table 16
Tumor Incidence Rates
Male Rats, Brain Granular Cell Tumor

Weeks	Control		LOW		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	1	0	0	0	1	0	0
51-80	0	1	0	0	0	0	1	3
81-109	0	3	0	8	0	10	0	8
Terminal	0	45	0	42	0	39	2	39
Total	0	50	0	50	0	50	3	50

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Pairwise : C vs H
P = 0.1594

Statistical Review and Evaluation
(An Addendum)

IND #: Date: MAR -8 1994

Applicant: Miles Inc.

Name of Drug: Nisoldipine

I. Background

The two animal carcinogenicity studies (one in rats and one in mice) included in this IND submission were reviewed and a statistical review report was issued on Dec. 3, 1992 by the Division of Biometrics. Our analyses showed that there were statistically significant positive linear trends in urinary bladder benign stromal tumor ($p = 0.0337$) and stomach inverted papilloma of para cutanea ($p = 0.0072$) in male mice and in reticulo-histiocytary system malignant lymphoma ($p < 0.00001$) in female mice. However, in the last case, if all of the malignant lymphomas in different organs of female mice are combined, then the linear trend became not significant. In rats study, results of tumor data analyses showed that there was a significant positive linear trend in brain granular cell tumor ($p = 0.0411$) in male rats. Dr. Xavier Joseph, HFD-110, who is the reviewing pharmacologist of this IND, requested the Division of Biometrics to test if the positive dose-response relationship is significant in any possible combinations of urinary bladder malignant stromal tumor, benign polypous stromal tumor, and benign stromal tumor in male mice. He also requested the reviewer to check the difference in the incidence rates of reticulo-histiocytary system malignant lymphoma in female mice between the sponsor's and the reviewer's results. Pairwise comparisons were also requested for tumors which have significant positive linear trends.

II. The Mice Study

The methods described in Peto et al. (1980) and the methods of age-adjusted exact permutation trend test were used to test the positive linear trend in different combinations of urinary bladder tumors in male mice and in malignant lymphoma in female mice. The time intervals 0-50, 51-80, 81-103 weeks, and terminal sacrifice were used in those methods. The actual dose levels 0, 100, 300, and 900 ppm were the scores assigned to the control, low, medium, and high dose groups, respectively.

The results showed that there was a statistically significant positive linear trend in urinary bladder benign stromal tumor (trend: $p = 0.0337$; pairwise: high vs control: $p = 0.1403$) in male mice. There was no statistically significant (at 0.05 level) trend in any other urinary bladder tumors in male mice. Noted that there are two male mice (animal numbers 68 and 189) developed urinary bladder polypous stromal tumors, one male mice (animal number 207) developed

urinary bladder malignant stromal tumor, and two male mice (animal numbers 234 and 242) developed urinary bladder benign tumors. The following table lists the p-values of the test results in urinary bladder tumors in male mice:

Tumors	P-values
Benign Stromal Tumor	0.0337 *
Polypous Stromal Tumor	0.5793
Malignant Stromal Tumor	0.6226
Benign Stromal Tumor & Polypous Stromal Tumor	0.0606
Benign Stromal Tumor & Malignant Stromal Tumor	0.0725
Polypous Stromal Tumor & Malignant Stromal Tumor	0.4289
Benign Stromal Tumor & Polypous Stromal Tumor & Malignant Stromal Tumor	0.1036

The incidence rates of urinary bladder benign stromal tumor, urinary bladder malignant stromal tumor, and urinary bladder benign polypous stromal tumor in male mice are given in Tables 1 to 3.

For the malignant lymphoma, as mentioned in the previous review, there was a statistically significant positive linear trend in reticulo-histiocytary system malignant lymphoma (trend : $p < 0.00001$; pairwise: high vs control: $p = 0.0001$; pairwise: high vs medium: $p < 0.0001$) in female mice. However, if all of the malignant lymphomas in different organs of female mice were combined, then the linear trend became not significant ($p = 0.2307$). Table 4 lists the incidence rates of reticulo-histiocytary system malignant lymphoma in female mice. Table 5 lists the incidence rates of reticulo-histiocytary system malignant lymphoma and all the malignant lymphomas in different organs of female mice. Noted that the sponsor did not include data of female low dose group in the computer diskettes. Hence, female low dose group was not included in the analysis of malignant lymphoma.

In the sponsor's submission dated on August 11, 1988, the tumor bearing animals were summarized according to location and tumor type and shown in Table 18 of their submission (see attached Table 6). Under "RH system malignant lymphoma", there are 18, 12, and 14 female mice developed this tumor in control, medium, and high dose groups, respectively. However, the reviewer found that the numbers are 0, 0, and 13 female mice in control, medium, and high dose groups,

respectively, recorded in the data diskette submitted on April 1, 1992. If the female animals developed malignant lymphoma in reticulo-histiocytary system and all different organs are combined, then the numbers are 18, 12, and 14 female mice in control, medium, and high dose groups, respectively.

There was a statistically significant positive linear trend in stomach inverted papilloma of para cutanea ($p = 0.0072$) in male mice. The pairwise comparison also showed a statistically significant difference between control and high dose male mice in this tumor ($p = 0.0435$). The incidence rates of this tumor are given in Table 7.

III. The Rats Study

Results of tumor data analyses showed that there was a significant positive linear trend in brain granular cell tumor ($p = 0.0411$) in male rats. However, the pairwise comparison between control and high dose male rats did not show any significant difference ($p = 0.1594$) in tumor rate between the two groups. The incidence rates of this tumor are given in Table 8.

IV. Summary

The statistical methods given in the paper of Peto et al. (1980) and an exact permutation trend test were used to test the positive linear trend in malignant lymphoma in female mice and in any possible combinations of urinary bladder malignant stromal tumor, benign polypous stromal tumor, and benign stromal tumor in male mice. The time intervals 0-50, 51-80, 81-103 weeks, and terminal sacrifice were used in those methods. The actual dose levels 0, 100, 300, and 900 ppm were the scores assigned to the control, low, medium, and high dose groups, respectively.

The results showed that there was a statistically significant positive linear trend in urinary bladder benign stromal tumor ($p = 0.0337$) in male mice. However, the pairwise comparison between control and high dose male mice did not show any difference ($p = 0.1403$) in urinary bladder benign stromal tumor rates between the two groups. There was no statistically significant (at 0.05 level) trend in any other urinary bladder tumors in male mice.

There was a statistically significant positive linear trend in reticulo-histiocytary system malignant lymphoma (trend : $p < 0.00001$; pairwise: high vs control: $p = 0.0001$; pairwise: high vs medium: $p < 0.0001$) in female mice. However, if all of the malignant lymphomas in different organs of female mice were combined, then the linear trend ($p = 0.2307$) became not significant. In Table 18 of the sponsor's submission dated on August 11, 1988, the "RH system malignant lymphoma" in female mice actually is the "combination of malignant lymphomas in reticulo-histiocytary system and all different organs".

There was a statistically significant positive linear trend in stomach inverted papilloma of para cutanea (p = 0.0072) in male mice. The pairwise comparison also showed a statistically significant difference between control and high dose male mice in this tumor (p = 0.0435). For the rat study, there was a significant positive linear trend in brain granular cell tumor (p = 0.0411) in male rats. However, the pairwise comparison between control and high dose male rats did not show any difference (p = 0.1594) in tumor rate between the two groups.

Daphne Lin

Daphne Lin, Ph.D.
Mathematical Statistician

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HFD-502/Assistant Director (Pharmacology)
HFD-715/DRU 2.1.1, Nisoldipine, Miles Inc.

Table 1
Tumor Incidence Rates
Male Mice, Urinary Bladder Benign Stromal Tumor

Weeks	Control		LOW		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	0	7	0	2	0	16
81-103	0	11	0	5	0	17	1	20
Terminal	0	36	0	35	0	31	1	10
Total	0	50	0	49	0	50	2	50

Trend test: p = 0.0337
Pairwise comparison: High vs Control p = 0.1403

Table 2
Tumor Incidence Rates
Male Mice, Urinary Bladder Malignant Stromal Tumor

Weeks	Control		LOW		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	0	7	0	2	0	16
81-103	0	11	0	5	1	17	0	20
Terminal	0	36	0	35	0	31	0	10
Total	0	50	0	49	1	50	0	50

Trend test: p = 0.6226

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 3
Tumor Incidence Rates
Male Mice, Urinary Bladder Polypous Stromal Tumor

Weeks	Control		Low		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	0	7	0	2	0	16
81-103	0	11	0	5	0	17	0	20
Terminal	1	36	0	35	1	31	0	10
Total	1	50	0	49	1	50	0	50

Trend test: p = 0.5793

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 4
Tumor Incidence Rates
Female Mice, Reticulo-histiocytary System Malignant Lymphoma

Weeks	Control		Medium		High	
	T	N	T	N	T	N
0-50	0	2	0	1	0	3
51-80	0	10	0	9	3	9
81-103	0	14	0	24	8	19
Terminal	0	22	0	16	2	18
Total	0	48	0	50	13	49

Trend test: $p < 0.00001$
Pairwise comparison: high vs control: $p = 0.0001$
: high vs medium: $p < 0.0001$

Table 5
Tumor Incidence Rates
Female Mice, Reticulo-histiocytary System Malignant Lymphoma & All Possible sites of Malignant Lymphoma

Weeks	Control		Medium		High	
	T	N	T	N	T	N
0-50	0	2	0	1	0	3
51-80	5	10	1	9	3	9
81-103	9	14	7	24	8	19
Terminal	4	22	4	16	3	18
Total	18	48	12	50	14	49

Trend test: $p = 0.2307$

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 18: Comparative summary of tumours occurring according to location, type, number and dignity $ (animals scheduled for terminal kill)

Sex	♂				♀			
Dose ppm	0	100	300	900	0	100	300	900
Lung:		-				-		
bronchiolo-alveolar adenoma	2		3	4	2		1	3
bronchiolo-alveolar carcinoma (malig.)	13		12	5	8		6	5
Stomach:								
papilloma	0	0	0	2	0	0	0	0
sarcoma (malig.)	0	0	2	1	0	0	0	0
Liver:								
hepatocellular adenoma	2	2	2	3	0	0	0	1
hepatocellular carcinoma (malig.)	3	4	5	8	0	0	0	1
haemangiosarcoma (malignant)	1	0	0	0	0	0	0	0
Kidneys:		-				-		
tubular carcinoma (malignant)	0		1	0	0		0	0
haemangiosarcoma (malignant)	0		1	0	0		0	0
Bladder:		-				-		
stromal tumour (benign)	1		1	2	0		0	0
stromal tumour (malignant)	0		1	1	0		0	0
Ovary:	-	-	-	-		-		
granulosa-theca cell tumour (ben.)					5		5	3
granulosa-theca cell tumour (malig.)					1		0	0
luteoma (benign)					2		2	0
tubular adeno-carcinoma (malig.)					1		0	0
Sertoli cell tumour (benign)					0		0	1
Uterus:	-	-	-	-				
adenoma					0	0	1	0
carcinoma (malig.)					1	0	0	0
fibroma					0	0	1	0
myoma					0	0	1	2
myosarcoma (malig.)					0	0	0	1
stromal tumour (benign)					3	0	2	2
stromal sarcoma (malignant)					1	3	2	2

Table 18 (continued):

Sex	♂				♀			
Dose ppm	0	100	300	900	0	100	300	900
Testes:	-				-	-	-	-
Leydig cell tumour (benign)	2		2	0				
adenoma of rete testis	1		0	0				
Pituitary:		-						
adenoma	2		0	0	0	3	3	1
Thyroid:		-				-		
follicle cell adenoma	0		1	0	0		0	0
papillary cyst-adenoma	1		0	0	0		0	0
Adrenals:		-				-		
cortical adenoma	3		3	1	2		0	0
phaeochromocytoma (benign)	1		0	0	1		0	2
phaeochromocytoma (malignant)	0		0	1	0		0	0
RH system:		-				-		
lymphoma (malig.)	7		3	1	18		12	14
lymph node sarcoma (malignant)	1		0	0	1		0	0
Skin/subcutis:		-				-		
epithelioma (malignant)	0		0	1	0		0	0
sarcoma (malig.)	1		0	0	0		3	0
Mammary gland:	-	-	-	-		-		
carcinoma (malig.)					3		0	0
adeno-ancanthoma (malignant)					0		1	1
Harder's gland:		-				-		
papillary adenoma	3		2	0	1		1	1
Spinal marrow:		-				-		
schwannoma (malig.)	0		0	0	0		1	0
Bones:		-				-		
osteosarcoma (malignant)	0		0	0	0		1	0
Abdomen:		-				-		
haemangiosarcoma (malignant)	0		0	0	0		1	0
Pelvic serosa:		-	-	-	-	-	-	-
sarcoma (malig.)	1							

- Organ not investigated § Bilateral tumours counted twice

Table 7
Tumor Incidence Rates
Male Mice, Stomach Inverted Papilloma of Pars Cutanea

Weeks	Control		Low		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	0	0	2	0	0	0	4
51-80	0	3	0	7	0	2	0	16
81-103	0	11	0	5	0	17	0	20
Terminal	0	36	0	35	0	31	2	10
Total	0	50	0	49	0	50	2	50

Trend test: p = 0.0072
Pairwise comparison: high vs control: p = 0.0435

Table 8
Tumor Incidence Rates
Male Rats, Brain Granular Cell Tumor

Weeks	Control		Low		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	1	0	0	0	1	0	0
51-80	0	1	0	0	0	0	1	3
81-109	0	3	0	8	0	10	0	8
Terminal	0	45	0	42	0	39	2	39
Total	0	50	0	50	0	50	3	50

Trend test: p = 0.0411
Pairwise comparison: high vs control: p = 0.1594

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

ATTACHMENT

Histopathology Incidence

Two-Year Dietary Study in the Rat

A Brief Coversheet for Carcinogenesis Study Review

NDA 20-356
Drug: Nisoldipine
Sponsor: Miles Inc. Pharmaceutical Division

1. Species & Strain	Rat, Wistar (TNO/W 74)
2. Name of Laboratory	
3. No./sex/group	50 (additional 10 rats/sex included in each group were sacrificed after 12 months of treatment)
4. Doses (C, L, M & H)	0, 50, 300 & 1800 ppm in diet
5. Basis for dose selection stated: yes (x) no ( )	4-week dietary dose rangefinding study in mice
6. Interim sacrifice	yes
7. Total duration	Two year
8. No. alive at termination (C, L, M & H)	Males: 46, 42, 39 & 39 Females: 42, 42, 35 & 37
9. Statistical methods used	U-test of Mann and Whitney and of Wilcoxon, and Fischer's exact test

10. Tumor and non-tumor data for each tissue attached.

List of neoplasms in the rats at the interim sacrifice

Control group, ♀

Animal No. 71 Thyroid: cystadenoma
No. 80 Pituitary: adenoma

Dose group 1800 ppm BAY k 5552, ♂

No. 399 Testis : Leydig cell tumour (benign)
No. 414 Brain (Cerebellum): Meningioma (benign)

Table : Number of the blastoma carriers in the individual dose groups

Sex	♂				♀			
Dose (ppm)	0	50	300	1800	0	50	300	1800
Total number of rats investigated	49	50	50	48	48	48	48	48
Number of the blastoma carriers	31	16	21	26	33	21	21	28
Number of rats with exclusively benign blastomas	28	10	12	18	24	15	14	18
Number of rats with exclusively malignant blastomas	2	3	8	4	6	3	4	6
Number of rats with benign and malignant blastomas	1	3	1	4	3	3	3	4
Number of blastoma carriers as % all rats investigated	63	32	42	54	69	44	44	58
Number of blastoma carriers with exclusively benign blastomas as % all rats investigated	57	20	24	38	50	31	29	38
Number of blastoma carriers with exclusively malignant blastomas as % all rats investigated	4	6	16	8	13	6	8	13
Number of blastoma carriers with benign and malignant blastomas as % all rats investigated	2	6	2	8	6	6	6	8

Table : List of all blastomas according to number, localisation, type and status (male rats)

(Dose ppm)	0	50	300	1800
Adenohypophysis*				
Adenoma	14	0	3	11
Carcinoma	0	2	0	0
Thyroids*				
C cell adenoma	6	0	0	0
C cell carcinoma	1	0	0	1
Follicular carcinoma	0	0	0	1
Adrenal cortex				
Adenoma unilateral	1	1	0	0
Adrenal medulla				
Phaeochromocytoma (b) unilateral	7	5	3	7
Phaeochromocytoma (b) bilateral	0	1	0	1
Phaeochromocytoma (m) unilateral	0	1	1	0
Parathyroids*				
Adenoma	3	0	0	0
Testes				
Leydig cell tumour (b) unilateral	4	5	6	7
Leydig cell tumour (b) bilateral	0	1	2	1
Pancreas* endocrine				
Adenoma	2	0	0	0
Pancreas* exocrine				
Adenoma	3	0	0	0
Heart*				
Endocardial fibromatosis (b)	2	0	0	1
Lung*				
Adenoma	0	0	1	0
Epididymis				
Sarcoma	0	0	0	1
Brain*				
Meningioma (b)	0	0	0	2
RHS*				
Malignant lymphoma	0	1	3	1
Histiocytary sarcoma	0	0	1	0
Skin*				
Cornified squamous cell carcinoma	0	1	1	1
Subcutis*				
Sarcoma	1	1	1	3
Haemangiosarcoma	0	0	1	1
Mesentery*				
Leiomyosarcoma	0	0	1	0
Abdomen*				
Fibrosarcoma	1	0	0	0
Sarcoma	0	1	0	0

b = benign
m = malignant
* This organ was not routinely histologically investigated in all the rats from the 50 and 300 ppm groups.

Table : List of all blastomas according to number, localisation, type (continuation) and status (female rats)

(Dose ppm)	0	50	300	1800
Adenohypophysis				
Adenoma	16	3	5	7
Carcinoma	1	1	1	0
Thyroids*				
C cell adenoma	6	1	2	1
C cell carcinoma	1	0	0	0
Follicular adenoma	0	0	0	1
Follicular carcinoma	1	0	0	0
Adrenal cortex				
Adenoma unilateral	1	1	0	0
Adrenal medulla				
Phaeochromocytoma (b) unilateral	0	0	0	1
Phaeochromocytoma (m) unilateral	0	0	0	1
Ovary				
Granulosa-theca cell tumour (b)	0	0	1	1
Granulosa-theca cell tumour (m)	0	0	0	1
Uterus				
Endometrial stromal tumour (polyp) (b)	8	12	11	13
Endometrial stromal sarcoma	2	0	2	2
Adenocarcinoma	1	4	2	2
Mammary gland				
Adenoma	0	2	1	2
Adenocarcinoma	1	1	1	0
Kidneys				
Adenoma	1	0	0	0
Sarcoma	1	0	0	0
Urinary bladder*				
Adenoma	1	0	0	0
RHS*				
Malignant lymphoma	0	0	0	1
Histiocytary sarcoma	0	0	0	1
Intestine*				
Fibroma	0	0	0	1
Mesentery*				
Malignant mesothelioma	0	0	1	0
Skin				
Papilloma	0	0	0	1
Subcutis				
Haemangiosarcoma	1	0	0	0
Sarcoma	1	0	0	2

b = benign
m = malignant
* This organ was not routinely histologically investigated in all the rats from the 50 and 300 ppm groups.

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group: 0 ppm, sex ♂

Animal No.	8	Pituitary	Adenoma
		Endocrine pancreas	Islet cell adenoma
No.	11	Testes	Leydig cell tumour, benign (b), unilateral
		Thyroid	C cell adenoma
No.	13	Pituitary	Adenoma
No.	14	Testes	Leydig cell tumour (b), unilateral
No.	18	Adrenals	Cortical adenoma, unilateral
No.	19	Pituitary	Adenoma
No.	20	Heart	Endocardial fibromatosis (b)
		Adrenals	Phaeochromocytoma (b), unilateral
		Parathyroids	Adenoma, unilateral
		Exocrine pancreas	Adenoma
No.	23	Testes	Leydig cell tumour (b), unilateral
		Thyroid	C cell carcinoma
No.	24	Pituitary	Adenoma
No.	26	Pituitary	Adenoma
No.	27	Pituitary	Adenoma
No.	30	Adrenals	Phaeochromocytoma (b), unilateral
		Parathyroids	Adenoma, unilateral
No.	33	Adrenals	Phaeochromocytoma (b), unilateral
No.	35	Pituitary	Adenoma
No.	36	Thyroid	C cell adenoma
No.	37	Pituitary	Adenoma
No.	39	Pituitary	Adenoma
No.	41	Adrenals	Phaeochromocytoma (b), unilateral
No.	42	Thyroid	C cell adenoma
No.	43	Subcutis (ear)	Sarcoma
No.	44	Thyroid	C cell adenoma
No.	45	Pituitary	Adenoma
No.	46	Endocrine pancreas	Islet cell adenoma
		Adrenals	Phaeochromocytoma (b), unilateral
No.	47	Pituitary	Adenoma
		Parathyroids	Adenoma, unilateral

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group: 0 ppm, sex ♂

Animal No.	49	Testes	Leydig cell tumour (b), unilateral
No.	50	Exocrine pancreas	Adenoma
		Pituitary	Adenoma
		Thyroid	C cell adenoma
No.	54	Pituitary	Adenoma
		Adrenals	Phaeochromocytoma (b), unilateral
No.	55	Heart	Endocardial fibromatosis (b)
No.	56	Pituitary	Adenoma
		Adrenals	Phaeochromocytoma (b), unilateral
No.	57	Abdomen	Fibrosarcoma
No.	60	Exocrine pancreas	Adenoma
		Thyroid	C cell adenoma

Dose group 50 ppm, sex ♂

Animal No.	124	Adrenals	Phaeochromocytoma (b), unilateral
No.	128	Adrenals	Phaeochromocytoma (b), unilateral
No.	132	Testes	Leydig cell tumour (b), unilateral
		Skin (head)	Cornifying squamous cell carcinoma
No.	136	Adrenals	Phaeochromocytoma (b), unilateral
No.	142	Testes	Leydig cell tumour (b), unilateral
No.	145	Abdomen	Sarcoma
No.	149	Adrenals	Cortical adenoma, unilateral
No.	151	Adrenals	Tumour. The neoplastic tissue has penetrated the organ capsule. The origin of the tumour cells cannot be determined with certainty due to autolysis. Presumably it is a malignant unilateral phaeochromocytoma.
		Reticulohistiocytary system (RHS)	Malignant lymphomas in the spleen, liver, lung and bone marrow
No.	153	Adrenals	Phaeochromocytoma (b), unilateral
No.	163	Testes	Leydig cell tumour (b), unilateral

List of blastomas found on histopathological examination of the rats in the 2-year feeding study with BAY k 5552

Dose group: 50 ppm, sex ♂		
Animal No. 165	Pituitary	Adenocarcinoma, infiltrating the brain
No. 168	Testes	Leydig cell tumour (b), unilateral
No. 169	Testes Subcutis (head)	Leydig cell tumour (b), bilateral Sarcoma
No. 170	Adrenals Pituitary	Phaeochromocytoma (b), unilateral Adenocarcinoma with multiple focal necroses
No. 175	Adrenals	Phaeochromocytoma (b), bilateral
No. 180	Testes	Leydig cell tumour (b), unilateral
Dose group: 300 ppm, sex ♂		
Animal No. 252	Adrenals	Phaeochromocytoma, the tumour cells have penetrated the organ capsule, malignant, unilateral
No. 253	RHS	Malignant lymphoma in the thymus (?) and spleen
No. 257	Testes	Leydig cell tumour (b), unilateral
No. 259	Adrenals RHS	Phaeochromocytoma, (b), unilateral malignant lymphoma in the spleen, lymph nodes, kidneys, lung, mesentery, muscles, urinary bladder, pancreas and bone marrow
No. 260	Pituitary	Adenoma
No. 262	Testes	Leydig cell tumour (b), bilateral
No. 263	Subcutis (rear limb)	Sarcoma infiltrating the skeletal muscle
No. 265	Pituitary	Adenoma
No. 267	Testes	Leydig cell tumour (b), unilateral
No. 272	Adrenals Testes	Phaeochromocytoma (b), unilateral Leydig cell tumour (b), bilateral

Mean Clinical Chemistry Parameters (Male Rats)					
		Dose Group (ppm in diet)			
Parameter	Week	0	50	300	1800
ALP	28	211	201	201	175*
U/L	54	182	174	186	145*
	79	180	156	176	136**
GOT		38.8	39.0	38.9	52.7*
U/L					
Bilirubin	28	3.6	3.1	4.0	4.8*
mcmol/L					
Creatinine	79	53	50	47*	51
mcmol/L	105	57	63	46**	50**
Urea	105	5.80	7.01*	5.69	5.27
mmol/L					
Cholesterol	28	1.98	2.16	2.21*	2.21
mmol/L					
Protein	54	66.5	64.2**	61.0**	61.4**
g/L	105	68.4	67.1*	66.3*	67.8
Sodium	28	142	143	140*	140*
mmol/L	54	141	142	142*	142
	79	140	139	138*	141
Potassium	79	4.8	5.0	5.1*	5.1
mmol/L					
Calcium	28	2.64	2.54*	2.49*	2.55*
mmol/L	79	2.76	2.66*	2.62**	2.63**
	105	2.69	2.66	2.63	2.58*
Aldosterone	55	349.7	360.2	334.9	245.1**
pg/mL					

** Significantly different from control at the 0.05 level*
*** Significantly different from control at the 0.01 level*

Mean Clinical Chemistry Parameters (Female Rats)					
		Dose Group (ppm in diet)			
Parameter	Week	0	50	300	1800
ALP	28	174	140	153	133*
U/L					
CPK	28	98	54*	72	85
U/L	79	43	75	64	77*
GPT	28	54.1	50.5	52.9	66.3*
U/L					
Bilirubin	54	3.0	3.1	3.2	4.3**
mcmol/L	105	4.7	2.9**	3.2*	2.9*
Creatinine	79	56	50	59	57*
mcmol/L	105	71	59	55**	61
Urea	28	7.54	7.27	6.56*	6.30**
mmol/L	79	6.22	5.96	6.51	7.56**
	105	6.09	6.43	6.67*	7.28*
Cholesterol	28	7.54	7.27	6.56*	6.30**
mmol/L	105	2.46	3.01*	2.76	2.96
Glucose	105	4.71	5.25	5.52*	5.22
mmol/L					
Sodium	54	140	138	135**	138
mmol/L					
Potassium	54	4.8	4.8	5.0	5.2*
mmol/L	105	4.5	4.6	4.8*	4.8*
Calcium	28	2.58	2.65	2.59	2.47*
mmol/L	54	2.71	2.65	2.58*	2.52**
Corticosterone	55	36.6	41.7	20.4	19.2*
mcg/DL					

** Significantly different from control at the 0.05 level*
*** Significantly different from control at the 0.01 level*

At the termination of the study, the relative mean weights of adrenals, heart, kidneys and liver of the high dose group (both sexes) were significantly higher than respective control values (page 50A). However, no significant differences were seen in the absolute weights of the above organs except for the increased mean kidney weight of the high dose males. At the interim sacrifice, relative heart and liver weights (high dose males and females) and relative adrenal and kidney weights (high dose females) were significantly increased without any significant changes in absolute weights.

No significant treatment related gross lesions were seen in this study.

At the interim sacrifice, no treatment-related histological findings were observed except for the moderate widening of the zona glomerulosa region of the adrenal cortex of high dose animals. The cells of this zone were large and contained a foamy cytoplasm. Four benign tumors [2 in control females (cystadenoma of thyroid in one and pituitary adenoma in the other) and 2 in high dose males (Leydig cell tumor of testis in one and meningioma of the cerebellum in the other)] were seen at the interim sacrifice.

[Note: The terms "blastoma" and "tumor" are used interchangeably in this NDA.]

The number of rats with benign and/or malignant tumors and the percent of these tumor carriers are given in Table 7. According to sponsor, no treatment-related increased incidence of tumor bearing animals was observed in this study. The incidence of various types of tumors observed at different locations are presented in Tables 8 and 9. Although the incidence of Leydig cell tumor of testes appears to be higher in treated male groups than in control, the differences were statistically not significant.

Analysis of the tumor data by FDA statisticians showed that there was a statistically significant (at 0.05 level) linear trend in brain granular cell tumor (listed also as meningioma in this NDA) in male rats ($p=0.0411$). The incidence of this tumor is as follows: control - 0/50, low dose - 0/50, mid dose - 0/50 and high dose - 3/50 (2 animals at the final necropsy and one at the interim sacrifice). However, pairwise comparison did not reveal any significant difference between control and high dose groups ($p=0.1594$). According to the sponsor, "the incidence rate for granular cell tumors among male rats at terminal kill in the study performed with BAY 5552 lay within the spontaneous range for male rats at terminal kill" (Table-page 50e). Spontaneous tumors of meningeal origin (meningioma, meningeal sarcoma or granular cell tumor) were seen in 7 out of 30 studies in male Wistar rats (39-50 rats/study). In 2 studies, 3 rats each were diagnosed with such tumors at terminal kill, whereas in 4 other studies, only one rat each had above tumors.

Mean Organ Weights of Male and Female Rats (Final Sacrifice - 107 Weeks)

Organ		Sex	Dose Group (ppm in drinking water)			
			0	50	300	1800
Body Weight (g)		M	416	410	413	396*
		F	274	275	261	216**
Adrenals	Absolute, mg	M	49	49	43**	50
	Relative, mg/100g	M	12	12	10*	13*
	Absolute, mg	F	67	66	63	61
	Relative, mg/100g	F	24	24	25	29**
Heart	Absolute, mg	M	1215	1205	1199	1234
	Relative, mg/100g	M	294	295	290	312**
	Absolute, mg	F	912	927	898	934
	Relative, mg/100g	F	334	338	347	434**
Kidneys	Absolute, mg	M	2588	2600	2638	2791**
	Relative, mg/100g	M	625	634	640	706**
	Absolute, mg	F	1875	1823	1809	1745
	Relative, mg/100g	F	690	668	700*	810**
Liver	Absolute, mg	M	14277	14661	15213	14831
	Relative, mg/100g	M	3476	3715	3736	3937**
	Absolute, mg	F	8157	7912	8315	8087
	Relative, mg/100g	F	3327	3343	3509	3773**
Lung	Absolute, mg	M	1380	1368	1460	1373
	Relative, mg/100g	M	337	347	358	365
	Absolute, mg	F	1028	998	1004	1008
	Relative, mg/100g	F	419	423	425	472**
Spleen	Absolute, mg	M	656	656	673	684
	Relative, mg/100g	M	160	166	165	181*
	Absolute, mg	F	447	444	438	419
	Relative, mg/100g	F	183	188	185	196
Testicles	Absolute, mg	M	3371	3618	3661	3149
	Relative, mg/100g	M	823	917*	899	831

* *Significantly different from control at 0.05 level*
** *Significantly different from control at 0.01 level*

Table 7 : Number of the blastoma carriers in the individual dose groups

Sex	♂				♀			
Dose (ppm)	0	50	300	1800	0	50	300	1800
Total number of rats investigated	49	50	50	48	48	48	48	48
Number of the blastoma carriers	31	16	21	26	33	21	21	28
Number of rats with exclusively benign blastomas	28	10	12	18	24	15	14	18
Number of rats with exclusively malignant blastomas	2	3	8	4	6	3	4	6
Number of rats with benign and malignant blastomas	1	3	1	4	3	3	3	4
Number of blastoma carriers as % all rats investigated	63	32	42	54	69	44	44	58
Number of blastoma carriers with exclusively benign blastomas as % all rats investigated	57	20	24	38	50	31	29	38
Number of blastoma carriers with exclusively malignant blastomas as % all rats investigated	4	6	16	8	13	6	8	13
Number of blastoma carriers with benign and malignant blastomas as % all rats investigated	2	6	2	8	6	6	6	8

Table 8 : List of all blastomas according to number, localisation, type and status (male rats)

(Dose ppm)	0	50	300	1800
Adenohypophysis*				
Adenoma	14	0	3	11
Carcinoma	0	2	0	0
Thyroids*				
C cell adenoma	6	0	0	0
C cell carcinoma	1	0	0	1
Follicular carcinoma	0	0	0	1
Adrenal cortex				
Adenoma unilateral	1	1	0	0
Adrenal medulla				
Phaeochromocytoma (b) unilateral	7	5	3	7
Phaeochromocytoma (b) bilateral	0	1	0	1
Phaeochromocytoma (m) unilateral	0	1	1	0
Parathyroids*				
Adenoma	3	0	0	0
Testes				
Leydig cell tumour (b) unilateral	4	5	6	7
Leydig cell tumour (b) bilateral	0	1	2	1
Pancreas* endocrine				
Adenoma	2	0	0	0
Pancreas* exocrine				
Adenoma	3	0	0	0
Heart*				
Endocardial fibromatosis (b)	2	0	0	1
Lung*				
Adenoma	0	0	1	0
Epididymis				
Sarcoma	0	0	0	1
Brain*				
Meningioma (b)	0	0	0	2
RHS*				
Malignant lymphoma	0	1	3	1
Histiocytary sarcoma	0	0	1	0
Skin*				
Cornified squamous cell carcinoma	0	1	1	1
Subcutis*				
Sarcoma	1	1	1	3
Haemangiosarcoma	0	0	1	1
Mesentery*				
Leiomyosarcoma	0	0	1	0
Abdomen*				
Fibrosarcoma	1	0	0	0
Sarcoma	0	1	0	0

b = benign
m = malignant
* This organ was not routinely histologically investigated in all the rats from the 50 and 300 ppm groups.

Table 9 : List of all blastomas according to number, localisation, type and status (female rats) (continuation)

(Dose ppm)	0	50	300	1800
Adenohypophysis				
Adenoma	16	3	5	7
Carcinoma	1	1	1	0
Thyroids*				
C cell adenoma	6	1	2	1
C cell carcinoma	1	0	0	0
Follicular adenoma	0	0	0	1
Follicular carcinoma	1	0	0	0
Adrenal cortex				
Adenoma unilateral	1	1	0	0
Adrenal medulla				
Phaeochromocytoma (b) unilateral	0	0	0	1
Phaeochromocytoma (m) unilateral	0	0	0	1
Ovary				
Granulosa-theca cell tumour (b)	0	0	1	1
Granulosa-theca cell tumour (m)	0	0	0	1
Uterus				
Endometrial stromal tumour (polyp) (b)	8	12	11	13
endometrial stromal sarcoma	2	0	2	2
Adenocarcinoma	1	4	2	2
Mammary gland				
Adenoma	0	2	1	2
Adenocarcinoma	1	1	1	0
Kidneys				
Adenoma	1	0	0	0
Sarcoma	1	0	0	0
Urinary bladder*				
Adenoma	1	0	0	0
RHS*				
Malignant lymphoma	0	0	0	1
Histiocytary sarcoma	0	0	0	1
Intestine*				
Fibroma	0	0	0	1
Mesentery*				
Malignant mesothelioma	0	0	1	0
Skin				
Papilloma	0	0	0	1
Subcutis				
Haemangiosarcoma	1	0	0	0
Sarcoma	1	0	0	2

b = benign
m = malignant
* This organ was not routinely histologically investigated in all the rats from the 50 and 300 ppm groups.

Historical Control Data - Spontaneous Tumors in Wistar Rats (1981-1989)

NERVOUS S.

MALES

Experiment No.		1	2	3			6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	total
BRAIN																																
No. of rats examined		50	50	39	48	40			50	50	50	50	50	50	50	50	49	48	50	50	50	50	50	50	50	50	50	50	49	50	50	1464
meningioma	b	0	0	0	0	0	0			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
meningeal sarcoma	m	0	0	0	0	0	0				0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	6
glioma	m	1	0	0	1	0	1	0			0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	8
granular cell tumour	b	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
granular cell tumour	m	0	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
sarcoma NOS	m	0	0	0	0	1	0	0	0			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
SPINAL CORD																																
No. of rats examined		50	50	39	40	40	49	50	50	50	50	50			50	50	49	48	50	50	47	50	47	50	50	50	50	50	49	50	50	1458
astrocytoma	m	0	0	0	0	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	2

FEMALES

Experiment No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	total
BRAIN																																
No. of rats examined		50	49	40	40	39	50	50	50	50	50	50	50	50	49	50	49	50			50	50	50	50	50	50	50	50	49	50	50	1465
ependymoma	b	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0	0	0	1
meningioma	b	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0	0	0	1
meningeal sarcoma	m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	0	1	0	0	1	0	2
glioma	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	1	0	0	1	0	0	1
glioma	m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0			0	0	0	0	0	1	0	0	3
granular cell tumour	m	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0	1
neurolemmal sarcoma	m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0			0	0	0	0	0	0	0	1

[Note: Granular cell tumors are believed to be of meningeal origin and are considered to be a subclassification of meningiomas.(Boorman et. al. 1990. eds. Pathology of the Fischer Rat. Academic Press, Inc.)]

Relevant nonneoplastic findings observed in this study included hypertrophy of the cells of the zona glomerulosa of the adrenal cortex of high dose males and females and increased incidence of progressive nephropathy in high dose females.

d. Two-Week Intravenous Toxicity Study

Testing Facility:

Study Number: Not provided (Pharma Report No.7721)

Study Date: October, 1977

GLP Compliance: Not addressed

Animals: SPF Wistar albino rats, individually housed in Type II Macrolon cages, weighed 125 to 130 g at the initiation of dosing.

Dose Levels: 0, 0.1, 0.3 and 1.0 mg/kg. BAY K 5552, dissolved in a 10%:90% mixture of Cremophor EL and physiological saline, was administered as a single iv bolus injection (caudal vein; 1 ml/kg) daily for 14 consecutive days.

Number of Animals: 10/sex/group

Parameters Evaluated: Appearance and behavior (daily), body weight and food and water consumption (weekly), hematology and clinical chemistry (at the termination of the study; 5 rats/sex/group), urinalyses (after 10th treatment), major organ weights and gross and microscopic pathology (more than 30 different tissues/rat; 5 rats/sex from control and high dose groups).

Results: High dose animals showed inertia and dyspnea for about 5 to 15 min following drug administration. Two high dose females died during the study, one after the third dose and the other after the ninth dose. No treatment-related clinical signs or mortalities were seen in low and mid dose group animals.

Intravenous administration of nisoldipine had no significant effect on body weight, food or water consumption and hematologic, blood chemistry and urinalyses parameters. There were no treatment-related gross or histopathological findings, or any evidence of local intolerance at dose levels tested in this study.

MOUSE STUDIES (X. Joseph)

a. 28-day Dietary Dose Rangefinding Study

Testing Facility:

Study Number: T 1003 576

Study Dates: June-July, 1981

GLP Compliance: Study was not conducted according to GLP regulations. The deviations were as follows: a. no phase 1-3 GLP audits, and b. no checking of physico-chemical properties of test substance.

Animals: SPF-bred NMRI mice, individually housed in Type I Macrolon cages, were 4-5 weeks old (average body weights: males-20.0 g, females-19.8 g) at the initiation of the study.

Dose Levels: Bay k 5552 (Batch No. 576,923) was mixed with powdered diet by the addition of peanut oil DAB 7 (1%) to obtain dietary drug concentrations of 0, 400, 800, 1200 and 1600 ppm.

Achieved Dose Levels:

Dose (ppm)	Average Drug Intake (mg/kg/day)	
	Male	Female
0	0	0
400	110	124
800	226	271
1200	348	383
1600	429	523

(Note: The drug intake was calculated from the average daily food intake/animal/group for the whole duration of the study.)

Number of Animals: 10/sex/group

Parameters Evaluated: Appearance and behavior (daily), body weight, food and water consumption (weekly), organ weights (heart, lungs, liver, spleen and kidneys) and gross pathology.

Results: No treatment-related clinical signs or mortalities were observed. Significant reductions in body weights (3-7%), compared to concurrent control, were seen in females throughout the study

at dose levels of 800 ppm and above except at 1200 ppm at the end of the study. In males, although body weights were lower than concurrent control (4-9%) at 1200 and 1600 ppm levels, the differences were statistically significant only at 1200 ppm. No significant differences were seen in food and water consumption between treated and control groups. Organ weight findings are given below.

Mean Organ Weights of Male and Female Mice

		Sex	Dose Group (ppm in diet)				
			0	400	800	1200	1600
Body Weight (g)		M	31.8	30.1	31.0	29.5*	30.3
		F	25.5	25.4	24.0*	24.7	24.5*
Heart	(Absolute, mg)	M	0.14	0.15	0.17**	0.15*	0.15
	(Relative, mg/100g)	M	0.44	0.52**	0.53**	0.53**	0.49*
	(Absolute, mg)	F	0.13	0.14	0.14	0.15*	0.14*
	(Relative, mg/100g)	F	0.51	0.54*	0.58**	0.61**	0.57**
Kidneys	(Absolute, mg)	M	0.46	0.46	0.49	0.49	0.48
	(Relative, mg/100g)	M	1.44	1.53	1.59	1.66*	1.58
	(Absolute, mg)	F	0.35	0.34	0.33	0.33	0.34
	(Relative, mg/100g)	F	1.35	1.32	1.38	1.33	1.37
Liver	(Absolute, mg)	M	1.93	1.84	1.82	1.74*	1.72*
	(Relative, mg/100g)	M	6.08	6.09	5.66	5.90	5.66*
	(Absolute, mg)	F	1.47	1.40	1.34	1.39	1.36
	(Relative, mg/100g)	F	5.72	5.53	5.55	5.64	5.56
Lung	(Absolute, mg)	M	0.23	0.26*	0.25	0.23	0.22
	(Relative, mg/100g)	M	0.72	0.88**	0.80**	0.78*	0.74
	(Absolute, mg)	F	0.22	0.21	0.20	0.23	0.22
	(Relative, mg/100g)	F	0.87	0.82	0.82	0.92*	0.89
Spleen	(Absolute, mg)	M	0.09	0.09	0.10	0.09	0.09
	(Relative, mg/100g)	M	0.28	0.31	0.32	0.30	0.29
	(Absolute, mg)	F	0.10	0.09	0.10	0.10	0.09
	(Relative, mg/100g)	F	0.38	0.37	0.41	0.38	0.38

** Significantly different from control at 0.05 level.*
*** Significantly different from control at 0.01 level.*

Relative heart weights in all treatment groups (both sexes) were significantly higher than control; however, absolute heart weights were significantly higher in males only at 800 and 1200

ppm and in females at 1200 and 1600 ppm levels. Both absolute and relative liver weights were lower than control in 1600 ppm males.

No significant gross findings were observed. Histopathological evaluations were not performed in this study.

Based on the results of this study, dietary dose levels of 100, 300 and 900 ppm were selected for the mouse carcinogenicity study.

b. 21 Month Carcinogenicity Study in Mice

Testing Facility:

Study Number: T7010709 (Sponsor's number)

Study Dates: Initiation of dosing - 10/7/81
Autopsy of last animal - 7/7/83

GLP Compliance: Studies were done in accordance with GLP regulations.

Animals:

Strain: Bor:NMRI (SPF HAN)
Sex: Both sexes
Age and Wt: 4 to 6 weeks old/20-22 g
Housing: Individually housed in Makrolon Type I cages.

Mode of Administration of Test Agent: Powdered diet.

Dose Levels: 0, 100, 300 and 900 ppm dosage levels of BAY k 5552 (Batch No. 662845, purity-98.1%) were used on the basis of results of the 28-day dietary dose rangefinding study. The stability and the concentration of drug in the diet were determined periodically. The concentrations of the drug in diet, at all intervals, were more than 89% of the theoretical values, and the compound was found to be stable in the diet for at least 10 days. However, there is no indication that concentrations of drug in diet were adjusted periodically to maintain a constant mg/kg body weight exposure.

No. of Animals: Equal numbers of males and females (50+20*/sex/dosage level) were used. *Additional 20 mice included in each group were sacrificed 12 months after the initiation of dosing for interim investigations.

Observations/Measurements:

Appearance/Behavior monitored twice daily and a detailed assessment of each individual animal was made on a weekly basis, with particular attention given to posture, general behavior, body surfaces, orifices and breathing and elimination products.

Body weight determinations were done at the beginning of the study, once a week until 27th week and every 2 weeks thereafter and also before the termination of the study.

Food intake was calculated on a weekly basis up to 23rd week and every two weeks thereafter.

Hematological (RBC, WBC, platelet and reticulocyte counts, differential white cell counts, hemoglobin, hematocrit, MCV, MCH and MCHC) and clinical chemistry [alkaline phosphatase, transaminases (ASAT and ALAT), plasma creatinine, urea, blood glucose, cholesterol, bilirubin and total plasma proteins] investigations were done at 12 months (interim sacrifice group) and also at the end of the study (10 animals/sex/treatment group).

Autopsies were done on all mice which died during the course of the study or that were killed in extremis, and also on those that were sacrificed at 12 months and at the termination of the study. Nine major organs were weighed and sections of various organs and tissues (about 38 different tissues/mouse) and gross lesions were preserved for histopathologic evaluation. At 12 months, these evaluations were done only on tissues from 0 and 900 ppm dosage groups and also on any tissue from 100 and 300 ppm groups which looked tumorous macroscopically. At the termination of the study, tissues from 0, 300 and 900 ppm dosage groups were examined histologically (only stomach, pituitary, uterus and liver were examined from 100 ppm group).

Statistical analysis on body weights, clinical laboratory values and organ weights were done using two-tailed U test according to Mann and Whitney, and Wilcoxon. The survival data were analyzed by the statistical software package using the generalized Wilcoxon test. Statistical analysis of tumor findings was done using the death rate method for malignant tumors and the prevalence method for benign tumors (Peto et al.). Because of the high mortality rate in high dose males, the death rate and the prevalence methods were used combined for the analysis of hepatocellular tumor data.

Interim Sacrifice:

Surviving animals from interim sacrifice groups (originally 20 mice/sex/dosage group) were killed at 12 months.

Achieved Dose Levels:

Average daily drug intake* (mg/kg body wt)

Sex	Dose (ppm) 0	100	300	900
Male		19.37	58.06	162.93
Female		24.99	74.36	217.28

(*The drug intake was calculated from the average daily food intake/animal/group for the whole duration of the study.)

Mortality:

Mortality data is summarized below and it is presented graphically in Figures 9 & 10.

Mortality of Mice Receiving Nisoldipine in Diet for 21 Months

Daily Dose (ppm in diet)	Number of Mice (M/F)	Number of Dead (M/F)	% Mortality (M/F)
6 Months			
0	50/50	0/0	0/0
100	50/50	2/0	4/0
300	50/50	0/0	0/0
900	50/50	3/1	6/2
12 Months			
0	50/50	0/2	0/4
100	50/50	3/5	6/10
300	50/50	0/3	0/6
900	50/50	8/5	16/10
18 Months			
0	50/50	5/21	10/42
100	50/50	11/19	22/38
300	50/50	7/20	14/40
900	50/50	29/21	58/42
21 Months			
0	50/50	14/28	28/56
100	50/50	15/34	30/68
300	50/50	19/34	38/68
900	50/50	40/32	80/64

The mortality rates in treated females (all groups) were not significantly different from controls at any given interval. However, the incidence of deaths in females was high in all groups including controls from 12 months onward. The mortality rates in males from 900 ppm group, especially at 21 months, were significantly higher ($p<0.001$) compared to controls or other

Fig.9 : Mortality curves of male mice which received 21 months BAY k 5552 in the diet

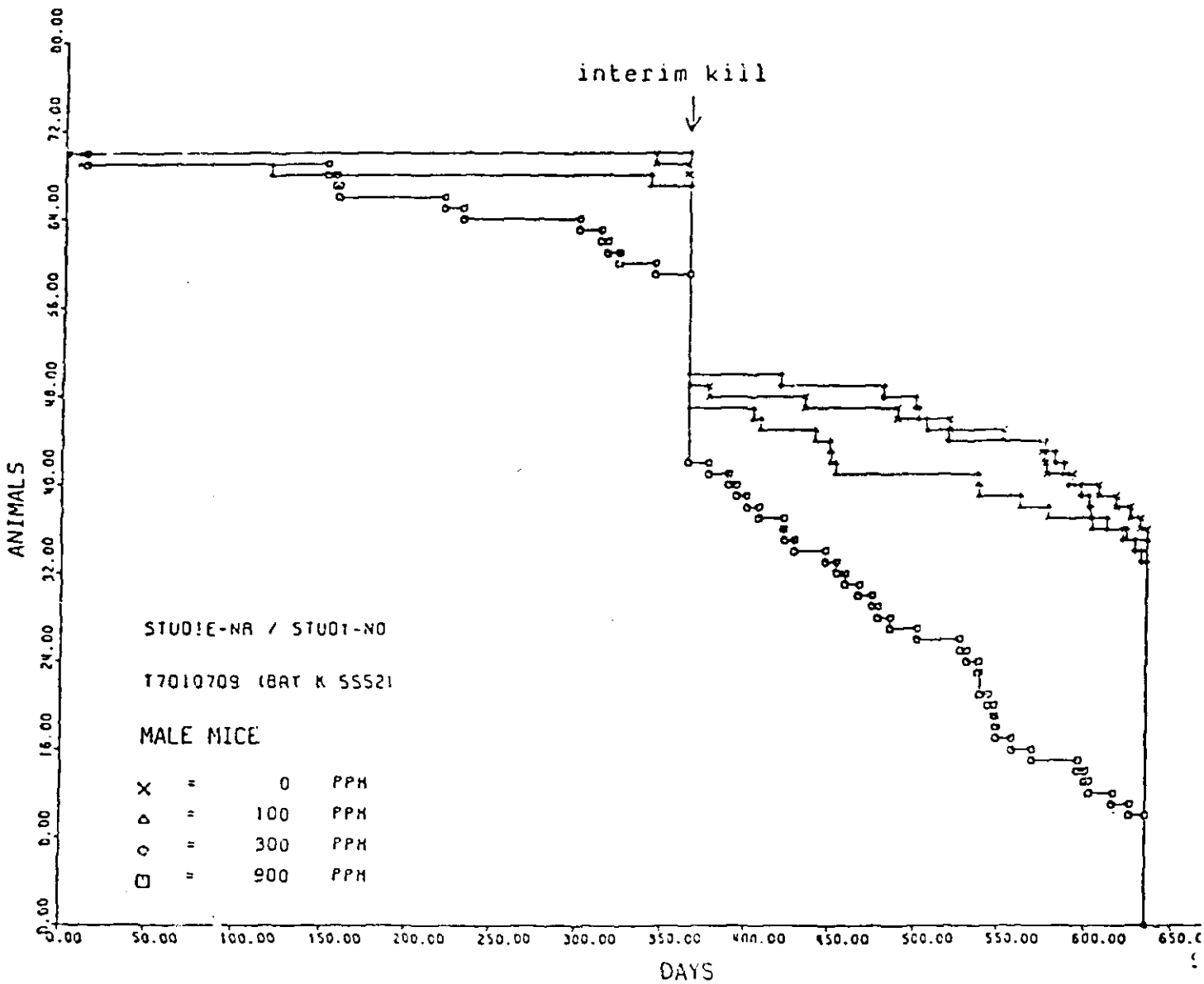
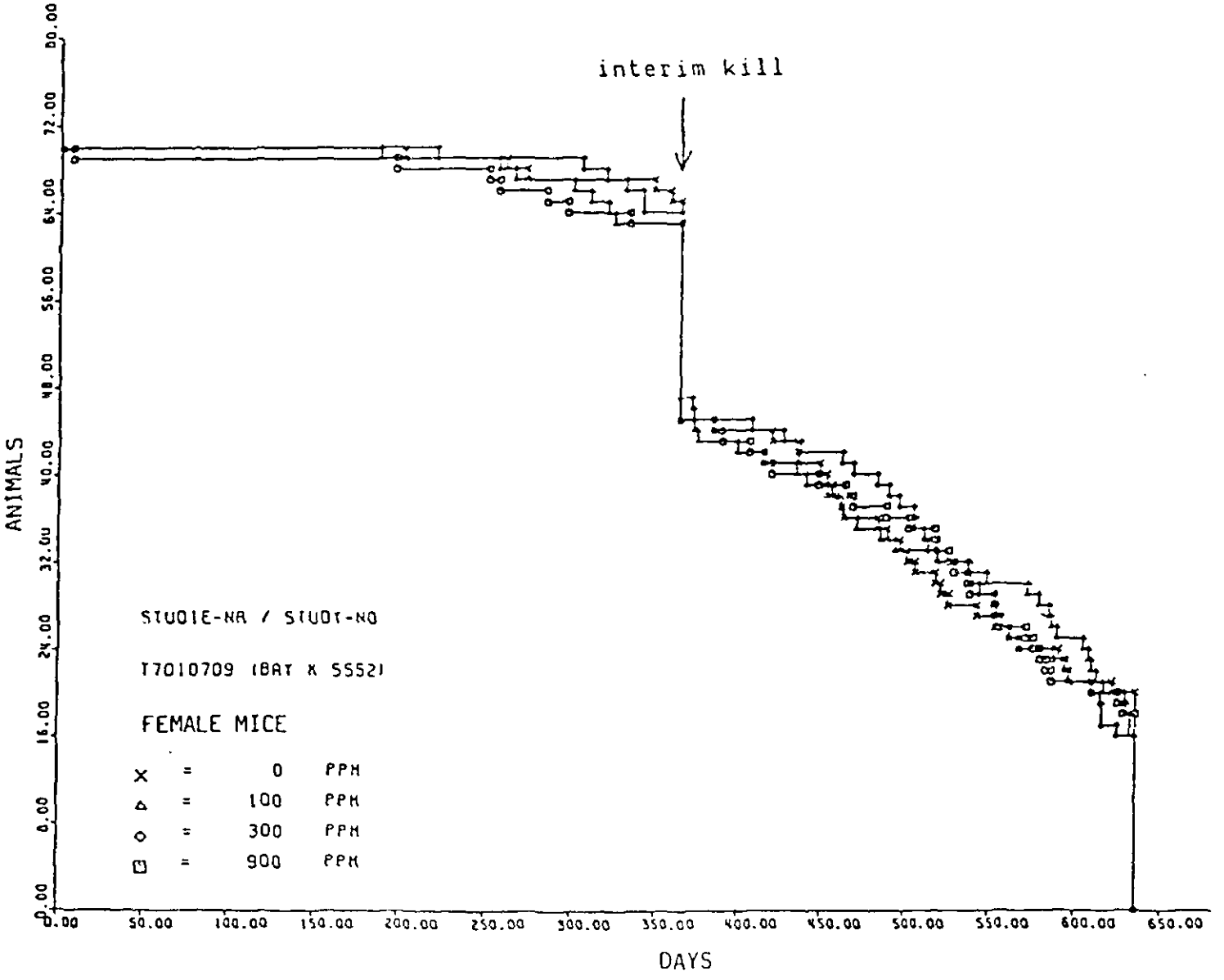


Fig.10: Mortality curves of female mice which received 21 months BAY k 5552 in the diet



lower dosage groups. No significant difference noticed in this parameter between males of low or middle dosage groups and controls. The increased mortality of males in the high dose group is partly attributed to pharmacodynamically induced colonic atonies. Autopsy of animals that died or were killed in extremis frequently showed that the large intestine was tightly filled with solid faeces resulting from colonic atony.

Using the Cox and the generalized Wilcoxon methods for testing the heterogeneity in survival distribution, FDA statisticians observed a statistically significant difference (at 0.05 level) in the survival distribution in males, but not in females (for both of the above tests, the p values for males were <0.00001).

Drug Associated Findings:

No treatment related clinical signs were seen in this study. The food intake in males from the 900 ppm group was about 9% less than in control males. Average body weights are presented graphically in Figures 11 & 12. Statistically significant reductions in body weights at certain weeks were seen especially in males of 900 ppm group and to a lesser extent in 100 ppm group. However, at the termination of the study, no significant body weight differences were seen between treatment and control groups (both sexes). Leukocyte counts at 21 months were significantly lower ($p<0.01$) in males (900 ppm) and females (300 and 900 ppm groups) compared to respective controls. However, differential counts did not show any significant variations in the proportion of different cell types between treated and control mice. The hemoglobin and hematocrit values in mid and high dose males were significantly lower at 12 months, but not at 21 months. The blood glucose concentration was significantly higher ($p<0.01$) in males (300 and 900 ppm) at 12 months and also at 21 months (all treatment groups). Females showed a similar increase only at 12 months. All these values were reported to be within the range of historical control values. Significant elevations in blood urea levels were seen only at 12 months in all treated male groups and in high dose females.

Macroscopically, swollen gastric mucous membranes were observed more frequently in treated males than in controls (0 ppm - 5; 100 ppm - 12; 300 ppm - 14; 900 ppm - 14). The incidence of enlarged hearts was more in males of 900 ppm group (0 ppm - 7; 100 ppm - 2; 300 ppm - 11; and 900 ppm - 23). In mice that died or were killed in extremis, the incidence of large intestines impacted with solid feces was higher in both sexes at the highest dosage level (0 ppm - males 5 and female 0; 900 ppm - male 13 and female 9).

The heart and liver weights (absolute and relative) in males (21 months) were significantly increased at 300 and 900 ppm dose levels, but the weights of adrenals (absolute and relative) were significantly decreased in all treated male groups compared to controls. In females, the heart and liver weights were

Figure11 : Body weight curves for male mice receiving BAY k 5552 in their food for 21 months

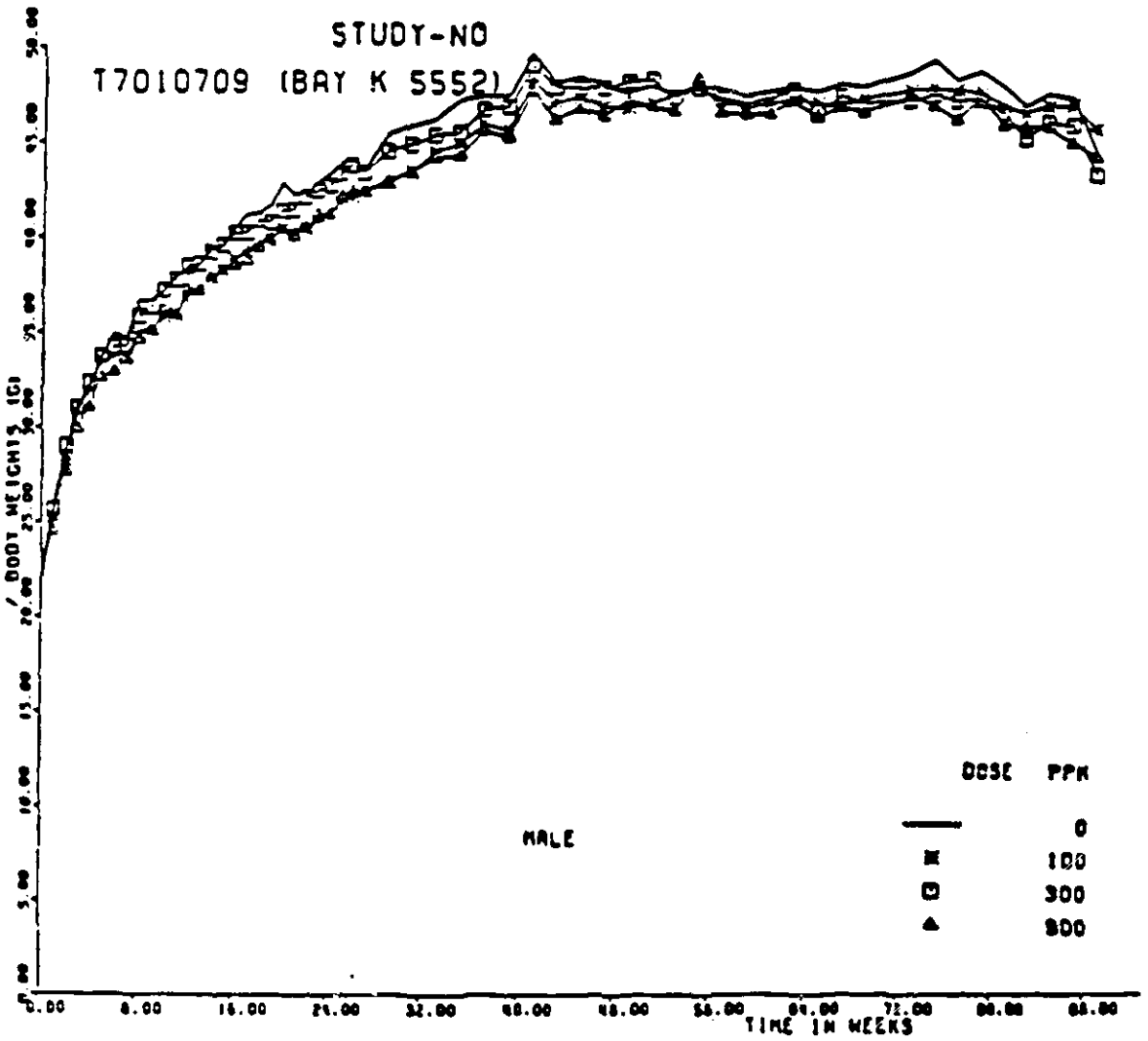
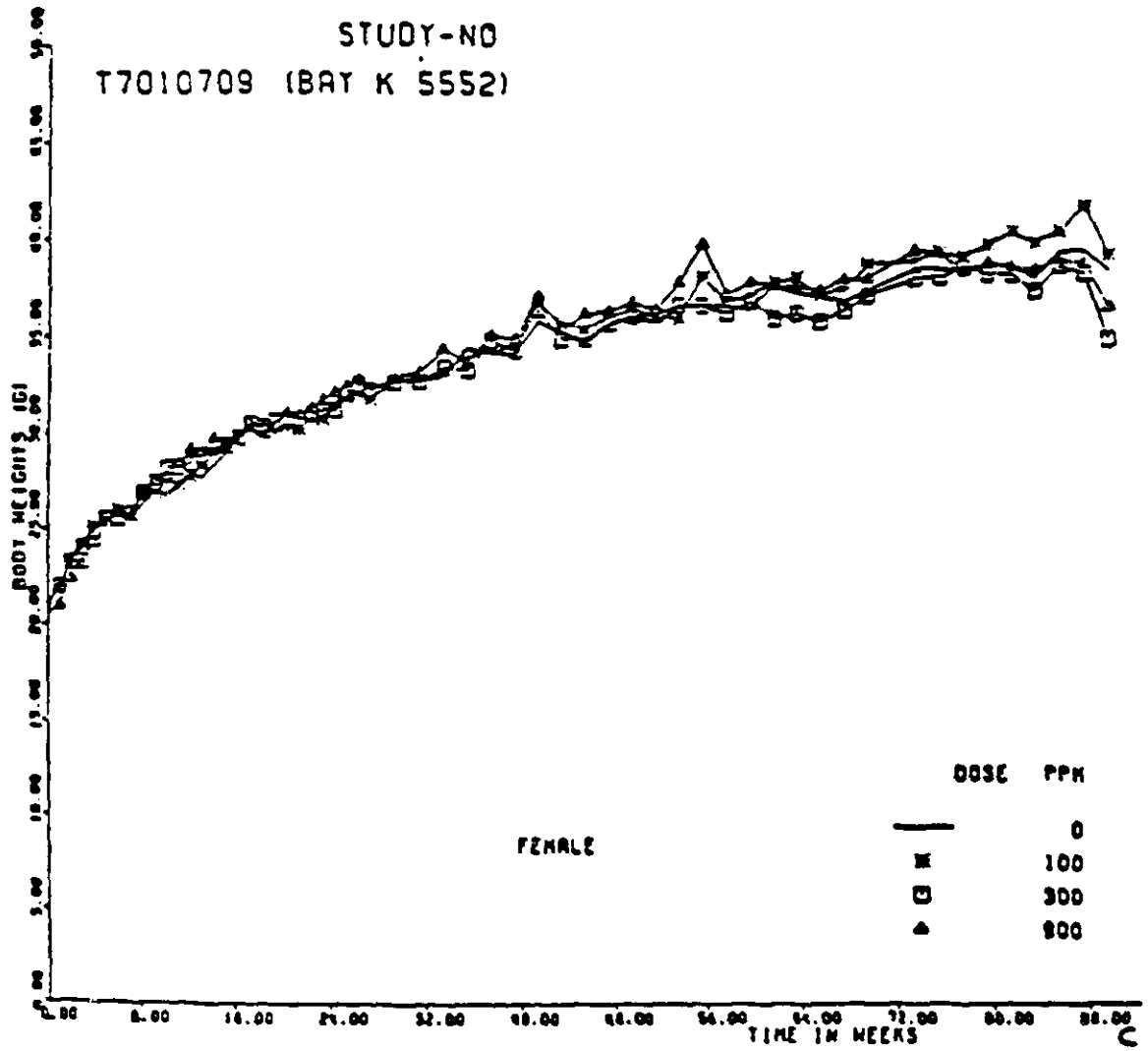


Figure12: Body weight curves for female mice receiving BAY k 5552 in their food for 21 months



significantly higher only at the highest dose level. Females that received either 300 or 900 ppm doses had significantly lower kidney weights at 21 months. Significantly increased liver weights (absolute and relative) were seen in mid and high dose females at the interim sacrifice.

Histologically, hyperplastic mucosa of the glandular stomach was found more frequent in treated males than in females. The incidence of this condition is given below.

Incidence of Hyperplastic Mucosa of the Glandular Stomach

Dose (ppm)	Percent affected	
	Male	Female
0	18	6
100	31	16
300	36	10
900	24	16

The above values are reported to be within the range of historical control values (Table 10).

A dose dependent increase in the occurrence of intracytoplasmic vacuoles near the nucleus was seen in the hepatic cells, more in females than in males. Round cell infiltrates in the kidney and senile nephropathy were predominantly found in females. The endometrium was often found to be hyperplastic (0 ppm - 13%; 100 ppm - 34%; 300 ppm - 33%; and 900 ppm - 28%). The above incidences are reported to be within the historical control ranges for this strain of mouse (Table). An increased incidence of pituitary hyperplasia was seen in females (0 ppm - 17%; 100 ppm - 13%; 300 ppm - 22%; 900 ppm - 42%).

The incidences of tumors observed at the interim sacrifice are given below.

Comparative Summary of Tumors at 12 Months According to Location, Type and Malignancy

Sex:	Males		Females	
Dose (ppm in diet):	0	900	0	900
Reticulocytary system: malignant lymphoma	1	1	2	4
Lung: alveologenic carcinoma (malignant)	0	2	1	2
Liver: hepatocellular carcinoma (malignant)	0	1	0	0
Stomach: kerato-acanthoma	0	0	1	0
Number of blastoma carriers	1	4	4	5
Number of malignant tumors	1	4	3	6
Number of benign tumors	0	0	1	0
Number of mice investigated	20	20	20	20

Table 10

Historic control values: NMRI mouse 1980 to 1984

	Number					
Test No.	1	2	3	4	5	6
Adenomatous gastric mucosal hyperplasias in males	9*	20	10	32	5	27
n	50	50	50	44	47	48
%	18	40	20	73	11	56
in females	1*	8	11	14	10	13
n	50	48	49	46	47	48
%	2	17	22	30	21	27
*classified as adenoma						
Liver tumour in males	7	3	9	5	1	6
n	50	50	50	45	46	48
%	14	6	18	11	2	12
Uterine hyperplasias	0	19	21	23	0	33
n	50	46	49	45	45	46
%	0	41	43	51	0	71

Glucose concentration in the plasma: 4.32 - 9.36 mmol/l (male)
4.51 - 7.75 mmol/l (female)
Urea concentration in the plasma: 5.96 - 15.12 mmol/l (male)
4.05 - 14.99 mmol/l (female)

n = Number of organs evaluated

The increased incidence of lymphoma of reticulohistiocytary system (RHS) observed in high dose females is considered to be incidental since the incidence of this tumor at 21 months was higher in the control group than in treated groups.

The overall incidences of benign and/or malignant tumors and the total number of tumor bearing animals (21 months) for both sexes at 0, 300 and 900 ppm dose levels are given in Table 11. There is no significant increase in the neoplasm incidence among treated animals of either sex compared to respective controls (sponsor's analysis). Moreover, there is also no difference in tumor occurrence between 300 and 900 ppm dosage groups. Incidence of tumors according to the location and the type is presented in Table 12. Because of the increased incidence of hepatocellular tumors in males especially in the 900 ppm group (only few females, 900 ppm group, had this type of tumor) at 21 months and also because of the occurrence of hepatocellular carcinoma in a male mouse from interim sacrifice, an additional investigation was carried out by examining more hepatic tissue sections (5 per animal) from male mice of each group for hepatocellular tumor occurrence. This second study showed additional cases of hepatocellular tumors as follows: 1 each from 100 and 900 ppm groups, 4 from 300 ppm and 1 from control groups. Combined incidences of these tumors (males) from the original and additional investigations (49-50 mice/group) and the p values from the trend test (death rate method) are given below. (The results of sponsor's statistical analyses of liver tumor data are summarized in Table 13.)

	Dose(ppm)				p value
	0	100	300	900	
hepatocellular adenoma	2	2	2	3	0.0735
hepatocellular carcinoma	3	4	5	8	0.0015
hepatocellular tumors (all)	5	6	7	11	0.0004

Thus, the sponsor's analysis showed significant positive linear trends (at 0.05 level) for the incidences of hepatocellular carcinoma and hepatocellular tumors (all) in male mice. Analysis of the tumor data by FDA statisticians showed that there were no significant positive linear trends for the incidences of hepatocellular carcinoma (p=0.0762) and hepatocellular tumors (p=0.0514) in male mice. According to FDA statisticians, the above discrepancies in p values observed in sponsor's and FDA analyses are attributed to "1. the sponsor did not apply the survival-adjusted method and 2. the ordinal dose levels 0, 1, 2 and 3 were used in sponsor's analysis."

Table II : Summary of number of male and female mice with benign and/or malignant tumours, as well as frequency of benign and malignant tumours-encountered

Sex	♂			♀		
Dose ppm	0	300	900	0	300	900
No. of animals investigated	50	50	49	48	50	50
No. of animals with tumours	27	28	24	34	31	30
No. of animals with only benign tumours	7	6	8	6	6	6
No. of animals with only malignant tumours	12	15	14	21	18	17
No. of animals with benign and malignant tumours	8	7	2	7	7	7
No. of animals with more than one primary tumour	15	11	5	13	9	8

Table 12 : Comparative summary of tumours occurring according to location, type, number and dignity $ (animals scheduled for terminal kill)

Sex	♂				♀			
Dose ppm	0	100	300	900	0	100	300	900
Lung:		-				-		
bronchiolar adenoma	2		3	4	2		1	3
bronchiolar carcinoma (malig.)	13		12	5	8		6	5
Stomach:								
papilloma	0	0	0	2	0	0	0	0
sarcoma (malig.)	0	0	2	1	0	0	0	0
Liver:								
hepatocellular adenoma	2	2	2	3	0	0	0	1
hepatocellular carcinoma (malig.)	3	4	5	8	0	0	0	1
haemangiosarcoma (malignant)	1	0	0	0	0	0	0	0
Kidneys:		-				-		
tubular carcinoma (malignant)	0		1	0	0		0	0
haemangiosarcoma (malignant)	0		1	0	0		0	0
Bladder:		-				-		
stromal tumour (benign)	1		1	2	0		0	0
stromal tumour (malignant)	0		1	1	0		0	0
Ovary:	-	-	-	-		-		
granulosa-theca cell tumour (ben.)					5		5	3
granulosa-theca cell tumour (malig.)					1		0	0
luteoma (benign)					2		2	0
tubular adeno-carcinoma (malig.)					1		0	0
Sertoli cell tumour (benign)					0		0	1
Uterus:	-	-	-	-				
adenoma					0	0	1	0
carcinoma (malig.)					1	0	0	0
fibroma					0	0	1	0
myoma					0	0	1	2
myosarcoma (malig.)					0	0	0	1
stromal tumour (benign)					3	0	2	2
stromal sarcoma (malignant)					1	3	2	2

Table 12 (continued):

Sex	♂				♀			
Dose ppm	0	100	300	900	0	100	300	900
Testes:		-			-	-	-	-
Leydig cell tumour (benign)	2		2	0		-		
adenoma of rete testis	1		0	0				
Pituitary:		-						
adenoma	2		0	0	0	3	3	1
Thyroid:		-				-		
follicle cell adenoma	0		1	0	0		0	0
papillary cyst-adenoma	1		0	0	0		0	0
Adrenals:		-				-		
cortical adenoma	3		3	1	2		0	0
phaeochromocytoma (benign)	1		0	0	1		0	2
phaeochromocytoma (malignant)	0		0	1	0		0	0
RH system:		-				-		
lymphoma (malig.)	7		3	1	18		12	14
lymph node sarcoma (malignant)	1		0	0	1		0	0
Skin/subcutis:		-				-		
epithelioma (malignant)	0		0	1	0		0	0
sarcoma (malig.)	1		0	0	0		3	0
Mammary gland:	-	-	-	-		-		
carcinoma (malig.)					3		0	0
adeno-ancanthoma (malignant)					0		1	1
Harder's gland:		-				-		
papillary adenoma	3		2	0	1		1	1
Spinal marrow:		-				-		
schwannoma (malig.)	0		0	0	0		1	0
Bones:		-				-		
osteosarcoma (malignant)	0		0	0	0		1	0
Abdomen:		-				-		
haemangiosarcoma (malignant)	0		0	0	0	-	1	0
Pelvic serosa:		-	-	-	-	-	-	-
sarcoma (malig.)	1							

- Organ not investigated $ Bilateral tumours counted twice

Table 13 : Statistical Analysis of Tumour Data

sex	target character	groups (ppm)	trend test	incidence	ż	p
male	hepatocellular tumours	0/100/300/900	death-rate	5/ 6/ 7/11	3.321	0.0004
male	hepatocellular tumours	0/100/300/900	prevalence	5/ 6/ 7/11	1.833	0.0334
male	hepatocellular tumours	0/100/300	death-rate	5/ 6/ 7	0.821	0.2059
male	hepatocellular tumours	0/900	death-rate	5/11	3.430	0.0003
male	hepatocellular tumours	0/300	death-rate	5/ 7	0.855	0.1964
male	hepatocellular tumours	0/100	death-rate	5/ 6	0.374	0.3541
male	tumour, benign	0/300/900	prevalence	15/13/10	0.286	0.3876
male	tumour, malignant	0/300/900	death-rate	20/22/16	2.692	0.0035
male	hepatocellular adenoma	0/100/300/900	death-rate	2/ 2/ 2/ 3	1.450	0.0[illegible]
male	hepatocellular carcinoma	0/100/300/900	death-rate	3/ 4/ 5/ 8	2.970	0.0015
male	hepatocellular carcinoma	0/100/300/900	prevalence	3/ 4/ 5/ 8	1.931	0.0267
male	hepatocellular carcinoma	0/100/300	death-rate	3/ 4/ 5	0.876	0.1905
male	hepatocellular carcinoma	0/900	death-rate	3/ 8	3.067	0.0011
male	hepatocellular carcinoma	0/300	death-rate	3/ 5	0.919	0.1790
male	hepatocellular carcinoma	0/100	death-rate	3/ 4	0.434	0.3321
female	tumour, benign	0/300/900	prevalence	13/13/13	0.040	0.4842
female	tumour, malignant	0/300/900	death-rate	28/25/24	-0.257	0.6015

Historical Control Data - Spontaneous Tumors in NMRI Mice (1981-1988)

Experiment No.		1		2		3		4		5		6		7		8		9		10	
Sex		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f
Stomach																					
No. of mice examined		48	48	47	47	50	49	50	48	45	46	50	50	49	49	50	48	48	48	49	45
papilloma	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
adenomatous polyp	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
adenoma	b	0	0	0	0	0	0	0	0	0	0	9	1	0	0	0	0	0	0	0	0
adenocarcinoma	m	0	0	0	0	0	0	0	0	0	0	0	0	6	1	0	0	0	0	0	1
spindle cell carcinoma	m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Experiment No.		11		12		13		14		15		16		17		18		total		
Sex		m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m+f
Stomach																				
No. of mice examined		48	47	49	50	50	50	49	48	50	49	49	47	47	45	47	46	875	860	1735
papilloma	b	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	2
adenomatous polyp	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
adenoma	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	1	10
adenocarcinoma	m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	2	8
spindle cell carcinoma	m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1

Historical Control Data - Spontaneous Tumors in NMRI Mice (1974-1979)

Number of tumors of the digestive system (salivary gland, liver, stomach, intestine).

Experiment No.	1		2		3		4		5		6		7		8		9		10		11		12	
	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f
Salivary gland																								
Squamous cell carcinoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Adenoma	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Adenocarcinoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Liver																								
Adenoma, hepatocellular	1	0	4	0	2	0	3	0	0	0	0	0	2	0	3	0	4	1	9	0	1	1	3	0
Carcinoma, hepatocellular	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
Sarcoma	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Forestomach																								
Papilloma	1	0	3	1	0	0	1	2	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	0
Squamous cell carcinoma	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0
Glandular stomach																								
Adenoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	0	0	0	0
Adenocarcinoma	0	0	0	0	0	0	0	0	2	0	0	0	1	1	2	0	1	0	0	0	1	1	0	1

Experiment No.	1	2	3	4	5	6	7	8	9	10	11	12
Number of male animals at start	58	75	75	40	40	40	50	50	50	51	50	50
Number of females animals at start	58	75	75	40	40	40	50	50	50	49	50	50
Number of male animals evaluated	56	75	75	40	40	36	48	47	46	51	50	50
Number of female animals evaluated	56	73	75	40	40	36	45	37	42	49	49	49

FDA analysis of tumor data showed a significant positive linear trend for inverted papilloma of pars cutanea of the stomach in male mice (p=0.0072). The incidence of the above tumor is as follows: 0 ppm - 0/50; 100 ppm - 0/49; 300 ppm - 0/50; and 900 ppm - 2/50. Pairwise comparison also showed significant difference between high dose and control groups (p=0.0435). Historical control data from 21 month studies in NMRI mice, conducted during a 7 year period from July 1981 to August 1988, showed that papillomas of the stomach occurred in 2 of the 18 studies evaluated (page 65e), in 1/49 males and 1/47 females examined (amendment to original application dated May 31, 1994). Moreover, incidence rates upto 4% were seen for the above tumor in NMRI control male mice in carcinogenicity studies conducted between 1974 and 1979 (page 65f). Although statistically significant, the incidence rate (4%) observed in the present study for the stomach papilloma is considered to be within the historical control range for NMRI mice.

Significant positive linear trends were also reported by FDA statisticians for the urinary bladder benign stromal tumor in male mice and RHS malignant lymphoma* in females. However, when the incidences of urinary bladder stromal tumors are combined (benign + malignant, benign + polypous, or benign + malignant + polypous tumors), no statistically significant trend was seen. In the case of RHS malignant lymphoma also, if all malignant lymphomas of different locations are combined, then, no significant linear trend was observed.

*Note: The sponsor has listed all malignant lymphomas, irrespective of locations, under RHS system; however, for some lymphomas, the anatomic site (organ) is specified (e.g. lymphoma of adrenal or heart etc.) but for others no site is given (listed only as lymphomas). By using the combined incidences of all lymphomas, no treatment-related increased incidence of this tumor was seen in sponsor's statistical analysis. [According to NTP guidelines (McConnel et al, 1986. Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. JNCI 76: 283-289), lymphomas of all types can be combined for statistical evaluation.]

DOG STUDIES (S.Stolzenberg)

a. 4-Week Oral Administration Study

Testing Facility:

Pharma-Report No: 7075
Study No: Not given

Study Dates: 11/8/76 to 12/9/76

GLP compliance: This study predates GLP compliance requirements.

Animals: Purebred beagles, 2 males and 2 females per group were used. At the start of dosing, the animals were 25 to 30 weeks oid, with body weights of 7.4 to 11.8 kg.

Dose Levels/Mode of Administration: The test substance (Batch 2/76) was administered at doses of 0, 1, 3 and 10 mg/kg, once daily, 4 to 6 hours before feeding, in a vehicle of polyethylene glycol 400, glycerol and water, in gelatin capsules.

Observations/Measurements: Body weights were obtained before the start of treatment and weekly thereafter. Appearance, behavior, body posture, appetite and feces were checked daily. Neurological investigations (pupillary reflex, patellar reflex and extensor postural reflex) and body temperature measurements (rectal) were conducted pretreatment and after 2 and 4 weeks. Ophthalmoscopy (direct) was performed at pre-treatment and after 4 weeks. ECG measurements (Leads I, II and III) were recorded on the 1st, 11th and 23rd day, immediately before administration and 1 and 24 hours after administration. Femoral artery blood pressure was measured on the 1st, 11th and 23rd day, before administration and 1 and 24 hours after, via a Stratham element, Hellige measuring bridge and Hellige recorder. Blood and 6 hour urine samples were obtained before treatment, then after 1 and 4 weeks, for hematology, blood chemistry and urinalysis. Post-mortem examination included weights of 12 or 13 major organs (including gonads and prostate), gross pathology and complete histopathology (31 or 32 organs).

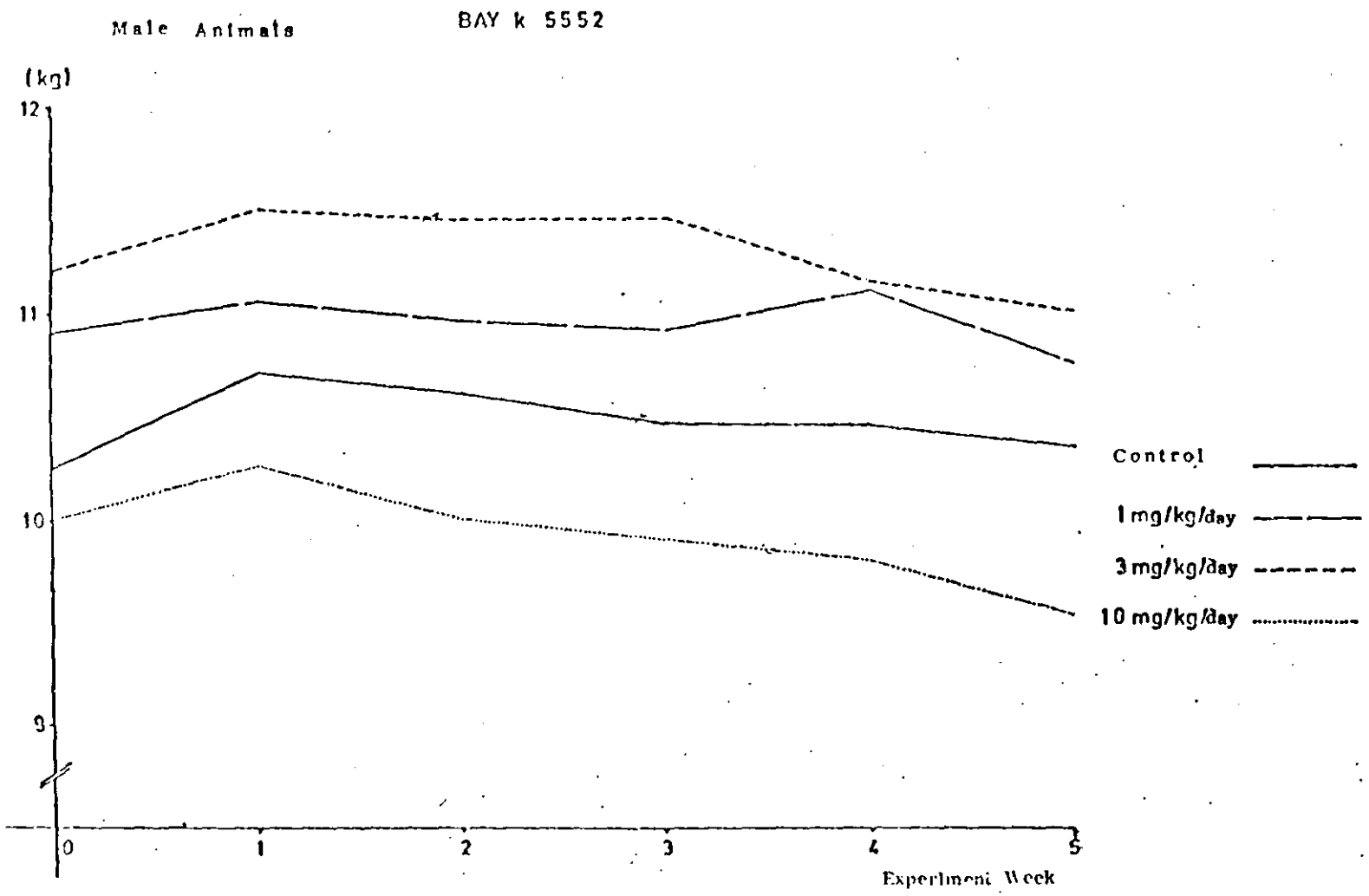
Mortality: There were no deaths.

Drug Associated Findings: Slightly reduced weight gain was observed in the high dose males, with a reduced food consumption in both high dose females and in one high dose male, from the middle of the third week to the end of the study. In the 10 mg/kg treated animals, a distinct ST drop (manifestation of a possible myocardial ischemia) was observed in one male 1 hour after the 1st and 23rd dose, and in one female 1 hour after the 1st dose. No treatment related effects on P or Q waves or QRS complex were observed at any time. Heart rates determined from

ECGs, showed dose dependent increases one hour after dosing on days 1, 11 and 23, and with the high dose, bradycardia was still evident 24 hours after dosing on days 11 and 23. Systolic and diastolic blood pressures at 1 hour post dosing were decreased by a mean of 30 to 50% in all treated groups (dose dependent) on days 1, 11 and 23. As a rule, blood pressures returned to pre-treatment levels by 24 hours after treatment, except after day 1, when they remained lower for the 1 and 10 mg/kg groups.

Although no gross pathology or organ weight changes due to treatment were noted, histopathology revealed that the hearts of both females and 1 of the 2 males on the high dose had myocardial scars in one or both left ventricular papillary muscles. The effect was attributed to hypoxic damage related to vasodilator-induced heart rate increase, "a known damage mechanism in the dog". The ST drops noted above were observed in two of the dogs with myocardial scars. The ST drops and the bradycardia (which was most pronounced in a male with the most severe lesions) were attributed to the heart muscle damage.

Weight Gains of the Male Dogs. The weights were measured in each case at the end of the experimental week.



BAY k 5552

Heart Rate (Beats per Minute)

(Average Values)

Dose mg/kg	Time of Investigation	Before Administration	1 Hour After Administration	% Deviation from 1-Hour Value	24 Hours After Administration
0	Preliminary Investigation	136			
	1st Administration	148	115	- 22	140
	11th Administration	118	108	- 8	125
	23rd Administration	123	123	0	118
1	Preliminary Investigation	158			
	1st Administration	143	253	+ 77	160
	11th Administration	120	235	+ 96	113
	23rd Administration	113	223	+ 97	110
3	Preliminary Investigation	128			
	1st Administration	133	195	+ 47	143
	11th Administration	113	213	+ 88	110
	23rd Administration	98	213	+ 117	90
10	Preliminary Investigation	133			
	1st Administration	133	210	+ 58	148
	11th Administration	68	223	+ 228	75
	23rd Administration	80	200	+ 150	81

Blood Pressure (mmHg)

(Average Values)

Dose mg/kg	Time of Investigation	Before Administration		1 Hour After Administration		% Deviation from 1-Hour Value		24 Hours After Administration	
		s	d	s	d	s	d	s	d
0	Preliminary Investigation								
	1st Administration	172	107	179	99	+ 4	- 7	181	101
	11th Administration	176	95	171	102	- 3	+ 7	174	110
	23rd Administration	181	78	191	96	+ 6	+ 24	187	97
1	Preliminary Investigation								
	1st Administration	171	95	115	63	- 33	- 34	116	83
	11th Administration	176	101	96	54	- 45	- 47	181	110
	23rd Administration	173	94	114	68	- 34	- 28	193	118
3	Preliminary Investigation								
	1st Administration	177	101	99	52	- 44	- 49	179	96
	11th Administration	178	106	78	49	- 56	- 54	176	109
	23rd Administration	178	99	107	53	- 40	- 46	188	96
10	Preliminary Investigation								
	1st Administration	177	94	114	51	- 36	- 47	149	74
	11th Administration	194	111	98	51	- 49	- 54	194	111
	23rd Administration	203	111	116	53	- 43	- 52	231	133

s = systolic pressure d = diastolic pressure

Histological Data

BAY k 5552, Oral, Dogs (2 Week Experiment)

Animal No.	Sex	Dose and Frequency of Administration	Heart	Lung	Liver	Spleen	Kidney	Adrenals
F 813	♂	Control (0 mg/kg)	0	Ici +	0	0	0	0
F 823	♂	"	0	Ici +	Ici +	0	0	0
F 800	♀	"	0	Ici +	Ici +	0	0	0
F 802	♀	"	0	Ici +	Ici +	0	0	0
F 807	♂	10 mg/kg	Fi2	Ici +	0	0	0	0
F 817	♂	"	0	Ici +	0	0	0	0
F 814	♀	"	Fi1-2	Ici1	Ici +	0	0	0
F 818	♀	"	Fi +	Ici2	Ici +	0	0	V+

List of Abbreviations

Histological Data

At	=	Atrophy
Cy	=	Cyst
Fi	=	Focal fibrosis with isolated mononuclear cells (Figures 4 and 5)
Ici	=	Cellular or inflammatory-cellular infiltration
P	=	Parasitic lesion (bore hole, granuloma, eosinophilic infiltration)
0	=	Finding within the normal variability, which, in particular, corresponds to the species and to the age of the experimental animals and to their conventional housing conditions
∅	=	Not investigated (section missing)
Pi	=	Yellow-green (hematogenous) pigment
Th	=	Thrombus
V	=	Cytoplasmic vacuoles

Intensity of the Changes

+	=	very slight, indicated
1	=	slight
2	=	moderate
3	=	severe

b. 13-Week Oral Administration Study in Dogs

Pharma-Report No: 10,380
Study No: B/K 5552/023

Performing Laboratory:

Dates Performed: 8/21/80 to 11/25/80

Quality Assurance: No statement of GLP compliance is included.

Test Animals: Purebred beagles, 3 males and 3 females per group, were used. At the start of dosing, the animals were 38 to 51 weeks old, with body weights of 6.8 to 10.8 kg.

Dose Levels/Mode of Administration: The test substance (Batch 576,923) was administered at doses of 0, 1, 2.5 and 6.25 mg/kg, once daily, 4 to 6 hours before feeding, in a vehicle of polyethylene glycol 400, glycerol and water, in gelatin capsules.

Observations/Measurements: Body weights were obtained before the start of treatment and weekly thereafter. Appearance, behavior, body posture, appetite and feces were checked daily. Neurological examinations (pupillary reflex, patellar reflex and extensor postural reflex), ophthalmoscopic examinations (direct) and body temperature measurements (rectal) were performed pretreatment and after 2, 5 and 12 weeks. Femoral artery blood pressure was measured at the time of the first dose, and in weeks 3, 6 and 13, before administration, and 1 and 24 hours after, via a Stratham element, Hellige measuring bridge and Hellige recorder; ECG measurements (Leads I, II and III) were recorded at the same time periods. Blood and 6 hour urine samples were obtained before treatment, then after 3, 6 and 13 weeks, for hematology, blood chemistry and urinalysis. Postmortem examination included weights of 12 (female) or 13 (male) organs, gross pathology and complete histopathology (31 or 32 organs for control and high dose, but all 3 doses for heart).

Mortality: There were no deaths.

Drug Associated Findings: Circumoral reddening of the skin and reddening of the conjunctiva in the mid and high dose groups, and ataxia in the high dose group, occurred regularly throughout the treatment period, around 1 hour after dosing. Blood pressure decreased (systolic decreased to a greater extent than the diastolic), and heart rate increased (data on heart rate not provided by sponsor) at 1 hour post dosing in all 3 treated groups. Neither of these two effects were considered to be dose related, and values returned to pretreatment levels by 24 hours post-treatment. No changes in ECG occurred at low and mid doses. One high dose male developed a ventricular tachycardia with a "bundle-branch-block-like deformation of the QRS complex",

diagnosed 1 hour after the first dose. For this animal, another ECG was taken on the following day 2 hours after dosing; the P wave was still elevated and the ST segment again showed sagging depression. "On the 19th day in this dog, no pathological finding in the ECG was observed" but this animal showed extra systoles and an elevated P wave. Serum chemistry effects included a small increase in GOT during week 6. The only compound related post-mortem finding noted was scaring of the left ventricular papillary muscles of 1 male and 1 female at the high dose and 1 female at the mid dose. Histopathology revealed focal fibrosis with isolated mononucler cells and a cellular and inflammatory-cellular infiltration.

Study No.: B/K 5552/023

Blood Pressure (mm Hg) - Average Values

Dose mg/kg	Time of Examination	Before administration		1 hour after administration		% Deviation of 1 hour value		24 Hours after administration	
		s	d	s	d	s	d	s	d
0	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration	210	130	180	110	-14	-15	180	110
	in the 3rd week	200	120	190	120*	- 5	0	205	125**
	in the 6th week	225	135	170	90	-24	-33	165	105**
	in the 13th week	200	120	205	130	+ 2	+ 8	195	120*
1.0	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration	190	115	140	75*	-26	-35	175	115
	in the 3rd week	195	115	135	75	-31	-35	190	120
	in the 6th week	185	115	85	50	-54	-57	175	100**
	in the 13th week	190	120	130	80	-32	-33	195	115**
2.5	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration	190	105	145	70	-24	-33	180	105
	in the 3rd week	195	115	115	60	-41	-48	200	120
	in the 6th week	195	115	100	50	-49	-56	175	105
	in the 13th week	195	120	130	70	-33	-42	175	110
6.25	Preliminary examination	-	-	-	-	-	-	-	-
	1st administration -	185	115	110	60	-41	-48	185	125
	in the 3rd week	190	115	110	55	-42	-52	170	110**
	in the 6th week	185	115	80	45	-57	-61	170	105
	in the 13th week	195	120	100	60**	-49	-50	190	125

s = systolic blood pressure; d = diastolic blood pressure *n = 5; **n = 4

BAY k 5552/Study 023

Animal No.	Sex	Dose	Esophagus	Stomach	Intestine	Mesenteric Lymph Nodes	Thymus	Gall-bladder	Urinary Bladder
K 103	♂	Control	0	0	0	0	0	0	0
K 109	♂	Control	0	0	0	ø	At3	0	0
K 121	♂	Control	0	0	0	0	0	0	0
K 108	♀	Control	0	0	0	0	0	0	0
K 112	♀	Control	0	0	0	0	At2	0	0
K 120	♀	Control	0	0	0	0	ø	0	0
K 93	♂	6.25 mg/kg	0	0	0	0	ø	0	0
K 115	♂	6.25 mg/kg	0	0	0	0	0	0	0
K 117	♂	6.25 mg/kg	0	0	0	0	ø	0	0
K 102	♀	6.25 mg/kg	0	0	0	P/Icl1	At1	0	0
K 104	♀	6.25 mg/kg	0	0	0	P/Icl2	0	0	0
K 118	♀	6.25 mg/kg	0	0	0	0	0	0	0

BAY k 5552/Study 023

Animal No.	Sex	Dose	Heart	Animal No.	Sex	Dose	Heart
K 103	♂	Control	0	K 113	♂	2.5 mg/kg	0
K 109	♂	Control	0	K 119	♂	2.5 mg/kg	0
K 121	♂	Control	0	K 123	♂	2.5 mg/kg	0
K 108	♀	Control	0	K 122	♀	2.5 mg/kg	F11 P1+
K 112	♀	Control	0	K 124	♀	2.5 mg/kg	0
K 120	♀	Control	0	K 126	♀	2.5 mg/kg	0
K 93	♂	6.25 mg/kg	0	K 79	♂	1.0 mg/kg	0
K 115	♂	6.25 mg/kg	F12-3 Ic11	K 105	♂	1.0 mg/kg	0
K 117	♂	6.25 mg/kg	0	K 107	♂	1.0 mg/kg	0
K 102	♀	6.25 mg/kg	(	K 110	♀	1.0 mg/kg	0
K 104	♀	6.25 mg/kg	F11	K 114	♀	1.0 mg/kg	0
K 118	♀	6.25 mg/kg	0	K 116	♀	1.0 mg/kg	0

c. 52-Week Oral Administration Study in Dogs

Study No: T 20 10 506

Performing Laboratory:

Dates Performed: July 13, 1981 to July 11, 1982

Quality Assurance: No statement of GLP compliance is included.

Test Animals: Purebred beagles, 4 males and 4 females per group. At the start of dosing, the animals were 38 to 51 weeks old, with body weights of 6.9 to 11.2 kg.

Dose Levels/Mode of Administration: The test substance (batch 57 69 23) was administered at doses of 0, 0.3, 1.0 and 3.0 mg/kg, once daily, 7 days per week, 4 to 6 hours before feeding, in a vehicle consisting of 85.3% polyethylene glycol 400, 4.8% anhydrous glycerol and 9.9% water, contained in gelatin capsules.

Observations/Measurements: Body weights were obtained before the start of treatment and weekly thereafter. General appearance was checked "several times a day". Neurological exams were conducted and body temperatures were checked pretreatment and after 3, 6, 17, 29, 39 and 50 weeks. Ophthalmoscopy was performed pre-treatment and after 12, 31, 38 and 51 weeks. Blood pressure and ECG were measured pre-treatment and after 3, 6 and 17, 29, 39 and 50 weeks, before dosing, then 1 and 24 hours after dosing. The methods and instruments used were the same as in the preceding dog studies. Blood and 6 hour urine samples were obtained before treatment, then after 3, 6, 13, 26, 39 and 52 weeks, for hematology, blood chemistry and urinalysis. Postmortem examination included weights of 11 or 12 organs, gross pathology and complete histopathology (31 or 32 organs for all animals on test). Liver enzyme induction of O-demethylase, N-demethylase and cytochrome P_{450} content of liver homogenates were measured.

Mortality: No deaths occurred.

Drug Associated Findings: Slight reddening of the mucosa and skin, observed in all nisoldipine treated groups, was considered to be due to the vasodilator effect of the drug. Dose related decreases in blood pressure and resultant increases in heart rate were observed. Twenty-four hours after dosing, all values had returned to normal. Slight ST segment depression, T wave inversion and QT segment shortening were observed, which were all reversible (data on ECG could not be found) and considered to be due to increased heart rate.

Study No: T 20 10 506

BLOOD PRESSURE (mm Hg)

(Means n = 8)

DOSE	TIME	before admin.	1 h after admin.	% difference **	24 h after admin.
0.0 mg/kg	1st admin.	195/112	198/113	+1.5/+0.9	199/114
	17th admin.	194/112	193/119	-0.5/+6.3	
	38th admin.	184/102	187/106	+1.6/+3.9	
	114th admin.	210/116	205/116	-2.4/0.0	
	200th admin.	199/108	204/113	+2.5/+4.6	
	269th admin.	211/108	195/110	-7.6/+1.9	
	347th admin.	201/118	204/119	+1.5/+0.8	
0.3 mg/kg	1st admin.	208/119	*167/ 97	-19.7/-18.5	193/115
	17th admin.	203/111	164/ 98	-19.2/-11.4	
	38th admin.	180/ 98	163/ 88	- 9.4/-10.2	
	114th admin.	219/118	171/ 98	-21.9/-16.9	
	200th admin.	191/113	153/ 86	-19.9/-23.9	
	269th admin.	205/114	168/ 90	-18.0/-21.1	
	347th admin.	215/124	184/105	-14.4/-15.3	

* n = 7

** Calculation using unrounded figures

Study No: T 20 10 506

BLOOD PRESSURE (mm Hg)

(Means n = 8)

DOSE	TIME	before admin.	1 h after admin.	% difference**	24 h after admin.
1.0 mg/kg	1st admin.	179/104	141/ 76	-21.2/-26.9	173/104
	17th admin.	177/101	136/ 76	-23.2/-24.8	
	38th admin.	181/ 98	116/ 68	-35.9/-30.6	
	114th admin.	184/106	133/ 75	-27.7/-29.0	
	200th admin.	178/107	136/ 79	-23.6/-26.3	
	269th admin.	196/109	135/ 73	-31.1/-33.0	
	347th admin.	195/115	161/ 93	-17.6/-19.1	
3.0 mg/kg	1st admin.	195/114	116/ 64	-40.7/-43.9	194/115
	17th admin.	200/127	133/ 71	-33.5/-44.1	
	38th admin.	186/112	*106/ 59	-43.0/-47.3	
	114th admin.	212/126	124/ 66	-41.6/-47.6	
	200th admin.	197/119	133/ 76	-32.5/-36.1	
	269th admin.	218/123	115/ 62	-47.2/-49.6	
	347th admin.	206/124	134/ 69	-35.0/-44.4	

* n = 7

** Calculation using unrounded figures

Study No: T 20 10 506

HEART RATES (beats/min)

(means, n = 8)

(calculation using unrounded figures)

DOSE (mg/kg)	Initial Figure	TIME OF INVESTIGATION													
		1st admin.			17th admin		38th admin		114th admin.		200th admin.		269th admin.		347th admin.	
		before	1 h	24 h	before	1 h	before	1 h	before	1 h	before	1 h	before	1 h	before	1 h
0.0	134*	120	113*	134	128	132*	136	133	133	121	129	138	119	119	101**	112
Diff %			-5.9		+3.1		-2.2		-9.0		+7.0		0.0		+10.9	
0.3	138	136	172	141	134	190	137	194	123	177	128	205	118*	178	113*	161
Diff %			+26.5		+41.8		+41.6		+43.9		+60.2		+50.8		+42.5	
1.0	147	134	191	135	130	209	131	218	106	221	113	220	96	207	111	153
Diff %			+42.5		+60.8		+66.4		+108.5		+94.7		+115.6		+37.8	
3.0	146	134	211	128	120	234	113	201	101	224	99	215	90	209	93	186*
Diff %			+57.5		+95.0		+77.9		+121.8		117.2		+132.2		+100.0	

*n = 7
**n = 6

CHRONIC TOXICITY STUDY ON DOGS

B A Y K 5 5 5 2 STUD.T2 010 506

SYNOPSIS OF GROUP MEANS

		HEART / HERZ	LUNG / LUNGE	LIVER / LEBER	KIDNEYS / NIEREN	SPLEEN / MILZ	TESTES / HODEN	OVARIES / OVARIEN	THYROID / SCHILD-DRUESE	ADREN. / NEBEN-NIEREN	THYMUS / THYMUS	PROSTA. / PRO-STATA	BRAIN / GEHIRN	PANC. / PAN-KREAS
ABSOLUTE ORGANWEIGHTS (G) / ABSOLUTE ORGANGEWICHTE (G)														
MALES / MN.TIERE														
CNTR./KONTR.		98.6	96.8	436.3	57.0	30.3	17.30	-	0.825	1.277	5.15	8.087	78.8	33.0
GROUP/GRUPPE	I	108.0	91.8	423.0	56.5	29.8	18.38	-	0.727	1.162	5.32	6.910	75.5	35.0
GROUP/GRUPPE	II	103.3	100.5	484.3	66.3	37.3	20.92	-	0.860	1.335	5.20	5.707	82.3	36.8
GROUP/GRUPPE	III	111.3	93.8	472.0	61.3	68.0	20.42	-	0.805	1.310	5.65	7.340	78.5	33.5
FEMALES / WB.TIERE														
CNTR./KONTR.		95.8	86.0	409.8	53.0	26.3	-	0.937	0.867	1.385	6.96	-	76.5	25.5
GROUP/GRUPPE	I	102.8	96.5	385.5	57.5	57.5	-	0.832	0.797	1.550	7.27	-	73.3	29.5
GROUP/GRUPPE	II	96.8	82.5	393.5	53.3	38.0	-	1.133	0.807	1.672	7.67	-	77.8	32.3
GROUP/GRUPPE	III	103.0	90.3	393.8	59.3	41.0	-	1.787	0.860	1.587	7.17	-	77.0	31.0
BOTH SEXES / ALLE TIERE														
CNTR./KONTR.		97.3	91.4	424.3	55.0	28.3	17.30	0.937	0.846	1.331	6.05	8.087	77.4	29.3
GROUP/GRUPPE	I	105.4	94.1	404.3	57.0	43.6	18.38	0.832	0.762	1.356	6.30	6.910	74.4	32.3
GROUP/GRUPPE	II	100.0	91.5	438.9	59.8	37.6	20.92	1.133	0.834	1.504	6.44	5.707	80.0	33.9
GROUP/GRUPPE	III	107.1	92.0	432.9	60.3	54.5	20.42	1.787	0.832	1.447	6.41	7.340	77.8	32.3
RELATIVE ORGANWEIGHTS (G/KG) / RELATIVE ORGANGEWICHTE (G/KG)														
MALES / MN.TIERE														
CNTR./KONTR.		9.12	9.00	40.25	5.25	2.85	1.667	-	0.0780	0.1200	0.480	0.7577	7.42	3.05
GROUP/GRUPPE	I	10.17	8.62	39.77	5.32	2.85	1.760	-	0.0687	0.1100	0.514	0.6585	7.15	3.30
GROUP/GRUPPE	II	9.15	8.87	42.67	5.85	3.30	1.842	-	0.0757	0.1175	0.462	0.5085	7.30	3.20
GROUP/GRUPPE	III	10.02	8.45	42.75	5.52	6.25	1.842	-	0.0730	0.1180	0.510	0.6627	7.10	3.05
FEMALES / WB.TIERE														
CNTR./KONTR.		9.50	8.55	40.77	5.25	2.50	-	0.0912	0.0865	0.1372	0.667	-	7.65	2.55
GROUP/GRUPPE	I	9.37	8.85	38.88	5.25	5.20	-	0.0755	0.0722	0.1415	0.652	-	6.70	2.65
GROUP/GRUPPE	II	9.72	8.25	44.17	5.32	3.77	-	0.0869	0.0812	0.1690	0.767	-	7.82	3.22
GROUP/GRUPPE	III	9.87	8.62	37.32	5.65	3.85	-	0.1642	0.0805	0.1535	0.670	-	7.45	3.05
BOTH SEXES / ALLE TIERE														
CNTR./KONTR.		9.31	8.77	40.51	5.25	2.67	1.667	0.0912	0.0822	0.1286	0.556	0.7577	7.54	2.80
GROUP/GRUPPE	I	9.77	8.74	43.28	5.29	4.02	1.760	0.0755	0.0705	0.1257	0.575	0.6585	6.92	2.97
GROUP/GRUPPE	II	9.44	8.56	43.42	5.59	3.54	1.842	0.0869	0.0785	0.1432	0.615	0.5085	7.56	3.21
GROUP/GRUPPE	III	9.95	8.54	40.74	5.59	5.05	1.842	0.1642	0.0767	0.1358	0.590	0.6627	7.27	3.05

REPRODUCTIVE TOXICITY STUDIES (S. Stolzenberg)

1. Fertility and Reproduction Ability in Wistar Rats

Bayer Study No: T0002152

This report is accompanied by a "first amendment to report no. 12691", dated 8/11/93. Tables in the original English translation of the report were of very poor quality, not legible, contained errors in translation and typing, and a few tables were not logically organized. Some of these faults are listed in the amendment under the "rationale for the first amendment".

Performing Laboratory:

Dates Performed: 2/81 to 9/81

Quality Assurance: No statement on GLP compliance was found in the original report. In the amendment, it is claimed, "...there were no legally binding GLP regulations in force" during the time that this study was performed.

Test Animals: Mura:WIST (SPF 67 HAN), 24 males and 60 females per group. At the start of dosing, males were 5-7 weeks old, and weighed 74-110 g, females were 8-10 weeks old and weighed 158-190 g.

Procedure: The test substance (batch 576 923) was administered at doses of 0, 3, 10 and 30 mg/kg, once daily, by oral gavage in a vehicle consisting of polyethylene glycol 400:glycerol:water in a ratio of 969:60:100. Males were dosed starting 10 weeks before mating and during the 3 week mating period, females were dosed for 3 weeks prior to mating until the 7th day of pregnancy. Except during mating and lactation, both the males and females were kept in individual Makrolon cages. Each male was paired with 2 or 3 females, which were placed together in a Makrolon cage each night and the females were examined for vaginal sperm in the morning. Half the pregnant females in each group, selected by "statistical methods", were C-sectioned on day 20 of gestation, the remaining half were allowed to litter and raise their young to postpatum day (PPD) 21. All C-sectioned fetuses were examined for external anomalies, 1/3 from each dam were examined for soft tissue anomalies (modified Wilson method) and 2/3 for skeletal malformations (alizarin red S). In addition to examining the F_0 parents for reproductive performance, the F_0 females for lactational performance and the F_1 offspring for survival and weight gain during lactation, one male and one female from each litter of the control group and of the highest dose group were reared to sexual maturity to determine F_1 reproductive capacity. The mated F_1 dams were allowed to litter, and testicular weights for F_1 males were obtained after mating.

Test substance administered was Batch 576 923. It is claimed that the preparations for oral gavage were tested for stability and concentration but the data and details for these tests were not included in the report.

There is no statement on why these doses were selected for this study.

Effects on *F_0 Males*

All treated and control males survived to scheduled necropsy and no compound related clinical signs were evident in males of any treated group. There were no effects on weight gain, mating behavior or fertility in males of any treated group compared to controls. There were no effects on gross pathology observed at necropsy (presumably sacrificed after mating and while still on drug treatment). The drug had no effect on testicular weights (See page which follows).

STUDY ON FERTILITY BAY K 5552 T0002152

BODYWEIGHTS [G] OF THE MALES BEFORE MATING
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEEK 10	90.5	87.3	91.0	89.9
	9.6	7.7	7.9	7.4
WEEK 9	136.5	132.5	135.4	131.7
	12.7	11.0	10.0	9.2
WEEK 8	175.0	172.0	174.3	173.3
	16.8	14.8	14.3	13.6
WEEK 7	215.5	212.3	215.7	213.5
	22.0	17.0	16.7	17.8
WEEK 6	252.5	249.6	249.6	252.1
	26.4	19.5	18.6	20.2
WEEK 5	279.0	276.3	276.1	280.6
	29.9	21.6	21.9	23.4
WEEK 4	291.5	291.5	287.6	294.5
	31.9	23.4	26.1	23.6
WEEK 3	311.4	309.9	308.8	314.0
	33.5	25.2	25.1	26.0
WEEK 2	331.5	329.7	327.1	331.6
	34.8	27.1	28.5	28.5
WEEK 1	344.5	345.9	341.6	346.9
	35.0	28.8	29.8	30.0
WEEK 0	360.7	358.9	354.7	360.4
	35.6	29.5	31.4	30.8

TESTICLE WEIGHTS [G]

GROUP MEAN VALUES AND STANDARD DEVIATIONS

0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
3.23	3.26	3.17	3.26
0.30	0.20	0.31	0.25

Effects on F_0 Females

Mortality: Deaths are listed only in the narrative portion of this report. In both the original report and the amendment, there is no indication of the time of death; not even if the deaths occurred before mating, during pregnancy or lactation. Deaths occurred in two rats at 3 mg/kg, in one at 10 mg/kg and in two at 30 mg/kg, but none of the deaths were attributed to treatment. Based on scrutinization of tables in the original report, the 2 animals in the 30 mg/kg group which died had both been assigned to "rearing animals". Deaths were attributed to misintubation for a low and mid dose rat, "gastrointestinal disorders" for the second low dose rat, to a lung tumor and to pneumonia for the two high dose rats. In addition, one dam in the control group, which had littered 12 pups and died shortly after birth, was not included in the results because at necropsy only 4 nidation sites were found.

Even in the amended tables for individual animal data, there is no indication of which animals died and the time of the deaths. Numerous animals were dropped from the study for a variety of reasons, which included, "not inseminated", "not pregnant", and for a few, there is a statement "animal dropped from the study" but no reason is given. Most summary tables do not specify the number of animals per group upon which the data are based. Therefore, the following table lists the total number of females in each group that were included in the results, based on a count taken from the individual animal body weight data.

Dosage Group	# C-Sectioned*	# Littered*
Control	25	22
3 mg/kg/day	23	27
10 mg/kg/day	24	20
30 mg/kg/day	27	22

* There were 60 mated females per group at initiation of the study, 30 of which were designated for C-section or littering.

Body Weight and Body Weight Gain: Mean body weight gains and body weights of pregnant females, those that were C-sectioned and those that were allowed to litter, are given on the two pages which follow. Body weights 3 weeks before mating (prior to initiation of treatment) and during pregnancy, were significantly lower for the high dose group of the C-sectioned animals, but there was no effect on body weight gain. Although a small increase in body weight gain was noted for the low dose C-sectioned group between days 7 and 20 of gestation, there was obviously no effect that could be attributed to treatment. No effects on mean body weight or body weight gain were observed in the females selected for delivery of litters during the 3 weeks prior to gestation, during gestation or during lactation.

STUDY ON FERTILITY BAY K 5552 T0002152

WEIGHT DEVELOPMENT [G] OF THE FEMALES UNDERGOING CESAREAN SECTION
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEIGHT GAIN				
DAY 0 - 7 P.C.	22.2	23.9	21.7	23.0
	4.7	5.1	5.1	5.6
DAY 7 - 20 P.C.	73.3	82.1*	76.2	71.4
	10.1	12.6	10.8	17.1
DAY 0 - 20 P.C.	95.5	106.0*	97.9	94.4
	12.4	15.5	12.6	18.1
BODYWEIGHTS BEFORE MATING				
WEEK 3	173.6	172.3	170.3	168.6**
	7.0	6.8	6.8	6.3
WEEK 2	187.1	187.1	186.0	184.9
	8.7	9.5	8.7	9.1
WEEK 1	198.9	197.3	198.3	194.9
	10.0	9.3	9.5	9.7
WEEK 0	209.7	210.2	210.8	204.8
	10.9	11.3	11.6	9.7
BODYWEIGHTS DURING GESTATION				
DAY 0 P.C.	223.8	223.2	223.9	213.5**
	11.5	13.9	13.6	12.4
DAY 7 P.C.	245.9	247.0	245.6	236.5*
	13.2	14.3	13.4	13.5
DAY 20 P.C.	319.2	329.1	321.8	308.0
	17.2	23.2	17.4	23.3

* SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.01$
** SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.005$

T0002152

WEIGHT DEVELOPMENT [G] OF THE DAMS
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEIGHT GAIN				
DAY 0 - 7 P.C.	23.7	19.9	23.7	21.4
	6.2	5.1	5.2	4.4
DAY 7 - 20 P.C.	73.5	70.3	77.0	71.7
	13.3	13.8	13.0	14.2
DAY 0 - 20 P.C.	97.2	90.2	100.7	93.1
	14.6	14.5	11.7	15.0
BODYWEIGHTS BEFORE MATING				
WEEK 3	173.7	171.6	172.5	172.4
	8.2	7.9	8.2	8.9
WEEK 2	186.1	186.1	188.8	187.6
	9.2	10.2	10.1	10.1
WEEK 1	194.7	196.8	200.2	198.6
	11.1	11.1	10.3	12.4
WEEK 0	206.3	208.7	211.0	208.7
	13.1	12.8	10.8	14.2
BODYWEIGHTS DURING GESTATION				
DAY 0 P.C.	216.4	225.0	224.1	217.5
	16.4	16.8	13.6	18.7
DAY 7 P.C.	240.1	244.9	247.8	238.9
	19.4	16.5	11.0	20.1
DAY 20 P.C.	313.5	315.3	324.8	310.6
	25.3	26.1	19.6	31.2
BODYWEIGHTS DURING LACTATION				
DAY 1 P.P.	244.8	250.0	253.7	240.3
	18.9	18.1	15.1	21.3
WEEK 1 P.P.	272.3	277.8	281.6	267.1
	20.5	19.4	14.3	24.4
WEEK 2 P.P.	272.5	278.7	283.3	271.7
	18.8	16.8	14.0	20.1
WEEK 3 P.P.	258.6	265.5	266.7	259.4
	19.0	17.8	15.3	18.2

C-Section *F_0 Females*

As seen in the tables which follow, there were no effects of compound treatment on number or percentages of animals inseminated, with implantations and with live fetuses, mean corpora lutea count, nidations, average number of male or female live fetuses, sex ratio or fetal loss. From these data, it is evident that there were no effects on pre- or post-implantation losses.

The mean fetal weights were significantly increased (apparently dose related) in the 10 and 30 mg/kg groups, and the mean placental weight was slightly but significantly increased in the 3 mg/kg group. The investigators claimed that the mean placental weight increase was incidental, and that the mean fetal weights for the mid and high dose groups were within the norm for this strain (given as 3.5 ± 0.27, based on 268 litters).

There were no compound related effects on mean numbers of gross, visceral or skeletal malformations, nor were there any effects on "underdeveloped forms" (fetuses weighing <3 g). There were also no effects on minor skeletal variations.

STUDY ON FERTILITY BAY K 5552

NUMBER OF ANIMALS - RESULTS OF THE STUDY

ANIMALS UNDERGOING CESAREAN SECTION

DOSE (MG/KG)	USED	NUMBER OF FEMALES INSEMINATED N	% OF THOSE USED	WITH IMPLANTATIONS N	% OF THOSE INSEMINATED	WITH FOETUSES N	% OF THOSE WITH IMPLANTATIONS
0	30	27	90.0	25	92.6	25	100.0
3	29	27	93.1	23	85.2	23	100.0
10	30	26	86.7	25	96.2	24	96.0
30	30	28	93.3	27	96.4	27	100.0

STUDY ON FERTILITY BAY K 5552 T0002152

RESULTS OF THE CESAREAN SECTION (MEAN VALUES)

DOSE [MG/KG]	WEIGHT GAIN [G] 0-20 P.C.	WEIGHT GAIN [G] 7-20 P.C.	NUMBER (PER DAM) OF CORP. LUTEA	IMPL.	MALE	FEM.	SUM	LOSS	MEAN-WEIGHT IN GRAMMS FETUSES	PLACENT.	NO. OF FOETUSES EXAMINED BY WILSON	DAWSON	FOETUSES WITH MINOR SKELETAL DEVIAT.	MALFORMATIONS	NO. OF RUNTS (<3G)
C	95.5	73.3	12.4	11.9	6.5	4.9	11.2	0.7	3.49	0.50	3.3	7.9	3.52	0.04	0.52
3	106.0**	82.1**	12.4	12.0	6.3	5.0	11.4	0.6	3.58	0.53*	3.5	7.8	3.48	0.09	0.30
10	97.9	76.2	11.4	11.0	5.6	5.0	10.6	0.4	3.60*	0.50	3.4	7.6	2.67	0.00	0.25
30	94.4	71.4	11.6	11.0	5.4	4.9	10.3	0.7	3.63**	0.52	3.0	7.3	3.22	0.00	0.19

* SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.025
** SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.01

F_1 Females Allowed to Litter

Pregnancy duration was slightly increased in all 3 treated groups (statistically significant for the 3 and 30 mg/kg groups; see table below). This effect was considered to be "incidental" because the mean durations for these groups were within the norm for this strain. No effects during lactation were noted.

Postpartum Examination of Pups: There were no significant effects on total number of live pups at birth per group, nor on number of viable pups after 1, 2 or 3 weeks postpartum (See table below). It was claimed there were no treatment related effects on number of stillborn pups, and on sex ratio at birth or at the 3 weekly intervals. Mean birth weight was slightly higher for all 3 treated groups (statistically significant for low and high dose), but mean weight and weight increase during the 3 weekly intervals were not influenced by treatment (See table below).

Maturational Development: There were no effects on age of pinna unfolding of the ears, hair coat, eye opening or normal gait.

Function Tests: There were no effects on sight or pupillary reflexes to light, hearing ("pinna twitch reflex", tested by means of a Galton whistle with a set frequency and duration). In a proprioreceptive reflex test (running roller brought from stationary position to 10 revolutions per minute) there was a decrease in performance at 30 mg/kg during the first test but no effect in the second or third test. The age of the animals when these tests were done was not indicated.

Fertility Test of F_1 Generation: There was no effect of treatment with 30 mg/kg on mating, fertility, duration of pregnancy, litter size, live and dead pups, sex ratio, mean weights of the pups or external anomalies at birth.

T0002152

\AMS

DOSE (MG/KG)	USED	N	INSEMINATED % OF THOSE USED	NUMBER OF FEMALES WITH IMPLANTATIONS N	% OF THOSE INSEMINATED	N	THAT LITTERED % OF THOSE WITH IMPLANTATIONS	THAT REARED THEIR PUPS N	% OF THOSE THAT LITTERED
0	29	24	82.8	22	91.7	22	100.0	22	100.0
3	29	28	96.6	27	96.4	27	100.0	27	100.0
10	29	22	75.9	20	90.9	20	100.0	20	100.0
30	28	26	92.9	22	84.6	22	100.0	22	100.0

DURATION OF PREGNANCY IN DAYS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
21.9	22.2*	22.1	22.2**
0.5	0.6	0.6	0.4

* SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.05
** SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.025

NUMBER OF IMPLANTATIONS OF THE DAMS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
10.8	10.4	11.0	11.2
3.0	2.9	2.4	2.8

PRENATAL LOSS OF DAMS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
0.5	0.6	0.4	0.9
0.7	0.9	1.0	1.1

STUDY ON FERTILITY BAY K 5552 T0002152

NUMBER AND WEIGHT DEVELOPMENT OF THE VIABLE PUPS
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION		0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
NUMBER OF PUPS					
AT BIRTH	TOTAL	10.4 3.0	9.8 2.8	10.4 2.5	9.7 2.7
	MALES	5.2 1.8	5.2 2.3	5.2 1.6	4.9 1.8
	FEMALES	5.2 2.3	4.6 1.7	5.3 2.0	4.8 2.1
AFTER 1 WEEK	TOTAL	10.2 3.0	9.7 2.7	10.4 2.5	9.4 2.9
	MALES	5.0 1.9	5.2 2.3	5.2 1.6	4.8 1.8
	FEMALES	5.1 2.3	4.5 1.7	5.3 2.0	4.5 2.3
AFTER 2 WEEKS	TOTAL	10.0 3.0	9.6 2.7	10.3 2.5	9.3 3.0
	MALES	5.0 1.9	5.1 2.2	5.1 1.7	4.7 1.9
	FEMALES	5.0 2.3	4.5 1.7	5.1 2.0	4.5 2.3
AFTER 3 WEEKS	TOTAL	10.0 3.0	9.6 2.7	10.2 2.5	9.3 3.0
	MALES	5.0 1.9	5.1 2.2	5.0 1.7	4.7 1.9
	FEMALES	5.0 2.3	4.5 1.7	5.1 2.0	4.5 2.3
WEIGHT [G] OF THE VIABLE PUPS					
AT BIRTH		5.9 0.5	6.2** 0.5	6.1 0.6	6.2* 0.6
AFTER 1 WEEK		14.0 2.2	14.8 1.6	14.9 1.8	15.0 2.2
AFTER 2 WEEKS		24.7 4.4	26.1 3.5	26.1 3.3	26.4 4.4
AFTER 3 WEEKS		38.1 6.1	40.4 5.8	39.7 5.8	41.5 7.4

* SIGNIFICANT DIFFERENCE TO CONTROL, $P \leq 0.05$
** SIGNIFICANT DIFFERENCE TO CONTROL, $P < 0.025$

2. Embryotoxic and Teratogenic Action in Long-Evans Rats

Pharma Report No: 7596 Study No: T2012540

Performing Laboratory:

This study was originally presented as a translation from with only a few brief summary tables; no individual animal data. Amendments received at CDER on 9/29/93 and 10/8/93 contain tables with individual animal findings and summaries. It is claimed that the study was carried out between January and May, 1977, "in accordance with FDA recommendations", but there is no statement of GLP compliance.

Procedure: Naturally inseminated Long-Evans female (strain FB 30) rats, 20 or 21 per group, 2.5 to 3.5 months of age and weighing 195 to 262 g prior to mating, received 0, 10, 30 or 100 mg nisoldipine/kg/day by oral gavage (batch 3/76, micronized), from days 6 to 15 of gestation. The drug was dissolved in polyethylene glycol 400/glycerol/water. A C-section was performed for each dam on day 20 of gestation and the fetuses were examined for external, visceral (Wilson technique) and skeletal (alizarin red stain) anomalies.

Effects on Survival and Body Weights of Dams: One control rat died on gestation day 13 or 14, due to improper intubation into lungs, and was excluded from results. There was no compound related effect on mortality, nor on "general appearance or behavior" of the dams, but there was a dose related decrease in mean weight gain (see table which follows).

Dose (mg/kg)	Weight Gain in Grams	
	Treatment Period	Total Pregnancy
0	62.3	152.4
10	56.1	140.3
30	53.0*	132.6*
100	49.6*	131.8*

*) Significant difference from the control, $P < 0.01$

C-Section of Dams: Of the 21 inseminated rats in each of the 3 compound treated groups, 20 were pregnant, and all 20 surviving rats in the control group were pregnant. All pregnant treated and control rats had live fetuses at necropsy on day 20 of gestation. Corpora lutea count for each rat was not determined in this experiment, but no statistically significant differences between the treated groups and control were found for mean number of implantations, mean number of fetuses, mean number of dead fetuses and resorbed embryos, mean fetal weight, underdeveloped forms (fetuses <3 g in weight), mean placental weight, frequency of fetuses with minor skeletal deviations, sex distribution (see page 94), nor on external, soft tissue or bone deformations (see table below on this page).

Group	Dam No.	Number of Malformed Fetuses	Malformation
Control	664	2	Rib dysplasia (hump formation)
10 mg/kg	681	1	Edematous head
30 mg/kg	—	—	None
100 mg/kg	605	1	Rib dysplasia (hump formation)
	639	1	Cryptorchidism
	647	1	Kinking of the tail
	683	1	Hydrops universalis, micrognathia, kinking of the tail

BAY k 5552 T2012540

Results of the Caesarean Section

Mean values of the groups and standard deviations

Note: The mean fetal and placental weights given in the report no. 7596 were calculated by adding all litter weights of the group and by dividing these sums by the number of fetuses or placentas per group. In the following table these mean values are marked with "a".
For the calculation of the standard deviation the mean fetal and placental weights per litter were calculated first and were used for further calculation. Mean fetal and placental weights obtained by this procedure are marked with "b".

Dose mg/kg	Weight gain (g) during pregnancy	Weight gain (g) during treatment period	Number (per dam) of fetuses impl.	male	female	total	res.**	Mean weight (g) of fetuses	Mean weight (g) of placentas	Number of fetuses with minor skeletal deviations	Number of fetuses with malformations	Number of fetuses runts < 3 g
0	152.4	62.3	11.6	5.8	5.4	11.1	0.4	4.26[a] 4.27[b]	0.57[a] 0.58[b]	2.95	0.10	0.00
	18.5	11.7	1.4	2.3	2.3	1.7	0.7	0.29	0.06	2.11	0.45	0.00
10	140.3	56.1	11.0	5.5	5.0	10.5	0.5	4.07[a] 4.07[b]	0.59[a] 0.59[b]	2.60	0.05	0.00
	23.9	9.7	2.9	1.8	1.6	2.8	0.8	0.29	0.11	2.19	0.22	0.00
30	132.6*	53.0*	11.3	5.3	5.1	10.3	0.9	4.08[a] 4.09[b]	0.57[a] 0.57[b]	3.50	0.00	0.05
	19.9	8.1	2.5	2.4	2.5	2.6	1.3	0.33	0.05	2.61	0.00	0.22
100	131.8*	49.6*	11.9	5.5	5.2	10.7	1.1	4.12[a] 4.14[b]	0.57[a] 0.57[b]	4.10	0.20	0.00
	21.2	11.2	2.7	2.4	2.1	2.7	1.4	0.23	0.05	2.45	0.41	0.00

* significant difference to control, $p < 0.01$ (WILCOXON-MANN-WHITNEY-U-TEST)

** Res. is the abbreviation for resorptions, which the sponsor defined as the total of resorbed embryos and dead fetuses

3. Teratology Study in Sprague Dawley (CD) Rats

Study No: Not provided. Report No. 87/0938

Performing Laborat

Sponsor:

Dates Performed: 8/5/87 to 12/2/87

Quality Assurance: A signed statement of GLP compliance is included.

Test Animals: Charles River CD (Sprague-Dawley derived) females, 9-10 weeks of age and weighing 200-248 g on the day of insemination, were mated on a 1:1 basis with stock males of the same strain.

Procedure: The test substance (batch number 500139) was administered to 32 inseminated females per group, once daily by oral gavage as a suspension in 0.5% aqueous Tylose, prepared fresh each day, from days 7 to 17 of gestation, at doses of 0, 10, 30 and 100 mg/kg. On day 20 of gestation, 21 dams per group were C-sectioned and the fetuses were examined for external anomalies; 1/2 from each dam were examined by free hand serial sectioning (Wilson technique) for soft tissue anomalies. The remaining half were first dissected (neck, thoracic and abdominal cavities) to evaluate for soft tissue anomalies, then were prepared by a modification of Dawson's alizarin staining technique for evaluation of skeletal malformations.

The remaining 11 dams/group were allowed to litter and raise their young to postpatum day 25. On PPD 4, litters with more than 8 were reduced to 8 by random culling, leaving, if possible, 4 of each sex per litter. After weaning, the offspring were housed on a litter basis, but the sexes were separated and there was a maximum of 5 of the same sex per cage. At approximately 5 weeks of age, following completion of behavioral and neuromuscular function tests, 20/sex/group were randomly selected for further assessment of physical, sexual maturation and reproductive performance; unselected ones were killed and grossly examined. At 9 or 10 weeks of age, F_1 males and females were paired 1:1 within treatment groups, avoiding sibling matings. All F_1 mated females were laparotomized on day 20 of gestation; the fetuses were examined only for external malformations and discarded. After gross examination of the F_1 females, F_1 males were killed, then examined externally and internally for macroscopic abnormalities.

Stability of Test Substance: Test formulations for all 3 concentrations, taken from the first and last weeks of treatment,

were generally found to be within approximately 82 to 90% of the target concentrations, and were stable for at least 4 hours after preparation.

Results

F_0 Females

Mortality and Clinical Signs: No data on mortality, and no statement in the text pertaining to mortality, were found; all rats apparently survived. It is claimed that one dam receiving 100 mg/kg showed flaccid muscle tone and piloerection during the early stages of treatment (possibly compound related), but other females in that group were not affected.

Body Weights/Food Consumption: Small reductions in body weight gain (not statistically significant) were evident in all 3 treated groups during the initial day or 2 of treatment (See table on the page which follows), and this was accompanied by significant reductions in food intake by the mid and high dose groups, limited to the first 3 days of treatment. Subsequent body weight gains in all 3 treated groups were not affected by treatment, but the body weights remained below control to the day of necropsy. The lower mean body weights of the mid and high dose groups compared to controls were statistically significant only on day 18 of gestation.

Laparotomy Observations: There were no effects on mean corpora lutea counts, total implantatiions, viable males or females. There was a slight increase in total resorptions ($P< 0.05$) predominently due to number of late resorptions, and in percent post-implantation loss ($P< 0.05$), in the high dose group. Fetal weights were depressed in all 3 treated groups; statistically significant and dose related at mid and high dose (See table two pages ahead).

Fetal Evaluation: There was an increased incidence of small fetuses (<2.7 g) and litters with one or more small fetuses in the 100 mg/kg group. An increased number of fetuses with slightly increased dilatation of lateral ventricles and/or space between the body wall and organs, occurred mainly in two litters, and this was associated with fetuses of low body weight in these two litters. The investigators considered this to be indicative of fetal immaturity. Also associated with the fetuses weighing <2.7 g in 2 litters of the high dose group and considered to be due to fetal immaturity, was an increased incidence of incomplete ossification of basisphenoid, first thoracic vertebral centrum, sacral vetebral arches, ischia, metacarpals and metatarsals.

Group mean bodyweights (g) of females during gestation

Group	:	1	2	3	4
Compound	:	Control	--- BAY k 5552		--
Dosage (mg/kg/day)	:	0	10	30	100

Group		Day of gestation														
		0	3	7	8	9	10	11	12	13	14	15	16	17	18	20
1	Mean	219	238	255	261	266	272	278	284	291	298	307	317	330	346	378
	SD	8	9	12	11	12	12	12	13	13	13	14	15	16	17	18
	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
2	Mean	217	237	251	256	261	267	273	279	285	292	300	312	323	338	368
	SD	10	12	14	12	13	12	14	13	14	14	15	16	16	17	18
	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
3	Mean	217	237	253	255	260	266	272	278	284	291	299	311	323	338*	367
	SD	11	13	14	13	14	14	14	15	15	15	17	17	18	19	21
	n	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
4	Mean	215	235	253	250	252	261	267	272	278	285	293	303	316	327***	361
	SD	8	9	12	12	12	13	12	12	13	13	13	16	17	18	20
	n	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31

SD Standard deviation.
n Number of pregnant animals.
* Bodyweight gain from Day 7 significantly different from Controls, $P<0.05$ (one way analysis of variance and Student's t-test).
*** Bodyweight gain from Day 7 significantly different from Controls, $P<0.001$ (one way analysis of variance and Student's t-test).

Group mean litter data - females killed on Day 20 of gestation

Group	: 1	2	3	4
Compound	: Control	--- BAY k 5552 ---		
Dosage (mg/kg/day)	: 0	10	30	100

Group	Number of pregnant animals		Corpora lutea count	Implant-ations	Viable young			Resorptions			Implantation loss (%)		Foetal weight (g)	Placental weight (g)
					M	F	Total	Early	Late	Total	Pre-	Post-		
1	21	Mean	17.1	15.6	8.0	6.9	14.9	0.5	0.1	0.7	9.4	4.3	3.50	0.53
		SD	1.7	1.3	2.3	1.9	1.1	0.7	0.4	0.8			0.06	0.01
2	21	Mean	16.9	14.9	7.0	7.0	14.0	0.8^{NS}	0.1	0.9^{NS}	11.6	6.1^{NS}	3.45	0.53
		SD	1.5	1.4	1.8	2.0	1.5	0.9	0.4	1.0			0.06	0.02
3	21	Mean	16.4	14.9	6.4	7.1	13.5	1.2^{NS}	0.1	1.4^{NS}	9.8	9.3^{NS}	3.34*	0.54
		SD	1.7	1.4	2.3	2.3	2.4	1.1	0.4	1.2			0.06	0.02
4	20	Mean	16.9	15.1	6.7	6.8	13.5	0.9^{NS}	0.8^{NS}	1.6^{+}	11.2	10.6^{+}	3.19***	0.52
		SD	1.9	3.3	2.3	2.6	3.6	0.9	0.9	1.3			0.09	0.03
Background control (159 studies)														
Mean			15.9	14.5	6.7	6.9	13.7	0.69	0.18	0.87	8.7	6.0	3.32	0.50
Low			13.9	12.0	5.2	5.6	11.1	0.05	0.00	0.25	1.6	1.7	3.00	0.43
High			19.0	16.7	8.2	8.7	15.3	1.68	0.58	1.79	16.5	12.7	3.55	0.57

SD Standard deviation.
* Significantly different from Control, P<0.05 (Nested analysis of variance and weighted t-test).
*** Significantly different from Control, P<0.001 (Nested analysis of variance and weighted t-test).
NS Not significant (Mann Whitney 'U'-test).
+ Significantly different from Control P<0.05 (Mann Whitney 'U'-test)

Summary of foetal observations at necropsy

Group	: 1	2	3	4
Compound	: Control	--- BAY k 5552		--
Dosage (mg/kg/day)	: 0	10	30	100

Group :	1	2	3	4	Control data	
External examination						
Number of foetuses (litters) examined:	313(21)	294(21)	284(21)	269(20)	39809 foetuses	159 studies
Number of male : female foetuses:	168:145	147:147	135:149	134:135		
Observations: % incidence[♦] (number of litters)					Mean	Study ranges
Small foetus (less than 2.70 g)	1.0(3)	0.7(2)	1.4(4)	11.9(6)	3.5	0 - 16.9
Large foetus (more than 4.00 g)	2.9(3)	1.4(3)	1.4(2)	-	1.3	0 - 8.7
Shiny pup	-	0.3(1)	-	1.1(1)	0.3	0 - 4.1
Pale pup	-	-	-	0.4(1)	0.02	0 - 1.1
Domed head	-	0.3(1)	-	-	0.01	0 - 0.4
Subcutaneous haemorrhage on chin	0.3(1)	-	-	-	0.1	0 - 0.7
Small placenta (less than 0.30 g)	-	-	-	0.4(1)	0.2	0 - 2.3
Large placenta (more than 0.70 g)	2.2(4)	2.0(6)	3.2(4)	3.0(3)	1.3	0 - 6.2
Conjoined placentae	-	0.3(1)	-	-	0.03	0 - 0.8
Dark green material surrounding placenta	-	0.3(1)	-	-	0.1	0 - 6.9
Short tail	-	-	-	0.4(1)	0.01	0 - 0.5
Threadlike tail	0.3(1)	-	-	-	0.02	0 - 0.8
Imperforate anus	0.3(1)	-	-	-	0.04	0 - 0.8

♦ One foetus may have more than one observation.

continued

Summary of foetal observations after free-hand serial sectioning

Group	:	1	2	3	4
Compound	:	Control	--- BAY k 5552 ---		
Dosage (mg/kg/day)	:	0	10	30	100

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined.*	156(21)	140(21)	143(21)	134(20)	12835	126
Number of males : females	82:74	78:70	70:73	68:66	foetuses	studies
Observations: % foetal incidence (number of litters affected)					Mean	Study ranges
Abdomen:						
Diaphragmatic hernia	0.6(1)	-	-	0.7(1)	0.1	0 - 1.9
Small additional liver lobe(s)	25.6(19)	32.4(18)	23.1(18)	28.4(17)	0.8	0 - 10.9
Hepatic haemorrhage(s)	9.0(8)	12.2(15)	7.7(7)	9.0(11)	10.3	0 - 27.7
Localised internal abdominal haemorrhage	1.9(3)	-	0.7(1)	2.2(3)	1.3	0 - 6.7
Haemorrhagic peritoneal fluid	0.6(1)	-	0.7(1)	-	2.4	0 - 18.0
Haemorrhagic abdomen	0.6(1)	0.7(1)	0.7(1)	-	1.7	0 - 8.0
Left kidney displaced slightly towards midline	-	-	-	0.7(1)	0.05	0 - 1.7
Small haemorrhage within capsule of right kidney	-	0.7(1)	-	-	0.02	0 - 1.0
Unilateral hydronephrosis	1.3(1)	3.4(3)	1.4(2)	1.5(2)	2.6	0 - 11.7
Bilateral hydronephrosis	-	1.4(1)	-	-	0.9	0 - 9.8
Unilateral hydroureter	13.5(12)	7.4(6)	7.7(6)	14.2(13)	6.7	0 - 24.2
Bilateral hydroureter	2.6(2)	7.4(4)	2.8(4)	4.5(4)	4.4	0 - 27.1
Testis(es) displaced slightly†	11.0(7)	12.8(7)	8.6(6)	10.3(7)	3.7	0 - 23.5
Fluid-filled vesicle at anal edge of genital tubercle	-	0.7(1)	-	-	*	*
Genital tubercle slightly elongated	-	-	-	3.0(2)	0.4	0 - 6.3
Blood in anus	-	1.4(2)	0.7(1)	1.5(2)	0.2	0 - 7.2
Threadlike tail; imperforate anus; displacement of adrenal glands and kidneys	0.6(1)	-	-	-	0.08	0 - 1.8
Tip of tail threadlike and hooked	0.6(1)	-	-	-	*	*

* One foetus may have more than one observation.
† Percentage calculated on number of male foetuses.
* No record in background control data.

continued

Summary of foetal observations after free-hand serial sectioning

Group	:	1	2	3	4
Compound	:	Control	---	BAY k 5552	--
Dosage (mg/kg/day)	:	0	10	30	100

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined.*	156(21)	148(21)	143(21)	134(20)	12835	126
Number of males : females	82:74	78:70	70:73	68:66	foetuses	studies
Observations: % foetal incidence (number of litters affected)					Mean	Study ranges
Thorax:						
Oesophagus displaced to right of trachea	-	-	-	0.7(1)	0.01	0 - 1.2
Left lobe of thyroid gland very reduced in size/absent	-	-	-	0.7(1)	0.1	0 - 2.2
Space between bodywall and organs	-	2.0(2)	-	11.2(5)	10.9	0 - 47.4
Retro-oesophageal right subclavian artery and misshapen thymus gland	-	0.7(1)	-	-	0.02	0 - 1.2
Blood-filled thoracic lymph duct	0.6(1)	1.4(2)	0.7(1)	-	0.3	0 - 2.9
Innominate artery reduced in length/absent	-	1.4(2)	1.4(1)	0.7(1)	0.1	0 - 1.4
Gross cardiovascular abnormality with valve defects	-	-	-	0.7(1)	*	*
Gross cardiovascular abnormality with double outlet right ventricle	-	-	-	0.7(1)	*	*
Slightly increased amount of pericardial fluid	1.3(2)	0.7(1)	1.4(2)	0.7(1)	0.3	0 - 5.3
Slightly haemorrhagic pericardial fluid	-	0.7(1)	1.4(1)	-	0.2	0 - 9.1
Haemorrhages on edge of lung lobes	-	-	-	1.5(2)	0.1	0 - 2.2

* One foetus may have more than one observation.
* No record in background control data.

continued

Summary of foetal observations after free-hand serial sectioning

Group	: 1	2	3	4
Compound	: Control	---	BAY k 5552	--
Dosage (mg/kg/day)	: 0	10	30	100

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined.*	156(21)	148(21)	143(21)	134(20)	12835	126
Number of males : females	82:74	78:70	70:73	68:66	foetuses	studies
Observations: % foetal incidence (number of litters affected)					Mean	Study ranges
Others:						
Subcutaneous haemorrhage(s):						
Lower/side of jaw	3.2(3)	7.4(4)	4.2(4)	5.2(3)	0.8	0 - 8.9
Submandibular	2.6(2)	3.4(5)	6.3(7)	6.0(6)	1.0	0 - 8.8
Nasal	1.3(2)	3.4(5)	0.7(1)	2.2(2)	1.1	0 - 5.9
Cranial	1.9(3)	4.1(6)	1.4(2)	2.2(3)	2.5	0 - 8.2
Ventral/dorsal cervical	0.6(1)	2.7(3)	1.4(2)	6.7(5)	1.1	0 - 5.8
Scapular	11.5(12)	17.6(11)	16.1(12)	15.7(11)	28.6	6.4 - 90.5
Lateral/ventral/dorsal thoracic	2.6(3)	5.4(7)	7.7(8)	5.2(5)	1.5	0 - 5.8
Fore-/hind-limb(s)	17.3(10)	13.5(7)	10.5(7)	16.4(12)	*	*
Lateral/dorsal abdominal	0.6(1)	2.0(3)	0.7(1)	0.7(1)	0.6	0 - 3.8
Anal region	-	0.7(1)	-	0.7(1)	0.5	0 - 10.0
Tail	-	-	0.7(1)	0.7(1)	0.7	0 - 10.6
Subcutaneous oedema - trunk	2.6(2)	6.1(4)	4.2(5)	9.0(8)	3.6	0 - 17.5

* One foetus may have more than one observation.
* No record in background control data.

continued

Summary of foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	-----	BAY k 5552	-----
Dosage (mg/kg/day)	:	0	10	30	100

Group :	1	2	3	4	Control data	
Number of foetuses (litters) examined:	157 (21)	146 (21)	141 (21)	135 (20)	19316 foetuses	129 studies
Observations :Grand Mean % foetal incidence @ (number of litters)					Mean	Study ranges
Vertebrae,limbs and girdles						
Ossification of ventral arch of 1st cervical vertebra.	7.0 (7)	4.8 (6)	7.1 (5)	2.2 (3)	6.94	0.0 - 22.2
Incomplete ossification, one or more cervical vertebral arches.	0.6 (1)	1.4 (2)	0.7 (1)	0.0 (0)	0.50	0.0 - 5.2
1st thoracic vertebral centrum unossified.	0.6 (1)	1.4 (1)	0.7 (1)	7.4 (2)	1.10	0.0 - 5.5
Incomplete ossification, one or more thoracic vertebral centra.	27.4 (15)	19.9 (16)	25.5 (17)	27.4 (14)	26.66	8.6 - 58.3
Incomplete ossification of one or more lumbar vertebral centra.	0.0 (0)	0.7 (1)	0.0 (0)	0.7 (1)	0.41	0.0 - 2.5
Incomplete ossification of one or more lumbar vertebral arches.	0.0 (0)	1.4 (2)	0.0 (0)	0.0 (0)	0.12	0.0 - 1.8
Incomplete ossification of sacral vertebral centra.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	0.01	0.0 - 0.5
Incomplete ossification of one or more sacral vertebral arches.	1.9 (3)	2.1 (3)	3.5 (5)	9.6 (4)	1.17	0.0 - 6.2
Short tail, tip thickened.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	*	
25 pre-sacral vertebrae.	1.3 (2)	0.0 (0)	2.8 (4)	0.7 (1)	0.81	0.0 - 6.7
Incomplete ossification of caudal vertebrae (less than 5).	1.3 (2)	1.4 (2)	1.4 (2)	14.1 (5)	2.96	0.0 - 14.5
Metacarpals/metatarsals 3/4.	69.4 (20)	56.2 (20)	80.1 (21)	74.1 (19)	67.30	28.6 - 86.9
Metacarpals/metatarsals 4/4.	29.3 (14)	43.2 (16)	19.9 (10)	16.3 (12)	30.65	6.2 - 71.4
Metacarpals/metatarsals incompletely ossified or unossified.	6.4 (5)	4.8 (5)	5.0 (6)	14.1 (6)	3.05	0.0 - 10.8
One or more phalangeal bones ossified.	1.3 (1)	6.2 (5)	2.1 (2)	0.7 (1)	1.89	0.0 - 8.1
Inner corners of one or both scapulae unossified.	5.7 (6)	2.7 (2)	9.2 (9)	5.2 (4)	3.59	0.0 - 14.4
Pubic bones incompletely ossified or unossified.	7.6 (6)	4.1 (6)	7.1 (5)	14.8 (8)	7.43	0.0 - 18.6
Incomplete ossification of one or both ischial bones.	1.9 (3)	2.1 (3)	5.0 (3)	5.2 (5)	0.90	0.0 - 4.7
Asymmetric pelvis, ilial bones associated with different sacral vertebrae.	0.0 (0)	0.0 (0)	1.4 (2)	0.7 (1)	0.49	0.0 - 3.7

@ One foetus may have more than one observation
* New parameter, no control data available

- continued

Summary of foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	-----	BAY k 5552	-----
Dosage (mg/kg/day)	:	0	10	30	100

Group :	1	2	3	4	Control data	
Number of foetuses (litters) examined:	157 (21)	146 (21)	141 (21)	135 (20)	19316 foetuses	129 studies
Observations :Grand Mean % foetal incidence @ (number of litters)					Mean	Study ranges
Sternebrae and ribs						
Incomplete ossification of 1 sternebra.	18.5 (13)	19.2 (12)	9.2 (8)	11.9 (9)	13.53	0.0 - 40.0
Incomplete ossification of 2 sternebrae.	66.9 (21)	65.1 (21)	75.9 (21)	48.9 (18)	66.88	43.3 - 84.8
Incomplete ossification of 3 sternebrae.	7.6 (8)	11.6 (7)	11.3 (11)	23.0 (14)	11.80	1.1 - 23.3
Incomplete ossification of 4 sternebrae.	4.5 (4)	3.4 (4)	1.4 (2)	7.4 (9)	3.68	0.0 - 17.5
Incomplete ossification of 5 sternebrae.	*0.0 (0)*	*0.0 (0)*	*2.1 (1)*	*3.0 (2)*	*0.00*	*0.0 - 3.8*
Incomplete ossification of 6 sternebrae.	1.3 (2)	0.0 (0)	0.0 (0)	5.2 (2)	0.53	0.0 - 6.7
1st sternebra cleft.	0.6 (1)	0.7 (1)	0.7 (1)	5.2 (4)	0.86	0.0 - 7.6
One or more sternebrae offset.	2.5 (4)	1.4 (1)	2.1 (3)	0.0 (0)	1.32	0.0 - 5.2
Ribs 13/13.	100.0 (21)	97.3 (21)	98.6 (21)	97.0 (20)	98.17	92.5 -100.0
Ribs 13/14.	0.0 (0)	1.4 (2)	1.4 (2)	1.5 (2)	1.20	0.0 - 4.2
Ribs 14/14.	0.0 (0)	1.4 (2)	0.0 (0)	1.5 (2)	0.51	0.0 - 3.5
14th rib enlarged.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	*	
13th rib or ribs reduced in length.	1.3 (2)	2.7 (3)	2.8 (3)	2.2 (2)	2.31	0.0 - 13.4
Slight medial thickening of one or more ribs.	0.0 (0)	0.7 (1)	0.0 (0)	0.0 (0)	0.02	0.0 - 1.4

@ One foetus may have more than one observation
* New parameter, no control data available

Summary of foetal observations at skeletal examination

Group	:	1	2	3	4
Compound	:	Control	------	BAY k 5552	-----
Dosage (mg/kg/day)	:	0	10	30	100

Group :	1	2	3	4	Control data	
Number of foetuses (litters) examined:	157 (21)	146 (21)	141 (21)	135 (20)	19316 foetuses	129 studies
Observations :Grand Mean % foetal incidence @ (number of litters)					Mean	Study ranges
Head						
Small anterior fontanelle.	1.9 (2)	0.0 (0)	0.7 (1)	0.0 (0)	1.33	0.0 - 11.2
Medium anterior fontanelle.	96.2 (21)	98.6 (21)	99.3 (21)	91.9 (19)	96.20	76.4 -100.0
Large anterior fontanelle.	1.9 (3)	1.4 (2)	0.0 (0)	8.1 (2)	2.46	0.0 - 23.6
Incomplete ossification of supra-occipital bone.	24.2 (12)	14.4 (10)	12.8 (11)	19.3 (11)	13.48	0.0 - 29.2
Incomplete ossification of interparietal bone.	49.0 (20)	40.4 (18)	40.4 (18)	41.5 (18)	28.40	4.9 - 91.0
Incomplete ossification of parietal bone.	0.6 (1)	1.4 (2)	0.0 (0)	2.2 (1)	1.30	0.0 - 7.1
Incomplete ossification of squamosal bone.	0.0 (0)	0.7 (1)	0.0 (0)	3.0 (2)	0.84	0.0 - 4.7
Incomplete ossification of frontal bone.	1.3 (2)	0.0 (0)	0.0 (0)	3.0 (2)	0.09	0.0 - 1.7
Discrete unossified area in frontal bone.	0.6 (1)	2.7 (3)	2.1 (3)	2.2 (2)	0.43	0.0 - 4.6
Incomplete ossification of nasal bone.	0.0 (0)	0.0 (0)	0.0 (0)	0.7 (1)	0.02	0.0 - 0.6
Discrete unossified area in basioccipital bone.	0.0 (0)	0.0 (0)	0.7 (1)	0.0 (0)	0.12	0.0 - 2.4
Incomplete ossification of basioccipital bone.	0.0 (0)	0.7 (1)	0.0 (0)	0.0 (0)	0.01	0.0 - 0.6
Incomplete ossification of basisphenoid, craniopharyngeal canal enlarged.	0.0 (0)	0.0 (0)	1.4 (2)	2.2 (2)	0.19	0.0 - 14.3
Incomplete ossification of basisphenoid bone.	4.5 (7)	7.5 (8)	11.3 (9)	13.3 (8)	0.94	0.0 - 12.6
Presphenoid bone incompletely ossified or unossified.	0.6 (1)	0.0 (0)	0.0 (0)	4.4 (2)	0.12	0.0 - 5.4
Fronto-nasal suture enlarged.	1.9 (3)	0.7 (1)	0.7 (1)	4.4 (2)	1.13	0.0 - 5.3
Incomplete ossification of hyoid bone.	7.0 (6)	5.5 (6)	9.9 (3)	5.9 (4)	7.28	0.0 - 25.0
Hyoid bone unossified.	12.7 (8)	11.6 (9)	7.1 (8)	5.2 (6)	8.56	0.0 - 18.5

@ One foetus may have more than one observation

F_0 Dams; Postnatal Phase

There was a slight increase in gestation length in the 30 mg/kg (n.s.) and 100 mg/kg ($P< 0.05$) groups, from a mean of 22.5 days in control to a mean of 23.0 days in the 100 mg/kg group). Although body weights of the drug treated animals tended to be higher than control, no significant intergroup differences in body weight were observed during lactation.

F_1 Offspring to 5 Weeks of Age

There were no compound related effects at any dose level on number of stillbirths, litter size or sex ratio at birth, number of implantation sites, survival indices throughout lactation, body weight or body weight gain during lactation.

There were no compound related effects on physical development (time of pinna unfolding, hair growth, testis descent, tooth eruption, eye opening and vaginal opening).

There were no effects of compound treatment on auditory and visual function (tested on PPD 25), within-cage activity (measured by means of electronic detectors and infra-red light apparently on PPD 26 to 27), learning ability (water filled Y-maze on PPD 27) and neuromuscular function (traversing flat and round rods, rotorod treadmill, mid-air righting reflex, fore- and hind-limb wire hanging and grid-griping ability on PPD 28-30). There were no effects on body weight or body weight gain to 5 weeks of age.

F_1 Offspring Between 5 and 10 Weeks of Age

The 20 males and 20 females per group, selected at 5 weeks of age, were assessed primarily for physical and sexual maturation and reproductive performance.

There were no compound related effects in males or females on appearance, behavior, body weights, mating performance at 9 or 10 weeks of age or on fertility.

In F_1 females killed on day 20 of gestation, there was no evidence of effects on implantation, embryo/fetal survival, fetal weights or placental weights.

No gross pathology abnormalities were observed in F_1 males or females that were considered to be related to treatment of the F_0 females.

4. Embryotoxic and Teratogenic Effects in Rabbits

Report No: 7595
Study No.: Not given

Performing Laboratory:

Dates Performed: 10/77 to 1/78 (first test)
1/78 to 4/78 (second test)

Quality Assurance: These studies were performed prior to the time that GLP compliance was required. The investigators in Germany claim that to the best of their knowledge, the study was performed "according to the state of the art".

Test Animals: Sexually mature female Himalayan rabbits, around 2 to 3.5 kg body weight, inseminated twice by means of copulation with males of the same strain and similar age.

Procedure: There were two separate but related studies in which batch 1/77, micronized Bay k 5552 were used. In the first study, the test substance was administered once daily by stomach tube, as a suspension in a vehicle consisting of "60 g anhydrous glycerol, 100 g demineralized water and polyethylene glycol 400 to make up 1129 g". Twelve or 13 does/group were treated from days 6 to 18 of gestation with doses of 0, 3, 10 or 30 mg/kg. On day 29, a C-section was carried out and each doe was examined for number of implantations (no data on corpora lutea count), number of live and dead fetuses and embryos, weight of litter and placentae. Each fetus was sexed, then examined for external, visceral and skeletal anomalies. Because of diarrhea in 4 animals of the 30 mg/kg group and spontaneous abortion in 2 of these 4 does, a second study limited to 0 and 30 mg/kg (n = 12 or 13/group), with a vehicle consisting of 0.5% aqueous Tylose, was performed. It was suspected that the vehicle contributed to the diarrhea in the first study. In all other respects, the procedure was the same as in the first study.

Results of the First Study

Maternal Survival, Clinical Signs and Body Weight Gain

One death at high dose (day 27 p.c.) was attributed to diarrhea on multiple days; 1 death at mid dose (day 9 p.c.) was attributed to an intubation accident.

The 30 mg/kg dose "caused" diarrhea in 4 animals (1, with multiple days of diarrhea, died; the remaining 3 had diarrhea on only 1 day, either on day 16 or 17 p.c.). Spontaneous abortions occurred in 1 doe at 3 mg/kg (day 25 p.c.), 1 at 10 mg/kg (day 28 p.c.), and 2 at 30 mg/kg (days 18 and 27; both had diarrhea). None of the control does aborted. Although there was only one

more spontaneous abortion in the high dose group than in the low or mid dose groups, the investigators nevertheless suggested that the incidence in the high dose group was increased, and attributed this effect to diarrhea associated with the vehicle and treatment. The abortions at low and mid doses were considered to be neither treatment related nor significant because the observed rates were considered normal for the strain of rabbit used.

Only females found to be pregnant were included in calculation of mean body weight values (N = 13, 12, 12 and 10 for control, 3, 10 and 30 mg/kg groups). Decreases in body weight gain between days 6 and 18 and over the entire period of gestation in all 3 treated groups were not statistically significant (See table on page 105A).

Laparoscopic Observations

There were no significant effects on mean numbers of implantations or resorptions per doe. The mean number of live male fetuses per doe was reduced in the 30 mg/kg group vs control ($P< 0.05$) resulting in a reduction in ratio of males:females but it was considered to be a random occurrence. There was no effect on fetal or placental weights, and no increase in number of "underdeveloped forms" (i.e. fetuses lower than 2.5 g body weight). There was an increase in malformation rate in the high dose group; the high dose malformations occurred in the offspring of the three animals that had diarrhea and were suggested to be the result of maternal stress (see pages 104 to 105A).

Results of the Second Study:

Maternal Survival, Clinical Signs and Body Weight Gain

Diarrhea or other clinical signs did not occur in does of the 30 mg/kg group, but one doe on 30 mg/kg died of an intubation accident (sometime between days 18 and 29, p.c., based on individual animal weight gain tables). Final results were based on 11 surviving does in each group. There was a decrease in mean body weight in the treated group, compared to an increase in control, between days 6 and 18 (treatment period), resulting in a compound related decrease in body weight gain over the entire gestation period.

Laparoscopic Observations

There were no compound related effects on pregnancy rate, spontaneous abortion rate, mean number of implantations (corpora lutea were not counted) or live fetal count, but mean fetal weight and placental weight were lower ($P<0.05$) and the number of "underdeveloped forms" was higher in the treated group. One of the dams on 30 mg/kg had 5 fetuses with "reduced motility". In this second study, there was no effect on sex ratio at birth, as had been observed in the first study. There was no increase in

external, visceral or skeletal malformations in any treated group vs control (See tables on pages 105B, C & D).

Comment: There were indications of fetal toxicity at 30 mg/kg, a dose that was maternally toxic. For example, there was a reduction in live male fetuses per dam and suggestions of an increase in total number of fetuses with malformations and the total number of litters with fetuses that had malformations, compared to control, in the first study. The total number of runts was higher and mean fetal weights were lower than control in the second study. However, there was no increase in incidence of any specific form or class of terata and no clear indication that this substance was teratogenic in rabbits.

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Incidence table of the findings of the fetuses#

Findings	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
MENINGOCELE	1(1)[a]			
TELENCEPHALON dysplasia	1(1)[a]			
CRAWN-HAND slight	1(1)[a]			
TONGUE small/sharp/thin		1(1)		
FORE-LIMB abnormal position				1(1)[a]
arthrogryposis	1(1)[b]			1(1)[a]/1(1)[b]
MULTIPLE MALFORMATION			1(1)	1(1)[a]/·
CLEFT PALATE				
MOTILITY reduced				5(1)[b]

# on individual basis; values in () or :r basis
a = first study / b = second study

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Individual clinical findings of the dams

Note: animals without findings are not listed

Dose [mg/kg]	Dam-No.	Findings
0 (1st study)	914	on day 26 p.c. no stool, from day 27 p.c. very reduced stool
3	899	abortion on day 25 p.c.: 3 placentas
10	876	abortion on day 28 p.c.: 4 fetuses with placentas
	896	found dead on day 9 p.c. (lung application)
30 (1st study)	877	abortion on day 27 p.c.: 3 fetuses and 3 centas
	881	on day 13 p.c. red bordered eyes on day 16 p.c. scratch wounds on day 17 p.c. diarrhea
	885	on day 17 p.c. diarrhea
	905	on day 18 p.c. diarrhea
	921	on days 17 and 18 p.c. die from day 24 p.c sick, re stool on day 25 p.c. bloody ion found dead on day 27
	925	abortion on day 18 3 fetuses
0 (2nd study)	-	-
30 (2nd study)	950	from d .c. sick, decreased feed consumption cool on ay between day 18 and 25 p.c. ea d dead on day 25 p.c

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R e t s o f t h e C a e s a r e a n S e c t i o n

Mean v of the groups and standard deviations 1st study

Note: The mean fetal and pl weights given in the report no. 7595 were caculated by adding all litter weights of the group and by div hese sums by the number of fetuses or placentas per group. In the following two tables these mean values fec with "a". For the calculation of the deviation the mean fetal and placental weights per litter were calculated first and were used for furth ulation. Mean fetal and placental weights obtained by this procedure are marked with "b".

Dose mg/kg	Weight gain [g] during pregnancy	Weight gain [g] during treatment period	Number [per f e t u impl.	male	female	of	res.**	Mean - weight [g] of fetuses	Mean - weight [g] of placentas	Number of fetuses with minor skeletal deviations	Number of fetuses with malformations	Number of fetuses runts < 25g
0	166.2	25.8	7.6	3.2	3.5	6.7		36.64[a] 36.96[b]	4.37[a] 4.37[b]	0.00	0.08	0.62
	176.4	140.2	1.8	2.0	1.5	2.0	1.	.12	0.61	0.00	0.28	1.33
3	109.2	5.0	6.2	2.3	2.9	5.2	1.0	36	4.50[a] 4.50[b]	0.00	0.08	0.17
	86.5	87.2	2.2	1.4	1.7	2.8	1.4	5.9.	1.08	0.00	0.29	0.58
10	123.8	7.5	8.0	3.0	3.9	6.9	1.1	34.70[a] 35.11[b]	*	0.00	0.08	0.25
	133.1	78.9	1.6	1.5	1.9	2.5	2.2	3.92	0..	0.00	0.29	0.87
30	112.5	0.5	5.9	1.5*	2.4	3.9	2.0	37.27[a] 37.44[b]	4.76[a] 4.76[b]	0	0.40	0.00
	135.5	135.6	1.9	1.1	1.9	2.6	2.7	3.94	0.77		0.70	0.00

* significant difference to control, $p < 0.05$

** Res. is the abbreviation for resorptions, which ponsor defined as the total of resorbed embryos and dead es

BAY k 5552 Report No. 7595

Results of the Caesarean Section

Mean values of the groups and standard deviations 2nd study

Dose mg/kg	Weight gain [g] during pregnancy	Weight gain [g] during treatment period	Num impl.	Num male	Num dams	Num fetuses total	Num res.**	Mean fetal weight [g] of fetuses	Mean fetal weight [g] of placentas	Number of fetuses with minor skeletal deviations	Number of fetuses with malformations	Number of fetuses runts < 25g
0	200.9	38.2	7.0	3.2	3.		0.6	36.94[a] 37.50[b]	4.25[a] 4.25[b]	0.00	0.09	0.00
	128.0	62.7	2.5	1.5	1.9		.7	3.49	0.45	0.00	0.30	0.00
30	131.8	-47.6	6.9	2.8	3.0	5.8		34.07[a]* .95[b]	3.77[a]* 3.77[b]	0.00	0.18	0.64*
	136.4	158.6	1.4	1.9	1.2	1.6	1.1	9	0.52	0.00	0.40	0.81

* significant difference to control, $p < 0.05$

** Res. is the abbreviation for resc ons, which the sponsor defined as the total of resorbed e s and dead fetuses

Individual necropsy findings of the dams

Note: animals without findings are not listed

Dose [mg/kg]	Dam-No.	Findings
0 (1st study)	-	-
3	879	gall bladder congested
	899	colon distended and filled with dark brown-red fluid, liver light and brittle, distinct lobulation, gall bladder congested
10	896	thoracic cavity filled with fluid, organs autolytic, pregnant
30 (1st study)	921	gall bladder congested, liver light brittle, intestines filled with g[illegible] liquid brown-red paste, pregnant
0 (2nd study)	-	-
30 (2nd study)	937	fatty tissue of the [illegible]s chondroid hardened
	938	uterus anomaly - [illegible] excluded from the study
	949	gall bladder c[illegible]ed, knobby surface
	950	died as a re[illegible]of lung application, pregnant
	955	subdermal [illegible] tissue chondroid hardened in the abdo[illegible] region
	956	subder[illegible]atty tissue chondroid hardened in the [illegible]nal region
	967	su[illegible]l fatty tissue chondroid hardened in [illegible]dominal region
	968	[illegible] bladder congested

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	Oral Dose (mg/kg)	Dam No.	No. of Malformed Fetuses	Malformation
First Test	0	902	1	Meningocele and telencephalic dysplasia.
	3	903	1	Dysplasia of the tongue.
	10	926	1	Abnormal skull form, twisting of the umbilical cord, dysplasia of a liver lobe, abnormal bone formation and dysplasia in the sternum.
	30	881	2	1st fetus: Cleft palate 2nd fetus: Abnormal position of the right front leg.
		885	1	Multiple malformation (including cleft palate, fissured chest and stomach, spina bifida, ectrodactyly, abnormality of the ears).
		905	1	Arthrogryposis of both front legs.
Second Test	0	951	1	Arthrogryposis of the left front leg.
	30	943	1	Abdomen, pelvic girdle, and rear limbs rudimentary; cleft palate, ectrodactyly or adactyly of the front.
		956	1	Arthrogryposis of the right front leg.

5. Teratogenicity Study in Cynomolgus Monkey

Bayer Study No: T 3 022 847

Performing Laboratory

Sponsor

Dates Performed: 2/23/87 to 6/16/87

Quality Assurance: A signed statement of GLP compliance was included. The statement notes two deviations from 21CFR 58: 1) "the stability of the test article/carrier mixture had not been determined at the time of the study", and 2) "the final report of the study does not contain all the information specified in subsection 58.185. In particular, no analytical data relating to the test article or test article/carrier mixture are included."

Justification for Species Selection: "...because of its similar hormonal profile during pregnancy to that of man and this particular non-human primate submits itself as a favourable species for reproductive toxicology studies."

Doses Tested: 0, 30 and 100 mg/kg

Procedure: Feral cynomolgus monkeys (Macaca fascicularis) were obtained from The females were "sexually mature", quarantined for "at least 2 weeks" and acclimatized to laboratory conditions for 3 weeks, before initiation of the study. There were 10 (30 mg/kg) or 12 females (0 and 100 mg/kg) per group that had tested positive for pregnancy (by a mouse uterotropic test); these animals weighed 2.6 to 3.8 kg on day 20 post-coitum. Pregnancy was subsequently monotored by rectal palpation on specified days between GD 30 and 86. Test substance was administered by intragastric intubation between gestation days (GD) 20 and 50, and necropsies for C-section were done on GD 100 ±1 day. Examination for fetal malformations included a comprehensive external examination, including head and body size measurements and appearance of externally observable organs. Soft tissue examination consisted of "a full necropsy of each fetus with visual macroscopic inspection" of the organs and weight measurements of 12 organs. After weighing, the organs were fixed in formalin, but not further evaluated. The skeleton was cleared and stained and examined for skeletal anomalies.

Test Substance: Batch No. 828 305. The vehicle consisted of 9.9% demineralized water, 4.8% glycerine and 85.3% Lutrin (polyethylene glycol 400). Test mixtures, consisting of separate solutions for each dose level, were prepared daily. The report notes that "Analysis of formulations and proof of absorption were not required by the study sponsor."

Results:

Spontaneous Abortions, Clinical Observations and Mortality: One control and 1 high dose monkey were found to be not pregnant, thus leaving 11, 10 and 11 monkeys in control, low and high dose groups, respectively. Incidence of abortions and deaths are summarized below.

Dose mg/kg	#/Gp*	# Abortions	# Deaths
Control	11	6 (GD 26-56)	3 (GD 21-99)
30	10	7 (GD 28-53)	1 (GD 48)
100	11	8 (GD 33-59)	5 (GD 27-88)

*Includes only pregnant animals.

Symptoms included heavy bleeding (followed by abortion), reduced food intake, diarrhea and vomiting. Symptoms were observed only during intervals of treatment, and included animals in the control group. Although all of the symptoms were generally more frequent or of longer duration in drug treated monkeys, they are considered to be due to treatment with the vehicle.

Two of the 3 control animals that aborted, subsequently died. The increased incidence of spontaneous abortions and deaths in the 100 mg/kg dose treated group were considered treatment related. (See comments on next page.)

The death of an additional animal in the high dose group which was found to be not pregnant and is not included in these results, was also considered to be treatment related. Causes of death in the 3 pregnant control monkeys included gastro-enteritis, catarrhal enteritis and "signs of asphyxia". Of the drug treated animals which died, the one in the 30 mg/kg group, and 4 of the 5 in the 100 mg/kg group, each had a volvulus (an intestinal obstruction due to twisting of the bowel); 3 of these animals (all high dose) exhibited abdominal distention shortly before death, and one of these 3 had acute catarrhal enteritis. A volvulus was considered to be a compound related cause of death; no volvulus was seen in any control animals.

Blood Analyses: Blood samples were taken from each monkey on GD 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90 and 97. However, data on blood analyses of any kind could not be found.

Fetal Examinations: External, visceral and skeletal findings for each surviving fetus are shown on the page which follows. In spite of the very few surviving fetuses (3 control, 2 mid dose and 1 high dose), the investigators concluded that the external and skeletal malformations seen in the sole surviving high dose fetus were drug related and were due to severe maternal toxicity. It was claimed that these malformations had never

before been observed in controls.

Fetal Organ Weights: Although weights of 10 or 11 different fetal organs with means (and standard errors only for controls) are presented in Table 4 and Appendix III of the report, statistical comparisons were obviously not possible (only one or two surviving fetuses in the treated groups).

Comments: It should be noted that the control incidence of symptoms, mortality and spontaneous abortions were "unusually high". Although the investigators attributed the higher incidence of abortions and deaths in the high dose group to treatment, there are no indications that the differences were statistically significant. The animals used in the present study were feral monkeys. It seems reasonable to suggest that there may have been an interaction between disease or parasitic infestations (often inherent in feral monkeys), stress of handling or control vehicle administration, and treatment with the high dose, which would confound the outcome of this study. The monkey is not a commonly used model for reproductive toxicity tests and there is limited background information. The limited number of offspring (usually one per monkey) is considered to be a disadvantage for using this species for this type of study. The high mortality and abortion rates, even in control animals, further limits the usefulness of this study. However, the only surviving fetus in the in the high dose group showed malformations "which were never before observed in control fetuses".

Group 1 - 0 mg/kg

Female number	Fetus alive	Type of defect		
		External findings	Visceral findings	Skeletal findings
33797	+	bent tail end	left adrenal severely enlarged	6th to 10th and 12th rib on the right side and 7th to 9th rib on the left side of uneven thickness; 4th to 6th sternebra not ossified, 7th sternebra incompletely ossified
33383	+	no abnormalities detected	no abnormalities detected	5th to 11th rib on the right side and 4th to 11th rib on the left side of uneven thickness; 1st to 7th sternebra not ossified
28980	+	no abnormalities detected	no abnormalities detected	5th to 11th rib on the right side and 6th to 10th rib on the left side of uneven thickness; 6th sternebra not ossified and 7th sternebra incompletely ossified
149	+	no abnormalities detected	no visceral investigation due to caesarian section on day 76 p.c.	no skeletal investigation due to caesarian section on day 76 p.c.

Group 2 - 30 mg/kg

Female number	Fetus alive	Type of defect		
		External findings	Visceral findings	Skeletal findings
34738	+	prepuce not patent	no abnormalities detected	displaced zygostyle; 5th to 11th rib on the right side and 8th to 11th rib on the left side of uneven thickness; 1st to 2nd and 6th to 7th sternebra not ossified
34733	+	bent tail end	no abnormalities detected	parietals incompletely ossified; 6th to 13th rib on the right side and 2nd to 3rd, 7th to 11th and 13th rib on the left side of uneven thickness; 2nd and 6th to 7th sternebra not ossified

Group 3 - 100 mg/kg

Female number	Fetus alive	Type of defect		
		External findings	Visceral findings	Skeletal findings
34040	+	left forelimb appears thinner than normal; tail shortened and inwards curved tail end; only three fingers on the left side and 3rd finger with two nails	no abnormalities detected	additional ossification site between the metacarpals of the 2nd and 3rd finger, additional fingernail on the 3rd finger, proximal phalanx of the 2nd and 3rd finger and medial phalanx of the 3rd finger on the left side abnormally developed; the last three coccygeal vertebrae asymmetrically and incompletely ossified; 5th to 11th rib on the right side and 7th to 12th rib on the left side of uneven thickness; 1st to 3rd and 6th to 7th sternebra not ossified

6. Perinatal and Postnatal Effect Following Oral Administration to Rats

Study No: T1002153

This report is accompanied by a "first amendment to report no. 12801 A", dated 8/14/93. Tables in the original English translation of the report were of very poor quality, not well organized, not legible, and contained errors in translation and typing. Some of these faults are listed in the amendment under the "rationale for the first amendment".

Performing Laboratory: [redacted]

Dates Performed: May 1981 to October 1981

Quality Assurance: No statement on GLP compliance was found in the original report. In the amendment, it is claimed, "...there were no legally binding GLP regulations in force" during the time that this study was performed.

Doses Tested: 0, 3, 10 and 30 mg/kg.

Test Animals: Mura:WIST (SPF 67 Han) female rats, 11-14 weeks of age and weighing 177-240 g, were mated with stock males of the same strain.

Procedure: Presumed pregnant females were dosed orally by gastric intubation from gestation day (GD) 16 to postpartum day (PPD) 21. Half the females in each group were C-sectioned on GD 20, the remainder were allowed to litter and rear their young to PPD 21. Of the C-sectioned animals, approximately 1/3 of all the fetuses in each group were examined for visceral anomalies by the Wilson technique, the remainder were evaluated for bone anomalies by staining with Alizarin red. The abdominal and thoracic organs of animals selected for bone anomaly examination were removed and "evaluated".

Test Substance: Batch No. 576 923. The vehicle consisted of Lutrol 400, anhydrous glycerol and demineralized water in a ratio of 969:60:100. A preparation of 0.6% nisoldipine was tested for stability and was found to be stable after 7 days.

Effects on All Pregnant Females: There were two deaths, one at 3 mg/kg (cause of death not determined), and one at 10 mg/kg (died of pneumonia), but no treatment related effects on mortality or clinical signs were evident. A small but significant decrease in body weight gain was found for the dams at 30 mg/kg (both the C-sectioned and rearing groups) between the first day of administration (GD 16) and GD 20 (See tables on pages 113 & 113A). Body weights of the low dose group of the C-sectioned rats were significantly higher than control throughout gestation, even on GD 0, but obviously this was not a compound related effect.

There were no compound related effects on reproduction parameters (percent inseminated, percent with implantations, etc.; see table on a page 114), nor were there any effects on gross pathology.

Examination of C-Sectioned Females: There were no effects found on mean number of implantation sites, live or dead fetuses or resorptions per dam. Furthermore, there were no effects of treatment on sex distribution, mean placental weight, number of runts (fetuses <3 g), or frequencies of external, visceral or bone deformations. A decrease ($P< 0.01$) in mean fetal weight at the 30 mg/kg dose was evident (See table on page 115). The table which follows immediately below is a summary of malformations found in all 4 groups, copied from the original report.

Group	Dam No.	Number of changed foetuses	Changes
Control	-	-	-
3 mg/kg	2995	1	no tail
10 mg/kg	2849	1	Otocephaly
	2852	2	slight dilation of the lateral ventricle of the brain
30 mg/kg	2991	1	Cryptorchism

Effects on F_0 Dams Allowed to Litter: There were no effects on duration of pregnancy at any dose level. The report indicates a significantly higher number of implantations per dam in the high dose group (probably incidental and not treatment related), and no effect on prenatal loss (implantations - surviving and dead pups). Complete litter losses were reported for 2 dams in the 30 mg/kg group (both did not suckle their young), and for 1 in the 10 mg/kg group (devoured its young during the 3rd week), but normal lactational behavior was observed in all other dams (not shown in tables but indicated narratively in the original report).

Effects on F_1 Offspring: There was an increase in number of stillborn pups, and a dose related increase in mortality of the newborn pups during the first week postpartum in the 10 and 30 mg/kg groups (See table on page 115B; no statistical analysis). The birth weight and the weight increase during the 3 weeks postpartum were both significantly reduced in the 30 mg/kg group vs control (See table on page 115C). The report claims no compound related effects on appearance or clinical signs of the F_1 offspring. In the maturational development tests, there were no effects of treatment on time to pinna unfolding, appearance of fur or eye opening, but there was a slight delay in time for normal walking in the 30 mg/kg group. For the functional tests, there was no effect on pupillary reflex ("following a light in a darkened room"; age when given is not stated), and no effect on hearing (stimuli from a Galton whistle "at the end of the lactation period"). Running performance on a running roller

(sensomotor behavior or proprioreceptor reflexes) was considerably reduced in the 3 mg/kg group, but this was considered an incidental finding because there was no effect at the 10 and 30 mg/kg dose levels. For the F_1 generation fertility test, 1 male and 1 female in each litter of the control and high dose groups were reared to 10 weeks of age, then mated. There were no differences between the 2 groups in rate of insemination or fertilization, duration of pregnancy, total number of live male or female pups at birth, body weight of pups or pups with external deformities.

PERI- AND POSTNATAL-STUDY BAY K 5552 T1002153

WEIGHT DEVELOPMENT [G] OF THE FEMALES UNDERGOING CESAREAN SECTION
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEIGHT GAIN				
DAY 0 - 16 P.C.	59.8	64.4	60.1	57.8
	9.6	9.9	11.1	10.3
DAY 16 - 20 P.C.	38.4	40.2	39.2	3[illegible]
	10.2	8.0	6.6	
DAY 0 - 20 P.C.	98.3	104.5	99.3	[illegible].8
	16.4	16.3	14.6	12.5
BODYWEIGHTS DURING GESTATION				
DAY 0 P.C.	201.0	210.0*	[illegible].8	203.1
	11.8	12.2	[illegible]0.1	11.3
DAY 16 P.C.	260.8	274.4*	261.9	260.9
	12.9	16	16.9	16.5
DAY 20 P.C.	299.3		301.1	293.9
	20.8	[illegible].0	20.6	16.8

* SIGNIFICANT DIFFERENC[illegible] ONTROL, P < 0.025
** SIGNIFICANT DIFFERENC[illegible] CONTROL, P < 0.01

PERI- AND POSTNATAL-STUDY BAY K 5552 T1002153

WEIGHT DEVELOPMENT [G] OF THE DAMS
GROUP MEAN VALUES AND STANDARD DEVIATIONS

INVESTIGATION	0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
WEIGHT GAIN				
DAY 0 - 16 P.C.	58.4	60.1	61.2	61.7
	11.6	7.8	12.8	10.7
DAY 16 - 20 P.C.	39.9	37.7	35.6	33
	8.2	7.6	8.0	
DAY 0 - 20 P.C.	98.4	97.8	96.8	.3
	15.2	12.5	16.4	13.6
BODYWEIGHTS DURING GESTATION				
DAY 0 P.C.	204.3	202.1	[illegible]	205.3
	10.6	10.0	1.6	11.1
DAY 16 P.C.	262.8	262.2	264.1	267.0
	17.6	15.[illegible]	20.1	15.6
DAY 20 P.C.	302.8	[illegible]	299.7	300.7
	21.0	[illegible]	23.1	16.8
BODYWEIGHTS DURING LACTATION				
DAY 1 P.P.	238.8	240.0	239.7	236.6
	15	11.7	17.7	17.7
WEEK 1 P.P.		267.6	264.1	262.6
	.0	16.8	21.3	18.8
WEEK 2 P.P.	270.3	274.5	272.8	276.3
	20.3	13.5	21.2	19.8
WEEK [illegible]	264.6	262.2	265.1	266.0
	15.4	13.5	17.5	14.6

[illegible]IGNIFICANT DIFFERENCE TO CONTROL, P < 0.005

PERI- AND POSTNA[illegible] 'UDY BAY K 5552 T1002153

NUMBER OF ANIMALS 'SULTS OF THE STUDY

DAMS

DOSE (MG/KG)	USED	N	INSEMINATED % OF THOSE USED	N	NUMBER OF FEMALES 'MPLANTATIONS 'F THOSE 'INATED	N	THAT LITTERED % OF THOSE WITH IMPLANTATIONS	N	THAT REARED THEIR PUPS % OF THOSE THAT LITTERED
0	25	25	100.0	21		20	95.2	20	100.0
3	25	25	100.0	20	8[illegible]	20	100.0	19	95.0
10	25	25	100.0	23	92.[illegible]	23	100.0	22	95.7
30	25	25	100.0	20	80.0	20	100.0	17	85.0

ANIMALS UNDERGOING CESAREAN SECTION

DOSE (MG/KG)	USED	N	INSEMINATED % OF THOSE USED	N	NUMBER OF FEM[illegible]S WITH IMPLANTATIONS % OF THOSE INSEMINATED	N	WITH FO[illegible] % OF TH[illegible] IMPLANTA[illegible]	TH
0	25	25	100.0	20	80.0	20	100.0	
3	25	25	100.0	20	80.0	19*	95.0	
10	25	25	100.0	21	84.0	19*	90.5	
30	25	25	100.0	23	92.0	22	95.7	

* ONE FEMALE DIED BEFORE CESAREAN SECTION

PERI- AND POSTNA[illegible]UDY BAY K 5552 T1002153

RESULTS OF THE CESAR[illegible] SECTION (MEAN VALUES)

DOSE (MG/KG)	WEIGHT GAIN (G) 0-20 P.C.	WEIGHT GAIN (G) 16-20 P.C.	NU IMPL.	(PER DAM) FEM.	O F SUM	O F LOSS	MEAN-WEIGHT IN GRAMMS FETUSES	MEAN-WEIGHT IN GRAMMS PLACENT.	NO. OF FOETUSES EXAMINED BY WILSON	NO. OF FOETUSES EXAMINED BY DAWSON	FOETUSES WITH MINOR SKELE-TAL DEVIAT	FOETUSES WITH MALFOR-MATIONS	NO. OF RUNTS (<3G)
0	98.3	38.5	11.0	5.1	10.3	0.7	3.50	0.50	3.1	7.1	4.80	0.00	0.60
3	104.5	40.2	11.4	5.1	5.[illegible]	0.8	3.52	0.52	3.3	7.2	6.11***	0.05	0.98
10	99.3	39.2	10.2	4.5	4.9	0.8	3.54	0.53	2.8	6.9	4.68	0.05	0.32
30	90.8*	33.0*	10.7	5.3	4.6	9.[illegible]	3.38**	0.49	3.1	7.2	4.73	0.05	0.91

* SIGNIFICANT DIFFERENCE TO CONTROL, P < [illegible]
** SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.[illegible]
*** SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.[illegible]

PERI- AND POSTNATAL-STUDY BAY K 5552 T1002153

PRENATAL LOSS OF THE REARING ANIMALS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
0.7	0.6	0.7	1.2
1.3	0.9	0.8	1.2

NUMBER OF IMPLANTATIONS OF THE [illegible]S

MEAN VALUES AND STANDARD [illegible]TIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	[illegible]0 MG/KG	DOSE 30 MG/KG
10.3	9.9	10.5	11.0*
2.8	0.9	0.8	1.2

* SIGNIFICANT DIFFEREN[illegible] CONTROL, P<0.005

DU[illegible]ATION OF PREGNANCY IN DAYS

MEAN VALUES AND STANDARD DEVIATIONS

DOSE 0 MG/KG	DOSE 3 MG/KG	DOSE 10 MG/KG	DOSE 30 MG/KG
21.8	21.9	22.0	22.0
0.7	0.7	0.6	0.8

Dose (MG/KG)	Total number of young (surviving + dead)	Number of still-born young	Total number of dead young after 1 week	2 weeks	3 weeks
Control	203	3	4	5	5
3	186	4	6	6	6
10	226	4	11	13	23
30	196	28	34	34	34

NUMBER AND WEIGHT DEVELOPMENT OF THE VIABLE PUPS
GROUP MEAN VALUES PER LITTER AND STANDARD DEVIATIONS

INVESTIGATION		0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
NUMBER OF PUPS					
AT BIRTH	TOTAL	10.0**	9.6**	9.6**	9.6**
		10.0	9.6	9.7	8.8
		2.5	2.2	1.9	3.1
AFTER 1 WEEK	TOTAL	9.9**	9.5**	9.3**	9
		9.9	9.5	9.3	
		2.5	2.1	2.0	
AFTER 2 WEEKS	TOTAL	9.9**	9.5**	9.2	9.5**
		9.9	9.5	9	9.5
		2.5	2.1		2.2
AFTER 3 WEEKS	TOTAL	9.9**	9.5**	9.2**	9.5**
		9.9	9.5	9.2	9.5
		2.5	2	2.0	2.2
WEIGHT (G) OF THE VIABLE PUPS					
AT BIRTH		5.9	6.1	6.0	5.2
		0	0.5	0.6	0.6
AFTER 1 WEEK			14.2	14.0	11.7
		.2	1.1	1.7	1.6
AFTER 2 WEEKS		24.2	25.2	25.0	20.6
		3.6	2.3	3.3	2.6
AFTER 3 WEEK		37.5	39.8	40.0	32.6
		6.1	3.8	5.1	4.4

** O[illegible]OM DAMS WHICH SURVIVED UNTIL THE END OF THE EXPERIMENT
AGE VALUE)

PERI- AND POSTNATAL-STUDY BAY K 5552 T1002153

NUMBER AND WEIGHT DEVELOPMENT OF THE VIABLE PUPS
GROUP MEAN VALUES PER LITTER AND STANDARD DEVIATIONS

INVESTIGATION		0 MG/KG	3 MG/KG	10 MG/KG	30 MG/KG
NUMBER OF PUPS					
AT BIRTH	TOTAL	10.0 2.5	9.6 2.2	9.7 1.9	8.8 3.1
	MALES	5.8 2.1	4.4 1.3	5.3 1.9	4.0 2.4
	FEMALES	4.2 1.6	5.2 1.8	4.4 1.6	4.8 2.1
AFTER 1 WEEK	TOTAL	9.9 2.5	9.5 2.1	9.3 2.0	9.5 2
	MALES	5.8 2.1	4.4 1.3	5.1 1.9	
	FEMALES	4.1 1.6	5.1 1.7	4.2 1.7	5.2 1.8
AFTER 2 WEEKS	TOTAL	9.9 2.5	9.5 2.1	9	9.5 2.2
	MALES	5.8 2.1	4.4 1.3	.8	4.4 2.2
	FEMALES	4.1 1.6	5.1 1.7	4.2 1.7	5.2 1.8
AFTER 3 WEEKS	TOTAL	9.9 2.5		9.2 2.0	9.5 2.2
	MALES	5.8 2.1	4 1.3	5.0 1.9	4.4 2.2
	FEMALES	4.1 1.6	5.1 1.7	4.2 1.7	5.2 1.8
WEIGHT [G] OF THE VIABLE					
AT BIRTH		6.1 0.6	6.1 0.5	6.0 0.6	5.2+ 0.6
AFTER 1 WEEK		13.4 2.2	14.2 1.1	14.0 1.7	11.7* 1.6
AFTER 2 WEEKS		24.2 3.6	25.2 2.3	25.0 3.3	20.6*** 2.6
AFTER 3 [illegible]		37.5 6.1	39.8 3.8	40.0 5.1	32.6** 4.4

* SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.01
** SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.005
*** SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.001
\+ SIGNIFICANT DIFFERENCE TO CONTROL, P < 0.0005

7. Supplemental Perinatal and Postnatal Study in Rats

Study No: T3008898

Sponsor

Dates Performed: February 1984 to March 1984

Quality Assurance: A signed statement of GLP compliance was included.

Doses Tested: 0 and 30 mg nisoldipine/kg/day. On the last 2 days of treatment, "several animals" received only 20 mg/kg due to an error, but the study results were not considered to have been affected by this error.

Procedure: Bor:WISW (SPF Cpb) naturally inseminated female rats (25 per group) were 12-14 weeks of age and weighed 178-216 g at the start of treatment. They were dosed orally by gastric intubation from gestation day (GD) 16 to postpartum day (PPD) 14. All the pregnant dams were allowed to litter and rear their young to PPD 14. The dams and offspring were evaluated for tolerance of the compound, effects of nisoldipine on birth and lactation and influence on post-natal development (similar to the observations in the main study). However, this study differed from the main study because the treatment and observation period extended to PPD 21 in the main study and only to PPD 14 in the present study.

Test Substance: Batch No. 907437. The vehicle consisted of Lutrol, glycerol and water.

Effects on the Dams: At 30 mg/kg, 1 died during parturition (with 6 dead fetuses). Treatment related effects noted were a highly significant decrease in weight gain between GD 16 and GD 20 (initial part of the treatment period), which resulted in a reduced mean body weight up to PP day 7, no longer evident by PPD 14, lightly colored feces, no longer evident after littering, and prolonged gestation (from 21.6 days in control to 22.1 days in the treated group; P< 0.001).

NDA 20-356

D. Roeder

SEP 27 1993

NDA20356 P1

DIVISION OF CARDIO- RENAL DRUG PRODUCTS
MEDICAL OFFICER'S NDA REVIEW

NDA: 20 356

DRUG: Nisoldipine Core- coat (BAY k 552)

SPONSOR: Miles Inc (Pharmaceutical Division)

DATE SUBMISSION: 3 March, 1993

DATE REVIEW: 20 August 1993

REVIEWER: Philip L. Dern M.D. P.L. Dern

RESUME:

This review deals entirely with safety aspects of the above submission; not efficacy. The primary approach is via examination of individual pools of data based on similar studies and provided by the Sponsor in this submission.

I. Hypertension

A. Exposure

Although the number of cases treated world-wide with nisoldipine exceeds 6000, according to the Sponsor, relatively few of these, N= 1292, were given nisoldipine core- coat (NIS cc) as shown below in completed studies:

PATIENTS EXPOSED TO NISOLDIPINE IN EACH CATEGORY

FORMULATION	NDA STATUS	INDICATION: ANGINA NON-US	ANGINA US	HYPERTENSION NON-US	HYPERTENSION US	HEART FAILURE NON-US	HEART FAILURE US	CLIN. PHARM. NON-US	CLIN. PHARM. US	OTHER NON-US	OTHER US	TOTAL
COAT-CORE	INCLUDED	824	474	518	778	142		210	183			2925
	EXCLUDED											0
	TOTAL	824	474	518	778	142	0	210	183	0	0	2925
IMMEDIATE RELEASE	INCLUDED	3472	521	2371	10	414	14	755	83	131		7771
	EXCLUDED	411		334		185		421		136		1487
	TOTAL	3883	521	2705	10	599	14	1176	83	267	0	9258
OTHER	INCLUDED	81				103		575				886
	EXCLUDED					31		126				157
	TOTAL	81	0	0	0	134	0	701	0	0	0	1043
TOTAL	INCLUDED	4104	995	2889	788	659	14	1540	266	131	0	11382
	EXCLUDED	411	0	334	0	216	0	547	0	136	0	1644
	TOTAL	4515	995	3223	788	875	14	2087	266	267	0	13025

STUDIES IN EACH CATEGORY

FORMULATION	NDA STATUS	INDICATION: ANGINA NON-US	ANGINA US	HYPERTENSION NON-US	HYPERTENSION US	HEART FAILURE NON-US	HEART FAILURE US	CLIN. PHARM. NON-US	CLIN. PHARM. US	OTHER NON-US	OTHER US	TOTAL
COAT-CORE	INCLUDED	4	3	4	7	2		11	6			37
	EXCLUDED											0
	TOTAL	4	3	4	7	2	0	11	6	0	0	37
IMMEDIATE RELEASE	INCLUDED	111	16	87	1	33	2	87	3	8		328
	EXCLUDED	36		21		14		36		7		116
	TOTAL	147	16	108	1	47	2	105	3	15	0	444
OTHER	INCLUDED	11				9		55				85
	EXCLUDED					3		13				16
	TOTAL	11	0	0	0	12	0	68	0	0	0	87
TOTAL	INCLUDED	116	19	91	8	44	2	133	9	8	0	430
	EXCLUDED	36	0	21	0	17	0	51	0	7	0	132
	TOTAL	152	19	112	8	61	2	184	9	15	0	562

The following table provides the total duration of treatment by total daily dose of longest duration for the US NIS CC (total controlled and uncontrolled) cases. The second table provides duration of treatment in the non- US studies.

TABLE 2
TOTAL DURATION OF TREATMENT WITH NISOLDIPINE
BY TOTAL DAILY DOSE OF LONGEST DURATION

POOL OF US CC HYPERTENSION
TOTAL CONTROLLED + TOTAL UNCONTROLLED

	ALL	DURATION									
		2-7 DAYS		8-30 DAYS		31 DAYS-60 DAYS		61 DAYS-180 DAYS		181 DAYS-360 DAYS	
	N	N	%	N	%	N	%	N	%	N	%
DRUG											
NIS CC 10MG QD	37			33	88.2	2	5.4	2	5.4		
NIS CC 20MG QD	303	9	4.4	37	18.2	67	33.0	64	31.5	26	12.8
NIS CC 30MG QD	139	1	0.7	32	23.2	48	34.8	39	31.0	28	20.3
NIS CC 40MG QD	166	3	1.8	13	8.8	55	34.6	81	48.4	16	8.5
NIS CC 60MG QD	199	6	3.0	11	5.5	68	40.2	23	11.6	61	30.7
NIS CC 90MG QD	11	4	36.4	6	54.5	1	9.1				
ALL	774	23	3.0	133	17.0	281	36.2	208	26.8	131	16.8

2. DURATION BY DOSE TABLE 10:22 TUESDAY, SEPTEMBER 22, 1992
NISOLDIPINE (BAY K 5522) - DATA POOL / NON US-STUDIES
TOTAL DURATION OF TREATMENT WITH NISOLDIPINE BY TOTAL DAILY DOSE OF LONGEST DURATION

POOL 80: CC - HYPERTENSION - TOTAL STUDIES

INSTITUTE OF BIOMETRY

DRUG	ALL	DURATION															
		NOT RECORDED		1 DAY		2-7 DAYS		8-30 DAYS		31-60 DAYS		61-180 DAYS		181-360 DAYS		> 360 DAYS	
	N	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
>5- <=10 MG	114	1	0.9	2	1.8	1	0.9	6	5.3	49	43.0	5	4.4	17	14.9	33	28.9
>10- <=20 MG	192	1	0.5					5	2.6	56	29.2	6	3.1	39	20.3	85	44.3
30 MG	106	1	0.9							47	44.3	1	0.9	22	20.8	35	33.0
40 MG	104									2	1.9	7	6.7	84	80.8	11	10.6
ALL IN POOL	516	3	0.6	2	0.4	1	0.2	11	2.1	154	29.8	19	3.7	162	31.4	164	31.8

In the non- US studies N= 326 received NIS CC for more than 6 months; N= 164 for more than 1 year. In the US studies the figure for greater than 6 months was N= 131 but, apparently, none were treated longer than 1 year.

Demographic characteristics in this safety evaluation will be related primarily to adverse effects and other safety- related features.

B.Safety

1. Deaths

No deaths occurred in the US NIS CC hypertension studies other than for a single subject receiving placebo. He was aged 68 years, collapsed, and failed to respond to resuscitation efforts. In the non-US NIS CC hypertension studies a placebo case died of cerebral hemorrhage; N= 2 NIS CC cases died, one due to an accident, another due to cerebral metastases from prostatic cancer.

2. Serious ADE

The next table shows the number and percentage of subject in the US NIS CC studies by dose for both cases with serious ADE, which display dose response, and for those withdrawing due to serious ADE, in whom dose response is probably present. The dose response for % of patients with ADE versus dose varys from 0.7% (10mg) to 9.1%(80mg).

Number (%) of Patients with Serious Adverse Events and Withdrawals from Study Participation because of Those Events by Dose of NIS CC

DOSE OF NIS CC (n)	10mg (151)	20mg (395)	30mg (244)	40mg (292)	60mg (199)	80mg (11)	Total (1292)
NO. OF PATIENTS WITH SERIOUS AEs (% OF PTS ON DOSE)	1 (0.7)	8 (0.2)	2 (0.8)	5 (1.7)	9 (4.5)	1 (9.1)	26 (2.0)
NO. OF PATIENTS WHO WITHDREW BECAUSE OF SERIOUS AE (% OF PTS WITH SAE)	0	3 (38)	0	4 (80)	5 (55)	1 (100)	13 (50)

3. Discontinuations due to ADE

Of the N= 1292 patients (combined US+ non- US NIS CC), N= 25 (2.0%) ADE reports were received. Of these cases N= 13 were withdrawn because of these events. N= 17 of the 25 reports occurred during the double- blind phase of trials.

These N= 13 cases, among others, have narrative comments in Table 15a Pool 6 Vol 521. Each of the narratives on these cases was examined by the Reviewer. The final diagnoses were angina, MI, cellulitis of legs, possible MI, CVA, possible MI, infection, flu, pleural effusion, cholelithiasis, CVA, chest pain, pituitary tumor and berry aneurysm, elevated liver enzymes, edema and erythema plus petechiae, pain in legs with elevated CPK, chest pain.

Of the non- US NIS CC completed studies N= 35 cases on NIS withdrew due to ADEs. A listing, Vol 523 Table 15, provides reasons for discontinuation in N= 11 cases: These include tinnitus, non-response, pheochromocytoma, headache plus edema, atrial fibrillation, impotence, edema(2), vertigo, lack of efficacy, non- compliance. In the remaining cases a cause for discontinuation was not given although co-start terms for side effects were. There were a variable number of such terms for different patients. Most were manifestations of vasodilatation. No withdrawals for laboratory abnormalities were listed.

In the N= 6 placebo- controlled trials (US and non- US) with NIS CC 55/828 (6.6%) discontinued. Another N= 68 cases (11.5%) discontinued from among N= 590 patients on long- term uncontrolled studies. Since all but one of the 6 trial was a US study, the Sponsor focused on them. The following table shows that a dose response exists, except at 30 mg, for withdrawal due to ADE in the US placebo- controlled trials. The Sponsor does not believe dose response is evident, but this reviewer's logistic regression (below) shows a slope coeff t of 3.5 std errors.

Number and Percent of Patients in US Placebo-Controlled Studies Withdrawing Due to Adverse Experiences, by Dose of NIS CC							
	PLA (n=280)	10mg (n=37)	20mg (n=180)	30mg (n=125)	40mg (n=184)	60mg (n=137)	80mg (n=15)
N (%)	9 (3.21)	2 (5.4)	13 (7.2)	5 (4.0)	15 (8.2)	15 (10.9)	3 (20.0)

Table 15a Vol 521, not attached, provides a complete listing of reasons for withdrawal in this group. What is striking is that many subjects have multiple reasons for withdrawal. When more than one reason is listed, no single one is given most weight in this table. There was a suggestion, based on inspection of the table, that peripheral edema and rash might be associated but the number of cases is not more than a few.

The following table shows the most frequently reported ADE in subjects withdrawing due to ADE in the controlled US studies.

Incidence (%) of Most Frequently Reported Adverse Events in Patients Withdrawn Due to Adverse Events in U.S. Placebo-Controlled Studies		
ADVERSE EVENTS	PLA (n=280)	ALL NIS CC (n=678)
Any Body System	3.2	7.8
Headache	0.4	3.8
Peripheral Edema	0.4	2.9
Vasodilatation	0	1.5
Nausea	0	0.9
Palpitation	0	0.9
Dizziness	0.4	0.7

The Sponsor reports that the ratio of the number of ADE to the number of patients discontinuing was greater at lower doses than at higher ones.

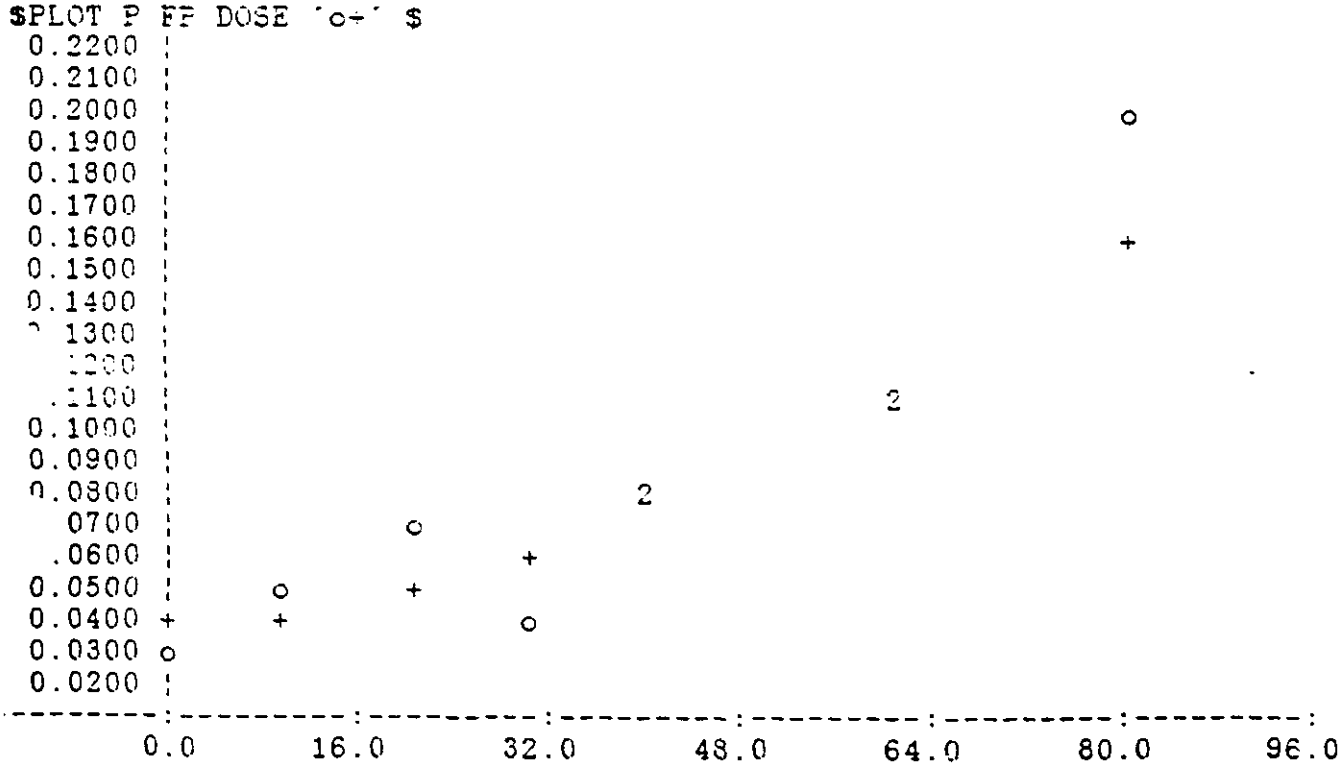
LOGISTIC REGRESSION OF WITHDRAWAL PROPORTION ON DOSE
Y AXIS: PROPORTION WITHDRAWN DUE TO ADE
X AXIS: DOSE MIS MG/DAY

Method: Iteratively- reweighted least squares (GLIM)
(analysis by reviewer)

o = OBSERVED

+ = PREDICTED

US PLACEBO- CONTROLLED HYPERTENSION TRIALS



The Sponsor suggests that multiple, but mild, events could be occurring at low doses with more severe ones, though fewer, at high doses. No analysis of this hypothesis is provided.

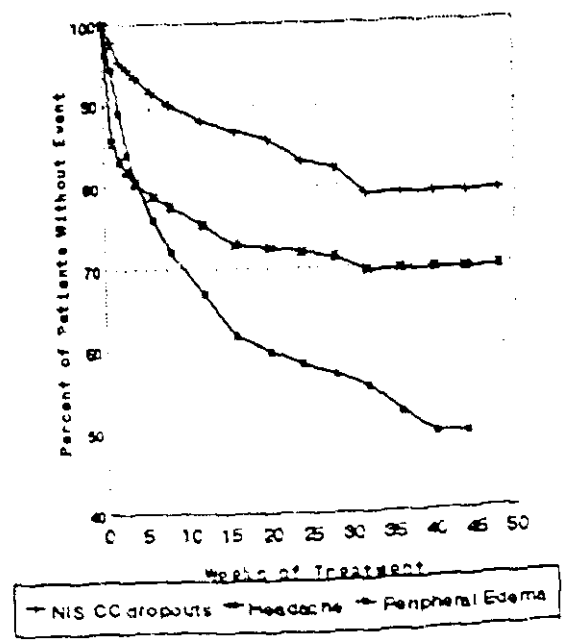
The following table shows cases withdrawing due to ADE from US uncontrolled studies. Patients are assigned the dose they were on for the longest time. There is a counterintuitive inverse association of withdrawal and dose. One possibility is that subjects on higher doses had the opportunity to have withdrawn on lower ones during the process of upward dose adjustment in these long- term studies.

Number and Percent of Patients in U.S. Uncontrolled Studies Withdrawing Due to Adverse Experiences, by Dose of NIS CC				
	20mg (n=55)	30mg (n=46)	40mg (n=34)	50mg (n=89)
N (%)	12 (21.8)	9 (19.6)	6 (17.6)	8 (9.0)

The most frequent ADE in patients withdrawn during the US uncontrolled studies, shown below, are ordered somewhat differently than in the short- term studies. In particular, peripheral edema is more frequent in the long- term trials probably because of a time-dependence for withdrawal such that headache occurs earlier than edema. This is shown in the following table and in a Kaplan-Meier plot.

Incidence (%) of Most Frequently Reported Adverse Events in Patients Withdrawn Due to Adverse Events in U.S. Uncontrolled Studies		
ADVERSE EVENTS	ALL NIS CC (n=224)	
Any Body System	15.6	
Peripheral Edema	12.1	
Headache	7.6	
Rhinitis	4.0	
Asthenia	3.6	
Dizziness	3.1	
Chest Pain	2.7	
Vasodilatation	2.7	

Kaplan-Meier analysis for dropouts due to adverse events and for incidence of headache and peripheral edema



The Sponsor states events causing discontinuation are more severe or higher doses, say 60 mg, than on lower ones. In the absence of a specific analysis taking account of the correct denominators, this may be questioned.

4. Most frequent ADE

The following table lists the most frequent ADE regardless of whether they were associated with withdrawal. Data from US and non- US NIS CC cases are given. The prominance of symptoms related to vasodilation is again seen. The relatively greater incidence of edema in the pooled controlled + uncontrolled studies is also shown for both US and non- US data. In addition, the rates in the non- US studies are overall less than in the US ones.

Incidence Rate (%) of Adverse Experiences (≥3%) in Patients Treated in U.S. and Non-U.S. Studies						
Study Location	Studies conducted in the U.S.			Studies conducted outside the U.S.		
Type of Studies	Placebo-Controlled		Controlled + Uncontrolled	Placebo-Controlled		Controlled + Uncontrolled
Adverse Events	PLA (n=280)	NIS CC (n=678)	NIS CC (n=776)	PLA (n=58)	NIS CC (n=150)	NIS CC (n=516)
Headache	15	22	23	21	23	18
Peripheral Edema	10	22	29	7	12	15
Dizziness	4	5	7	9	4	5
Asthenia	4	4	6	0	1	3
Vasodilatation	2	4	5	0	3	5
Palpitation	1	3	3	3	4	3

5. ADE by Demographic features

ADE by demographic sub- groups is examined in the US placebo- controlled trials. The incidence is given below of ADE selected by the Sponsor for "common observance" with the type of compound used. These are mostly those with higher incidence. There is no aggregation by sex though one might have expected this, say, for edema in females

Breakdown by Gender of the Incidence (%) of Selected* Adverse Events† in US Placebo-Controlled Studies				
	NIS CC		PLACEBO	
ADVERSE EVENT	Male (n=424)	Female (n=254)	Male (n=172)	Female (n=108)
Any Body System	67	69	50	58
Headache	20	24	14	18
Peripheral Edema	22	21	8	14
Dizziness	5	5	2	6
Asthenia	4	4	4	3
Vasodilatation	4	4	1	4
Palpitation	3	4	1	1

* The above events were selected because of their common observance with dihydropyridine therapy

† The US data includes both adverse events and intercurrent illnesses

In the non- US placebo- controlled trials there was an increase in edema in females treated with NIS CC but data for the placebo group is not provided. The incidence of edema in treated females is about the same as in the US placebo group, above. The more frequent ADEs are shown below by race in all placebo- controlled studies. Rates tend to be higher for headache and edema in Caucasians.

Breakdown by Gender of the Incidence (%) of Selected* Adverse Events† in Non-US Placebo-Controlled Studies		
	NIS CC	
ADVERSE EVENT	Male (n=65)	Female (n=85)
Any Body System	33.8	38.8
Headache	23.1	22.4
Peripheral Edema	7.7	15.3
Dizziness	3.1	4.7
Vasodilatation	< 3	4.7
Palpitation	3.1	4.7

* The above events were selected because of their common observance with dihydropyridine therapy

ADE by age are shown below for placebo- controlled studies. Headache is more frequent in younger subjects, interestingly, though edema may be less frequent than in older cases. These trends are present in both US and non- US studies.

Breakdown by Age of the Incidence (%) of Selected Adverse Events† in US and Non-US Placebo-Controlled Studies				
	US Studies		Non-US Studies	
ADVERSE EVENT	≤ 65 Years (n=601)	> 65 Years (n=77)	≤ 65 Years (n=131)	> 65 Years (n=19)
Any Body System	69	57	37	37
Headache	24	5	24	11
Peripheral Edema	21	27	12	16
Dizziness	5	5	4	5
Asthenia	4	0	< 3	5
Vasodilatation	4	4	4	< 3
Palpitation	3	1	5	< 3

† The US data includes both adverse events and intercurrent illnesses

Peripheral edema was more frequent in heavier subjects than in the lighter ones shown below both in US and non- US studies.

The table below shows selected adverse events with incidence rate ≥ 3% broken down by median weight in the US and non-US placebo-controlled studies.

Breakdown by Weight of the Incidence (%) of Selected Adverse Events[1] in US and Non-US Placebo-Controlled Studies				
	US Studies		Non-US Studies	
ADVERSE EVENT	≤ 165 lbs (n = 290)	> 165 lbs (n = 398)	≤ median weight (n = 61)	> median weight (n = 69)
Any Body System	64	70	33	39
Headache	20	23	24	22
Peripheral Edema	16	26	10	15
Dizziness	5	5	4	4
Asthenia	4	4	< 3	< 3
Vasodilatation	5	4	< 3	6
Palpitation	3	3	< 3	6

[1] The US data includes both adverse events and intercurrent illnesses

A table, not attached, shows that when ADE are stratified by baseline BP below and above 108 mmHg diastolic, that cases with lower BP tended to have more vasodilatation. The respective rates, 5/537 and 1/141. These are not very impressive.

6. Hemodynamic safety

A. Hypotension

ADE suggestive of hypotension, syncope and "hypotension" were sought. Asymptomatic hypotension was determined by "first dose effect", by trough/peak ratios from in- clinic and 24hr ambulatory BP readings, and by examining supine and standing BP plots. No cases (Sponsor) of syncope in the US NIS CC trials on NIS. N= 6 patients in the US placebo- controlled studies had either "hypotension" or "postural hypotension" on NIS CC. The next table shows data from US placebo- controlled trials.(INSERT tp15 v309)
Note that only a few of the cases show orthostatic hypotension in casual blood pressures. It seems worth while to point out that "dizziness" occurred in about 7% of all the US NIS CC studies and that it is possible that a number of cases had this symptom due to hypotension. Clinical experience shows that "dizziness" is not often distinguished from light- headedness without vertigo due to inadequate questioning of patients.

A first- dose effect was examined in two studies without showing adverse symptomatology but with BP reductions. The following table shows the results of in- clinic BP monitoring. Dose related peak effects are seen.

Mean Pre-dose and Peak Supine and Standing Blood Pressure Changes During In-Clinic Monitoring Periods in US Placebo-Controlled Studies							
		Mean Change in Supine Blood Pressures (mmHg)		Time to peak (hr)	Mean Change in Standing Blood Pressures (mmHg)		Time to peak (hr)
Drug Group	n	Pre-dose	Peak		Pre-dose	Peak	
STUDY D88-054 (24-hour period, BPs every hour)							
Placebo	8	-0.9/-3.4	-5.0/-8.8	3	-2.3/-3.5	-6.3/-7.0	22
NIS CC 10mg	7	-11.5/-10.3	-12.6/-11.1	8	-17.4/-8.7	-9.7/-10.5	9
NIS CC 20mg	7	-8.0/-8.3	-7.1/-12.0	14	-9.6/-4.6	-7.1/-10.5	11
NIS CC 30mg	5	-8.8/-12.0	-18.0/-15.5	12	-5.9/-7.4	-14.8/-16.2	11
STUDY D89-039 (12-hour period, BPs every two hours)							
Placebo	35	-4.9/-5.0	-3.4/-5.5	8	-5.7/-3.6	-1.6/-4.9	8
NIS CC 30mg	37	-11.7/-10.8	-16.7/-13.5	8	-14.3/-8.3	-15.2/-12.5	8
NIS CC 40mg	38	-18.0/-12.9	-19.1/-15.5	4	-18.2/-12.1	-22.9/-16.9	4
VER SR	40	-14.4/-14.8	-17.4/-15.1	2	-16.2/-14.9	-21.0/-15.8	8
STUDY D90-019 (12-hour period, BPs every hour)							
Placebo	27	-1.9/-7.2	-8.0/-8.1	7	-7.1/-6.0	-4.8/-7.5	4
NIS CC 30mg	32	-13.3/-13.6	-19.0/-14.4	8	-14.9/-12.8	-16.9/-15.8	5
NIS CC 60mg	28	-19.2/-17.9	-16.9/-18.*	9	-18.1/-15.2	-21.*/-22.3	7

Symptomatic Hypotensive Adverse Events
Occurring in US Placebo-Controlled Studies

Study Number	Patient No. / Drug & Dose	Investigator Term	Days on Drug	Duration (days)	Intensity	Baseline BPs (supine & standing)	BPs (day and blood pressures)
			SYNCOPE				
D89-039	16020 / PLA	Vasovagal Attack	43	1	Moderate	165/107 168/105	day 43: 142/99 143/90
D90-019	1012 / PLA	Feeling Faint at Times	30	>13	Mild	149/107 141/102	day 28: 155/113 171/113
			HYPOTENSION				
D89-039	13012 / NIS 20mg	Hypotension Post-Dose	62	1	Mild	136/95 135/97	day 62: 129/82 108/70
D89-039	16015 / VER SR 240mg bid	Hypotension	23	>5	Severe	129/100 115/91	day 22: 114/93 98/74
			POSTURAL HYPOTENSION				
D89-039	1005 / NIS 20mg	Dizziness upon standing	30	12	Mild	129/95 130/99	day 41: 136/91 135/85
D90-019	15004 / NIS 30mg	Dizziness (Postural upon standing up)	10	9	Mild	165/105 162/106	day 14: 151/99 146/98
D89-039	1008 / NIS 40mg	Postural Hypotension	16	1	Mild	155/98 141/99	day 16: 129/89 129/85
D89-039	12007 / NIS 40mg	Postural Hypotension	25	1	Mild	157/97 150/97	day 25: 146/89 136/88
D90-019	16007 / NIS 60mg	Postural Dizziness	28	>6	Mild	170/105 168/101	day 33 145/89 147/90
D89-039	4001 / ATN + PLA	Dizziness upon standup	3	5	Mild	187/105 146/90	day 6: 183/98 150/93 day 14 153/94 93/67

N= 5 ambulatory monitoring studies were done. The distribution of doses by study is provided. The second table shows the trough/peak ratios from these studies.

Number of Patients Undergoing 24-Hour ABPM by Dose of NIS CC						
Study & (location)	PLA	10mg	20mg	30mg	40mg	60mg
D88-054 (US)	18	12	11	12		
D89-029* (US)	31		32		32	30
D89-039 (US)	28		24		24	
DSU-006 (non-US)	33	33	32	37		
D90-019 (US)	31			39		25

* This study was conducted on the background of atenolol 50mg qd

Trough/peak ratios from the 24-hr ambulatory data are provided below. Note that for systolic BP two methods are used depending on whether peak systolic is a) determined at the time of peak diastolic BP or, b) whether the true peak is used. These ratios are consistant with peaks that are not substantially below the trough values. Note, however, that if in a given subject the trough readings are quite low that a small, further drop at peak might be hypotensive. For that reason trough/peak ratios may not be very good means of exploring for BP reductions for safety purposes.

TABULATION OF TROUGH-TO-PEAK RATIOS ANALYZED DURING 24-HOUR AMBULATORY BLOOD PRESSURE MONITORING (BASELINE- AND PLACEBO-SUBTRACTED RESULTS)						
	DOSE OF NIS CC					VER SR
PARAMETER	10mg	20mg	30mg	40mg	60mg	240mg bid
Diastolic BP Trough/Peak Ratio (%)	73	75	93	100	97	86
Systolic BP* Trough/Peak Ratio (%)	75	83	114	100	101	78
Systolic BP† Trough/Peak Ratio (%)	75	83	93	100	89	78

* Peak values correspond to the time of peak diastolic effect.
† Peak values are the actual maximum systolic effect.

The table below shows the percentage of patients having either a change of 20 mm from baseline or a BP below 100mm Hg. There appears to be a fairly consistant percentage of cases with a fall

in systolic BP regardless of dose except for low numbers in the small sample on 40 mgm NIS. Note that a substantial number of placebo cases also show this degree of fall. Subtraction of the placebo values gives about 4- 8% of cases with reduction below 100 mm systolic. Thus supine reductions of note occurred in some subjects.

TABULATION OF SAFETY PARAMETERS ANALYZED FROM 24-HOUR AMBULATORY BLOOD PRESSURE MONITORING (BASELINE-SUBTRACTED RESULTS)

SAFETY PARAMETER	DOSE OF NIS CC					PLA	VER SR
	10mg (n=45)	20mg (n=67)	30mg (n=98)	40mg (n=24)	60mg (n=29)	(n=106)	240mg bid (n=29)
Diastolic BP Change > 20mmHg from Baseline for at least 1 Hour (% of patients)	82	86	78	79	96	62	96
Systolic BP < 100mmHg for at least 1 Hour (% of patients)	24	26	22	4	20	16	24

The last method of assessing hypotension was to compare supine and standing blood pressures at trough. The correlation was near 1.0 and consistant with little orthostatic hypotension.

B. Reflex tachycardia

One of the effects of a vasodilator is reflex tachycardia. The Sponsor examined this by dose response of pulse rate; by frequency of tachycardia as an ADE; and by ECG HR.

In the US monotherapy studies the mean placebo- subtracted change in HR varied from -1.53/min to +0.48 over the dose range of 10 to 60mgm NIS CC. The change in HR for the combined doses was 0.52. Note that these are not specified as standing readings.

In the US placebo- controlled studies, tachycardia was an ADE in 1% of NIS CC patients. One patient in these studies withdrew for supraventricular tachycardia.Three patients in the US uncontrolled studies had tachycardia contributing to withdrawal. The Sponsor analyzed the transition from normal or low heart rate to high values in the US controlled trials and found this to have occurred in 1.4% of NIS CC patients and in 0.9% of placebo cases. Again, none of these readings are specified as taken standing, a position that might have exaggerated pulse change.

C. Rebound hypertension

A placebo- controlled study D90-022 in hypertensive patients treated for as long as 21 days sought evidence for rebound blood pressure elevations by a 72- hr follow-up after discontinuation of NIS CC. Examination of the Sponsor's table showed that the group means for diastolic BP show no evidence of rebound. However, the systolic BP values are higher at 72 hrs than at baseline except for the highest dose level, 120mg NIS. The systolic BP mean for the placebo group is also elevated at 72 hrs so that it is difficult to ascribe the increase in systolic BP to "rebound". It is more likely that loss of both the placebo and therapeutic effects are involved.

7. Clinical Laboratory Tests

A. US NIS CC placebo- controlled studies

1. Incidence rates of "high" lab abnormalities

The Sponsor provides Table 17a (not attached), in which the rates for "high" abnormalities are given by dose level from 0 mgm (PLAC) to 80mg. Sample sizes are very small for the lowest active dose, 10mgm and the highest, 80mgm. Examination of these rates show no evidence for dose response nor is it likely they would given the exceedingly small rates for the data pooled over doses. In particular, for items with overall higher rates including blood glucose, no dose response trend is seen. BUN has a 3% rate at 40mg NIS, 2% at 60mg, and 0% for placebo. No such trend is seen for creatinine. No trend is seen for increase with dose of serum calcium, alkaline phosphatase, or SGPT is seen.

Rates by dose/body weight are also provided but do not show trends of interest except, possibly, for alkaline phosphatase, which has a rate of 5% at the highest dose/weight level, >.55- <1.2 mgm/kg, versus a placebo rate of 2% and rates in lower active dose/weight levels of 1%.

For "low" values the hematologic values for all US NIS CC studies showed 2% of NIS patients with neutrophils below 1700/microl and 1% in the placebo group. The rate was also 2% in the pooled controlled and uncontrolled studies. No patients with platelets below 100,000/microl are shown. Thus addition of cases with long- term followup did not increase the rate of low values for these two tests.

Hematopoietic Parameter Abnormalities from Studies Conducted in the US

BLOOD CELL LINE	Placebo-Controlled		Controlled + Uncontrolled
RBC	PLA	NIS CC	NIS CC
	n=250	n=588	n=680
% PATIENTS LOW ABNORMAL	3	3	5
WBC	PLA	NIS CC	NIS CC
	n=232	n=550	n=636
% PATIENTS LOW ABNORMAL	5	4	5
% PT WITH NEUTROPHILS < 1700/μL	1	2	2
PLATELETS	PLA	NIS CC	NIS CC
	n=267	n=655	n=751
% PATIENTS LOW ABNORMAL	0	0	0
% PT WITH PLATELET<100,000/μL	0	0	0

This submission contains a tabulation of mean difference from baseline for both NIS CC (N= 650) and placebo (N= 280) for US placebo- controlled subjects. A tabulation on the following page contains selected laboratory tests from the larger tables. The larger table, Table 21 Vol 522, also has standard deviations. The Reviewer calculated the mean difference +/- 2 SE limits for NIS cc and for placebo for hematocrit, platelets, %neutrophils, glucose, BUN, alkaline phosphatase, and SGPT. All of the 2SE limits for the NIS group overlapped those for the PLAC group for these particular tests so that the treatment groups are not likely to differ by this method.

Examination of 10 lowest or highest values of selected laboratory tests in all US studies(controlled and uncontrolled for individual subjects showed, among the lowest 10, N= 3 instances in which falls in hematocit occurred. None were associated with the lowest 10 values for total wbc or %neutrophils. Respective baseline and lowest values were 34,29; 36,30; and 36,33. The baseline values tended towards being low. The subject with the lowest hematocrit,29, was on many medicines at baseline including Naproxin, insulin, enalapril,labetalol, and glyburide. During the

Renal Function Parameter Abnormalities from Studies Conducted in the US			
RENAL FUNCTION TEST	Placebo-Controlled		Controlled + Uncontrolled
CREATININE	PLA	NIS CC	NIS CC
	n=271	n=646	n=739
% PATIENTS HIGH ABNORMAL	0	0	1
BUN	PLA	NIS CC	NIS CC
	n=269	n=645	n=738
% PATIENTS HIGH ABNORMAL	0	1	1

Hepatic Parameter Abnormalities from Studies Conducted in the US			
LIVER FUNCTION TEST	Placebo-Controlled		Controlled + Uncontrolled
SGOT	PLA	NIS CC	NIS CC
	n=248	n=603	n=692
% PATIENTS HIGH ABNORMAL	3	2	3
% PT >3X NORMAL	0	0	0
SGPT	PLA	NIS CC	NIS CC
	n=224	n=562	n=647
% PATIENTS HIGH ABNORMAL	3	2	4
% PT >3X NORMAL	0	0	0
ALKALINE PHOSPHATASE	PLA	NIS CC	NIS CC
	n=252	n=623	n=713
% PATIENTS HIGH ABNORMAL	2	2	4
% PT >1.25X NORMAL	0	0	0
LDH	PLA	NIS CC	NIS CC
	n=257	n=628	n=717
% PATIENTS HIGH ABNORMAL	3	2	4

All cases of high blood glucose on treatment were high at baseline, usually above 200mg%.

N=7 instances of mildly elevated BUN on treatment were found. Only one of these (value =27) was associated with an increased serum creatinine(1.4,2.0).
Serum calcium increased in association with treatment in N=3 cases baseline, on treatment:9.8,10.5; 10.0,10.5;9.1,10.4) but alkaline phosphatase was not in the highest N=10 for any of these. The last of the above N=3 cases had a low phosphate (2.3,1.9).

Although a number of cases had elevation of alkaline phosphatase during treatment, all were high at baseline. One instance of notable change (112,248), but with a subsequent fall to 150, despite some increase at baseline was more closely examined. The patient was a 64- year-old female with diabetes mellitus, hyperlipidemia, and edema. During a long-term extension trial she was on concomittant medications including atenelol, niacin, and glyburide. A slow rise in alkaline phosphatase over 1 and 1/2 years occurred with 3 high readings.

N=3 instances of elevation of SGPT with concomittant elevation of SGOT were noted (baseline, on treatment: 25,384; 39,209;35,88). The first of these also had elevated total bilirubin (0.6,3.3). This patient was a 63-yr-old male with a history of elevated transaminases on ACE inhibitors.During treatment he received Lovastatin. Despite continued treatment with NIS the final day SGPT and SGOT were well within normal limits though the previous two values, both obtained within a one-week period, were elevated. An additional N=2 instances of elevation of SGOT occurred in the absence of enough rises in the other enzymes to reach the level of the highest 10 values.

increases in serum bilirubin occurred in N=4 cases. One of these had enzyme elevations described just above. Total CPK was elevated during treatment in N=2 patients. In one the MM and BB fractions were normal.

In the N=516 (depending on test) non- US controlled plus uncontrolled studies hematocrits were low in N= 3 cases but in N=2 they tended to increase subsequently. Total leucocytes were low in N=4 cases. In two of these the baseline value was also low. None of these cases were among the 10 lowest %granulocyte values. N=8 low platelet counts occurred in N= 8 cases. In N= 2 of these the baseline values were normal. Except for N=2 cases the on- Rx values were not very low, and though below the assigned normal range, were all above 100,000. In the one of N=2 cases with platelets below 100,000 total wbc was low both at baseline and on treatment. In the other the wbc count was not among the N=10 lowest.

In N= 10 cases elevation of SGOT occurred but baseline values were elevated in these. In two cases use of country- specific normal values might have reduced baseline values to normal. N=7 cases with elevated alkaline phosphatase values occurred. In N= 5 of these the baseline values were also elevated. These elevations ere not associated with values for SGOT in the highest 10. N=6 cases of elevated serum bilirubin were found; in N=4 baselines were high. None were associated with SGOT values in the highest 10.

Serum creatinine was not elevated above normal in any of the values in the highest 10.

8. ECG

A. Background

Because of the finding of t-wave changes in study D90-022 (hypertension), the Sponsor examined that study and three phase III NIS CC trials for such alterations. The Sponsor has provided background information from the medical literature. T wave inversion or flattening occur during rapid reduction of BP with vasodlators. These reductions in BP do not appear to be associated with wall motion abnormalities on 2D echocardiography. Long- term treatment with minoxidil has been carried out with improvement in the initial t-wave changes.

B. The phase II trial D90-022

This was a trial in N= 26 patients with mild to moderate hypertension, mean age about 60 years. This was a forced titration trial 30- 120mg NIS CC with the first two doses given for 4 days each; the next two for 7days each. N=8 subjects were randomized to NIS CC and N=5 developed t- wave flattening on the ECG. The N=120mg dose level was discontinued due to poor tolerance (severe peripheral edema, ECG changes).A second group, N= 10, was randomized to NIS. N=6 of these cases developed t wave flattening and/orinversion with occurrence equal, N=2 cases, at each of the doses, 30,60, and 90 mg. No angina occurred. Thallium scans were reported as negative in N=5 of the first cohort.

The percent with T-wave changes and dose were, respectively, 0%(PLAC); 22%(30mg); 39%(60mg); 64%(90mg); 80%(120mg). The respective sample sizes were 5,18,18,11,5. n.b. The excess over the N=23 that were randomized must represent titration steps.

Stratifying results into cases with normal and abnormal ECG and into 6 and 24 hrs after dosing, showed significant differences between BP falls at 6hrs between the two ECG groups. Differences at 24 hrs (trough) were not significant.

Stratifying normal and abnormal ECGs by AUC and Cmax showed that the abnomal ECG group had larger pharmacokinetic parameters.

The Sponsor relates the ECG changes to the forced- titration design and, by analogy, to the literature reports of T- alterations in rapidly- induced hypotension.

C. Other Phase III trials

ECGs from trials D89-029,039,and D90-019 coded blindly and read by a cardiologist. These ECGs were usually taken at trough. Peak ECGs were obtained in one study. Dosage was up to 80mg NIS CC (N=494 in the pooled studies) or placebo. Mean age was about 55. One study had background atenolol.

In these titration studies the dose assigned to an ECG was, for one analysis, that to which a patient was randomized, not to, say, a lower dose they might have transited from. Another analysis assigned the dose as that on which the event occurred. Two analyses were done; one ignoring baseline ECG events and another eliminating cases with these. It seems likely that more than one event per person could occur since rates were calculated from the "total number of events" divided by the number of cases at risk for the first type of analysis. In the second analysis all subjects were at risk. One analysis was done for peak ECG responses.

The table below shows some evidence of dose response except for the small sample at 80mg.

STUDY D89-039						
ECG abnormality	PLA	ALL NIS CC	NIS CC 20 mg	NIS CC 40 mg	NIS CC 80 mg	VER SR
T flattening	5/70 (7%)	10/155 (6%)	3/71 (4%)	7/69 (10%)	0/15 (0%)	4/71 (6%)
T inversion	3/73 (4%)	11/157 (7%)	4/72 (6%)	6/71 (8%)	1/14 (7%)	3/72 (4%)
either	8/74 (11%)	20/166 (12%)	7/75 (9%)	12/76 (16%)	1/15 (7%)	7/76 (9%)

Similar results were obtained when *'all patients' (regardless of baseline findings) were studied and* when the number of ECG tracings was used as the denominator. n.b. there tends to be a dose response in each of the above results except at 80mg.

Results for ECGs taken at peak were unrevealing. The sample sizes were far too small, about N=5 per active dose group.

Study DS029. This is the study with background atenelol in addition to NIS CC.The doses of NIS were 0,20,40, and 60mg.

The respective rates of T flattening or inversion at these doses were 18%,7%,20%13% so there was not much evidence of dose response.

For all cases, regardless of baseline status, there was a significant difference among doses for T-flattening (PLAC 21%; 20 21%; 40mg 41%; 60mg 27%).

For events per number of ECGs results were weaker.

Study D90-019. The rates for T-wave flattening or inversion by dose were PLAC 13%; 30mg 9%; 60mg 5%. Therefore no dose response is seen.

Rates using all patients or number of ECGs in the denominator were not more useful.

ST- segment elevation or depression: Rates for these were very low in each of the three studies (1-2%). Comparing the incidence in the PLAC and pooled NIS CC groups by ST depression and by elevation showed that placebo and active dose rates were each no more than 2%.

There is little evidence from these three studies, taken together, of a dose response for the primary ECG T-wave changes of interest. However, the findings in the Phase II trial with forced titration do show a trend for dose response as well as effects of BP reduction and plasma NIS concentrations on T abnormalities. Thus the findings in the Phase III trials may just be at the opposite end of a spectrum of effects.

Conclusions for Hypertension Safety (NIS CC):

In placebo- controlled trials there is a dose- related incidence of ADE over the range 0 to 80 mgm of NIS CC. The most frequent ADE are those related to the vasodilatory action of the drug - headache and edema. The occurrence of these two ADE is time- dependent with headache occurring relatively early versus edema.

Marked symptomatic hypotension was not prominant with NIS CC although "dizziness" occurred and may have been a manifestation of hypotension. Asymptomatic hypotension in trough readings was not frequent though supine systolic BP values in the region of 100mm Hg were not infrequent in 24- hr ambulatory BP readings. Rebound hypertension was not present overall during monitored withdrawal. ECG T- wave abnormalities, inversion and flattening, occurred during forced titration in a phase II study but in studies in which dosage was increased slowly was not prominant. There was an association of these ECG changes to the degree of BP reduction and to drug blood levels in the forced titration study ECG S-T alteration was infrequent.

Clinical laboratory abnormalities: In the US, placebo- controlled (shorter- term) studies, evaluations by overall rates of abnormality, transition from normal, and overall rates by dose were not very revealing. present. Examination of the N= 10 highest or lowest values, as appropriate, in all US studies, controlled or uncontrolled (long-term), showed a few instances of falls in hematocrit, total wbc count, and %neutrophils. Several instances of increased transaminases, one with increased bilirubin were found. An instance of substantial elevation of alkaline phosphatase occurred. Serum calcium increased in three cases without increases in alkaline phosphatase.

In the non- US controlled and uncontrolled trials (approximately N= 516 NIS cases) several instances of decreases in hematocrit or wbc occurred. Platelet counts fell in some cases but not below 100,000. A number of cases had increases in SGOT but from elevated baseline levels. Serum creatinine was not elevated in the highest ten values.

Pages
16-33

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Contain
CONFIDENTIAL
COMMERCIAL
INFORMATION

III. Congestive heart failure (NIS CC)

Safety information for this indication is based on two, non- US, placebo- controlled trials. Approval is not being sought for this indication though the safety information is useful since patients with hypertension may develop CHF.

A. Duration of exposure/ demography

Of N= 142 total CHF cases N= 68 were exposed to NIS CC for at least 1 month. Not quite half were exposed for 1- 2 months. Almost all cases, 90%, were male and about 3/4 were younger than 66 yrs of age. Most were NYHA class 1- 2. About half the cases in the placebo and active Rx groups were receiving beta blockers.

B. The following table shows ADE occurring in at least 1% of subjects. Oddly, headache is less frequent in the NIS CC group. Notably, dyspnea is also less frequent.

NIS CC IN PATIENTS WITH CONGESTIVE HEART FAILURE
TREATMENT EMERGENT ADVERSE EVENTS WITH INCIDENCE ≥ 1%

ADVERSE EVENTS	NIS CC n=142 %	PLACEBO n=71 %	PLACEBO SUBTRACTED
Chest pain	8.5	9.9	0
Dyspnea	7.7	12.7	0
Dyspepsia	6.3	5.6	0.7
Dizziness	5.6	7.0	0
Angina Pectoris	4.2	19.7	0
Palpitation	4.2	5.6	0
Vasodilatation	2.8	4.2	0
Headache	2.8	11.3	0
Peripheral Edema	2.8	4.2	0
Hypotension	2.1	4.2	0
Asthenia	1.4	4.2	0
Pain	1.4	0.0	1.4
Increased Cough	1.4	1.4	0

C. Discontinuations associated with ADE

N= 3 cases discontinued placebo treatment in association with ADE (angina, CHF, and ventricular fibrillation) and N= 2 NIS CC cases stopped (CHF, angina). Total discontinuations, regardless of assigned cause, were N=3 in each Rx group. No deaths were reported.

D. Biochemical tests

The submission contains tabulations including one for the percent of cases with "low" abnormalities. The sample sizes for NIS CC and PLAC are about N= 70 for the wbc and platelet data. N= 1 instance of low platelet count per each Rx group is recorded. No instances of low wbc counts are listed. SGOT and SGPT were more frequently elevated in the placebo group. Alkaline phosphatase levels are not listed. No elevations of serum creatinine are listed in either treatment group.

Cases with the ten lowest and ten highest lab values were examined by the Reviewer. One cases had a fall in hematocrit from 40 to 35.2. The lowest total wbc count was 3.9 and one instance occurred in each of the Rx groups. The lowest platelet counts were 75,000 (PLAC), 120,000 (NIS CC). The highest SGOT and SGPT on NIS CC were each 57 units.

Comment: In considering the above CHF safety data, note that the experience is limited to NYHA Class I and II cases.

IV. Congestive heart failure (CC IR)

A. Non- US studies, controlled plus uncontrolled

1. Exposure/ demography

Data was provided on N= 314 patients. Duration of exposure for about half the cases was 1- 2 months and for about 15% of cases was 6- 12 months. Most cases were NYHA Class II or III. Nearly half the patients were on concomittant diuretic therapy.

2. Patient completion status

Of NIS IR cases 60% (189/314) and 50% (29/58) of placebo cases completed therapy. Completion status was not available for 29% of NIS IR and 40% of placebo cases. There were N=2 and N= 1 deaths in those respective treatment groups.

a. Narratives of deaths in NIS IR cases

Case 1. This patient had pain in the right arm during the day prior to death during sleep. Death occurred on day 15 of NIS IR 20 mgm/daily.

Case 2. After N=12 days of NIS IR 20mgm/day the patient developed dypsnea at rest and was dropped from the study and put on an ACE inhibitor. Death occurred 35days later due to "protracted pump failure".

3. Adverse Events

Adverse events (ADE) occurring in at least 3% of cases of the NIS IR group included headache(6.1%); "vasodilatation"(5.4%); peripheral edema (3.8%); and "dizziness"(4.4%). Of N= 14 cases discontinuing in association with ADE relatively few were apparently due to increasing congestive heart failure or development of angina -perhaps one of each. Tachycardia occurred in N= 4 cases. In one case edema, angina, and decreased exercise tolerance all were listed as occurring on day 14. Non-US and US trial results often differ in this submission.

4. Biochemical tests

The Sponsor pooled the results of IR and solution data to study biochemical test results. Sample

sizes were very small [illegible] in the placebo- controlled studies evaluation of elevation of SGOT could be made [illegible] cases and N= 12 placebo ones (11.8 and 8.3%, respectively). Alkaline phosphatase [illegible] in 2/17 (11.8%) of NIS cases and 0/13 (0.0%) of PLAC ones. No cases in either tre[illegible] "elevated" serum creatinines or decreased total wbc count. One NIS case had a [illegible]atelet count.

V. Clinical pharmacology studies (safety)

The Sponsor has gathered information from NIS CC (US, non- US) ; NI IR (US); NI IR + other formulations (non- US). Information in the last of these three groups is quite incomplete since safety information was often not recorded. In N=8 ongoing, non- US NIS CC trials in N= 135 subjects there were reports of two dropouts due to edema, both from a trial in subjects with hypertension and renal failure.

A. Exposure/demography

1. NIS CC US Studies

N= 183 cases received NIS CC; N=25, placebo. N= 65 healthy volunteers and N= 118 were patients, mostly hypertensives or those with renal impairment. A few cases were cirrhotic. Females made up 26% of the cases. Duration of exposure was between 7 and 30 days for N= 34 hypertensives and between 1 and 7 days for the rest.

2. NIS CC non- US Studies

N= 215 cases received NIS and N= 40 placebo. Of these, 17% were female. Most of the studies were of short duration, N= 7 days or less.

3. NIS IR US studies

N=83 healthy volunteers participated in these trials, which were all cross- over studies.

4. NIS IR non= US studies

N= 349 cases were exposed to NIS IR tablets and N= 297 to NIS IR solution. Females made up 24% of the total. N= 98 subjects were exposed at least 98 days; of these N+ 20 were exposed 61- 180 days.

B. Adverse event rates

1. NIS CC US Studies

Events occurring in at least 3% of cases included for PLAC and then for NIS, respectively, headache 48%; 64.5%; edema: 0%; 7.7%; dizziness/lightheadedness: 8%;4.9%; tachycardia: 0%; 4.4%.

N=7 NIS subjects discontinued in association with the following ADE: headache, tachycardia,peripheral edema(3), t-wave flattening, and tremor. All discontinuations occurred at NIS 60mgm or higher. The case with t- wave flattening occurred in the forced titration study discussed under ECG abnormalities, hypertension studies. ADE, characterized as "serious" also occurred in the same trial, were edema(2), ECG changes (2), tremor.

2. NIS CC non- US Studies

Headache: 55%; Flushing 6%; tiredness 3.3%. Most of these studies had no placebo group so none is listed.

N= 7 of 215 NIS cases discontinued in association with angina, headache(5), transient atrial fibrillation, extrasystole.

3. NIS IR U.S. Studies

Headache occurred in 54% of subjects. Lightheadedness (13%) and drowsiness (3.6%) occurred. No discontinuations in association with ADE were reported.

4. NIS IR non- US Studies

Headache (5.7%) and "vasodilatation"(flushing)(4.9%) were two of the more frequently reported adverse effects. Headache was substantially less frequent than in the other groups of studies just discussed. N= 9 discontinuations in association with ADE were reported from among N= 646 cases treated with the IR formulation. These ADE were fluid retention and ankle swelling; headache; weakness and depression (2); and one case of arrhythmia. There were N= 4 additional discontinuations in cases receiving an IV formulation in doses of 0.12- 0.36 mgm. These ADEs were chest pain; "self-limiting" ventricular tachycardia; hypotension; and ECG ST changes.

C. Deaths

No deaths were reported in NIS CC studies in this category. One death occurred in a foreign study involving solution.

VI. Other safety information

A. Ongoing studies

1. Diabetic renal disease

About N= 1400 patients are involved in on- going, blinded studies. Most of these are taking part in a large U.S. trial of NIS CC in diabetic patients with or without hypertension and using enalapril as a positive control. Projected enrollment is N= 1200 per blood pressure category. The primary objective of the trial is to determine if intensive antihypertensive therapy is more effective than only moderate degrees of BP reduction in preventing deterioration of renal function. Additional antihypertensive drugs may be used if goal BP is not reached at doses of the assigned drugs causing intolerance. Note that in the material below data is still blinded.

a. Deaths

One case of suicide occurred in a subject with a history of two previous attempts. One death occurred at day 10 of active therapy in a case with a massive CVA.

b. "Serious" ADE

Most of these, N= 28, were cardiovascular (chest pain/angina: n=10; cardiac arrest/myocardial infarction: n= 4; CVA: n= 6; CHF: n= 2; deep vein thrombosis: 1; diagnostic procedure: n= 2).

c. Withdrawal "due to ADE"

There were N= 78 such ADE among N= 43 cases withdrawing. The most frequent events were edema (11), headache (9), chest pain/angina (5), flatulence (4), and hypertension (4). Cases withdrawing in association with angina/chest pain did not have antecedent headache noted in listings of multiple ADE.

2. Studies in various indications

These were foreign studies using CC IR in a total of N= 97 patients. Results were pooled over the indications for the safety review in the Sponsor's submission. Indications were peripheral vascular disease, post- MI, pulmonary hypertension, and renal hypertension.

a. Exposure/demography

About N= 90 cases were exposed for 1 month; N= 50 for 2- 6 months; and about N= 15 for more than 12 months. Only 25% of cases were females. About 58% of cases completed the study though the status is unknown for 37%. Only 1% are cited as dropping out for adverse effects

b. Adverse effects

Headache occurred in 7.2% of N= 97 NIS IR cases; vasodilatation in 5.2%; peripheral edema in 2.1%; skin rash in 2.1%.

There was one death reported- a patient in a post- MI study who had ventricular fibrillation on day 10 of treatment with NIS IR 10mg daily. Only N= 1 case dropped out due to ADE (for depression, headache, and rash on day 3 of treatment with NIS IR 20mg/day. N= 1 case dropped out (for malaise) of the trials called "supplemental" studies by the Sponsor consisting of trials in peripheral vascular disease in about N= 30 cases.

No biochemical data was available for these trials.

B. Post- marketing surveillance (PMS 1)

1. Exposure/demography

N= 8788 patients were treated in this German NIS IR study. About N= 6000 cases were treated for 2- 6 months. Women made up 40% of the patients.

2. Adverse events

a. Dropouts associated with ADE

Of N= 231 such dropouts 2% (7) had chest pain; 29% (67) had vasodilatation; 15.2%(35) had nausea; 13.4%(31) had peripheral edema; 0.4%(1) had lung edema; rash 1.3%(1). The Reviewer examined listings of associated signs/symptoms of the cases with chest pain/angina and found that 4/7 had either headache or vasodilation at the time of withdrawal. Biochemical data was not required in this study and none was presented.

b. Deaths

The following four pages provide a listing with comments on deaths in this study. N= 8 cases died from myocardial infarction; N= 4 from malignancy; N= 3 from heart failure; and N= 6 from other causes.

C. Post- marketing surveillance study (PMS 2)

1. exposure/demography

This second German study involve N= 640 NIS IR cases. Women comprised 38% of subjects. About 20% of cases were treated for more than one month and about 60 cases treated from 2- 6 months.

2. Dropouts associated with ADE

N= 18 cases dropped out under this condition. Of those 5.6% (1) had angina pectoris; 11.1%(2) had vasodilation; 16.7% (3) had hypotension. The patient with angina pectoris did not have cocomittant headache or vasodilatation in listings of multiple symptoms/signs. There were N= 6 deaths- N=3 due to myocardial infarction; N= 2 due to "cardiac insufficiency" with or without ventricular fibrillation. Clinical laboratory data was not assessed in this survey.

D. Post- marketing surveillance study (Japan)

1. exposure/demography

N= 1850 cases received NIS IR of whom about N= 1300 were treated from 2- 6 months. About half the cases were females.

2. Adverse effects

No deaths were reported from this study. Among the N= 50 dropouts for ADE 34% had headache, 26% had vasodilatation; 4% had chest pain; 4% hypotension;8% edema; 4% rash.

E. Experience after marketing

The Sponsor presents N= 10 Spontaneous Reports from marketing of the IR formulation in countries outside the U.S. These are one case each of anaphylaxis with edema of tongue and larynx; agranulocytosis; myocardial infarction (2); rash plus hyperglycemia; hyponatremia; jaundice with taste perversion and anorexia; photosensitivity; dypsnea plus headache; gastroenteritis with "non- cardiac pulmonary edema".

F. ADE literature search

The sponsor did a literature search using a database that periodically scans more than a dozen data bases. The cut- off date was June 15, 1992. Of N= 2941 articles, N=459 referred to adverse effects and of these N= 41 articles were selected as not containing data expected to overlap that in this submission. The Sponsor tabulated ADE by individual article (vol 322). Symptoms/signs were very nearly limited to those found in the NDA submission. One article dealt with increased insulin production.

004003

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NARRATIVES OF POST-MARKETING SURVEILLANCE EVENTS
POOL 10A PMS1 (GERMANY)
PATIENTS WHO DIED

Twenty one of the 8788 patients enrolled in this surveillance died during or after discontinuing the study and details are available on the following patients.

1999 **Cause of death: Bladder carcinoma.** This 58-year old male patient had participated in the surveillance for 83 days when he died from bladder carcinoma. His final dose of NIS IR was 10mg daily.

2474 **Cause of death: Myocardial infarction.** This 77-year old male patient with a 10-year history of CHD, no previous MI, arterial occlusive disease, and bronchitis was concomitantly taking phenprocoumon, theophylline, and digitalis in addition to NIS IR. During therapy with NIS IR, He died after 30 days on NIS IR 10mg daily from a myocardial infarction.

2541 **Cause of death: Cardiogenic shock.** This 82-year old male patient with a 15-year history of CHD, one previous MI 15 years prior to study participation, concurrent heart failure was taking metoprolol and mononitrate. He was observed to have a reduction in on NIS IR 20mg daily. Ten days later, as a consequence of a myocardial infarction, this patient died because of cardiogenic shock.

2542 **Cause of death: Encephalomalacia during coronary angiography.** This 68-year old female patient died nine days after discontinuing 20mg daily of NIS IR (total of 17 days on NIS IR). No additional information is available.

2899 **Cause of death: Acute heart failure.** This 69-year old male patient had a 7-month history of CHD with a myocardial infarction nine months prior to participating in this surveillance. In addition to CHD, this patient also had hypertension, cardiac dysrhythmias (Lown IVa) and was concomitantly taking metoprolol. He had two follow-up visits, after 39 and 68 days on NIS IR 10mg daily, which noted He died 10 days later because of acute heart failure with suspected recurrence of myocardial infarction resulting in cardiac dysrhythmias.

2957 **Cause of death: Sudden cardiac death with calcifying coronary sclerosis.** This 60-year old male patient with a history of CHD, no previous MI, status post-renal transplant and with renewed renal impairment had received 10mg daily of NIS IR for 30 days when he was hospitalized and drug was discontinued. He died four days later.

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4008 **Cause of death:: Breast cancer.** This 74 year old female patient with a history of died from breast cancer after having completed the surveillance with 10mg daily of NIS IR.

4373 **Cause of death: Myocardial infarction.** This 75-year old male patient with a 12-year history of and two prior MIs (2 and 8 years before study entry) was concomitantly taking digitalis and nitroglycerine spray along with his NIS IR 10mg daily. His first follow-up visit was done after 8 days on NIS IR
He died 3 days later as a result of a myocardial infarction.

4381 **Cause of death: Cardiac arrest.** This 48-year old male patient with a history of and no previous MI appears to have taken only one dose (10mg) of NIS IR during the study. He died two days later in the ambulance after cardiac arrest. No additional information is available.

4435 **Cause of death: Myocardial infarction.** *This 66-year old male patient with a 6-year* history of and a prior infarction 12 years prior to study participation also had hyperlipidemia and hypertension and was taking dinitrate, metoprolol and bezafibrate when he enrolled in the surveillance. His participation lasted for 61 days on a final daily dose of 10mg NIS IR. He died from a myocardial infarction 11 days after having completed the study.

6357 **Cause of death: Bronchial carcinoma.** This 54-year old male patient with a history of and no previous MI died from bronchial carcinoma after having participated in the study for 83 days on a daily dose of 10mg NIS IR.

6599 **Cause of death: Sudden cardiac death.** This 62-year old female patient with a 15-year history of and Type II diabetes mellitus was taking concomitant glibenclamide, digitalis, captopril, and furosemide. She participated in the study for 28 days on a daily dose of 10mg NIS IR. Thirty-nine (39) days after discontinuing NIS IR, she was admitted to the hospital (reason unknown) and died.

8016 **Cause of death: Car accident.** No additional information is available on this 74-year old female patient who had participated in the surveillance for 92 days and was taking 10mg NIS IR daily at the time of her death.

8601 **Cause of death: Post-operative after CABG, renal failure, and pneumonia.** This 76-year old male patient with a 3-year history of and no previous MI, was to undergo ACVB owing to the unstable condition of his at the start of surveillance. Follow-up visits were done 22 and 59 days after start of surveillance and showed no deterioration in . He was admitted to the hospital for purposes of the CABG and discontinued the NIS IR 20mg daily. Thirteen (13) days later, he died from sequelae of surgery.

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9891 **Cause of death: Status post-aneurysm of the abdominal aorta and circulatory failure.** This 86-year old female with a 10-year history of and no previous MI had follow-up visits done 64 and 78 days after starting NIS IR and completed the surveillance after 106 days with the final dose of NIS IR being 10mg daily. During the surveillance period, she had an operation for an aneurysm of the abdominal aorta and probably as a result of this surgery she died nine days after completing the study.

9916 **Cause of death: Myocardial infarction.** This 61-year old male patient with a 15-year history of wo previous MIs (15 and 3 years prior to study entry), and Type II diabetes mellitus was taking glibenclamide, nitroglycerine spray, mononitrate, and nifedipine/atenolol combination at the beginning of surveillance. During treatment with NIS IR 20mg daily, . He died 23 days into study as a result of a myocardial infarction.

10704 **Cause of death: Renal carcinoma.** This 57-year old male patient died from renal carcinoma after having participated in the study for 47 days and having taken 20mg daily of NIS IR. No additional information is available.

1095 **Cause of death: Acute heart failure.** This 73-year old male patient with a 11-year history of with 2 previous MIs (11 and 10 years prior to study) also had hypertension, cerebrovascular processes, and Parkinson's disease. At the start of surveillance, he was taking aspirin, potassium replacement, metixen, diltiazem, and mononitrate in addition to NIS IR. His participation in the study lasted for 41 days hile on 10mg daily of NIS IR. Thirty-four (34) days after his last dose of NIS IR, he died from acute heart failure.

11499 **Cause of death: Posterior wall infarction.** This 76-year old male patient with a 10-year history of and previous MI (3 years prior to study participation), also had hyperuricemia, prostatic hyperplasia, and heart failure, and was taking dinitrate, flecainide, phenprocoumon, digitalis, and magnesium/potassium replacement along with NIS IR. Improvement in symptoms was noted at his first follow-up visit after 19 days on NIS IR 10mg daily. He was hospitalized the same day for acute cholecystitis and NIS IR was discontinued. Four (4) days later, despite showing clinical improvement in cholecystitis, the patient died from a myocardial infarction.

12650 **Cause of death: Malignant hypertension.** This 60-year old male patient with a 6-year history of also had hypertension, cerebrovascular insufficiency, and heart failure, and was taking mononitrate, clonidine, indapamide, enalapril, and digitalis. His participation in the study lasted 66 days with a final NIS IR dose of 20mg daily during . He died 3 days after completing the study from malignant hypertension.

NIS CC SAFETY SUMMARY
10 February 1993

13765 **Cause of death: Acute re-infarction.** This 51-year old male patient had a 4-year history of previous MI (4 years prior to study participation), and hypercholesterolemia and was taking mononitrate and metoprolol. His follow-up visits occurred 20 and 49 days after starting NIS IR and he completed the study on day 50 on a final dose of 20mg daily of NIS IR. He died 27 days later from acute re-infarction.

G. A pharmacodynamic- clinical model for adverse reactions

If, in fact, a drug increases cardiac work unfavorably at some phase of its administration in a subset of patients and that increase in cardiac work is the factor responsible for determining the onset of ischemia, it is possible that the distribution of times to drop out for angina or chest pain would approximate that for withdrawal due to "vasodilation" phenomena other than angina such as headache, tachycardia etc. Perhaps in some patients the increase in cardiac work associated with vasodilation might increase rather than improve ischemia. If so, then the timing of both phenomena should be similar.

For US controlled- trial angina patients Table 17 page 20 of this review shows that 3% of NIS CC patients discontinued due to angina or myocardial infarction versus 1% for placebo cases.
Table 18 on p21 of this review shows that for non- US placebo- controlled trial cases, the percentage of cases discontinuing for these indications is similar in NIS CC and placebo cases.

For US, placebo- controlled trials, the narrative comments (this review page 20) show that the times to discontinuation for worsening angina or MI were relatively soon after starting treatment. For the non- US trials the days to discontinuation were also relatively early.

The Kaplan- Meier "survival" curve for the endpoint headache (see this review page 5) shows that the percentage of patients without headache falls abruptly early (in about two weeks) to 85% but takes until week 30 to fall another 15%. This early discontinuation for headache would be occurring during dose escalation and/or early exposure to nisoldipine and is most probably a concomitant response to the pharmacologic action of the drug.

Note that in the long- term trials the times of discontinuation vary widely and may be a number of months. Such cases might represent the "background" incidence of events in a population with from which some cases with had been removed due to drug exposure during the short- term phases of long- term studies. In some patients may be due to sporadic increases in dosage. To reduce the complexity of multiple causes of withdrawal, this reviewer has used the short- term studies for the graphical analyses to be shown subsequently.

Note that either 1) the similarity of timing of withdrawal for ischemia and for "vasodilation" and 2) withdrawal for both in the same patient would be supportative of an association between the action of the calcium channel blocker and ischemia. Although instances were not infrequent of withdrawal for both in the same patient, they were not observed often enough to provide strong evidence. This may be due to a tendency to emphasize a single cause for withdrawal in clinical trials. Therefore, the reviewer reports on the similarity of the withdrawal- time distributions for ischemia and vasodilation.

Graphical analysis of withdrawal:

Pages
41
41A
3
C
D
E
PURGED AS
CONFIDENTIAL COMMERCIAL
INFORMATION

Summary of Withdrawals from Hypertension Trials With Particular Reference to Chest Pain (N events due to this reviewer's tally using submitted safety pool data):

HYPERTENSION TRIALS

CONTROLLED TRIALS:

STUDY	NIS EVENT/N	PLAC EVENT/N	%NIS	%PLAC	DOSES
D89026	0/82	0/40	0.0	0.0	
D88054	0/91	0/31	0.0	0.0	10-30
D89029	2/189	0/62	1.1	0.0	20-60
D89039	3/167*	0/75	1.8	0.0	20-80
D90019	1/149	1/72	0.7	1.4	30-60

* N=1 with vasodilation (see definition below)

UNCONTROLLED TRIALS:

X89039	3/136	na	2.2	na	20-40
X90019	0/88	na	0.0	na	30-60

Comment: Even in the uncontrolled (long- term) trials withdrawal for angina/cad was relatively infrequent in the hypertension data. Probably this is a reflection of small rates of serious CAD in the population sampled such that any tendency for NIS Rx to accentuate manifestations was also minimal.

Overall Conclusions for Safety of Nisoldipine:

Two major findings in this submission are 1) a substantial incidence of withdrawal associated with signs/symptoms of vasodilation and 2) an increased rate of withdrawal for angina/cad in In the short- term trials the latter withdrawals tended to occur early and to be associated with signs/symptoms of vasodilation but, in the long- term trials they were primarily manifested by an increased rate of withdrawal.

In the US, placebo- controlled, hypertension trials there was a significant dose response for overall withdrawal of about 11% at 60mg NIS and 5.4% at 10 mg. The incidence of headache, not necessarily associated with withdrawal was about 20%. In the long- term, uncontrolled hypertension studies, about 20% of cases had withdrawn by 30 weeks and the cumulative incidence of peripheral edema was more than 40%. Thus whatever blood pressure reduction that is achieved is associated with substantial side effects, most of which are due to the pharmacologic action of the drug in causing vasodilation or to the compensatory mechanisms such as tachycardia.

One might expect some myocardial ischemic phenomena if the vasodilation and tachycardia increased cardiac work out of proportion due the benefits of reduction of afterload through lowering of blood pressure. There was little evidence that the balance was unfavorably affected since, in the hypertension studies the incidence of withdrawal due to angina/cad was about 1%. However, there were instances, especially in the phase 2 rapid, forced- titration trial, of the development of t wave abnormalities.

It is in patients that evidence for ischemic effects of nisoldipine are of greater concern. Even in the short- term, US placebo- controlled trials the rates of withdrawal for angina/chd exceed those on placebo. In addition, it is of particular interest to note that a high proportion of such withdrawals occurred very early at times close to those for withdrawal due to vasodilation. Thus the close similarity of the distribution of withdrawal for vasodilation and that for angina/cad supports a similar mechanism for both. Symptoms of vasodilation were not prominant in the but in those the number of events was higher, about 10%. Thus for the short- term trials one may use the rates, the timing of withdrawal and/or association of vasodilation; for the long- term trials only the rates are useful. Note that the use of timing of withdrawal and/or any associated vasodilation constitutes an "internal" control.

From a purely safety standpoint, this reviewer is not in favor of approval for the

It is possible that a combination of a beta- blocker and nisoldipine would allow use of the latter drug in a single study (0702) carried out in Canada, US, and Israel had very few withdrawals due

to ADE. N= 1 subject withdrew for [redacted] angina and N= 1 for myocardial infarction out of N= 200 NIS+ atenelol cases. The experience with this combination is as yet insufficient to recommend this combination but it may be a justifiable treatment to explore in future trials.

Note that if the NIS formulation dumps early, one would expect to see early withdrawal or the early occurrence of signs/symptoms of vasodilation.

The hypertension indication, at least for subjects without notable coronary heart disease, is supported by the safety data. However, if the spectrum of patients selected for treatment includes patients whose hypertension is associated with CHD or as hypertensive subjects develop CHD, some of these may be unable to tolerate NIS therapy. Alternative therapy should be considered in such cases.

The ECG findings in rapid- dose escalation (intervals of less than a week) in hypertensive subjects, while not clearly established as adverse, are in an unfavorable direction and suggest that titration be carried out over the longest intervals consistant with the patient's need for blood pressure control.

Summary of safety recommendations:

1. Nisoldipine, as studied, not suitable

2. Further studies with beta blocker + NIS of interest

3. Subjects with asymptomatic coronary disease constitute a somewhat difficult group with respect to suitability for NIS therapy since many hypertensives responding to this treatment undoubtedly have this condition. Perhaps some clinical judgement needs to be invoked here.

4. In view of the findings of ECG T abnormalities on rapid titration in hypertensive patients, it is suggested that dosage increments be made at the greatest intervals consistant with the need for BP control. The Sponsor's dosing recommendations do not specify the interval between dosage increments. Intervals of only a few days may be too frequent since they were associated with "adverse" ECG changes in hypertensives.

5. Although mono- therapy for hypertension with NIS appears justifiable due to the lack of serious drug- induced effects, there is still a substantial incidence of troublesome symptoms of vasodilation that might be diminished with combination of NIS and beta- blockade. This may also be a suitable and informative area for further trials.

6. Pharmacokinetic studies by the Sponsor show that the mean Cmax was 48% higher when NIS was administered with a meal. It is not clear whether this explains the very early occurrence of vasodilation after dosing and,
[redacted] It may not be correct to state that it is known that there are no clinical consequences from dose dumping.

7. Since elderly subjects have a 2- 3 fold higher plasma NIS concentration than younger ones, it may be best to follow these subjects more closely and increase dosage slower.

Philip L. Dern M.D.

cc:original
HFD-110
HFD-110/CSO
HFD-110/pld

JUL - 7 1994

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
MEDICAL OFFICER'S NDA REVIEW

NDA: 20- 356

DRUG: Nisoldipine

SPONSOR: Miles

DATE SUBMITTED: 17 August, 1993

DATE REVIEWED: 7 July, 1994

REVIEWER: Philip L. Dern M.D.

RESUME:

This is a 120- day safety review and includes both completed and uncompleted trials, foreign and domestic. For uncompleted trials, data is still blinded and treatment is listed as "either drug 1 or drug 2" if these are the possibilities for a particular patient.

I. Deaths
A. Completed US studies

One death is recorded for this update and was also included in the NDA for an on-going trial.

Study # D90- 029-06; Pt 6004.

This 44- yr- old female with a qualification, single- blind BP of 190/111 and a randomization BP of 141/97 died suddenly at home on day 24 of the study. She was on NIS 40 mg qd and HCTZ 25 mg qd. Serum potassium at baseline and last visit were, respectively, 3.9 and 3.6 mEq/l. Autopsy revealed moderate coronary atherosclerosis without thrombi.

B. Completed Non- US studies

Study 752. No deaths.

C. On-going Studies (all non- US)

Study 764; Pt 128.
This 83-yr- old female was being treated with either NIS or lisinopril (LIS). She died at home of acute pulmonary edema and has an associated abdominal infarction.

Study 769; pt 16002.

This 53 yr- old male was being treated with either NIS or atenelol (ATN). The patient had a TIA on 9/91. Baseline BP was 162/105 after three weeks of placebo. He was admitted to hospital on 5/18/92 after 53 days of treatment. He died the following day of a CVA. Bp at last clinic visit was 177/83.

Study 769; pt 17013.

This patient, a male aged 73, was being treated with either NIS or ATN. After 5 days of therapy the patient developed diarrhea, nausea, and vomiting, and, three days later, a fatal MI.

Study 769; pt 54002.

The patient, a male aged 66, was on either NIS or ATN. Baseline BP was 155/93. After 25 days of treatment he developed a CVA and died. BP at last clinic visit was 145/83.

II. Discontinuations due to adverse experiences

A. Completed US studies

REASON WITHDRAWN	DAY	DRUG & DOSE
Edema,erythema	24	NIS40
headache	2	NIS20
headache	1	NIS20
tachycardia,vasodil	1	NIS20
Dizzy, n & v,headach	2	NIS20
Gout	5	NIS20
Faint at phlebotomy	10	NIS40
edema	13	NIS40
edema	19	NIS40
Abn Liver function*	35	NIS40, HCTZ25
Cough	8	NIS40+ LIS20
card. arrest	24	NIS40, HCTZ25
sinus tachycardia	10	NIS40, HCTZ25

* Also abnormal during pre- NIS phase

n.b. There is a notable number of cases withdrawn relatively early in the active dose phase especially for those signs and/or symptoms likely to be due to the vasodilatory action of nisoldipine.

B. Ongoing trials (all non- US):

SELECTED SIGNS/SYMPTOMS	N*
edema	23
migraine	3
headache	28
angina	1
MI	2

* often sign/symptom occurred with others

The above table concentrates on typical findings on nisoldipine therapy (edema, headache) but notes occurrence of migraine. Number of cases of angina and MI is small. Total number of cases withdrawn due to syncope (1), postural hypotension (1), hypotension (1), suggests that excessive BP fall was uncommon. N=1 case was withdrawn for thrombocytopenia.

Among 1370 cases in these foreign studies, 100 were withdrawn due to adverse experiences.

Conclusions:

The withdrawals for adverse effects were often due to the pharmacologic action of nisoldipine and consequent to vasodilation (headache, vasodilation). In this hypertensive population few cases of angina or MI occurred. However, the data base for this report is not cumulative and the number of cases in the completed US trial is too small to provide a good estimate of the risk of angina/MI. The database for the foreign studies is larger and, even though treatment assignment is in doubt, the small number of angina/MI cases is notable (See this Reviewer's review of the hypertension safety segment of the NDA for comments on the association of symptoms/signs of vasodilation and angina/MI).

cc: HFD/110
HFD/110 orig
HFD/110 CSO ✓
HFD/110 pld

Docket

OCT 26 1993

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
MEDICAL OFFICER'S REVIEW

NDA: 20-356 (Nisoldipine Coat-Core tablets for exertional angina; Bay K 5552:

Sponsor: Miles Inc. Pharmaceuticals Div.

Submission: NDA 120 day Safety Update

Submission date: 17 August 1993.

Receipt date: 19 August 1993.

Review date: 26 October 1993.

Reviewer: N. Stockbridge, M.D., Ph.D.

1. US trials

There is only 1 ongoing US trial, #X90-015, an open-label, long-term follow-on to Study #90-015 (q). There are no new safety data for this trial. However, two subjects who completed this trial and continued to receive nisoldipine coat-core under an individual investigator's IND experienced serious adverse experiences.

Subject was a 69 year old Caucasian female who had generally received 20 mg q.d. The dose was reduced because of dizziness and light-headedness. He experienced chest pain or discomfort for two weeks prior to admission at 734 days for unstable angina. Enzymes did not indicate myocardial infarction and she was discharged after 3 days. She was readmitted with similar history at 770 days, at which time hiatal hernia was diagnosed.

Subject was a 68 year old Caucasian who suffered myocardial infarction while receiving 30 mg q.d. Three months later he was readmitted for severe angina while on 40 mg.

2. Non-US trials

2.1. Study #697

This is a randomized, double-blind, parallel group study being conducted in Germany and Italy. The groups are nisoldipine 40 mg q.d. (n=138) and diltiazem 60 mg t.i.d. (n=136).

Subject #11-009 was a 72 year old female who discontinued after 8 months because of allergic exanthema.

Subject #41-005 was a 55 year old female who discontinued after 3 months because of resting tachycardia.

2.2. Study #701

This is a randomized, double-blind, parallel group study being conducted in Germany. The groups are nisoldipine coat-core 20 mg q.d. (n=70) and nisoldipine immediate release 10 mg b.i.d. (n=72).

Subject 0101 was a 58 year old female with a 2-year . She discontinued at 5 days with severe burning sensation of the skin, headache, and vasodilation, all of which began with the first dose.

Subject #0115 was a 68 year old female with a 6-month She discontinued at 4 months with nausea, inner "trembling", and pressure and heat sensation in hands and feet.

2.3. Study #718

This is an ongoing randomized, double-blind parallel group trial with nisoldipine 20 and 40 mg q.d., and diltiazem 60 and 120 mg b.i.d. and t.i.d.

Subject #303 was a 51 year old male with a 6-year He withdrew after

4 days because of chest pressure and palpitations.

Subject #306 was a 54 year old male with an 8-year . During month 7, he complained of flatulence. After 8 months, he withdrew because of hypotension, malaise, and fatigue.

Study #771

This is an ongoing randomized, double-blind, comparison of nisoldipine 20 to 40 mg q.d. with diltiazem 60 mg t.i.d. or q.i.d.

Subject #3307 was a 55 year old male with a 7 month and myocardial infarction 12 years previously. He discontinued at 5 months with atrial fibrillation which resulted in prolonged hospitalization; outcome unknown.

Subject #3602 was a 78 year old male with 2 month . He suffered cramps from the onset of treatment and discontinued after 4 weeks with the onset of fasciculations.

Subject #3703 was a 63 year old male with a 1-year h He discontinued after 3 weeks because of vertigo.

2.4. Study #781

This is an ongoing randomized, double-blind trial in Germany comparing 20 and 40 mg q.d. nisoldipine with ISDN 20 and 40 mg b.i.d.

Subject #213 was a 70 year old female with a She discontinued after 3 weeks because of severe headaches and nausea.

Subject #214 was a 62 year old female with a . She discontinued after 6 weeks because of leg edema.

Subject #603 was a 64 year old female with a She discontinued after 2 weeks because of severe headaches, diarrhea, restlessness, and general malaise.

Subject #906 was a 69 year old female with a She discontinued after 2 months because of hair loss.

Subject #1004 was a 68 year old male with a and myocardial infarction 1 year previously. He was discontinued after 1 week because of non-compliance.

Subject #1016 was a 58 year old male with and possibly 2 previous myocardial infarctions. He was discontinued after 2 months because of non-compliance.

2.5. Study #761

This is an ongoing open-label trial in Israel with nisoldipine 10 to 60 mg q.d.

Subject #122 was a 59 year old Caucasian male who discontinued after 6 months because of unstable angina.

Subject #404 was a 54 year old Caucasian male who suffered a myocardial infarction at 6 months.

Subject #414 was a 73 year old Caucasian male who discontinued after 4 months because of unstable angina.

Subject #617 , was a 95 year old (?) Caucasian male who suffered a myocardial infarction after 7 months.

Subject #1004 was a 72 year old Caucasian male who discontinued for constipation after 6 months.

Subject #1017 was a 66 year old Caucasian male who discontinued after 4 months because of intermittent claudication and fissure following prostatectomy.

2.6. Study #762

This is an ongoing randomized, double-blind trial in Italy comparing 20 mg q.d. nisoldipine coat-core with 10 mg b.i.d. nisoldipine immediate release.

Subject #402 was a 21 year old Caucasian male with a who had a myocardial infarction at 1 month.

Subject #1504 was a 61 year old Caucasian male with an . He

discontinued after 4 weeks because of unstable angina.

Subject #605 , was a 69 year old Caucasian male with an He discontinued after 2 weeks because of unstable angina.

Subject #607 was a 64 year old Caucasian male with c He discontinued after 2 weeks because of rash and hypotension.

Subject #615 was a 58 year old Caucasian male with a He discontinued after 2 weeks with rhinitis, edema of the legs, erythema, and pruritus which began on the second or third day of treatment. Symptoms resolved 2 days after withdrawal.

Subject #810 , was a 60 year old Caucasian male with a He dropped out after 3 weeks because of unstable angina.

2.7. Study #10011 (X90-010)

This is an ongoing open-label study in Israel with doses 10 to 60 mg q.d.

Subject #101 , was a 56 year old Caucasian male who developed thyroiditis and tonsillitis after 4 months.

Subject #103 was a 63 year old Caucasian male who discontinued after 1 month because of unstable angina.

Subject #207 was a 64 year old Caucasian male who was hospitalized after 5 weeks because of unstable angina.

Subject #420 , was a 69 year old Caucasian male who discontinued after 3 months because of pedal edema.

Subject #805 was a 63 year old Caucasian male who experienced severe prolonged angina and tachycardia 9 days after beginning treatment. He was off study drug for a short period and later completed 12 months.

Subject #910 was a 70 year old Caucasian female with a history of ophthalmological disease. She had intraocular hypertension for 9 months during study.

Subject #911 was a 65 year old Caucasian male was hospitalized for severe angina after 2 weeks. He subsequently completed 6 months, with complaints of decreased libido. The reason for discontinuation is not explicitly stated.

Subject #913 was a 61 year old Caucasian male with He complained of flank pain at week 2; the complaint resolved.

Subject #915 was a 52 year old Caucasian male who discontinued after 3 months because of facial flushing.

Subject #100-) was a 71 year old Caucasian male who was hospitalized for unstable angina at 7 months. He completed 12 months of treatment.

Subject #1018 was a 60 year old Caucasian male. He was twice hospitalized for chest pain, the second was after about 12 months of treatment.

Subject #1107 , was a 57 year old Caucasian male who developed severe chest pain and underwent CABG 4 days after completing 12 months treatment.

3. Summary

Headache, vasodilation, and peripheral edema remained the common treatment-related adverse events.

Several of the cases described appear to represent acute worsening of angina during treatment. Subject #1107 in Study #10011 may represent a rebound phenomenon.

The information provided in this 120-day safety update do not materially affect conclusions made with the Medical Officer's review (2 August 1993) of safety and efficacy

Chemistry Review

SEP 16 1994

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-356 **CHEM.REVIEW #:** 4 **REVIEW DATE:** 09-Sep-94

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
ORIGINAL	31-Mar-93	05-Apr-93	05-Apr-93
AMENDMENT	20-Jun-94	21-Jun-94	24-Jun-94
	29-Jul-94	03-Aug-94	05-Aug-94
	29-Jul-94	03-Aug-94	05-Aug-94

NAME & ADDRESS OF APPLICANT: Miles Inc.
Pharmaceutical Division
400 Morgan Lane
West Haven, CT 06516-4175

DRUG PRODUCT NAME

Proprietary:	Not yet established
Nonproprietary/USAN:	Nisoldipine
Code Name/#:	BAY k 5552, CAS-63675-72-9
Chem.Type/Ther.Class:	1 S

Patent Status: Patents which claim the drug, Nisoldipine, (BAY 5552) and its use are as follows:

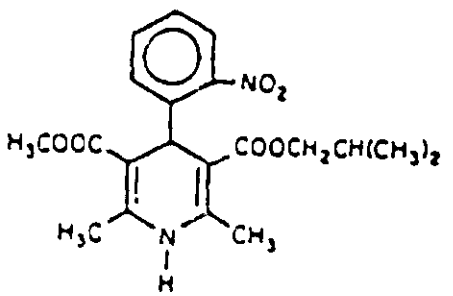
U.S. Patent No. 4,154,836	Expires May 15, 1996 and covers the compound, pharmaceutical compositions for increasing coronary perfusion; and claims methods for increasing coronary perfusion.
U.S. Patent No. 4,892,741	Expires January 9, 2007, covers the coat-core tablet.
U.S. Patent No. 4,600,778	Expires July 15, 2003. covers the preferred process for providing Nisoldipine.

PHARMACOL.CATEGORY/INDICATION: Hypertension

7/29/94 Angina indication was withdrawn

DOSAGE FORM: Coat core (extended release) Tablets
STRENGTHS: 10, 20, 30 and 40 mg
ROUTE OF ADMINISTRATION: Oral
DISPENSED: __X__ Rx _____ OTC

STRUCTURAL FORMULA, CHEMICAL NAME, MOLECULAR FORMULA, MOLECULAR WEIGHT:



Chemical name(s):

3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-methyl-2-methylpropyl ester, (±)

(±)-Isobutyl methyl 1,4-dihydro-2,6-dimethyl-4-(o-nitrophenyl)-3,5-pyridinedicarboxylate

Molecular Formula: $C_{20}H_{24}N_2O_6$ Molecular Weight: 388.42

SUPPORTING DOCUMENTS:

RELATED DOCUMENTS (if applicable):

CONSULTS: EA was requested on 6/11/93, amended 8/4/93.

REMARKS/COMMENTS:

The nisoldipine CC tablets consist of a tablet core with a rapid active ingredient release in a compressed press-coating with controlled, delayed active ingredient release. To achieve protection from light, the tablets are film-coated.

Nisoldipine, racemate, will be used in the preparation of the drug product. Studies using enantiomers of nisoldipine were performed. (+)-Nisoldipine was found to be 10-20 times more potent than (-)-nisoldipine in hypertensive rats. There was no relevant difference in oral efficacy between (+)-nisoldipine and the racemate in either rats or dogs. (+)-Nisoldipine binds to isolated membranes with an affinity 100 times higher than (-)-nisoldipine.

In general, (+)-nisoldipine (BAY R 1224) shows a spectrum of activities in standard safety pharmacology testing similar to the racemate, but at lower dose levels.

EER requested on 6/4/93. Acceptable on 12/16/93.

Methods validation - requested of DDA on 12/14/93. DO will be assigned when additional sample will be picked up. (Foreign manufacturing facility)

July 29, 1994 amendment - response to deficiencies.

July 29, 1994 amendment - change in dissolution specifications.

Proposed expiration date - 24 months.

Dissolution specifications (3 hours - 6 hours - 12 hours - nlt is acceptable (if Biopharm reviewer agrees).

CONCLUSIONS & RECOMMENDATIONS:

Responses to the deficiencies were satisfactory.

cc:
Orig. NDA 20-356
HFD-110/Division File
HFD-110/CunninghamD/9/9/94
District
HFD-110/CSO
HFD-102/CKumkumian [#1 only]
R/D Init by: SUPERVISOR

Danute G. Cunningham, Review Chemist
filename: 20356R04.NDA

9-16-94

PHARMACOLOGY REVIEW

DR

NDA 20-356

SEP - 2 1994

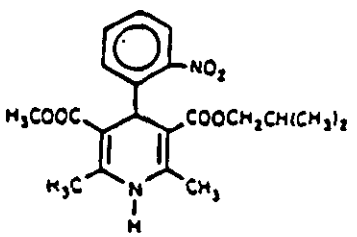
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Sidney Stolzenberg, Ph.D.
Xavier Joseph, D.V.M.

ORIGINAL NDA DATED: March 31, 1993
CENTER RECEIPT DATE: April 1, 1993
REVIEWERS RECEIPT DATE: April 9, 1993

SPONSOR: Miles Inc. Pharmaceutical Division
400 Morgan Lane, West Haven, CT 06516

DRUG: Proprietary name - not established
Generic name - nisoldipine
Code name - BAY k 5552



M.W. 388.4

FORMULATION: Coat core (extended release) tablets containing 10, 20, 30 or 40 mg of nisoldipine are formulated with following inactive ingredients: hydroxypropylcellulose, lactose, corn starch, crospovidone, microcrystalline cellulose, sodium lauryl sulfate, povidone and magnesium stearate (core and outer coat); hydroxypropylmethylcellulose, polyethylene glycol , ferric oxide and titanium dioxide (film coat).

PHARMACOLOGICAL CLASS: Calcium channel blocker

PROPOSED INDICATION: Treatment of hypertension

PROPOSED DOSAGE REGIMEN: 10-40 mg once daily

IND UNDER WHICH CLINICAL TRIALS WERE CONDUCTED:

Table of Contents

SUMMARY OF PHARMACOLOGICAL STUDIES (X. Joseph)

A. Studies Related to Therapeutic Indications

1. Effects on Blood Pressure

a. Rats

The effects of nisoldipine on blood pressure and heart rate were studied and compared with appropriate reference drugs (nifedipine, nicardipine and hydralazine) in normotensive (NT) and spontaneously hypertensive (SH) rats. Single oral doses of nisoldipine (3-30 mg/kg) produced a dose-dependent decrease in blood pressure in normotensive rats. Although the hypotensive effect at 3 mg/kg was statistically not significant, doses of 9 and 30 mg/kg produced significant reductions in blood pressure lasting for 2 and 4 hr, respectively, after nisoldipine administration (Table 1). A significant dose-dependent increase in heart rate was observed in all nisoldipine treated groups for 2-6 hr postdose (Table 2). Reference drugs also produced similar dose dependent hypotensive effects and increased heart rates. However, in normotensive rats, hypotensive effects produced by reference drugs at 9 mg/kg were almost equivalent to the effect produced by the high dose level, 30 mg/kg, of nisoldipine (Table 1).

Nisoldipine produced a more pronounced antihypertensive effect in SH rats than in normotensive rats. Dose dependent significant reductions in blood pressure were seen at all levels of nisoldipine tested (3-30 mg/kg, po), beginning at 30 min and lasting until 4 hr (3 mg/kg) or 6 hr (9 mg/kg and above) postdose (Table 3). Heart rate was significantly increased up to 2 hr postdose in all nisoldipine treated groups and until 6 hr at the highest dose level (Table 4). At 24 hr, no significant differences in blood pressure or heart rate were seen between control and treated groups. Reference drugs also produced dose dependent decrease in blood pressure and increases in heart rates in SH rats.

Studies in other experimental animal models of induced chronic hypertension (renal hypertensive rats and deoxycorticosterone-NaCl hypertensive rats) also revealed a dose related hypotensive effect for nisoldipine.

The doses of nisoldipine and reference drugs required to decrease blood pressure by 20% or increase heart rate by 20% of the initial values (ED20 values) in normotensive and SH rats and also in other experimental animal models of hypertension are given in Table 5.

Table 1: Effects of nisoldipine and reference drugs on blood pressure in normotensive rats.

Drugs	Dose (mg/kg)	Mean blood pressure (mmHg) ± S.E.M.						
		Before	30 min	60 min	120 min	240 min	360 min	24 h
Control	-	108 ± 5	109 ± 5	110 ± 4	190 ± 5	180 ± 5	109 ± 5	109 ± 5
Nisoldipine	3	109 ± 6	99 ± 5	100 ± 5	101 ± 5	103 ± 6	107 ± 6	109 ± 6
	9	111 ± 3	94 ± 6	93 ± 5*	92 ± 4*	99 ± 5	103 ± 5	112 ± 3
	30	112 ± 5	87 ± 4**	83 ± 2**	85 ± 2**	89 ± 2**	97 ± 3	114 ± 3
Nifedipine	1	112 ± 1	103 ± 1	105 ± 1	107 ± 1	107 ± 1	108 ± 1	113 ± 2
	3	109 ± 3	92 ± 3*	91 ± 3**	94 ± 3*	92 ± 2*	94 ± 2*	109 ± 3
	9	107 ± 5	79 ± 4**	76 ± 2**	77 ± 3**	77 ± 3**	80 ± 2**	108 ± 4
Nicardipine	3	113 ± 2	104 ± 2	106 ± 2	106 ± 1	109 ± 2	113 ± 2	115 ± 2
	6	112 ± 3	98 ± 3*	98 ± 3*	98 ± 2	100 ± 2	103 ± 3	113 ± 3
	9	109 ± 3	75 ± 2**	77 ± 2**	84 ± 2**	91 ± 1**	98 ± 1	111 ± 1
Hydralazine	3	113 ± 1	95 ± 3*	99 ± 2	101 ± 1	105 ± 1	107 ± 1	113 ± 1
	6	113 ± 2	90 ± 2**	89 ± 2**	90 ± 1**	91 ± 2**	91 ± 2**	112 ± 1
	9	108 ± 3	82 ± 4**	84 ± 4**	84 ± 3**	87 ± 3**	89 ± 2**	107 ± 3

Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$.
In the control group, the vehicle alone (0.5 % CMC suspension) was administered.

Table 2: Effects of nisoldipine and reference drugs on heart rate in normotensive rat.

Drugs	Dose (mg/kg)	Mean heart rate (beats/min) ± S.E.M.						
		Before	30 min	60 min	120 min	240 min	360 min	24 h
Control	-	361 ± 17	358 ± 17	358 ± 15	355 ± 12	350 ± 10	350 ± 7	356 ± 15
Nisoldipine	3	359 ± 9	417 ± 20*	405 ± 7*	400 ± 9*	391 ± 17	376 ± 15	356 ± 7
	9	359 ± 7	451 ± 24**	469 ± 24**	469 ± 25**	413 ± 22*	385 ± 13*	356 ± 9
	30	353 ± 10	498 ± 15**	500 ± 14**	484 ± 20**	468 ± 14**	433 ± 11**	342 ± 20
Nifedipine	1	349 ± 7	395 ± 17	389 ± 17	374 ± 17	367 ± 12	362 ± 9	349 ± 7
	3	351 ± 7	489 ± 13**	491 ± 14**	477 ± 14**	462 ± 20**	429 ± 15**	350 ± 7
	9	352 ± 17	487 ± 11**	491 ± 11**	483 ± 13**	476 ± 11**	463 ± 12**	349 ± 12
Nicardipine	3	363 ± 12	443 ± 20**	400 ± 16	389 ± 11	381 ± 10	368 ± 9	349 ± 12
	6	354 ± 20	461 ± 12**	451 ± 14**	429 ± 17**	413 ± 12**	387 ± 17	357 ± 17
	9	366 ± 9	532 ± 15**	513 ± 15**	476 ± 10**	446 ± 16**	402 ± 10**	364 ± 9
Hydralazine	3	361 ± 17	446 ± 12**	439 ± 12**	424 ± 10**	411 ± 22**	387 ± 17	361 ± 9
	6	357 ± 17	479 ± 22**	460 ± 12**	446 ± 13**	429 ± 12**	399 ± 22	359 ± 9
	9	366 ± 7	514 ± 18**	473 ± 17**	449 ± 15**	433 ± 13**	423 ± 12**	368 ± 9

Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$.

Table 3: Effects of nisoldipine and reference drugs on blood pressure in spontaneously hypertensive rats.

Drugs	Dose (mg/kg)	Mean blood pressure (mmHg) ± S.E.M.						
		Before	30 min	60 min	120 min	240 min	360 min	24 h
Control	-	181 ± 5	179 ± 7	183 ± 6	182 ± 5	175 ± 4	176 ± 5	184 ± 4
Nisoldipine	3	180 ± 5	157 ± 4*	159 ± 4*	159 ± 4**	158 ± 4**	166 ± 3	181 ± 5
	9	180 ± 5	140 ± 5**	144 ± 4**	144 ± 5**	146 ± 5**	150 ± 4**	181 ± 5
	30	180 ± 7	115 ± 6**	117 ± 6**	127 ± 5**	131 ± 5**	136 ± 5**	179 ± 7
Nifedipine	1	188 ± 3	171 ± 6	167 ± 5	172 ± 5	170 ± 6	171 ± 2	199 ± 5
	3	177 ± 2	147 ± 5**	152 ± 2**	152 ± 5**	153 ± 2**	162 ± 4	175 ± 4
	9	176 ± 4	137 ± 8**	134 ± 7**	131 ± 7**	144 ± 11*	140 ± 10**	168 ± 9
Nicardipine	3	180 ± 5	157 ± 8	160 ± 6*	162 ± 6*	159 ± 7	164 ± 6	180 ± 4
	6	180 ± 5	145 ± 6**	152 ± 7**	154 ± 7**	160 ± 5*	169 ± 4	180 ± 5
	9	180 ± 4	122 ± 9**	120 ± 7**	134 ± 7**	142 ± 5**	153 ± 5**	172 ± 4
Hydralazine	3	184 ± 8	160 ± 10	151 ± 5**	149 ± 5**	141 ± 7**	147 ± 7**	169 ± 6
	6	179 ± 2	133 ± 10**	132 ± 6**	133 ± 8**	132 ± 6**	139 ± 6**	163 ± 4
	9	178 ± 5	82 ± 8**	80 ± 7**	86 ± 7**	92 ± 7**	102 ± 5**	146 ± 4

Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$.

Table 4: Effects of nisoldipine and reference drugs on heart rate in spontaneously hypertensive rats.

Drugs	Dose (mg/kg)	Mean heart rate (beats/min) ± S.E.M.						
		Before	30 min	60 min	120 min	240 min	360 min	24 h
Control	-	397 ± 12	357 ± 15	352 ± 9	356 ± 11	369 ± 15	363 ± 12	403 ± 15
Nisoldipine	3	400 ± 16	402 ± 12*	394 ± 12*	398 ± 10*	404 ± 10	387 ± 10	415 ± 6
	9	400 ± 11	445 ± 21**	446 ± 18**	426 ± 17**	400 ± 10	403 ± 16	403 ± 9
	30	400 ± 9	423 ± 25*	435 ± 17**	452 ± 19**	436 ± 9**	410 ± 14*	390 ± 19
Nifedipine	1	385 ± 18	341 ± 10	343 ± 14	344 ± 13	351 ± 15	363 ± 12	403 ± 15
	3	399 ± 30	403 ± 11*	400 ± 16*	360 ± 15	373 ± 16	369 ± 18	391 ± 12
	9	413 ± 22	424 ± 26*	403 ± 21*	425 ± 24*	400 ± 15	400 ± 18	393 ± 15
Nicardipine	3	400 ± 9	395 ± 22	397 ± 21	389 ± 22	369 ± 12	358 ± 15	381 ± 5
	6	400 ± 14	453 ± 16*	406 ± 21*	414 ± 10*	419 ± 5*	396 ± 5*	419 ± 11
	9	397 ± 13	541 ± 9**	515 ± 27**	504 ± 19**	491 ± 15**	433 ± 27**	385 ± 10
Hydralazine	3	409 ± 6	461 ± 11**	443 ± 13**	425 ± 8**	422 ± 6**	413 ± 7**	416 ± 7
	6	394 ± 13	472 ± 8**	446 ± 9**	453 ± 12**	445 ± 3**	430 ± 13**	423 ± 6
	9	429 ± 20	537 ± 9**	508 ± 12**	504 ± 18**	500 ± 15**	470 ± 12**	457 ± 13

Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$.

Figure 1

Antihypertensive effect of nisoldipine (BAY k 5552) after oral administration to spontaneously hypertensive rats.

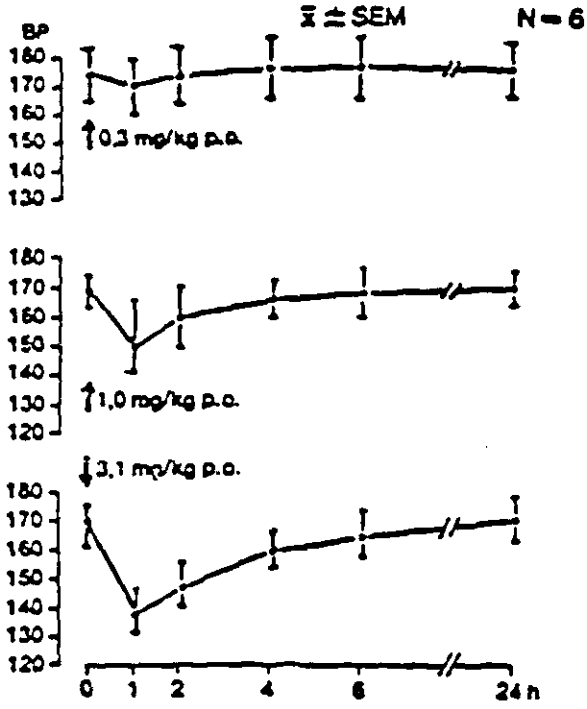


Figure 2

Antihypertensive effect of nisoldipine (BAY k 5552) by oral administration to one-kidney renal hypertensive rats.

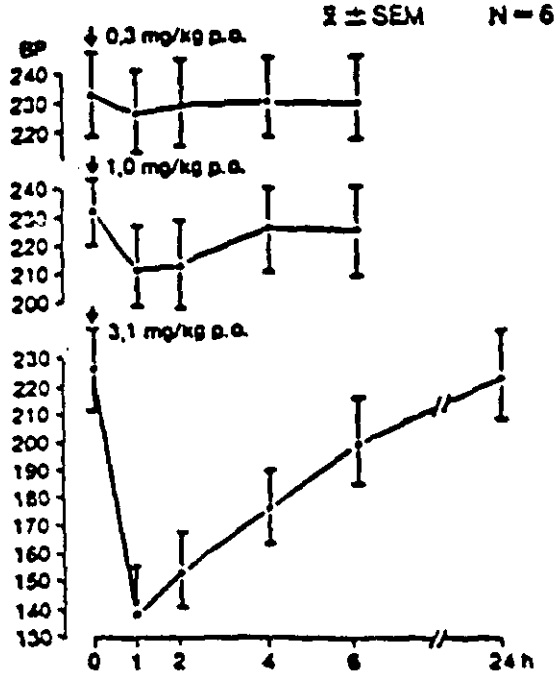


Table 5. Comparative effects (ED_{30}) of nisoldipine and reference drugs on blood pressure (BP) and heart rate (HR) in certain types of hypertensive rats and in normotensive rats.

Drugs	ED_{30} (BP) (mg/kg p.o.)				ED_{30} (HR) (mg/kg p.o.)			
	NR	SHR	DNR	RHR	NR	SHR	DNR	RHR
Nisoldipine	12.0 (1)	4.0 (1)	7.21 (1)	4.04 (1)	3.80 (1)	1.40 (1)	>30.00 (1)	1.60 (1)
Nifedipine	4.10 (0.34)	3.60 (0.90)	1.48 (0.21)	1.50 (0.37)	1.40 (0.37)	9.20 (1.46)	11.5 (0.38)	5.61 (0.37)
Nicardipine	6.80 (0.57)	4.00 (1)	2.52 (0.35)	1.93 (0.48)	3.00 (0.79)	5.00 (0.79)	9.68 (0.32)	7.80 (0.51)
Hydralazine	4.20 (0.37)	4.10 (1.03)	2.90 (0.40)	1.91 (0.47)	1.60 (0.42)	1.80 (0.29)	5.23 (0.17)	4.41 (0.29)

Relative values of ED_{30} are dipicted in parentheses (nisoldipine = 1).

NR=normotensive rat, SHR=spontaneously hypertensive rat, DNR= DOCA-NaCl hypertensive rat, RHR=renal hypertensive rat.

In terms of blood pressure lowering effect, nisoldipine was about equipotent to nifedipine, nicardipine and hydralazine in SH rats; however, it was less potent than the other drugs in other hypertensive models and in normotensive rats. The positive chronotropic effects of nisoldipine were less remarkable than those of reference drugs except in SH rats.

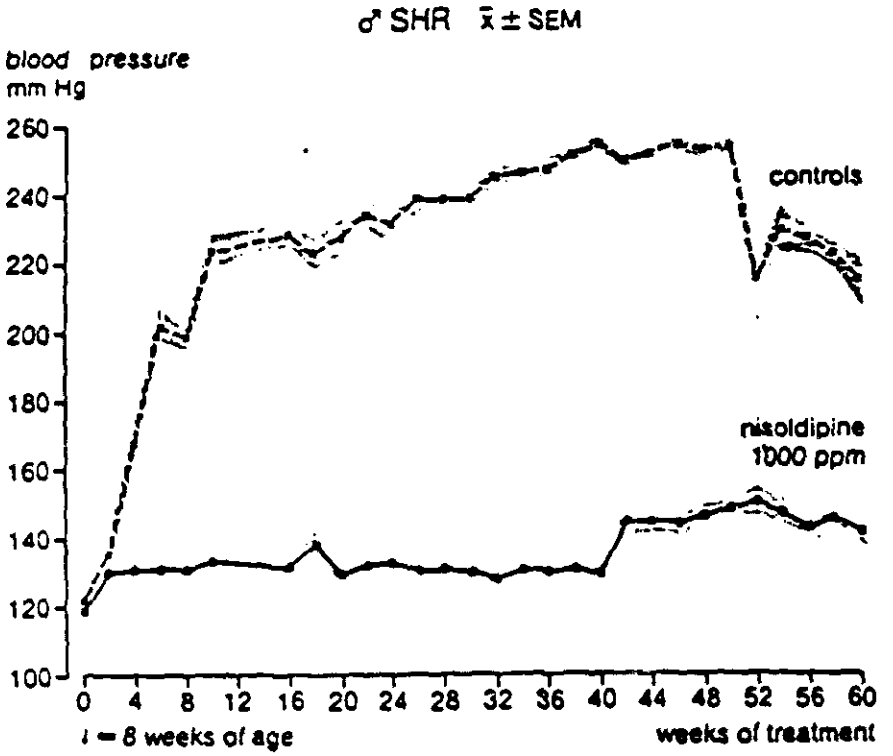
In another study, single oral doses of nisoldipine (0.315, 1.0 and 3.15 mg/kg) produced a dose dependent reduction of systolic blood pressure in conscious female SH rats (Fig.1). Although the lowest dose (0.315 mg/kg) produced only a slight reduction in blood pressure (3% reduction from the base value), doses of 1.0 and 3.15 mg/kg reduced blood pressure 12 and 18%, respectively. The maximum effect, at all dose levels, was seen at 1 hr after drug administration and the blood pressure returned completely or nearly to pretreatment level by 6 hr postdose. When the above doses of nisoldipine were given orally to one-kidney renal hypertensive rats, a significant decrease (39%) in blood pressure was seen at 3.15 mg/kg and moderate (9%) and slight (3%) reductions were observed at 1 and 0.315 mg/kg, respectively (Fig.2).

In conscious normotensive rats, nisoldipine (0.3 mg/kg po) significantly reduced systemic vascular resistance (0.58 to 0.38 mm Hg/kg/min/ml) and mean arterial pressure (122 to 108 mm Hg), and increased heart rate (395 to 447 beats/min), stroke volume (0.57 to 0.72 ml/beat/kg) and cardiac index (225 to 326 ml/min/kg). Left ventricular end-diastolic pressure (LVEDP) was slightly decreased (9.6 to 8.8 mm Hg, $p<0.05$) but no significant change in left ventricular systolic pressure was seen.

The effect of chronic dietary administration of nisoldipine on the development of hypertension was studied in SH rats.

Fig. 3

Effect of Long-term Treatment (60 weeks) with Nisoldipine on Systemic Blood Pressure in SH Rats



Shaded areas indicate SEM.

TABLE 6. *Preventive Experiment: The Effect of Long-term Treatment (60 weeks) with the Calcium Antagonist Nisoldipine on Systolic Blood Pressure, Plasma irANP, Relative Heart Weight, Body Weight, PRA, and Plasma Aldosterone Concentration in SHR and WKY*

	SHR		WKY	
Parameter measured after 60 weeks	Controls ($n = 7$)	Nisoldipine ($n = 10$)	Controls ($n = 8$)	Nisoldipine ($n = 8$)
SBP (mm Hg)	214 ± 7	141 ± 3‡	145 ± 3‡	137 ± 3
Plasma irANP (pg/ml)	470 ± 38	139 ± 35‡	88 ± 23‡	107 ± 29
Relative heart weight (mg/100 g body wt)	376 ± 29	313 ± 4*	277 ± 16†	284 ± 16
Body wt (g)	388 ± 8	377 ± 9	380 ± 16	381 ± 20
PRA (ng ANG I/ml/hr)	2.9 ± 0.3	1.9 ± 0.4	3.3 ± 0.4	2.4 ± 0.7
PAC (pg/ml)	332 ± 26	242 ± 16†	369 ± 30	454 ± 28

Values are means ± SEM. SBP = systolic blood pressure; irANP = immunoreactive ANP; ANG I = angiotensin I; PAC = plasma aldosterone concentration.
*$p < 0.025$; †$p < 0.01$, ‡$p < 0.001$, compared with values in untreated SHR.

Administration of nisoldipine to male SH rats (8 weeks old at the initiation of treatment) at 1000 ppm (50-100 mg/kg/day in diet) for 60 weeks prevented the development of hypertension during the treatment period [mean systolic blood pressure of 141 mm Hg in the treated group vs 214 mm Hg in the control group at the end of the study (Fig.3)]. The final blood pressure of treated SH rats was nearly the same as that of treated or untreated normotensive Wistar Kyoto (WKY) rats (Table 6). (However, it is noted that blood pressure in treated SH rats rapidly increased to the untreated control level when the treatment was stopped.) On the other hand, in untreated concurrent control SH rats, blood pressure increased progressively till week 48, to a maximum of 250 mm Hg, and declined thereafter to 214 mm Hg at the termination of the study. In WKY rats, no significant treatment related blood pressure changes were seen in the nisoldipine treated group compared to the control group. Furthermore, the results of the above study also showed that long term treatment with nisoldipine significantly decreased plasma atrial natriuretic peptide-like immunoreactivity (ANP-IR) and plasma aldosterone concentrations (PAC) and attenuated cardiac hypertrophy in SH rats (Table 6).

It was also shown that a 10 week dietary treatment with nisoldipine (50-100 mg/kg) in old SH rats (69 weeks old) with end-stage hypertensive disease caused significant reductions in systolic blood pressure [from 210 to 169 mm Hg (20%)], ANP-IR (20%) and relative heart weight (20%).

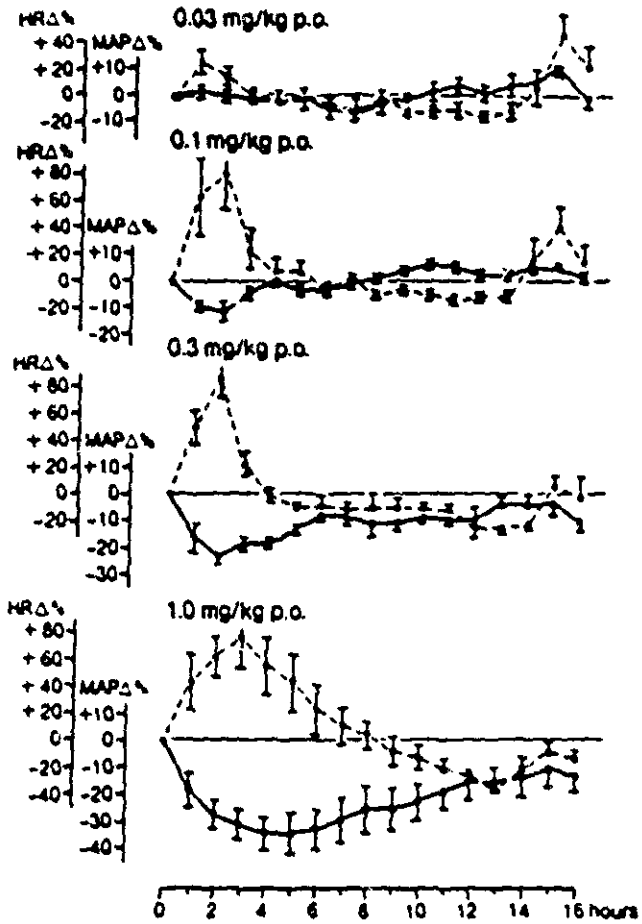
In inbred Dahl salt sensitive (DS) rats on a high salt diet (8% NaCl), dietary administration of nisoldipine at 1000 ppm (100 mg/kg/day) for 5 weeks produced significant reductions in systolic blood pressure (168 mm Hg in nisoldipine treated group vs 236 mm Hg in control DS rats) and plasma ANP-IR and renin activities. No nisoldipine treatment related effects were seen in Dahl salt resistant rats. Although treatment with the arteriolar vasodilator minoxidil (10 mg/kg, in drinking water) caused reduction of blood pressure in DS rats, the plasma ANP-IR levels and heart weights were significantly increased in treated rats compared to control DS rats.

In diabetic SH rats (streptozotocin induced), nisoldipine (9 mg/kg po for 10 weeks) significantly reduced blood pressure and inhibited the progress of renal lesions. No nisoldipine treatment related effects were seen on blood glucose, body weight gain, heart rate and heart and kidney weights.

Nisoldipine (0.3-0.6 mg/kg in food for 20 weeks) prevented the development of hypertension in rats subjected to 5/6 th nephrectomy (147 mm Hg in treated vs 237 mm Hg in untreated group).

Nisoldipine infusion (0.7 µg/min) decreased mean arterial pressure in anesthetized normotensive rats from 107 to 76 mm Hg; and in conscious rats, a bolus administration of nisoldipine (100 µg) caused a reduction of blood pressure from 1[illegible]2 to 76 mm Hg.

Figure 4



Effect of nisoldipine on mean arterial blood pressure (MAP; ●——●) and heart rate (HR; ○- - -○) of conscious, unrestrained renal hypertensive dogs.

Fig.5

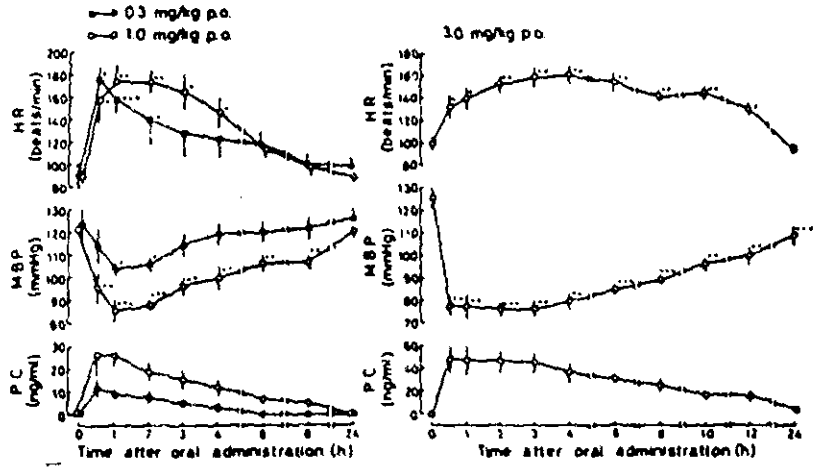


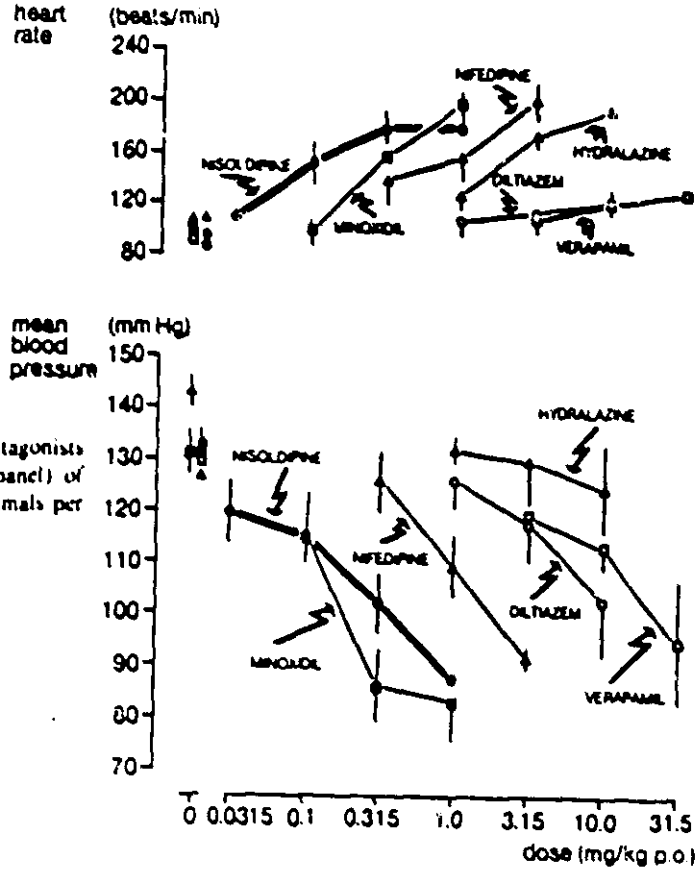
Fig. 5: Time course of the effects of single oral administration of nisoldipine on mean blood pressure (MBP), heart rate (HR) and plasma concentration (PC) in conscious renal hypertensive dogs. Values are expressed as the mean ± S.E.M. from 4 to 6 dogs. Asterisks indicate significant difference from the pre-drug values indicated at the zero time: *P < 0.05, **P < 0.01 and ***P < 0.001.

b. Dogs

The effects of oral nisoldipine on blood pressure and heart rate were studied in conscious, unrestrained, renal hypertensive (unilateral renal artery stenosis) beagle dogs using a radio-telemetric method, and compared with effects of other calcium antagonists (nifedipine, diltiazem and verapamil) and vasodilators (hydralazine and minoxidil). Single oral doses of nisoldipine (0.03-1.0 mg/kg) produced a dose-dependent decrease in mean arterial blood pressure in renal hypertensive dogs (Fig.4). At 0.3 mg/kg, a marked reduction in blood pressure (24%) was produced within 2 hr after drug administration and the hypotensive action lasted for 12 hours. A reflex tachycardia, lasting for about 3 hr, occurred at the above dose level. A more pronounced hypotensive effect was seen at 1 mg/kg. Nifedipine produced about the same degree of hypotension as that produced by 0.3 mg/kg po of nisoldipine at a 10 fold higher dose level (3.15 mg/kg po). The hypotensive effect and the reflex tachycardia lasted for 6 hr. Diltiazem and verapamil produced comparable antihypertensive effects at higher dose levels with slight to moderate increase in heart rates. [At the highest tested dose level of verapamil (31.5 mg/kg po), 3/5 dogs showed marked bradycardia.] The anti- hypertensive effect of minoxidil was more pronounced (34% blood pressure reduction at 0.3 mg/kg po) than that of hydralazine (about 15% reduction at 10 mg/kg po), and persistent reflex tachycardia was seen for the entire period of blood pressure reduction in both cases.

The ED20 (mg/kg) values (the dose that causes a 20% reduction in mean blood pressure) and the dose response curves for nisoldipine and other calcium antagonists and vasodilators are given below.

	ED20 (mg/kg)
nisoldipine	0.14
nifedipine	1.68
diltiazem	6.21
verapamil	8.39
minoxidil	0.14
hydralazine	>10.00



Dose response curves for the influence of nisoldipine and some other calcium antagonists and vasodilators on heart rate (upper panel) and mean blood pressure (lower panel) of conscious, unrestrained renal hypertensive dogs. Given are means ± S.E. of 4–6 animals per dose. Pre drug levels of heart rate and blood pressure are indicated by 0.

The above ED20 data indicate that nisoldipine and minoxidil are more potent antihypertensive agents in dogs than the other drugs studied. However, the dose response curves show that the antihypertensive effect of minoxidil is markedly more steep than that of nisoldipine. While diltiazem, verapamil and hydralazine are shown to be much less potent than nisoldipine and minoxidil in reducing blood pressure in renal hypertensive dogs, the antihypertensive action of nifedipine is rated as intermediate between nisoldipine or minoxidil and the other reference drugs studied.

Nisoldipine (31-315 µg/kg po) decreased MAP and TPR, and increased HR in anesthetized normotensive dogs. One hour after 100 µg/kg nisoldipine, MAP and TPR were decreased 20 and 45%, respectively, HR increased 76%, and LVEDP was unchanged. In another study in anesthetized dogs, nisoldipine (0.3 µg/kg iv) decreased TPR by 20% and increased stroke volume 28% without decreasing MAP, however, a dose of 30 µg/kg iv decreased MAP by 14% and TPR by 66%, increased HR 110% and stroke volume by 38% without changing EDP.

The antihypertensive effects and the pharmacokinetics of nisoldipine were compared with those of nifedipine, nimodipine, nicardipine and hydralazine in conscious, renal hypertensive (one-clip, two-kidney type hypertension of Goldblatt et al) male mongrel dogs. Single oral doses of nisoldipine (0.3, 1.0 and 3.0 mg/kg with Cmax values of 13, 33 and 60 ng/ml, respectively, or AUC values of 36, 132 and 523 ng/ml/hr, respectively) produced dose-dependent reductions in mean arterial blood pressure, which were significantly different from pre-drug values at 30 min (1 and 3 mg/kg), with maximum effect seen at about an hour after dosing (Fig.5). At 1.0 and 3.0 mg/kg dose levels of nisoldipine, mean blood pressure reductions of 36 and 50 mm Hg, respectively, were observed. Significant antihypertensive activity lasted up to 24 hr after the 3.0 mg/kg dose. Although not dose dependent, increased heart rate was seen at all dose levels and remained significantly higher than pre-drug levels for 4 (1 mg/kg) to 12 hr (3.0 mg/kg) after dosing. Peak plasma concentrations of nisoldipine were seen 0.5 hr after oral administration and the antihypertensive activity significantly correlated with plasma concentrations of the drug ($r=0.727$, $p<0.001$). Like nisoldipine, other calcium antagonists (nifedipine, nimodipine or nicardipine) also dose-dependently lowered mean blood pressure, attaining peak effects at 1-2 hr after dosing. Hydralazine had a slow onset and its effect peaked 3 hr postdose. In the above study, it was found that nisoldipine was 5-6 times more potent than nifedipine, nicardipine and nimodipine and its antihypertensive effect lasted 3-6 times longer.

In another study in conscious renal hypertensive beagle dogs, single doses of nisoldipine (0.03-1.0 mg/kg po) produced the following dose-dependent decreases in mean arterial blood pressure (MAP) and increases in heart rate (HR).

Dose (mg/kg po)	MAP (% decrease)	HR (% increase)
0.03	n.s.	30
0.1	20	45
0.3	30	47
1.0	45	82

In conscious normotensive coronary artery occluded mongrel dogs, infusion of nisoldipine (1 and 3 µg/kg/min) for 15 min produced the following changes in blood pressure and heart rate.

	Control	(1 µg/kg/min)	(3 µg/kg/min)
SBP, mmHg	**133±3**	**126±4**	**119±6***
DBP, mmHg	**92±2**	**74±3***	**59±5***
MAP, mmHg	**105±2**	**91±3***	**79±4***
HR, bpm	**103±10**	**146±9***	**163±12***

***Significantly ($p<0.05$) different from control.**

In conscious, nonsedated, chronically instrumented mongrel dogs, iv administration of nisoldipine (10-100 µg/kg) decreased MAP by 11 and 29 mm Hg at 30 and 100 µg/kg dose levels, respectively.

In anesthetized mongrel dogs with cardiac tamponade, nisoldipine (2 µg/kg/min, iv for 15 min) caused mean blood pressure to fall from 120 to 71 mm Hg and reduced heart rate from 212 to 167 bpm.

c. Cats, Pigs and Sheep

In conscious cats, oral nisoldipine at 0.1 and 0.5 mg/kg dose levels produced 21 and 18% reductions in mean blood pressure and 23 and 63% increase in heart rates, respectively.

Nisoldipine (10, 30 and 60 µg, iv) dose-dependently reduced MAP and total peripheral resistance (TPR) and increased cardiac output in anesthetized cats.

In anesthetized Yorkshire pigs, infusions of nisoldipine (0.25, 0.5 and 1.0 µg/kg/min for 10 min) produced dose-dependent decreases in arterial blood pressure (30%), systemic vascular resistance (30%) and left ventricular filling pressure (15%), and increases in heart rate (25%) and LV dP/dt max (20%). Cardiac output was not significantly affected.

Nisoldipine (0.6 mg/kg/day iv for 4 days) prevented the development or reversed established hypertension induced by ACTH in sheep.

d. Antihypertensive Activity of Enantiomers

The antihypertensive activities of orally administered stereoisomers of nisoldipine were compared with the antihypertensive activity of the racemic compound in conscious SH rats and renal hypertensive dogs. In SH rats, (+)nisoldipine (BAY R 1224, ED20=2.1 mg/kg) was only slightly more potent (1.4 times) than the racemic compound but was about 20 times more potent than the (-)nisoldipine (BAY R 1223). No significant difference in antihypertensive activity was seen between (+)nisoldipine and the racemic compound in dogs.

In a study in anesthetized normotensive dogs, 10 and 30 µg/kg po (+)nisoldipine decreased MAP by 10 and 38% respectively, while (-)nisoldipine had no significant effects at doses up to 300 µg/kg.

e. Antihypertensive Activity of Metabolites

(Unless otherwise noted, the following studies were done in anesthetized normotensive dogs.)

BAY R 9590, a major metabolite of nisoldipine, showed weak peripheral vasodilator activity at iv doses of 1 mg/kg and above. No significant changes in blood pressure were seen at dose levels (0.3 to 3 mg/kg iv) tested in this study.

BAY O 3199, another metabolite, had relatively minor peripheral vasodilator activity at doses of 0.3 and 1.0 mg/kg iv in dogs. Slight blood pressure reduction was noted following the 1 mg/kg dose.

Metabolites BAY S 1869 and BAY S 4755 had no hemodynamic effects (total peripheral resistance, cardiac output and LV dP/dt) at 1 mg/kg iv and had only minor peripheral vasodilator effect at 3.0 mg/kg.

BAY R 9425, a dihydropyridine metabolite of nisoldipine, caused a dose-dependent drop in blood pressure at doses of 10 µg/kg iv and above, the effect lasting about 60 min at 30 µg/kg. Tachycardia and increased cardiac output were seen at the above dose level. BAY R 9425 (iv administration) was found to be 1/3rd to 1/10th as potent as nisoldipine in dogs.

In conscious renal hypertensive dogs, oral doses of BAY R 9425 (1 mg/kg) had a much weaker and shorter duration of action than the parent compound.

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3. **Mechanism of Action**

Nisoldipine is a dihydropyridine calcium channel blocker which binds with very high affinity to L-type calcium channels. By blocking calcium entry into vascular smooth muscle cells, it inhibits muscular contractions, thereby causing vasodilation of peripheral and coronary vasculatures.

The receptor binding characteristics of nisoldipine (BAY K 5552) and its optically pure enantiomers, BAY R 1224 (+ isomer) and BAY R 1223 (- isomer), were studied in rat cerebral cortical membranes using labelled nitrendipine. The concentration that causes 50% inhibition of ^{3}H nitrendipine binding (IC50), the inhibition constant (Ki) and the Hill Coefficient (nh) for the above compounds and the reference drugs are given below.

Substance	IC_{50} [nM]	K_i [nM]	nH	N
Bay K 5552 (Nisoldipine)	1.2 ± 0.1	0.769 ± 0.064	1.0 ± 0.05	34
Bay R 1224 ((+) enantiomer)	1.1 ± 0.08	0.705 ± 0.051	1.0 ± 0.09	34
Bay R 1223 ((-) enantiomer)	109 ± 0.15	70 ± 10	1.0 ± 0.01	34
Nifedipine (Reference)	2.7	1.7	1.15	
Bay K 8644 (Reference)	16	10	0.76	
Verapamil (Reference)	173	110	0.6	

Nisoldipine showed competitive displacement of ^{3}H nitrendipine with a Ki of 0.769 nM. The (+) enantiomer has a comparable Ki of 0.705 nM, while the affinity of the (-) enantiomer was reduced 100 fold to 70 nM. The Hill Coefficient for all three compounds was same, representing a linear Scatchard Plot and a homogenous population of binding sites. The above data indicate that the biologically active component of nisoldipine seems to be the (+) enantiomer.

Further studies in guinea pig ileal smooth muscle have confirmed that the receptor binding was at high affinity, saturable, reversible and stereoselective with high structural specificity, and correlated well with the pharmacologic activities. Binding studies in partially purified rat brain membranes using ^{3}H

nimodipine showed Ki values of 0.24 and 7 nM for nisoldipine and nifedipine, respectively. Studies in rat and rabbit ventricular membranes showed that nisoldipine differs from nifedipine in its high affinity binding (about 20 times greater), slow dissociation rate and large partition coefficient. The binding characteristics of both drugs are given below.

Comparison of Binding Characteristics of Nisoldipine and Nifedipine

	(+)Nisoldipine	Nifedipine
K_d, nM	0.04	0.81
Dissociation $t_{1/2}$, min	12	1.2
B_{max}, pmol/mg	0.69	0.17
Association Rate ($\times 10^6 M^{-1}min^{-1}$)	6.7	3.1
Entropy of binding	large positive	negative
Partition coefficient into biological membrane	6,000–27,000	2,900

The above biochemical and biophysical differences can be expected to result in a longer duration of action for nisoldipine.

Studies in isolated rat aorta revealed that there is good agreement among binding affinity and the IC50 values both for inhibiting ^{45}Ca influx and aortic contraction. The IC50 values for the inhibition of rabbit aortic ring contraction (potassium stimulated) were 1.8, 0.15 and 81 nm for racemic nisoldipine, (+) isomer and (-) isomer, respectively, indicating that these findings were in reasonable agreement with the results of the ligand binding studies.

Voltage-clamp studies in isolated calf Purkinje fibers have shown that nisoldipine binding is one thousand times stronger to inactivated channels (Kd=1 nm) than to resting channels (Kd=1.3 μM), indicating that nisoldipine blocks calcium channel current in a voltage dependent manner. Nisoldipine (10 μM) completely blocked the slow inward current and contractile activation in the above tissue. Moreover, nisoldipine reduced the transient outward, but not the delayed rectifier K^+ current, in calf cardiac Purkinje fibers.

Nisoldipine was a more potent inhibitor of BHT 920 (α2-adrenoceptor agonist)-induced contraction of isolated aortic rings when these rings were taken from stroke-prone SH rats than when they were taken from normotensive WKY rats (IC50=0.15 nM vs 7 nM).

Several studies have shown that the degree of nisoldipine inhibition of calcium channel currents (as well as contractions) increases with membrane depolarization. In patch-clamp studies in isolated smooth muscle cells from canine coronary artery, membrane depolarization from -65 mV to -30 mV increased the apparent affinity of nisoldipine binding about 9 fold (in the presence of 1 μM Bay K 8644, a calcium agonist). The calculated dissociation constant in this study was 0.07 nM, which was identical to the value obtained with radioligand binding

studies. In rabbit mesenteric artery, depolarization from -100 to -55 mV decreased the concentration of nisoldipine needed for 50% inhibition from 12 to 1.9 nM. Because of the very high affinity binding of nisoldipine to depolarized membranes, it is suggested that nisoldipine could preferentially bind to those depolarized arteries which increase total peripheral resistance of hypertension, and also to those producing coronary spasms.

B. **Additional Cardiovascular Studies**

1. Effects on Other Vascular Beds

Under conditions of controlled blood flow, nisoldipine infusion (1 µg/min) dilated the hindquarters vascular bed of anesthetized cats and inhibited vasoconstrictor responses to sympathetic nerve stimulation, norepinephrine, tyramine, methoxamine and BHT 933 (α2-adrenoceptor agonist).

The effects of nisoldipine on vascular resistance and vasoconstrictor responses were studied in the pulmonary vascular bed of anesthetized cats under conditions of controlled blood flow. Nisoldipine (1 µg/min) infused into the lobar artery caused only a small reduction in basal lobar resistance. It reduced pulmonary vasoconstrictor responses to methoxamine, BHT 933 and U46619 (thromboxane A2 mimetic).

2. Other Myocardial/Cardiovascular Effects

In isolated Langendorff-perfused rat hearts, nisoldipine (30 nm) prevented the ischemia-reperfusion-induced depletion of the cardiac stores of norepinephrine. In the above hearts, pretreatment with the drug (10 nm) 2 min before the onset of ischemia significantly improved cardiac output following global ischemia (20 min) and reperfusion (5 min). Pretreatment with nisoldipine (1 µM) for 5 min before ischemia prevented transcoronary macromolecular leakage after ischemia-reperfusion in isolated rat hearts.

In isovolumic coronary artery-perfused ferret hearts subjected to global ischemia for 3 min followed by 10 min reperfusion, nisoldipine (10 nm) significantly reduced the ischemia-induced rise in diastolic and systolic intracellular free ionized calcium (FIC, determined with the bioluminescent protein aequorin), and lessened the decrease in contractile function. Moreover, nisoldipine significantly accelerated the decline in FIC during reperfusion and improved recovery of contractility and relaxation.

Nisoldipine (5 µM) had no significant effect on dopamine-induced inhibition of nerve stimulated vasoconstriction of isolated perfused rabbit ear artery.

In anesthetized rats, nisoldipine (3 mg/kg po, 1-1.25 hr before acute coronary ligation) greatly reduced the duration of ventricular tachycardia and fibrillation occurring in the first 30 min postligation period. None of the treated animals died compared with a 40% mortality in controls.

Long-term dietary administration of nisoldipine (50-100 mg/kg for 22 weeks) prevented the rarefaction of myocardial capillarization in SH rats. Drug treated rats had lower arterial blood pressure and decreased left ventricular and septal weights compared to untreated rats.

In conscious chronically instrumented dogs, nisoldipine did not influence cardiac impulse formation or impulse conduction at 10 and 30 µg/kg iv, but at 100 µg/kg iv (strongly hypotensive dose), it produced reflex increase in the rate of atrioventricular conduction.

In dogs subjected to occlusion of the left anterior descending coronary artery for 1.5 hr followed by reperfusion, nisoldipine (3.5 µg/kg iv 10 min before the occlusion and again 10 min before reperfusion) suppressed the ischemia-induced increase in phospholipid breakdown as well as the increase in serum CPK activity. The drug also prevented ischemia-induced myocardial hemorrhage and premature ventricular contraction in a similar study.

Cumulative 10 min infusions of nisoldipine (0.05, 0.1, 0.25 and 0.5 µg/kg/min) in pigs with chronic left ventricular dysfunction (produced by the ligation of the left circumflex coronary artery 2-3 weeks before the study) improved ventricular function to the same extent as pimobendan, a phosphodiesterase inhibitor (2.5, 5, 12.5 and 25 µg/kg/min). Both drugs normalized cardiac output and exhibited similar cardiovascular effects (systemic vasodilation, reduction in left ventricular filling pressure, and increased heart rate) except for the significantly greater increase in left ventricular dP/dt max with pimobendan (85%) than with nisoldipine (45%). Thus, nisoldipine, despite of its lack of inotropic properties, improved ventricular function to about the same extent as pimobendan.

Infusion of nisoldipine (10 µg/kg over a 5 min period, 30 min before coronary artery occlusion) in open-chest pigs subjected to the occlusion of the left anterior coronary artery, completely prevented the increase in lipid peroxidation products associated with ischemia.

C. General Pharmacological Studies

1. Central Nervous System Effects

The analgesic activity of nisoldipine (25-500 mg/kg po) was assessed in female Wistar rats by the failure of the animal to withdraw its tail within 20 seconds after exposure to a focused heat ray. Nisoldipine had no analgesic activity at 25 mg/kg; however, at higher dose levels (100 mg/kg and above) 40-60% of animals showed evidence of analgesic activity.

To study the effects of nisoldipine on orientation motility, mice were placed in cages in the dark and their orientation motility was assessed at 5 min intervals for a total of 25 min after the light was turned on. Nisoldipine (25, 100 and 250 mg/kg po) had no significant effect on orientation motility in this test. However, in a separate study in which mice were kept initially in a dark chamber and then exposed to daylight after drug treatment, nisoldipine at 10 or 31.5 mg/kg po, but not at 3.15 mg/kg, reduced orientation motility by 20% in mice. The above dose levels had no effect on spontaneous motility.

The balancing ability of male mice to maintain their position on a round horizontal wood bar (diameter 8 mm) was tested at 60 min after oral treatment with nisoldipine (3.15, 10 or 31.5 mg/kg). The drug inhibited the balancing ability by 10% at 31.5 mg/kg. The ability of the mice to grasp a horizontal metal bar (diameter 3 mm) was not inhibited at the above dose levels.

There was no evidence of any muscle relaxant or sedative effects for nisoldipine in mice, as assessed by the measurement of the holding and climbing ability on a horizontal bar with at least one hind paw within 5 sec after they were suspended on the bar by their front paws, at 25, 100 and 250 mg/kg po dose levels.

The anticonvulsant activity of nisoldipine was determined by the ability of the drug to antagonize either electroshock- or pentylenetetrazole (PTZ)-induced tonic convulsions in mice. Nisoldipine showed no anticonvulsant activity when administered 30 min before electroshock at 25, 100 or 250 mg/kg po. In the PTZ test, the drug (25-250 mg/kg po, 30 min before PTZ administration) had a dose-dependent anticonvulsant effect with an estimated ED50 of 98 mg/kg.

In a subsequent study, nisoldipine (3.15, 10 or 31.5 mg/kg po), given 60 min before electroshock or PTZ administration, antagonized the electroshock-induced tonic convulsions in 20% of mice at 3.15 and 10 mg/kg and in 30% at 31.5 mg/kg. PTZ-induced convulsions were antagonized by nisoldipine at 10 (30%) and 31.5 (80%) mg/kg, but not at 3.15 mg/kg.

The depth of hexobarbital-induced anesthesia was not affected by nisoldipine at 3.15, 10 and 31.5 mg/kg po, but the duration of

anesthesia was prolonged at 31.5 mg/kg.

To evaluate the possible tranquilizing effect of nisoldipine on defensive behavior, fighting episodes were induced in mice by weak electric foot shocks of 0.2 msec duration at a frequency of 5 per min. Nisoldipine at 25 mg/kg po was ineffective, but a dose-dependent antagonism of fighting was observed at higher doses (50 to 400 mg/kg po) with an ED50 of 82 mg/kg.

The (-)enantiomer (BAY R 1223) had no significant CNS effects in mice at oral doses of 3, 10 and 30 mg/kg. The (+)enantiomer (BAY R 1224) at 10 and 30 mg/kg po impaired motor coordination of mice in the balance rod test and increased the threshold dose of PTZ required to produce convulsions. The no-effect dose was 3 mg/kg. No significant effects were seen in mice on traction ability, analgesic and anticonvulsive responses or depth of hexobarbital-induced anesthesia at 3-30 mg/kg po dose levels. In rats, administration of BAY R 1224 (10 and 30 mg/kg po) produced ptosis, salivation, sedation, hypothermia, prone position, reduced muscle tone and ataxia during walking.

2. Gastrointestinal Effects

Nisoldipine (3, 10 and 30 mg/kg po) significantly reduced the intestinal transit time in mice, as measured by the length of the intestine covered by charcoal which was given orally 40 min after nisoldipine administration, at all tested dose levels with an estimated ED50 of 17.19 mg/kg.

(-)Nisoldipine, at the above dose levels, had no effect on intestinal transit time in the rat, whereas (+)nisoldipine significantly reduced transit time dose-dependently.

Nisoldipine or its stereoisomers (10 or 30 mg/kg po) did not induce any gastric lesions in rats.

At 3, 10 and 30 mg/kg po, (-)nisoldipine had no effect on indomethacin-induced ulcers in rats, while (+)nisoldipine significantly reduced these lesions at all dose levels.

Nisoldipine (3 or 30 mg/kg intraduodenal) or (+) nisoldipine (3, 10 or 30 mg/kg id) had no significant effects on basal gastric acid secretion, whereas (-)nisoldipine significantly reduced basal gastric acid secretion at 30 mg/kg id.

Nisoldipine antagonized acetylcholine- and histamine-induced spasms of isolated guinea pig ileum at 1 mg/L and barium chloride-induced spasms at 3 mg/L.

3. Metabolic Effects

In fasted rats nisoldipine at 10 and 30 mg/kg po significantly and dose dependently elevated blood glucose at 1, 2 and 4 hr after drug administration, while the serum triglyceride

concentration was slightly reduced in a dose-dependent manner. At 3 mg/kg po, the drug had no significant effect on either blood glucose or triglycerides.

In fed rats nisoldipine elevated blood glucose and lowered serum triglyceride concentrations at 3, 10 and 30 mg/kg po.

4. Effects on Respiration

Nisoldipine (0.26 nm to 26 µM) had no effect on resting tone of the isolated guinea pig trachea. Histamine- and LTD4-induced contractions were significantly reduced by nisoldipine at 26 nM.

Nisoldipine (0.32, 1.0 and 3.2 µg/kg iv) had no significant effect on spontaneous respiration in anesthetized dogs.

The enantiomers of nisoldipine ((3, 10 and 30 mg/kg po) had no effect on airway resistance or lung compliance, and did not modify histamine-induced increases in lung resistance in anesthetized, spontaneously breathing guinea pigs.

5. Renal Effects

The diuretic activity of nisoldipine was tested in normotensive male Wistar rats loaded with 0.5% methylhydroxyethylcellulose (10 ml/kg). Nisoldipine at 1.0, 3.15, 10.0 and 31.5 mg/kg po produced no significant effects on urine volume or urinary excretion of Na^+ or K^+ over a 6 hr collection period. However, at 100 mg/kg, the drug reduced urinary volume and Na^+ and K^+ excretion. Urinary pH was not changed over the entire dose range.

In another study in liquid-loaded rats (with a solution containing 0.2% NaCl, 0.4% KCl and 0.1% tragacanth), nisoldipine (10, 30 and 100 mg/kg po) increased urinary volumes and excretion of Na^+ and K^+ at all dose levels, the effects being more pronounced at 10 mg/kg than at higher dose levels.

BAY R 1224, the (+)enantiomer of nisoldipine, had no significant effects on urine volume or electrolyte excretion in normotensive rats at 3 and 10 mg/kg po; however, at 30 mg/kg, it significantly reduced urine volume and the excretion of Na^+ and K^+. BAY R 1223, the (-)enantiomer, had no significant effects on the above parameters.

In stroke-prone SH rats, administration of nisoldipine (0.315 to 10 mg/kg po) significantly increased urine volumes and excretion of Na^+ at 3.15 and 10 mg/kg. Although excretion of K^+ was increased after 10 mg/kg of nisoldipine, the increase failed to achieve statistical significance.

To elucidate the mechanism of diuretic effects of nisoldipine in SH rats, renal clearance and micropuncture studies were carried out in moderately saline-loaded animals. At dose levels of 0.1, 0.15 and 0.2 µg/kg/min iv for 15 min, the drug produced a

significant fall in blood pressure accompanied by increased sodium excretion and glomerular filtration rate (GFR). This natriuretic effect was attributed to the suppression of distal tubular sodium reabsorption. In another study in SH rats, nisoldipine (10 µg/kg/hr iv) produced diuretic and natriuretic effects without any changes in GFR. It was also shown that the diuretic and saluretic effects were considerably more pronounced in hypertensive rats than in normotensive Wistar-Kyota rats.

Nisoldipine (10 nm) completely reversed the norepinephrine-induced reduction in GFR in the isolated perfused rat kidney.

In the rat acute renal failure model (glycerol induced), nisoldipine treatment (10 mg/kg b.i.d for 2 days) increased urine volumes and significantly reduced glycerol-induced increases in serum creatinine and urea and renal tissue calcium levels.

In conscious dogs, nisoldipine at 0.1 mg/kg po had no significant effect on renal function (GFR and inulin and paraaminohippurate clearances). However, at 0.3 mg/kg the drug reduced all measured parameters of renal function, the maximum effects observed 20 min after drug administration.

The interaction of nisoldipine with angiotensin II (AII) or norepinephrine (NE) was studied in anesthetized mongrel dogs. Intrarenal infusion of nisoldipine (2, 10 or 50 ng/kg/min for 20 min) produced a dose-dependent increase in urine flow and urinary excretion of sodium, chloride and potassium, although no significant change in GFR was seen. Renal blood flow was significantly increased only at the highest dose level. AII and NE reduced renal blood flow and urine volumes. The decreased urine flow induced by AII, but not by NE, was completely blocked by nisoldipine, while the effect of AII on renal blood flow was only partially antagonized.

6. Hematological Effects

Collagen-induced platelet aggregation, coagulation time, thrombus elasticity and partial thromboplastin time were not affected by nisoldipine administration (10, 30 or 100 mg/kg po; blood sampling 90 min postdose) in rats. Nisoldipine (2.6 nm) had no effect on factor XIII activity in bovine plasma.

Neither (+) nor (-) enantiomer (3, 10 and 30 mg/kg po) had effects (60 min postdose) on hematological parameters in rats (hemoglobin, hematocrit, platelet count, fibrinogen levels, sedimentation rate, thrombin or thromboplastin time and collagen-induced platelet aggregation).

7. Antiatherogenic Activity

In rabbits fed a cholesterol (2.5%) supplemented diet, administration of nisoldipine (1 mg/kg/day for 7 weeks) significantly reduced the serum and aortic concentrations of cholesterol and

preserved endothelium-dependent relaxation of the isolated aortic rings.

8. Antiinflammatory Effects

Oral administration of nisoldipine (5 to 315 mg/kg, 1 hr before kaoline injection) showed antiinflammatory activity against edema (caused by intraplantar injection of kaolin into the hind paw) in rats at dose levels of 10 mg/kg and above (ED50=46 mg/kg po).

9. Effects on Histamine Release

Nisoldipine (0.26 to 260 µM) had no significant effect on histamine release from rat peritoneal mast cells in vitro and also did not modify antigen-induced histamine release from these cells.

D. **Antidote Studies**

In anesthetized rats, 100 µg/kg/min iv infusions of nisoldipine produced pronounced decreases in arterial blood pressure, heart rate, cardiac output and peripheral resistance, followed by death within about 55 min after the initiation of the infusion. EKG changes included sinus bradycardia, partial or complete AV block and shifting of the pacemaker to the AV node. Infusion of calcium gluconate (15 mg/kg/min), isoproterenol (20 µg/kg/min) or dopamine (100 µg/kg/min) simultaneously with nisoldipine prolonged survival time by more than 100%. Norepinephrine (20 µg/kg/min) had no significant effect.

In anesthetized dogs, calcium gluconate (100 mg/kg iv) reversed the hypotension and tachycardia produced by 10 µg/kg iv nisoldipine, but exacerbated the hypotension produced by 30 and 100 µg/kg.

E. **General Pharmacological Studies of Metabolites**

Nisoldipine was shown to be about 21 times and the BAY R 9425 (a dihydropyridine metabolite of nisoldipine) was twice as potent as diphenhydramine (reference compound) in inhibiting histamine-induced spasms of isolated guinea pig ileum. The other metabolites (BAY 03199, BAY R 9590, BAY S 1869 and BAY S 4755) had no significant effect on the above parameter.

BAY R 9425 had no significant CNS (rat and mouse), pulmonary (guinea pig), hematologic (rat), gastrointestinal (rat) and urinary (rat) effects. This metabolite at 0.26 to 26 µM did not induce histamine release from rat peritoneal mast cells in vitro; however, at the same concentration range, it significantly inhibited the antigen-induced histamine release from these cells.

BAY R 9425 inhibited LTD4-induced contraction of isolated guinea pig trachea at 260 nM, but not at 26 nM. This compound was about 10 times less potent than nisoldipine in inhibiting K^+-induced contractions of isolated pregnant (IC50=86 nM) or nonpregnant (IC50=138 nM) rat uterus. Other metabolites (BAY O 3199, BAY R 9590, BAY S 1869 and BAY S 4755) were effective only at very high concentrations (above 50 µM).

F. Interaction of Nisoldipine with Propranolol

Administration of nisoldipine (0.315 mg/kg po) in conscious unrestrained renal hypertensive dogs after ß-adrenoceptor blockade by propranolol (3.15 mg/kg po) increased the maximal reduction in systolic blood pressure from 15 to 23% and reduced the maximal reflex increase in heart rate from 109 to 50%.

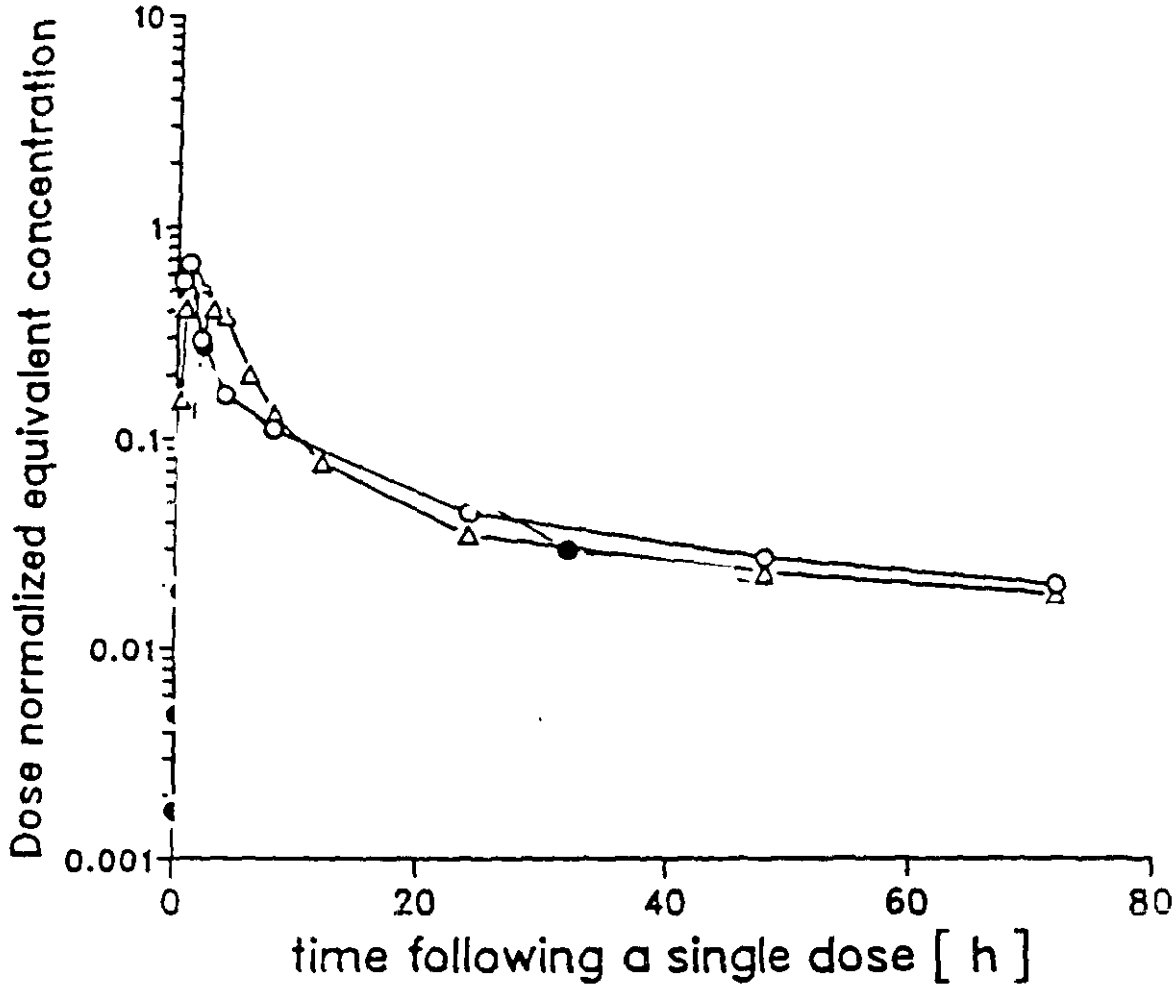
Propranolol (2 mg/kg iv) attenuated the reflex increase in heart rate produced by nisoldipine (2.5 to 25 µg/kg/min iv) in conscious instrumented dogs and prevented an increase in the heart rate-systolic pressure product (an index of myocardial oxygen consumption). Propranolol potentiated the hypotensive effect of nisoldipine (5-25 µg/kg/min), but did not further increase mean coronary blood flow.

Propranolol (4.4 mg/kg po) attenuated both the positive chronotropic and inotropic effects and the changes in systolic wall thickening caused by exercise in pigs with coronary artery stenosis. Nisoldipine (0.5 mg/kg po) alone did not modify cardiovascular effects of exercise, but it further improved wall function in the presence of propranolol.

SUMMARY OF PHARMACOKINETICS STUDIES (S. Stolzenberg)

1. Absorption and Excretion: Nisoldipine was measured by gas chromatography and electron capture. A summary of urinary, fecal and CO_2 recoveries following administration of a single dose of ^{14}C-labelled nisoldipine in four species, including man, is given on the page 30 of this review. In the rat, dog and pig, the primary route of excretion was the biliary-fecal route, whereas in the monkey and man, the urinary route predominated. Based on the ratio of the % of Dose Excreted ir urine for i.v./p.o. routes multiplied by 100, nisoldipine was considered to be rapidly and almost completely absorbed in male rats (107%), dogs (107%) and man (89.7%). For the pig, monkey (rhesus) and rat, where i.v. doses were not given, the figure under this column represents the amount excreted in the urine, which is regarded as "the lower limit of absorption".

2. Plasma Pharmacokinetics: The figure which follows illustrates the plasma concentrations vs time, following a single oral dose in rats, dogs and monkeys. The table on selected plasma pharmacokinetics on page 31 reflects these observations and includes results from i.v. administration in the same 3 species and in man. After oral administration, no distinct species differences were noted in the rat, dog or monkey; $CEQ_{max,norm}$ (normalized to 1 mg/kg dose, based on radioactivity equivalence of parent compound) ranged between 0.49 - 0.79 $kg*l^{-1}$, but was 4.7 to 7.5 times higher in man (3.7 $kg*l^{-1}$). AUC_{norm} for total radioactivity was also similar in the 3 species, but was higher in man by a factor of 6 to 10. $C_{max,norm}$ and AUC_{norm} for unchanged nisoldipine following oral administration were very low in all four species, including man, which was attributed to an extensive first pass effect, known for this compound. In humans given immediate release tablets of 2.5, 5, 10 and 20 mg, or core coated tablets of 10, 20, 40 and 60 mg, dose proportionality was observed for plasma nisoldipine C_{max} and AUC_{0-24}. At steady state (8th day of dosing) in humans, both AUC_{0-24} and C_{max} showed a "dose dependent and broadly dose proportional increase" at 30, 60, 90 and 120 mg. Correspondingly, both systolic and diastolic blood pressures "showed a general dose related decrease from baseline at steady state". Despite the high rates of absorption, bioavailabilities (F) of parent compound after oral doses were correspondingly low; 2.7, 11.7 and 8.4% in the rat, dog and man, respectively. The core coated preparation had an F value of 5.5% in man. In the dog, it was shown that both the gut wall and the liver contributed to the first pass effect and resulting low F values for the parent compound. The plasma half-life of unchanged drug was essentially similar in dog, monkey and man (2.3 to 4 h). The shorter $t_{1/2}$ of unchanged compound listed in the table for rats following oral or i.v. dosing does not represent a true terminal half-life because plasma levels were measured for only 2 hours post-dosing. In rats, the pharmacologically more potent (+)- enantiomer had a five fold higher bioavailability than the (-)- enantiomer.



Dose-normalized equivalent concentrations of total radioactivity after single oral administration of [^{14}C]nisoldipine to male rats (n = 5), female dogs (n = 3) and female monkey (n = 1) (mean of each).

o = 5 mg·kg^{-1}, rat

Δ = 5 mg·kg^{-1}, dog

● = 10 mg·kg^{-1}, monkey

ABSORPTION/EXCRETION OF NISOLDIPINE IN ANIMAL SPECIES AND MAN FOLLOWING ADMINISTRATION OF [^{14}C]NISOLDIPINE.

Species	Route	Dose (mg/kg)	% of Dose[1] Excreted in: urine	feces	CO_2	% absorbed (minimum)
Rat (M)	p.o.	5.0	30.8 (2.4)[a]	75.0 (0.7)[a]	0.5[b]	107.0
	i.v.	1.0	28.7 (3.5)[a]	71.8 (6.5)[a]	0.6[b]	-----
Rat (F)	p.o.	5.0	41.9 (2.9)[a]	62.0 (2.8)[a]	-----	41.9
Dog (F)	p.o.	5.0	38.9 (5.1)[d]	55.2 (2.3)[d]	-----	107.0
	i.v.	0.5	36.3 (5.2)[d]	59.1 (8.2)[d]	-----	-----
Man (M)	p.o.	12.0 mg	73.7 (5.4)[f]	12.3 (2.4)[f]	-----	89.7
	i.v.	0.8 mg	82.2 (7.4)[f]	14.4 (9.5)[f]	-----	-----

[1]Values are arithmetic means (sd)

n = 5 (rat), 3 (dog) and 10 (man). For monkey and pig, n=1

Collection periods:

a) 48 h. d) 144 h

b) 24 h e) 240 h

c) 72 h f) 96 h

SELECTED PLASMA PHARMACOKINETIC PARAMETERS IN SEVERAL SPECIES FOLLOWING ADMINISTRATION OF [^{14}C]NISOLDIPINE

Species	Rat (M)		Dog (F)		Monkey (F)	Man (M)	
Route Dose (mg/kg)	i.v. 1	p.o. 5	i.v. 0.5	p.o. 5		p.o. 12*	i.v. 0.8*
Radioactivity							
$CEQ_{max,norm}$ (kg*l^{-1})	---	0.49	---	0.59		3.7	---
t_{max}(h)	---	0.87	---	1.45		0.77	---
AUC_{norm}(kg*h*l^{-1})	13.6	4 0	4.2	5.9		40.1	50.2
$t_{½term}$(h)	23.9	14.9	37.9	54.4		80.3	85.8
Parent							
$C_{max,norm}$ (kg*l^{-1})	---	0.009	---	0.017		0.054	---
t_{max}(h)	---	0.5	---	1.0		0.42	---
AUC_{norm}(kg*h*l^{-1})	0.36	0.0097	0.46	0.054		0.077	0.954
$t_{½}$(h)	0.36	0.70	4.0	2.3		3.8	3.8
F(%)	---	2.7	---	11.7		8.4	---

n = 5 (rat), 3 (dog), 1 (monkey), 12 (man)
Rat data for parent (p.o.) from 1 mg/kg dose.

*Total mg dose per volunteer

Immediate Release

Dose (mg)	C_{max} (ng/mL)	AUC_{0-24} (ng.h/mL)
2.5	0.43 (58)	1.26 (84)
5	0.85 (46)	2.89 (48)
10	1.44 (59)	6.52 (46)
20	3.42 (57)	14.5 (42)

Coat Core

Dose (mg)	C_{max} (ng/mL)	AUC_{0-48} (ng.h/mL)
10	0.90 (43)	15.2 (33)
20	1.45 (39)	27.1 (37)
40	3.07 (49)	54.3 (47)
60	4.28 (52)	83.3 (43)

Study D90-022

Pharmacokinetic Parameters at Steady-State (Mean ± SD)

Dose (mg)	N	AUC(0-24h) (ng • h/ml)	C_{max} (ng/ml)	T_{max} (h)
30	18	74.28 ± 7.96	4.79 ± 0.68	7.22 ± 0.93
60	18	129.76 ± 12.74	8.48 ± 0.81	9.08 ± 1.97
90	9	199.31 ± 16.45	13.02 ± 1.20	6.78 ± 2.30
120	3	226.58 ± 12.41	14.92 ± 2.01	4.00 ± 1.00

Study D90-022

L.S. Mean Supine BP Change from Baseline at Steady-State (mmHg)

		Systolic		Diastolic	
Dose (mg)	N	8h post dose	24h post dose	8h post dose	24h post dose
30	18	-16.4	-14.0	-8.4	-10.2
60	18	-20.8	-16.8	-13.2	-15.0
90	9	-22.1	-23.0	-12.1	-13.4
120	3	-30.7	-44.3	-25.0	-19.0

3. Tissue Distribution: Quantitative tissue distribution in Sprague-Dawley rats was determined after 0.5, 1, 4, 8, 24, 48 and 72 hours (although the tables which follow provide 4, 24 and 72 hour values only). After oral administration, maximum concentrations in virtually all organs were reached within one hour, with liver, fat, kidney and adrenal gland generally containing the highest levels, brain and skeletal muscle the lowest. After a single dose, terminal elimination half-lives ranged from 42.2 h for plasma to 123 h for brain. With repeated daily dosing (5 mg/kg for 3 weeks), steady state was reached within 8 days. The CEQ at 24 h after the last (21st) dose and the AUC_{1-24} (based on radioactivity) were increased by a factor of 5 to 9, compared to a single dose. After 21 days of dosing, a slower elimination phase, characterized by half-lives of 2.66 days for plasma, 6.13 days for lung and up to 28 days for brain (generally 6-11 days in most other organs), was found.

After a single dose, there was no indication for changes in organ distribution pattern at later time intervals (up to 72 hours). By 72 hours, 1.1% of the administered radioactivity remained in the body of the rat (excluding the intestinal tract). In beagle dogs, tissue levels were measured only after 72 hours and the pattern of distribution was found to be similar to that in rats. Corresponding residue values in the body of dogs after 3 days were around 1% (i.v.) or 2% (oral) of the administered dose.

In pregnant rats following single oral or intravenous dosing, placental transfer was observed, with total radioactivity in the fetus reaching 17% of maternal plasma and 39% of the average maternal tissue concentration within one hour.

In lactating rats, secretion of nisoldipine and its metabolites into milk was noted after an oral dose of 5 mg/kg, with concentrations in milk being lower than in plasma at all time periods up to 48 hours post-dosing.

Whole-body autoradiography indicated rapid tissue distribution and penetration of the blood-brain barrier within 5 minutes after an intravenous dose. In addition, autoradiography essentially confirmed the widespread tissue distribution that was observed with the quantitative tissue measurements, including placental transfer and secretion into milk.

Placental transfer and milk secretion studies in rats are summarized in the 11/7/89 review of [redacted] by X. Joseph, D.V.M., Ph.D.

QUANTITATIVE[1] TISSUE DISTRIBUTION OF TOTAL RADIOACTIVITY IN THE RAT AFTER ORAL (MALE AND FEMALE) AND INTRAVENOUS (MALE ONLY) ADMINISTRATION OF [^{14}C]NISOLDIPINE.

Time post-dose	4 h			24 h		
Route	i.v. (male)	p.o. (male)	p.o. (female)	i.v. (male)	p.o. (male)	p.o. (female)
Dose (mg*kg-1)	1	5	5	1	5	5
Organ/Tissue:						
body excl. g.i.t.	0.11	0.11	0.29	0.026	0.021	0.032
plasma	0.35	0.16	0.36	0.13	0.044	0.056
erythrocytes	0.09	0.05	0.14	0.038	0.015	0.018
liver	0.54	0.56	1.8	0.13	0.11	0.36
kidneys	0.26	0.19	0.44	0.057	0.034	0.031
lungs	0.17	0.11	0.26	0.068	0.027	0.034
heart	0.09	0.07	0.20	0.029	0.016	0.016
brain	0.04	0.03	0.046	0.012	0.011	0.0062
adrenal gland	0.13	0.15	0.32	0.11	0.034	0.043
testes	0.06	0.04	----	0.019	0.014	-----
ovaries	----	----	0.38	----	----	0.029
renal fat	0.33	0.32	0.70	0.031	0.025	0.025
skin	0.06	0.06	0.16	0.022	0.018	0.021
skel. muscle	0.05	0.03	0.11	0.011	0.011	0.0081
resid. carcass	0.10	0.09	0.22	0.018	0.015	0.015

[1]Mean dose-normalized equivalent concentrations ($kg*l^{-1}$). n = 5 per group.

QUANTITATIVE[1] TISSUE DISTRIBUTION IN THE RAT AND DOG AT 72 H FOLLOWING ADMINISTRATION OF [^{14}C]NISOLDIPINE.			
Species	Dog (F)		Rat (M)
Time after application	72 h		72 h
Route of administration	i.v.	p.o.	p.o.
Dose [mg*kg^{-1}]	0.5	5	5
body excl. g.i.t.	0.0095	0.017	0.012
plasma	0.011	0.017	0.020
erythrocytes	0.0083	0.011	0.0095
liver	0.078	0.090	0.057
kidney	0.020	0.034	0.017
lungs	0.016	0.024	0.009
heart	0.0057	0.014	0.009
brain	0.0016	0.0034	0.008
adrenal gland	0.032	0.072	0.025
renal fat	0.016	0.026	0.015
skin	0.0071	0.012	0.012
skel. muscle	0.0034	0.010	0.007
uterus	0.0080	0.018	-----
ovary	0.0090	0.016	-----

[1]Mean dose-normalized equivalent concentrations (kg*l^{-1}). n = 3 (dog) and 5 (rat) per group.

organ/	CEQ_{max} [$\mu g \cdot g^{-1}$]	t_{max} [h]	$AUC_{0.5-72\ h}$ [$mg \cdot h \cdot kg^{-1}$]	$t_{1/2}$ [h]
body excl. g.i.t	1.75	1.0	12.3	59.5
plasma	3.35	1.0	21.4	42.2
erythrocytes	3.550	1.0	7.58	72.8
liver		0.5	66.7	50.6
kidney	1	0.5	30.4	48.0
lungs	2.6	0.5	13.0	45.5
heart	1.45	0.5	8.76	53.6
brain	0.265	0.5	4.8	123
adren. gland	1.55	5	16.5	74.9
testes	0.470		6.09	73.4
renal fat	4.40	2.	17.8	65.1
skin	1.15	1.0	8.54	82.1
skel. muscle	0.600	0.5	4.76	65.1
resid. carcass	1.60	2.0	7.44	58.6

Pharmacokinetic parameters in different organs and values obtained for total radioactivity after single oral administration of mg [^{14}C]nisoldipine per kg body weight to male Sprague Dawley rats (N = 5).

organ/tissue	parameters								
	CEQ (24 h) [$\mu g \cdot g^{-1}$]			AUC (1-3 d) [$mg \cdot h \cdot kg^{-1}$]			$t_{1/2}$ (1-3 d) [h]		
	21 doses	1 dose	F	21 doses	1 dose	F	21 doses	1 dose	F
body excl. g.i.t.	0.837±[illegible]	0.105±0.010	8.0	33.0	3.78	8.7	78.9	59.5	1.3
plasma	0.547±0.0[illegible]	.220±0.035	2.5	17.1	7.08	2.4	35.8	42.2	0.85
erythrocytes	0.484±0.038	[illegible]5±0.010	6.5	19.8	2.9[illegible]	6.8	96.6	72.8	1.3
liver	2.59 ±0.39	[illegible].100	4.7	93.2	18.5	5.0	59.4	50.6	1.2
kidney	0.938±0.140	0.1[illegible]20	5.5	36.8	5.82	6.3	80.1	48.0	1.7
lung	0.650±0.052	0.135±[illegible]	4.8	25.2	4.50	5.6	62.3	45.5	1.4
heart	0.435±0.049	0.080±0.[illegible]	[illegible].4	18.2	2.80	6.5	72.0	53.6	1.3
brain	0.285±0.032	0.055±0.005		12.8	2.30	5.6	109	123	0.89
adrenal gland	2.58 ±0.29	0.170±0.020	1[illegible]	111	7.43	15	110	74.9	1.5
testes	0.350±0.028	0.070±0.005	5.[illegible]	15.1	2.57	5.9	90.2	73.4	1.2
renal fat	2.41 ±0.49	0.125±0.025	19.3	[illegible]	4.44	24	168	65.1	2.6
skin	0.826±0.076	0.090±0.010	9.2	[illegible]	3.48	9.4	79.0	82.1	0.96
muscle	0.492±0.056	0.055±0.005	8.9	[illegible]	1.91	11	61.7	65.1	0.95

Comparison of pharmacokinetic parameters for the total radio[illegible]ty in different organs and tissues between 1 and 3 days after single oral administration and termi[illegible] of repeated (21x) oral administration of 5 mg [^{14}C]nisoldipine per kg body weight to male [illegible]e Dawley rats. Values represent means (± s.d.) of n = 5. Factors F give the ratios of the [illegible]eters after 21 doses and 1 dose.

organ/ti	parameters		
	CEQ_1 [/ug·g^{-1}]	$t_{1/2}$ [d]	AUC (1 - 10 d) [mg·h·kg^{-1}]
body excl. g.i.t.	0.753	9.74	109
plasma	0.548	2.66	35.8
erythrocytes	424	22.3	75.6
liver		4.49	262
kidney	0.	6.30	113
lung	0.61	6.13	73.2
heart	0.406	9.77	59.5
brain	0.266	28.0	49.7
adrenal gland	2.70	7.96	370
testes	0.335	7	50.4
renal fat	2.41		401
skin	0.709	1[illegible]	118
muscle	0.414	17.8	72.7

Pharmacokinetic parameters for the total radioactiv[illegible] different organs and tissues between 1 and 10 days after termination o[illegible]ated (21x) oral administration of 5 mg [^{14}C]nisoldipine per kg body weigh[illegible]ale Sprague Dawley rats.

4. Protein Binding: As determined by equilibrium dialysis, ^{14}C-nisoldipine was highly bound to plasma proteins of the rat (97.8-99.1%), dog (97.6-97.1%) and man (>99.4%) and was not influenced by sex in any of the three species. In humans, ^{14}C-labelled drug was bound predominently to the serum albumin, and the extent of binding was not influenced by plasma concentration over a broad range; i.e., between 0.1 and 10 ug/ml. Similar levels of protein binding were found in human plasma whether it was measured by equilibrium dialysis or ultrcentrifugation, and degree of binding of the (+) and (-) ^{14}C-enantiomers was similar (around 99.4%), with no indication of preferential stereospecific binding. In the "expert opinion" on pre-clinical pharmacokinetic studies, it is claimed that when protein binding was measured *ex vivo* (dialysis method) in rats and dogs after i.v. or oral administration, nisoldipine was highly bound initially, but the protein bound fraction dropped to 50 to 80% between 30 and 180 minutes after dosing, indicating lower binding affinities for the metabolites.

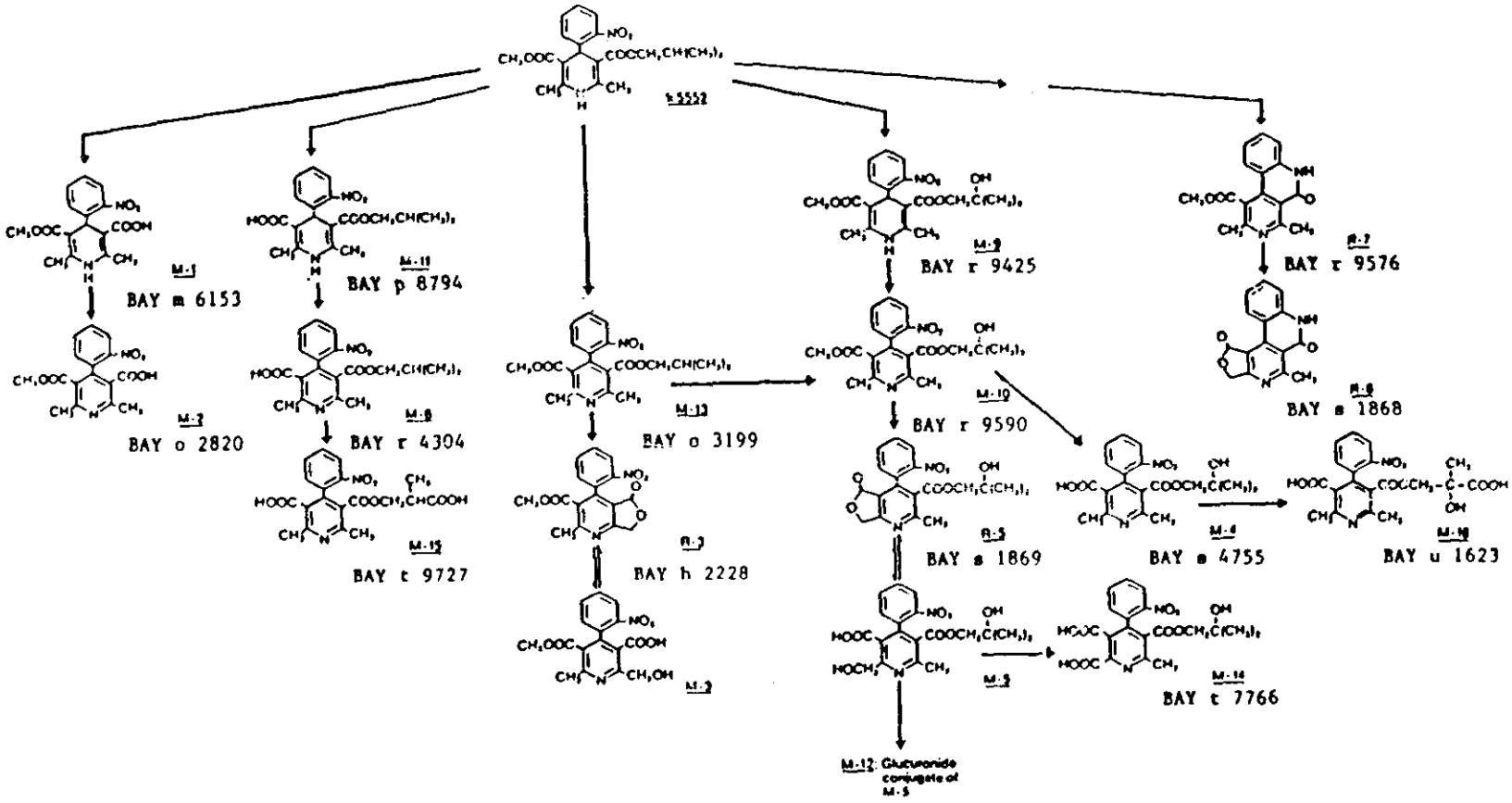
5. Metabolism

a. Biotransformation: Nisoldipine is rapidly and extensively metabolized in the rat, dog, monkey and man. Only a small percentage of unchanged ^{14}C-labelled substance could be found in the circulation of the rat or dog at 30 and 60 minutes after oral administration, when plasma radioactivity was at the maximum level. No unchanged drug was eliminated in the urine or feces of all 4 species, or in bile of bile duct cannulated rats that received the drug either by i.d. or i.v. route. Partial enterohepatic recirculation of metabolites was demonstrated in rats. A schematic for the biotransformation of the drug is shown on the page which follows. The investigators describe the biotransformation steps as follows:

-hydroxylation of the isobutyl ester
-dehydrogenation to the pyridine derivative
-cleavage of the ester to form the carboxylic acid
-reduction of the nitro group to the amino group
-glucuronidation (phase II enzymatic reaction)

In urine of all 4 species, at least 12 biotransformation products were detected, with 6 of them, M-1 to M-5 and M-12, accounting for 80% of radioactivity in urine; all other metabolites were minor products. M-5 was the major urinary metabolite, accounting for 24 to 46% of the renal eliminated radioactivity in all 4 species. Only 1 metabolite, M-12, was hydrolyzable with B-glucuronide, to give M-5 as the aglycone. Metabolic profile in urine was essentially similar in all 4 species.

In bile, metabolic profiles of rats "were quantitatively identical *in vitro* (isolated perfused rat liver model) and *in vivo* following intraduodenal administration". At least 24 metabolites have been detected, but only 8 of them, M-3 to M-5, M-10, M-12, M-14 to M-16, were quantitatively important. The



PROPOSED METABOLIC PATHWAYS OF NISOLDIPINE

major metabolic products in bile of rats were M-5 and its conjugate, M-12.

In serum, at least 12 metabolites were observed in rats within 30 minutes of dosing, and the main ones were M-2 and M-5, together with their gamma-lactones, R-3 and R-5. In dogs, at least 11 biotransformation products were isolated from the serum, and a similar pattern was seen as in rats, with M-2 and M-5 being the main products.

A total of 18 biotransformation products have been identified in urine and serum. The main biotransformation products in all 3 animal species and humans, based on findings in the urine and serum (also in the bile of rats), are M-5 plus M-12 which is the glucuronide of M-5, and R-5 which is the gamma-lactone of M-5.

main metabolites M-5, M-12 and R-5 in urine and serum

	rat serum	rat urine	dog serum	dog urine	monkey urine		
					0-7 h	7-11 h	11-24 h
	[%]	[%]	[%]	[%]	[%]		
M-5	13.3	34.9	46.5	44.9	27.2	23.7	26.7
M-12		3.1		11.7	30.8	31.1	15.5
R-5	11.7						
total:	25	38	46.5	56.6	58	54.8	42.2

In studies with dogs, the liver and gut wall were identified as the primary sites of biotransformation (Arzneim. Forsch./Drug Res. 38: 1093, 1988) after oral administration. It was estimated that around 60% is metabolized pre-hepatically in the gut and 30% in the liver.

b. Effects on Hepatic Enzymes: In two experiments with male rats, nisoldipine was administered orally at doses of 0, 10, 50 and 200 mg/kg for 2 weeks, followed by a 1 week recovery period in the second experiment. The positive control was phenobarbital at 25 mg/kg. The hepatic levels of cytochrome P-450, aminopyrine N-demethylase and aniline hydroxylase activities were decreased at mid and high dose, whereas phenobarbital caused significant increases in levels of all 3 enzymes. The decreases in all 3 enzyme levels were found to be reversible after a 1-week recovery period.

SUMMARY OF TOXICOLOGICAL STUDIES

A. **Acute Toxicity Studies** (X. Joseph)

Acute oral and iv toxicity studies were done in mice, rats, rabbits and dogs at

For oral toxicity studies, the drug (suspended in a solution of glycerol, lutrol and demineralized water) was given via stomach tube (20 ml/kg) to mice, rats and rabbits; and in dogs the drug was given in gelatin capsules. For iv studies, the drug suspended in the above solution was given at a volume of 5 ml/kg for rodents and at 1 to 4 ml/kg for dogs. After drug treatment animals were observed for a period of 14 days. No clinical signs or mortality were seen after oral administration. However, tonic/clonic convulsions, gasping, cyanosis, exophthalmos and respiratory disturbances (all species) were seen after iv administration. All deaths occurred either during or within 10-20 min of drug administracion. The surviving animals were free of symptoms within 1 (mice, rabbit and dog) to 48 hr (rat) postdose. The autopsies (dead or sacrificed at the end of the study) showed no pathological findings. The LD50 values for different species are below.

ACUTE TOXICITY OF NISOLDIPINE IN MICE, RATS, RABBITS, AND DOGS

Species	Sex	Route of Administration	LD_{50} - mg/kg (95% Conf. Limits)
Mouse (CFW1/W)	M	p.o.	> 10,000
Mouse (CFW1/W)	M	i.v.	2.20 (2.0-2.5)
Rat (Wistar)	M	p.o.	> 10,000
Rat (Wistar)	F	p.o.	> 10,000
Rat (Wistar)	M	i.v.	2.32 (2.06-2.65)
Rat (Wistar)	F	i.v.	1.86 (1.77-1.97)
Rabbit (Lge Chinchilla)	M,F	p.o.	> 5000
Rabbit (Lge Chinchilla)	M,F	i.v.	ca. 2.5
Dog (Beagle)	M,F	p.o.	> 5000
Dog (Beagle)	M,F	i.v.	ca. 2.0

A separate study showed that pretreatment with propranolol (1 mg/kg ip for 4 or 5 days) had no effect on the acute iv toxicity of nisoldipine in male Wistar rats (iv LD50 = 1.6 mg/kg with or without propranolol pretreatment).

B. **Subchronic, Chronic and Carcinogenicity Studies**

RAT STUDIES (X.Joseph)

a. Four Week Dietary Dose Rangefinding Study

Testing Facility:

Study Number: B/K 5552/024

Study Dates: August - September, 1980

GLP Compliance: Study was not conducted according to GLP regulations. The deviations were as follows: 1. no phase 1-3 GLP audits. 2. no checking of physico-chemical properties of test substance.

Animals: Wistar strain TNO-74 rats, individually housed in Macrolon cages, Type II, were 5-6 weeks old (average weights: males - 131 g; females - 112 g) at the initiation of dosing.

Dose Levels: BAY k 5552 (Batch No. 576,923) was mixed with powdered rat diet to obtain drug concentrations of 0, 300, 1000, and 3000 ppm.

Achieved Dose Levels:

Dose (ppm)	Average Drug Intake (mg/kg/day)	
	Male	Female
0	0	0
300	26	26
1000	86	89
3000	258	265

(Note: The drug intake was calculated from the average daily food intake for the whole duration of the study. No significant difference in drug intake was seen with time.)

Number of Animals: 10/sex/group

Parameters Evaluated: Appearance and behavior (at least once daily), body weight and food consumption (weekly), organ weights (heart, liver, kidneys and adrenal glands) and gross pathology. (No histopathological examinations were conducted.)

Results: No treatment-related clinical signs or mortalities were observed in this study. A significant reduction in body weight, compared to concurrent controls, was observed throughout the treatment period in high dose males (10-18%) and females (6-10%) except in females at week 4. No significant body weight differences were seen between control and mid or low dose groups (both sexes). Food consumption was reduced in the high dose group. Though not measured quantitatively, water consumption appeared to be increased in all treated groups. Organ weight findings (both absolute and relative) are given below.

Mean Organ Weights of Male and Female Rats

		Sex	Dose Group (ppm in diet)			
			0	300	1000	3000
Body Weight (g)		M	225	219	224	196*
		F	142	140	144	131
Adrenals	(Absolute, mg)	M	36	38	38	39
	(Relative, mg/100g)	M	16	18	17	21*
	(Absolute, mg)	F	50	49	53	49
	(Relative, mg/100g)	F	36	35	37	38
Heart	(Absolute, mg)	M	663	669	675	677
	(Relative, mg/100g)	M	295	304	301	349**
	(Absolute, mg)	F	490	517	555**	542
	(Relative, mg/100g)	F	346	371	387*	417**
Kidney	(Absolute, mg)	M	1489	1392	1409	1295**
	(Relative, mg/100g)	M	663	634	630	669
	(Absolute, mg)	F	986	978	1036	975
	(Relative, mg/100g)	F	697	702	719	749
Liver	(Absolute, mg)	M	8219	8074	8599	8699
	(Relative, mg/100g)	M	3650	3655	3825	4436**
	(Absolute, mg)	F	5300	5452	5857	6105*
	(Relative, mg/100g)	F	3761	3895*	4064*	4652**

** Significantly different from control at 0.05 level.*
*** Significantly different from control at 0.01 level.*

Relative mean heart and liver weights of the high dose group (both sexes) were significantly increased with no significant changes in absolute weights except for the mean liver weight of high dose females which was significantly higher (15%) than the control value. Both absolute and relative heart weights were increased in mid dose females.

It is stated that dietary dose levels, 0, 50, 300 and 1800 ppm, for the 2 year carcinogenicity study in rats were selected on the basis of the above study, and also based on the previous experience from long-term studies with other dihydropyridines [amendment (no serial number) dated May 31, 1994].

b. Three Month Oral (Gavage) Toxicity Study in Rats

Testing Facility:

Study Number: Bay k 5552/025

Study Dates: September - December, 1980

GLP Compliance: Not addressed.

Animals: Wistar (TNO/W 74, SPF) rats, individually housed in type 11 Makrolon cages, were 7-8 weeks old (males 115-155 g; females 120-145 g) at the initiation of dosing.

Mode of Administration: Bay k 5552 (Batch No. 576923) dissolved in a solvent mixture, containing Bay a 1040 placebo solution (polyethylene glycol, glycerol and distilled water) and distilled water, was given by oral intubation. It is stated that the test formulation was stable at room temperature for over a week.

Dose Levels: 0 (vehicle control), 10, 30 and 100 mg/kg/day (5 ml/kg)

Number of Animals: 15/sex/group

Parameters Evaluated: Appearance and behavior (daily), body weight and food and water consumption (weekly), hematology, blood chemistry and urinalysis (5 rats/sex/group; weeks 4/5 and 13), major organ weights and gross and microscopic pathology (more than 20 different tissues/rat; control and high dose groups).

Major Findings: Two low dose females (on days 8 and 68) died during the study. Gross pathology findings in the above animals included enlarged kidneys, bladder and adrenals in one rat and discolored lung and pulmonary emphysema in the other. Labored breathing was noticed in high dose rats during the first 5 weeks of treatment. No significant differences in body weights were seen between treated and control groups except for the lower body weights observed in the high dose group, compared to concurrent controls, during the first one or two weeks of treatment. Food consumption was unaffected. Water intake of high dose females was about 20% higher than that of concurrent control. Although hemoglobin and hematocrit values in mid and high dose males were lower than concurrent control values at 13 weeks, it is stated that all values were within the historical control range for Wistar rats. High dose females had significantly higher plasma urea levels, compared to control, after either 4 or 13 weeks of treatment. In mid and high dose males, both absolute and relative thymus weights were significantly higher than control. Heart and liver weights (both absolute and relative) in high dose females were significantly higher than control; absolute and/or relative

weights of these organs in mid dose females and mid- and high-dose males were also higher than control. There were no significant histopathological findings in this study.

Mean Organ Weights of Male and Female Rats

		Sex	Dose Group (mg/kg)			
			0	10	30	100
Body Weight (g)		M	338	347	330	326
		F	206	207	200	204
Adrenals	(Absolute, mg)	M	36	39*	37	37
	(Relative, mg/100g)	M	11	11	11	11
	(Absolute, mg)	F	52	55	53	56
	(Relative, mg/100g)	F	25	27	26	27*
Brain	(Absolute, mg)	M	1788	1860*	1849	1843
	(Relative, mg/100g)	M	530	538	569	568**
	(Absolute, mg)	F	1652	1676	1649	1685
	(Relative, mg/100g)	F	802	815	829	829
Heart	(Absolute, mg)	M	955	1022*	1000*	1004
	(Relative, mg/100g)	M	283	295	304**	308**
	(Absolute, mg)	F	688	704	706	758**
	(Relative, mg/100g)	F	334	342	354**	372**
Liver	(Absolute, mg)	M	10966	11179	11777	11523
	(Relative, mg/100g)	M	3243	3220	3602*	3537*
	(Absolute, mg)	F	6394	6822	6907	7588**
	(Relative, mg/100g)	F	3100	3304	3460*	3716**
Lung	(Absolute, mg)	M	1172	1202	1000*	1004
	(Relative, mg/100g)	M	347	347	366	366*
	(Absolute, mg)	F	900	937	913	911
	(Relative, mg/100g)	F	436	455	457*	447
Thymus	(Absolute, mg)	M	196	222	235*	233*
	(Relative, mg/100g)	M	58	64	72*	72*
	(Absolute, mg)	F	206	213	180	185
	(Relative, mg/100g)	F	100	104	90	91

* *Significantly different from control at 0.05 level.*
** *Significantly different from control at 0.01 level.*

c. Two Year Carcinogenicity Study in Rats

Testing Facility:

Study Number: T 1000876

Study Dates: November 1980 - November 1982

GLP Compliance: Study was conducted in accordance with GLP regulations

Animals: Wistar strain TNO/W 74 rats, individually housed in type II Makrolon cages, were 5-6 weeks old (mean body weights: males - 79 g; females - 75 g) at the initiation of the study.

Dose Levels and Mode of Administration: Bay k 5552 (Batch No.662836, purity - about 99.2%) was mixed, weekly, with powdered rat diet at concentrations of 0, 50, 300 and 1800 ppm. The stability and the concentration of the test substance in diet were determined pretest and then every three months. The concentrations of the drug in diet were found to be in good agreement with theoretical values. However, there is no indication that concentrations of drug in diet were adjusted periodically to maintain a constant mg/kg body weight exposure.

Number of Animals: 50/sex/group (An additional 10 rats/sex included in each group were sacrificed after 12 months of treatment - interim sacrifice.)

Observations/Measurements: Rats were observed at least once daily for general appearance, behavior and clinical signs. Body weights were recorded weekly until week 27 and biweekly thereafter. Food and water consumption were determined weekly and once every 3 months, respectively. Hematological [erythrocyte, leucocyte (total and differential), platelet and reticulocyte counts, hemoglobin, hematocrit, MCV, MCH and thromboplastin time (only at the termination of the study)] and blood chemistry (alkaline phosphatase, transaminases, creatine kinase, urea, creatinine, blood sugar, cholesterol, total bilirubin, total protein, corticosterone, aldosterone and serum electrolytes) evaluations and urinalyses were conducted on 10 rats/sex/group (selected at random) at 6, 12, 18 and 24 months. Complete autopsies were performed on animals that were sacrificed at 12 months and at study termination. Animals were examined grossly and heart, lung, liver, spleen, kidneys, adrenals and testes were weighed. All protocol specified tissues (more than 30 different tissues/rat) and gross lesions were fixed in buffered formalin. In addition, left liver lobe from all rats was fixed in formol-calcium, and lower jaw from 5 rats/sex/group was fixed in buffered formalin. Autopsies were also performed on rats that died or were sacrificed in extremis and all evaluable tissues were preserved. All protocol specified tissues from control and

high dose groups, all tissues from animals that died or were sacrificed moribund, as well as adrenals, genital organs, areas of skin change and kidneys (females) of low and mid dose animals and all grossly abnormal tissues were examined histologically.

Differences between treated and control groups were analyzed using the significance test (U-test) of Mann and Whitney and of Wilcoxon. Mortality and tumor data were analyzed by Fischer's exact test.

Achieved Dose Levels:

Dose (ppm)	Average Drug Intake (mg/kg/day)	
	Male	Female
0	0	0
50	2.15	2.78
300	13.13	18.04
1800	82.40	110.68

(Note: The drug intake was calculated, at the termination of the study, from the average daily food intake/animal/group for the whole duration of the study. Periodical drug intake determinations were not made in this study.

Results: No treatment-related clinical signs were seen in this study. The mortality data (cumulative) at different intervals are given below and it is presented graphically in Figures 6 and 7.

Mortality of Rats Receiving Nisoldipine in Diet for 24 Months

Daily Dose (ppm in diet)	Number of Rats (M/F)	Number of Dead (M/F)	% Mortality (M/F)
	12 Months		
0	50/50	1/1	2/2
50	50/50	0/1	0/2
300	50/50	1/0	2/0
1800	50/50	0/2	0/4
	18 Months		
0	50/50	2/3	4/6
50	50/50	0/2	0/4
300	50/50	2/5	4/10
1800	50/50	5/4	10/8
	24 Months		
0	50/50	4/8	8/16
50	50/50	8/8	16/16
300	50/50	11/15	22/30
1800	50/50	11/13	22/26

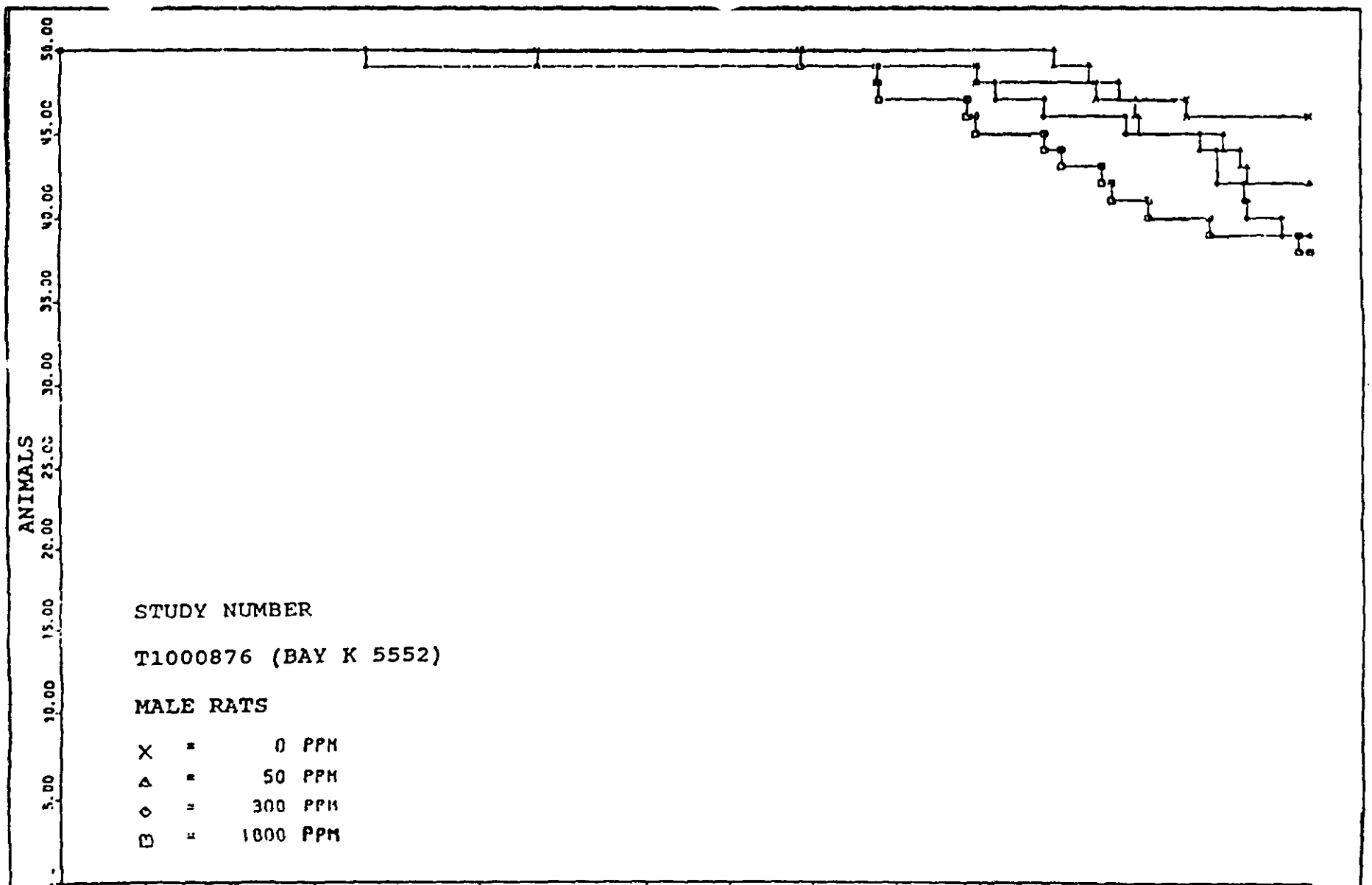


Fig. 6: Mortality curves of male rats which received Bay k 5552 for 24 months in their feed

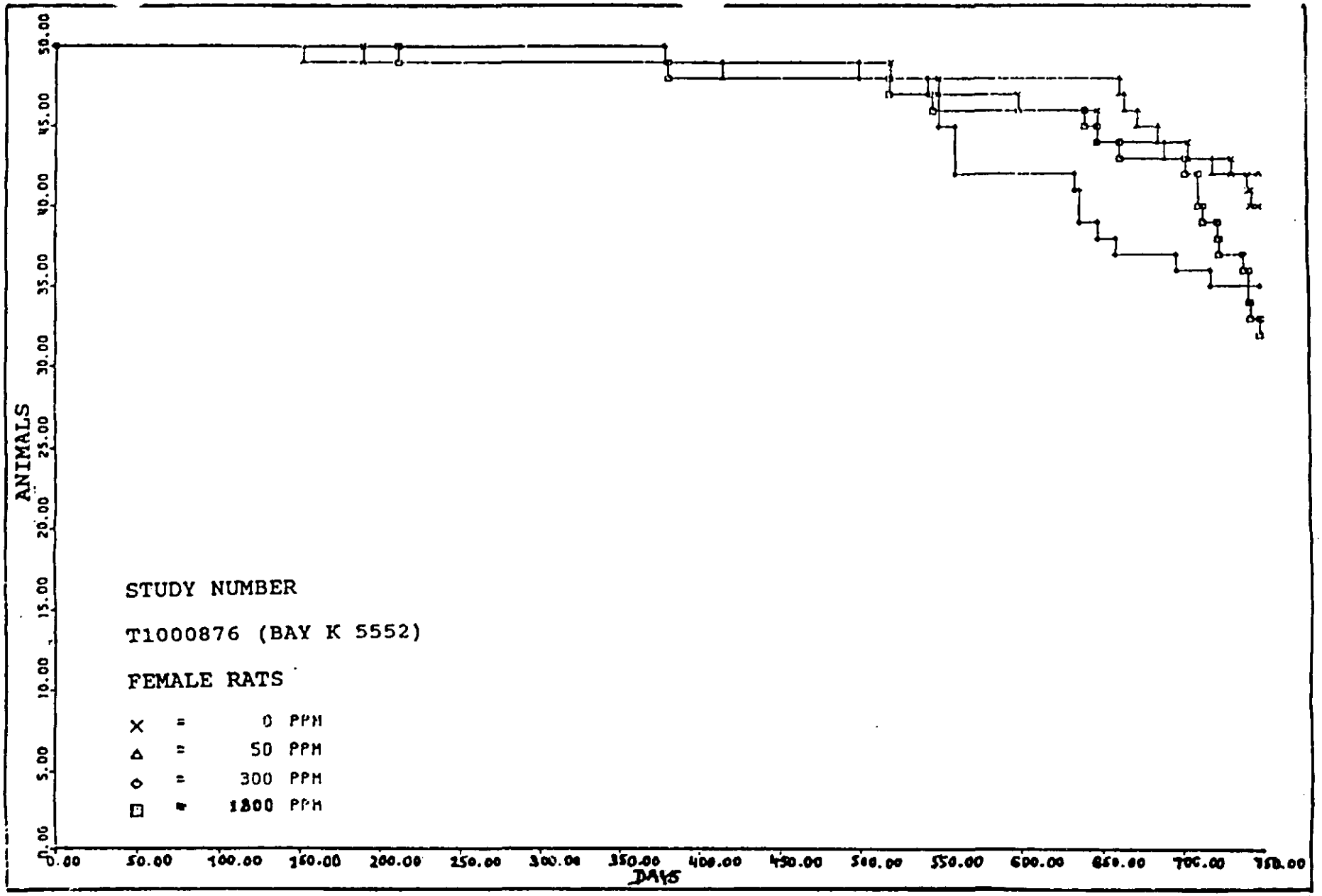


Fig. 7 : Mortality curves of female rats which received Bay k 5552 for 24 months in their feed

Although more animals (both sexes) died during the study in mid and high dose groups than in the concurrent control group, the differences were statistically not significant (sponsor's analysis - Fischer's exact test).

When tested for heterogeneity in survival distribution, FDA statisticians observed that there was no significant difference (at 0.05 level) in the survival distribution for either sex (both Cox test and generalized Wilcoxon test). Additionally, no significant linear trend (at 0.05 level) was seen in the intercurrent mortality rate for either males or females.

Mean body weight values (presented graphically in Fig.8) for the high dose group (both sexes) were significantly lower than control values for the whole duration of the treatment period, except on four occasions (weeks 25, 29, 51 and 63) in males. At the termination of the study, body weights of high dose males and females were 5.6% and 22%, respectively, lower than concurrent control. The body weight gain values of high dose males and females were 7% and 31%, respectively, lower than concurrent control. No significant treatment-related reductions in body weight were seen in low and mid dose groups except on a few occasions (weeks 13, 14 and 39 for males and weeks 77, 79, 85, 87 and 89 for females) at mid dose level. While food consumption was unaffected, water consumption in high dose animals, especially in females, was increased.

Although statistically significant hematological findings were occasionally observed in drug treated groups, no dose dependence or consistency at different intervals was observed. Statistically significant clinical chemistry findings and the time points of their occurrence are given on pages 48 & 49. In the high dose group, significant reductions in alkaline phosphatase levels (both sexes) and increases in GOT (males), GPT and CPK levels (females) were seen. Although a dose-dependent increase in bilirubin levels was seen in both sexes during the early part of the study, these levels in treated females were lower than that of control (no significant difference in males) at the termination of the study. Blood urea levels in mid and high dose females were significantly higher than control at the end of the study; however, these levels were lower than control during week 28. Decreased calcium levels were seen in treated animals, especially at the high dose level (both sexes). Plasma aldosterone levels in high dose males and plasma corticosterone levels in high dose females were significantly lower than respective control values at week 55.

A significant increase in urinary protein excretion was seen in high dose females. While urinary calcium excretion was decreased in treated female groups, especially in mid and high dose groups, urinary potassium levels were increased in high dose males. Urinary aldosterone excretion was significantly higher in high dose males than in control males.

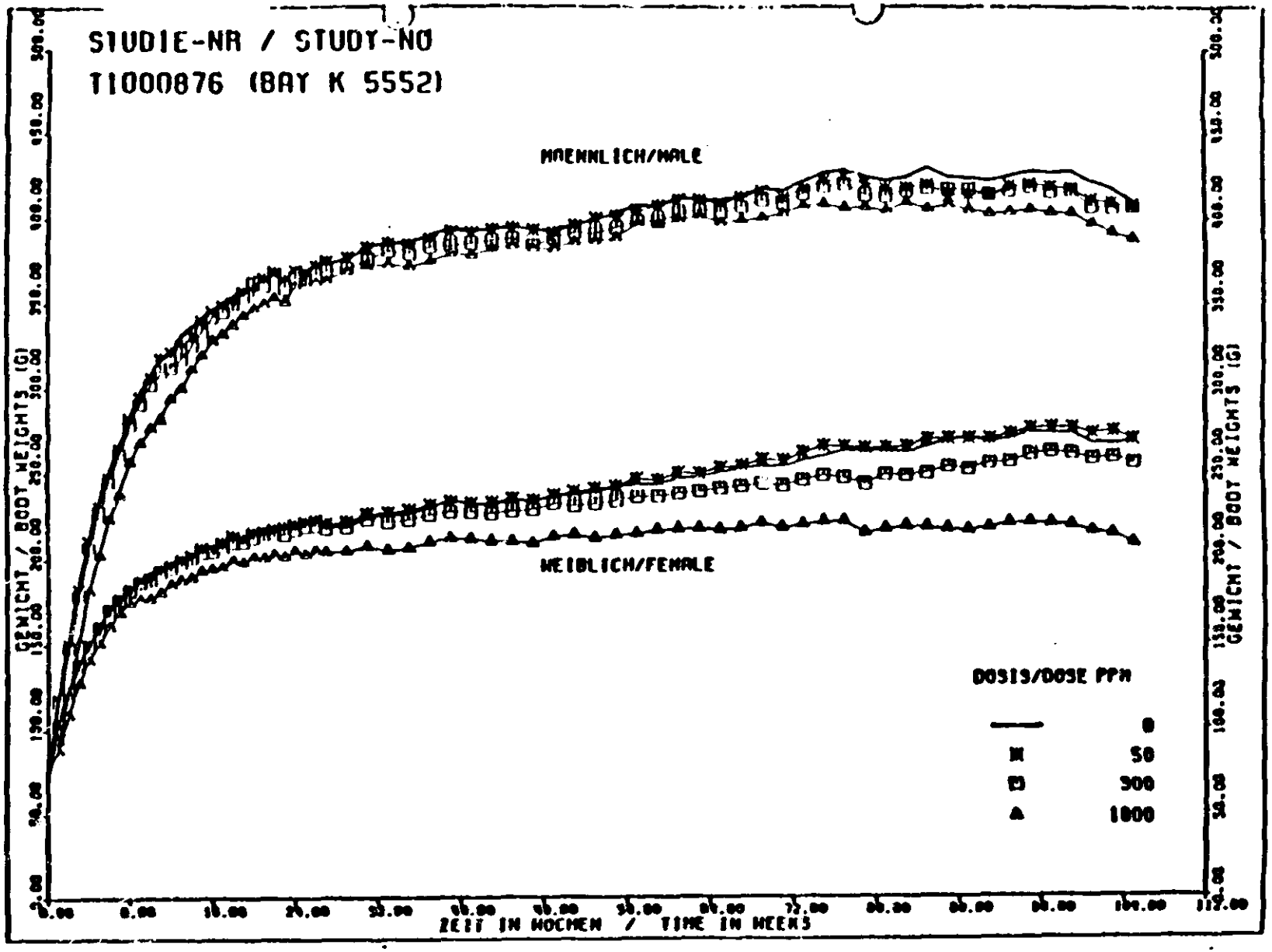


Fig.8 : Body weight curves for male and female rats which received BAY k 5552 with the feed for 24 months.

NDA 20-356

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg B	Reg B or C	Reg BC or D	Reg BCD or E	Reg BCDorE	
Placebo n	38	35	37	34	31	38
Baseline LSMean	101.45	101.31	101.20	100.52	100.79	101.45
LS Mean Change	-3.04*	-4.89*	-3.39*	-4.42*	-3.36*	-1.16
SE of Chang	0.99	1.10	1.26	1.21	1.20	1.29
p Values						
Drug	0.0323	0.2234	0.0001	0.0001	0.0001	0.0001
Drug-Center	0,1582	0.2604	1.381	0.0539	0.0472	0.0332

P Significantly different from placebo
* Significant Change from baseline

Values for supine systolic blood pressure are given in the following table:

Supine Systolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg B	Reg B or C	Reg BC or D	Reg BDC or E	Reg BCD or E	
Nisoldipine n	79	76	76	70	68	79
Baseline LS Mean	153.11P	153.39	153.41	153,35	153.42	153,11p
LS Mean Change	-8.67*	-10.123*p	-15.04*p	-14.10*p	-15.62*p	-14.73*p
SE of Change	1.33	1.61	1.61	1.61	1.64	1.89
Placebo n	38	35	37	34	31	38
Baseline LS Mean	159.59	158.99	158.23	157.78	159.71	159.59
LS Mean Change	-4.96*	-2.39	-1.90	-4.91*	-5.51*	-0.00
SE of Change	1.92	2.38	2.33	2.31	2.43	2.72

P-Values	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug	0.1140	0.0074	0.0001	0.0015	0.0008	0.0001
Drug Center	0.6059	0.0481	0.0416	0.1372	0.1224	0.0726

p Significantly different from placebo
* Significant change from baseline

Standing Diastolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg.B	Reg.B or C	Reg. BC or D	Reg BDC or E	Reg BCD or e	
Nisoldipine n	79	76	76	70	68	79
Baseline LSMean	100.40	100.31	100.31	100.21	100.27	100.40
LS Mean Change	-5.03*p	-6.24*p	-8.62*p	-10.18*p	-8.29#p	-7.86*p
SE of Change	0.75	0.75	0.85	0.83	0.98	1.00
Placebo n	38	35	37	34	31	38
Baseline SL Mean	102.15	101.85	101.85	101.22	100.89	102.15
LS Mean Change	-2.05	-1.99	-1.87	-4.27*	-3.65*	-1.18
SE of Change	1.08	1.11	1.22	1.20	1.45	1.44
P Values						
Drug	0.0253	0.0021	0.0001	0.0001	0.0095	0.0002
Drug-Center	0.2060	0.0052	0.6446	0.5733	0.4304	0.3160

p Significantly different from placebo
* Significant change from baseline

Standing Systolic

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	End-point
Drug Group	Reg B	Reg B or C	Reg BC or D	Visit BCD	or E Visit BCD	orE
Nisoldipine	n 79	76	76	70	68	79
Baseline						
LS Mean	149.22p	149.54	149.56	149.41	149.57	149.22p
LS Mean						
Change	-8.37*	-10.78*p	-14.18*p	-14.84*p	-13.76*p	-13.*p
SE of Change	1.34	1.49	1.78	1.73	1.66	1.77
Placebo n	38	35	37	34	31	38
Baseline						
LS Mean	156.22	155.79	155.30	154.22	155.46	156.22
LS Mean						
Change	1.94	2.20	2.55	2.49	2.48	2.57
P values						
Drug	0.0832	0.0005	0.0001	0.0002	0.0002	0.0001
Drug-Center	0.1162	0.0979	0.1092	0.2475	0.0099	0.0330

P Significantly different from placebo * Significant change from baseline

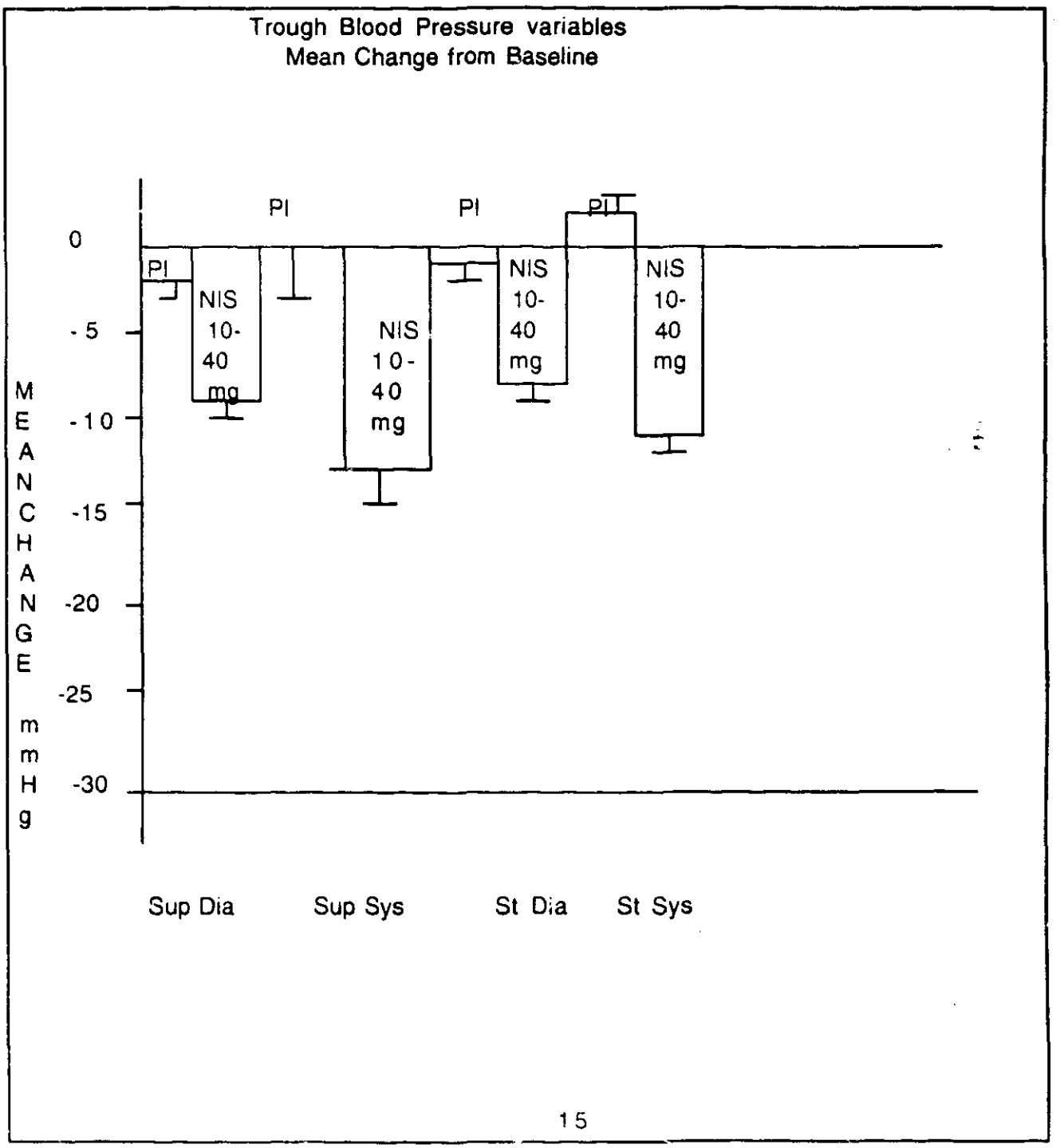
The effect of Nisoldipine on trough supine and standing systolic and diastolic blood pressure at study endpoint in all Nisoldipine treated patients is shown in the following table :

Change from Baseline to Endpoint in Trough Blood Pressures
Mean and SEM in mmHg

	Placebo n=38	Fisoldipine n-79
Supine Diastolic Blood Pressure	-1.16±1.29	-0.51±0.89*
Supine Systolic Blood Pressure	-.000±2.72	-14.73±1.89*
Standing Diastolic Blood Pressure	-1.18±1.44	-7.86±1.00*
Standing Systolic Blood Pressure	+0.89±2.57	-13.00±1.77*

* Significantly different from placebo. $p<0.05$

The change from baseline to endpoint in the primary efficacy blood pressure parameter supine diastolic blood pressure, as well as the 3 secondary blood pressure parameters is shown for placebo and all Nisoldipine doses in the figure below :



Conclusion. This was a titration study in which doses of Nisoldipine 10 mg, 20 mg, 30 mg, 40 mg and placebo were evaluated. In the course of the study most patient were moved to the higher doses in order to decrease the blood pressure and very few patients remained in the lower doses (see flow sheets pages 7, 8, and 9). Therefore a dose-range study could not be carried and only a global evaluation was possible. Such assessment demonstrated that Nisoldipine was very effective in lowering the blood pressure (pages 10-15).

Protocol D89-029

Title of Study : " Double-Blind Randomized Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine 20, 40 and 60 mg (2X30 mg) Core-Coat Tablets vs Placebo in Combination with Atenolol 50 mg in Hypertensive Patients ".

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Objectives : The objectives of this study were to determine the dose response and safety of 20 mg, 40 mg, and 60 mg Nisoldipine tablets as compared to placebo when administered once daily as additive treatment for hypertensive patients not controlled on once daily Atenolol 50 mg.

Inclusion Criteria. Ambulatory patients, male or female, of age 21 or older, with a history of essential hypertension were eligible for enrollment in the placebo run-in period.

Exclusion Criteria. Criteria foe exclusion were : labile hypertension, renal failure (plasma creatinine > 2.0 mg/dl), significant liver disease, insulin-dependent diabetes mellitus, history or presence of bronchial asthma, obstructive pulmonary disease, significant peripheral vascular disease, recent (3 months) myocardial infraction, cerebrovascular accident, or clinical signs suggesting impending myocardial infarction or cerebrovascular disease. Also excluded were patients with heart failure, major arrhythmias, conduction disturbances greater than first degree block, sinus bradycardia, failure of a major organ system, malignancy, psychosis, impaired absorption (such as chronic diarrhea), pregnancy,

women with childbearing potential, abuse of alcohol or drugs, allergy to dihydroperidines or beta blockers and participation in an investigational drug study within the past 30 days.

Qualifications for Randomization. Patients discontinued all previous antihypertensive medication and were given a single-blind placebo once daily in a 2-week qualifying period (Regimen A). Patients with a mean SUDBP 100-119 mmHg at the end of the placebo run-in period were given 1 capsule containing 50 mg Atenolol and 2 placebo tablets under single-blind conditions for 4 weeks (Regimen B). Patients with mean SUDBP 95-114 mmHg after 4 weeks of single-blind Atenolol were randomly assigned to 1 to 4 treatment groups and given double-blind drug.

Drug-Regimen Protocol (Regimen C). Patients who qualified for randomization received Atenolol 50 mg + Nisoldipine (20 mg, 40 mg, or 60 mg) or Atenolol 50 mg + placebo for 6 weeks.

Drugs for the double-blind period (Regimen C) contained encapsulated Atenolol 50 mg with one of the following :

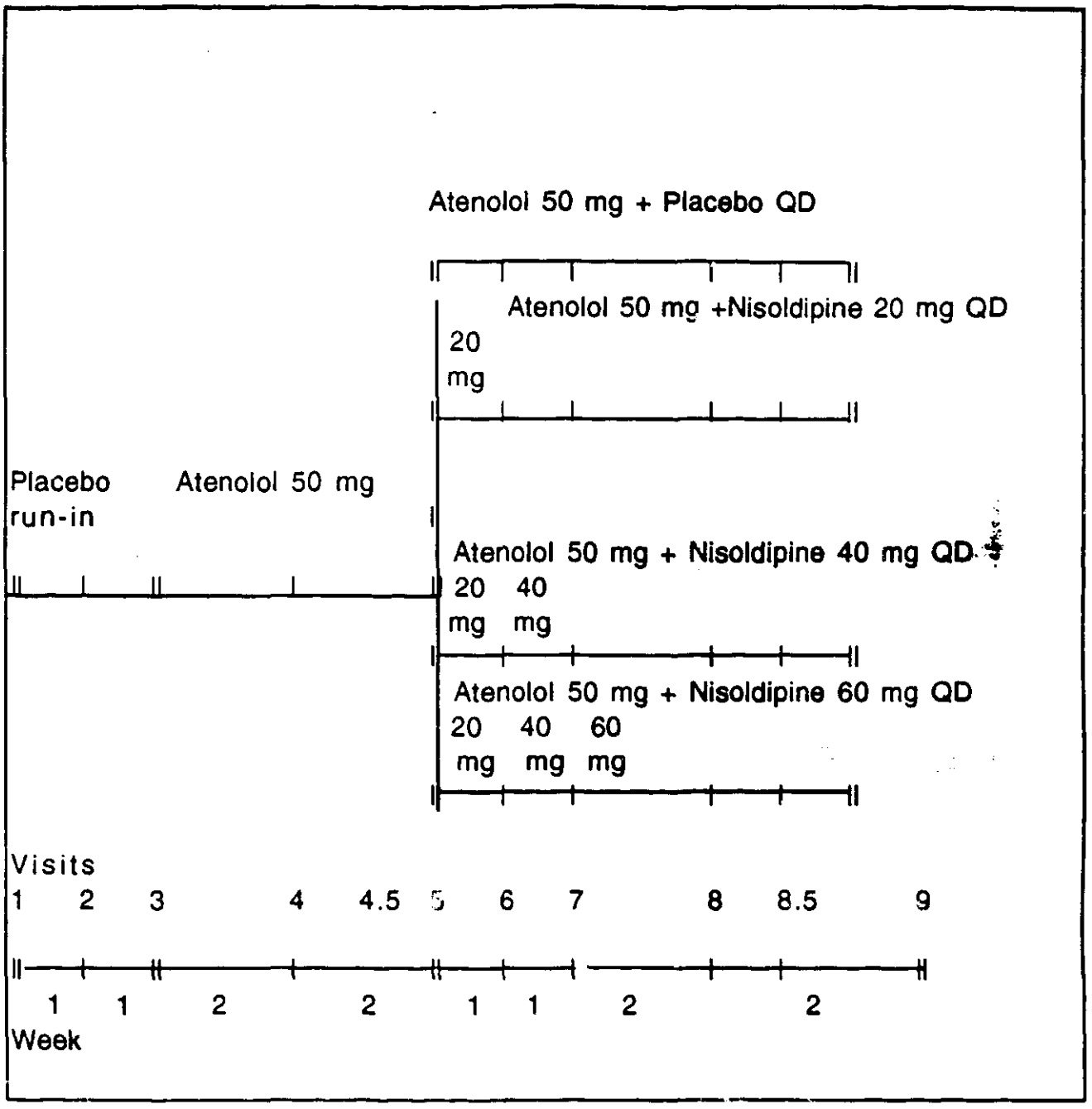
One Nisoldipine 20 mg tablet and
one placebo tablet once daily for 6 weeks

One Nisoldipine 20 mg tablet and
One placebo tablet once daily for 1 week
Forced titrated to
One Nisoldipine 40 mg tablet and
One placebo tablet once daily for 5 weeks

One Nisoldipine 20 mg tablet and
One placebo tablet once daily for 1 week
Forced titrated to
One Nisoldipine 40 mg tablet and
One placebo tablet once daily for 1 week
Forced titrated to
Two Nisoldipine 30 mg tablets once daily for 4 weeks

Two placebo tablets once daily for 6 weeks

The study design is illustrated in the following graph :



Removal of patients from Study or Analysis. Patients could leave the study at any time if they so wished. Patients could be discontinued if they had significant physical or laboratory abnormalities, or if they had significant concurrent illness or deterioration ot their condition. Patients could also be withdrawn if they were blatantly non-compliant. Patients with significant adverse events and those patients with elevations in SUDBP > 114 mmHg were also discontinued from the study.

Statistical Methods. All statistical tests were two-tailed and were conducted at a significant level of 0.05. Pairwise comparisons and within-group changes were tested via the least square means estimated by the model.

Results. . Demographic Characteristics. The demographic characteristics are given in the following table :

	Atenolol+ Nis 20 mg n=61	Atenolol+ Nis 40 mg n=59	Atenolol+ Nis 60 mg n=59	Atenolol+ Placebo n=59
Mean Age (years)	52	54	56	54
Mean wt (lbs)	201	198	198	195
Baseline BP (mmHg)				
Supine	159/101	159/101	162/101	156/110
Standing	154/102	157/103	156/103	152/101
% Male	79	73	70	64
% Caucasian	61	53	58	53
% Diabetic	8	9	14	12
% Mild Hypertensive	60	56	51	59
% Moderate Hypertensives	40	44	49	41

The sponsor states that there were no statistically significant differences between the groups for any of the characteristics examined.

Assessment. Patients were seen in the morning at weekly and biweekly intervals. A history complete physical examination and 12-lead electrocardiogram were taken in the firs visit, at baseline (after 4 weeks of Atenolol) and at the last visit on double-blind drug. Electrocardiograms

were included in visits 7 and 8. Twenty-four hour ambulatory electrocardiograms were taken at some centers. on weeks 4.5 after 3 weeks of single Atenolol and at 8.5 weks of double-blind therapy. Chest X-ray were taken after 2 weeks on placebo.

Laboratory tests performed in the course of the study included blood hematology, serum electrolytes, battery of liver function tests, and urinalysis.

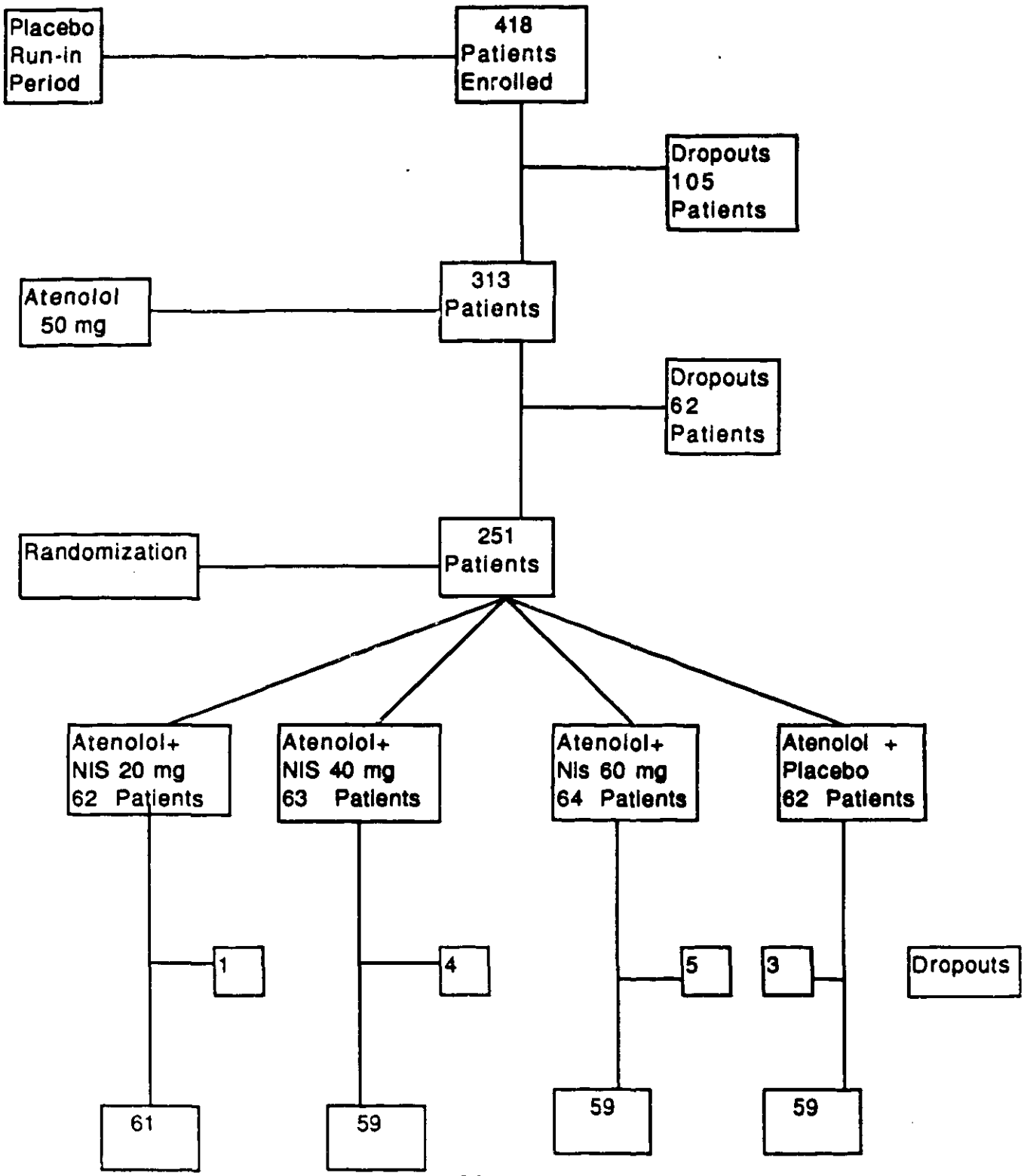
At the end of the single blind Atenolol phase and at the end of the double-blind phase blood was taken at trough for Nisoldipine assay.

At each visit vital signs were taken.

Criteria for Effectiveness. The change from baseline in SUDBP was the primary efficacy variable in this study. The primary time point was the endpoint which was defined as the last double-blind visit for all valid patients. A valid patient was one who had at least 3 weeks of double-blind drug. This criterion was later amended before braking the random code to 19 days. The overall treatment efficacy was determined by the change from baseline in trough SUDBP at endpoint between the average of the three Atenolol-Nisoldipine groups and the Atenolol-Placebo group. Secondary efficacy parameters were supine systolic blood pressure change at trough, standing blood pressure changes at trough, and ambulatory blood pressure trough/peak ratios.

An average decrease in diastolic blood pressure of at least 5 mmHg more than placebo was considered to be clinically meaningful. The actual power for the study was >95 %.

The disposition of the patients is given in the following flow-sheet :


Placebo Run-in Period
418 Patients Enrolled
Dropouts 105 Patients
Atenolol 50 mg
313 Patients
Dropouts 62 Patients
Randomization
251 Patients
Atenolol+ NIS 20 mg 62 Patients
Atenolol+ NIS 40 mg 63 Patients
Atenolol+ Nis 60 mg 64 Patients
Atenolol + Placebo 62 Patients
1
4
5
3
Dropouts
61
59
59
59

The reasons because the patients were withdrawn from the placebo run-in period are given in the following Table :

Mean SUDBP at visit 3 <100 mmHg or > 119 mmHg	=58
Mean SUDBP >119 mmHg during placebo run-in period	= 6
Adverse event during placebo run-in period	=10
Other illness	= 3
Abnormal laboratory value	= 4
Abnormal electrocardiogram	= 2
Noncompliance	= 3
Investigator discretion	= 5
Consent withdrawn	= 9
Lost to follow-up	= 5
Total	105

Patients withdrawn during the single Atenolol period and therefore not randomized :

Mean SUDBP at visit 5 <95 mmHg or >114 mmHg	=37
Mean SUDBP > 114 mmHg on 2 consecutive visits after placebo run-in	= 4
Adverse Event	= 4
Other illness	= 2
Abnormal electrocardiogram	= 1
Noncompliance	= 1
Investigator discretion	= 4
Consent withdrawn	= 4
Lost to follow-up	= 2
Enrolled after enrollment date	= 3
Total	62

The reasons for invalidity for patients that were withdrawn during the treatment period are given in the following table :

Drug Group	Number of Patients	Reasons for Invalidity
Atenolol + NIS 20 mg	1	Less than 19 days on double-blind drug
Atenolol + NIS 40 mg	4	Less than 19 days on double-blind drug
Atenolol + NIS 60 mg	4	Less than 19 days on double-blind drug
	1	Visit 5 diastolic BP not between 95 and 114 mmHg
Atenolol + Placebo	2	Less than 19 days on double-blind drug
	1	Visit 5 diastolic BP not between 95 and 114 mmHg
Total	13	

Effectiveness. The change from baseline in trough blood pressure by treatment for all patients valid for analysisof efficacy are given in the following tables

Supine Diastolic

Drug Group	Visit 6 Week 1	Visit 7 Week 2	Visit 8 Week 4	Visit 9 Week 6	Endpoint
ATN+NIS 20 mg					
N	58	61	61	61	61
Baseline BP	100.56	100.56	100.56	100.57	100.56
Mean Change	-8.53*C	-9.80*C	-9.10*AB	-10.09*	-10.09*
SE	0.85	0.78	C	ABC	ABC
ATN+NIS 40 mg			0.89	0.88	0.88
N	57	58		57	58
Baseline BP	100.75	100.75	58	100.77	100.75
Mean Change	-8.52*C	-11.26*C	100.75	-12.75*C	-12.69*C
SE	0.86	0.81	-12.87*C	0.92	0.91
ATN+NIS 60 mg			0.92		
N	56	59		57	59
Baseline BP	101.12	101.11	58	101.27	101.11
Mean Change	-9.72*C	-12.15*C	101.00	-14.36*C	-14.24*C
SE	0.87	0.80	-12.82*C	0.91	0.90
ATN+PL			0.92		
N	58	59		59	59
Baseline BP	99.93	99.93	59	99.93	99.93
Mean Change	-4.00*	-4.31*	99.93	-4.29*	-4.28*
SE	0.85	0.80	-4.61*	0.90	0.90
			0.91		

Drug Group	Visit 6 Week 1	Visit 7 Week 2	Visit 8 Week 4	Visit 9 Week 6	Endpoint
ATN+NIS 20 mg					
N	61	61	61	61	61
Baseline BP	158.71	158.70	158.70	158.71	158.70
Mean Change	-10.82*C	-12.96*	-13.30*	-13.14*	-13.15*
SE		ABC	BC	ABC	ABC
ATN+NIS 40 mg					
N	58	58	58	57	58
Baseline BP	158.78	158.77	158.77	158.97	158.77
Mean Change	-12.93*C	-19.04*C	-19.03*C	-20.09*C	-19.83*C
SE	2.01	2.11	2.29	2.05	2.05
ATN+NIS 60 mg					
N	58	59	58	57	59
Baseline BP Mean	161.46	161.58	161.70	162.01	161.58
Mean Change	-12.29*C	-20.38*C	-21.00*C	-23.43*C	-23.09*C
SE	2.00	2.08	2.28	2.03	2.02
ATN+Pla					
N	59	59	59	59	59
Baseline BP Mean	156.30	156.29	156.29	156.28	156.29
Mean Change	-3.78	-2.51	-0.02	-0.87	-0.85
SE	1.99	2.09	2.26	2.01	2.02

Standing Diastolic

ATN+NIS 20 mg					
N	61	61	61	61	61
Baseline BP	102.15	102.16	102.16	102.16	102.16
Mean Change	-7.50*C	-8.46*C	-7.90* ABC	-8.93* ABC	-8.93* ABC
SE	0.87	1.00	0.89	0.89	0.89
ATN+NIS 40 mg					
N	58	58	58	57	58
Baseline BP	103.42C	103.43C	103.43C	103.45C	103.43C
Mean Change	-8.55*C	-12.23*C	-12.85*C	-15.00*C	-14.93*C
SE	0.89	1.02	0.92	0.93	0.91
ATN+NIS 60 mg					
N	58	59	58	57	59
Baseline BP	102.97	102.94	102.97	103.18	102.94
Mean Change	-8.55*C	-12.23*C	-12.85*C	-15.00*C	-14.93*C
SE	0.89	1.02	0.91	0.91	0.91
ATN+Pla					
N	59	59	59	59	59
Baseline BP	101.13	101.13	101.13	101.12	101.13
Mean Change	-3.29*	-4.24*	-3.19*	-1.96*	-1.95*
SE	0.89	1.02	0.91	0.91	0.91

Standing Systolic

Drug Group	Visit 6 Week 1	Visit 7 Week2	Visit 8 Week4	Visit 9 Week 6	Endpoint
ATN+NIS 20 mg					
N	61	61	61	61	61
Baseline BP	152.33	152.23	152.23	152.20	154.37
Mean Change	-10.69*C	-11.17* ABC	-11.20* ABC	-10.97* ABC	-10.97* ABC
SE	2.00	2.10	2.17	2.04	2.00
ATN+NIS 40 mg					
N	58	58	58	57	58
Baseline BP	156.89	156,87	156.87	156.85	156.87
Mean Change	-13.25*C	-17.66*C	-21.52*C	-22.35*	-22.38*C
SE	2.10	2.18	2.25	2.13	2.11
ATN+NIS 60 mg					
N	58	58	58	57	59
Baseline BP	156.17	156.30	156.33	156.73	156.30
Mean Change	-11.16*C	-19.76*C	-20.50*C	-22.36*C	-22.10*C
SE	2.08	2.15	2.24	2.12	2.08
ATN+Pla					
N	59	59	59	59	59
Baseline BP	152.23	152.23	152.23	152.20	152.23
Mean Change	-3.17	-1.42	-0.90	1.88	1.89
SE	2.07	2.15	2.22	2.08	2.09

P-values

	Visit 6 Week1	Visit 7 Week2	Visit 8 Week 4	Visit 9 Week 6	Endpoint
Drug* Center	0.0203	0.6905	0.1363	0.1813	0.1478
NiS vs PLA	0.0001	0.0001	0.0001	0.0001	0.0001
20 mg vs PLA	0.0002	0.0001	0.0004	0.0001	0.0001
40 mg vs Pla	0.0003	0.0001	0.0001	0.0001	0.0001
60 mg vs Pla	0.0001	0.0001	0.0001	0.0001	0.0001

A: Significantly different from ATN+NIS 40 mg QD
B. Significantly different from ATN+NIS 60 mg
C. Significantly different fron ATN+Pla
* Significant change from baseline

The effect of Nisoldipine on trough SUDBP during the course of the double-blind treatment is shown in the following table :

Placebo Substracted Change in SUDBP
Mean in mmHg

NIS Dose	Week 1	Week 2	Week 4	Week 6
20 mg	-4.53*	-5.49*	-4.49*	-5.80*
40 mg	-4.52*	-6.95*	-8.26*	-8.46*
60 mg	-5.72*	-7.84	-8.21*	-10.07*

* Denotes values when Nisoldipine blood pressure responses are significantly different from placebo, <0.05

The changes from baseline to endpoint in trough blood pressure, men and SEM in mmHg are given in the following table :

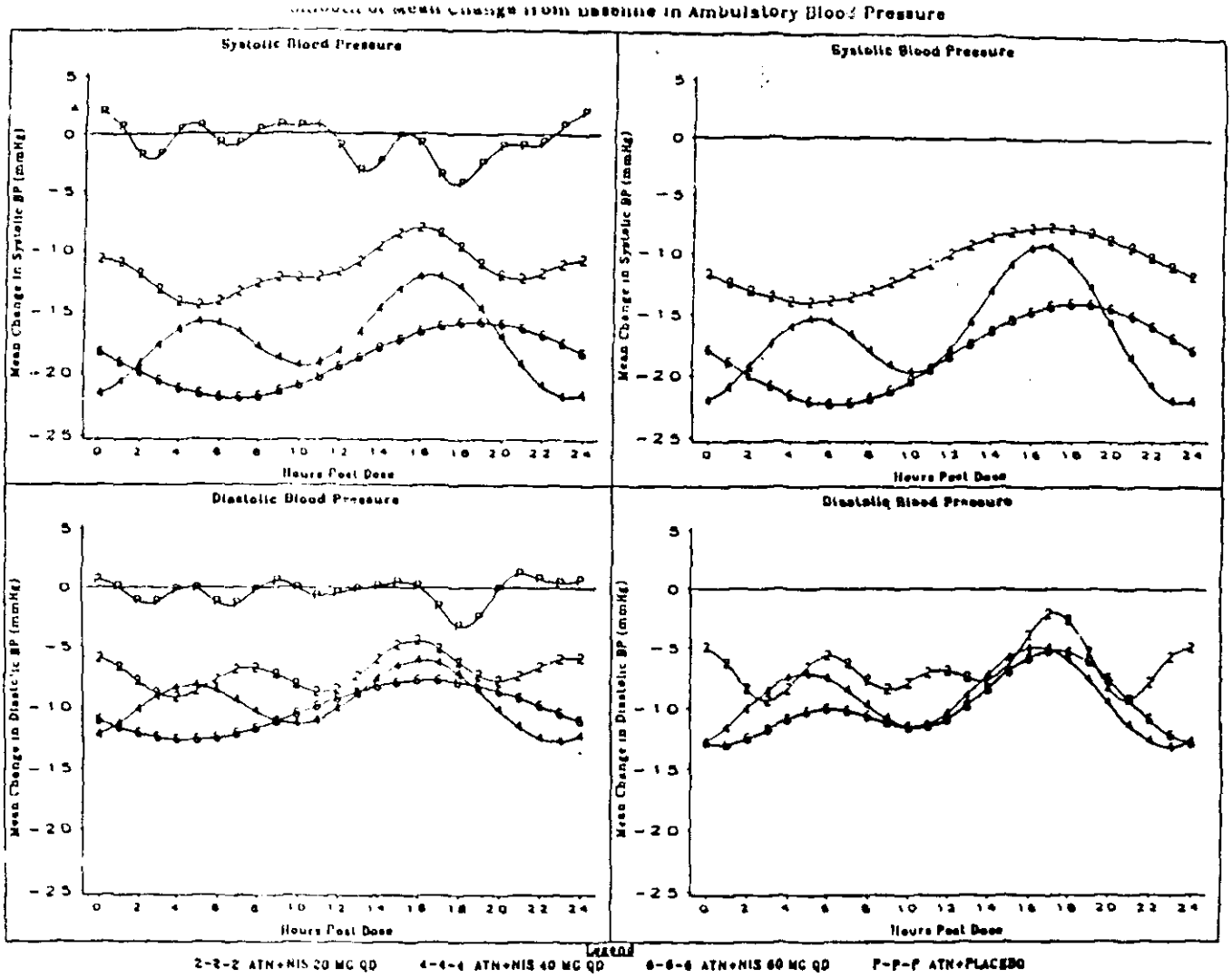
	ATN+PLA n=59	ATN + NIS 20 mg n=61	ATN + NIS 40 mg n=58	ATN + NIS 60 mg n=59
SUDBP	-4.28±0.90	-10.08±0.9[B]	-12.69±0.9*	-14.24±0.9*
SUSBP	-0.85±2	-13.15±2[AB]	-19.83±2*	-23.1±2*
STDBP	-1.95±0.91	-8.93±0.9[AB]	-13±0.92*	15±0.91*
STSBP	+1.89±2.09	-11±2.03*	-22.38±2.1*	-22.10±2.1*

A denotes values NIS 20 mg significantly different from placebo,p<0.05
B denotes values NIS 20 mg significantly different from Nisoldipine 60 mg<0.05.

	ATN+PLA	ATN+NIS 20 mg	ATN+NIS 40 mg	ATN+NIS 60 mg
Trough response mmHg	% Patients	% Patients	% Patients	% patients
SUDBP ≤90	32.2	55.7*	67.8*	66.1*
Fall in SUDBP ≥10	23.7	50.8*	67.8*	74.6*
SUDBP≤90 or Fall in SUDBP ≥10	39	63.9*	74.6*	78
SUDBP ≤90 and fall in SUDBP ≥ 10	16.9	42.6*	61*	62.7*

* p <0.01 vs placebo

At 8 centers ambulatory blood pressure monitoring (ABPM) was done after 3 weeks on single blind Atenolol and after 5 weeks of double-blind therapy. Smoothed and unsmoothed means for the ambulatory data are shown in the graphs in the following two pages :



2-2-2 ATN+NIS 20 mg 4-4-4 ATN+NIS40 mg 6-6-6 ATN+NIS 60 mg
P-P-P ATN+Placebo

D30-029

Figure 10

Mean Change from Baseline in Ambulatory Blood Pressure

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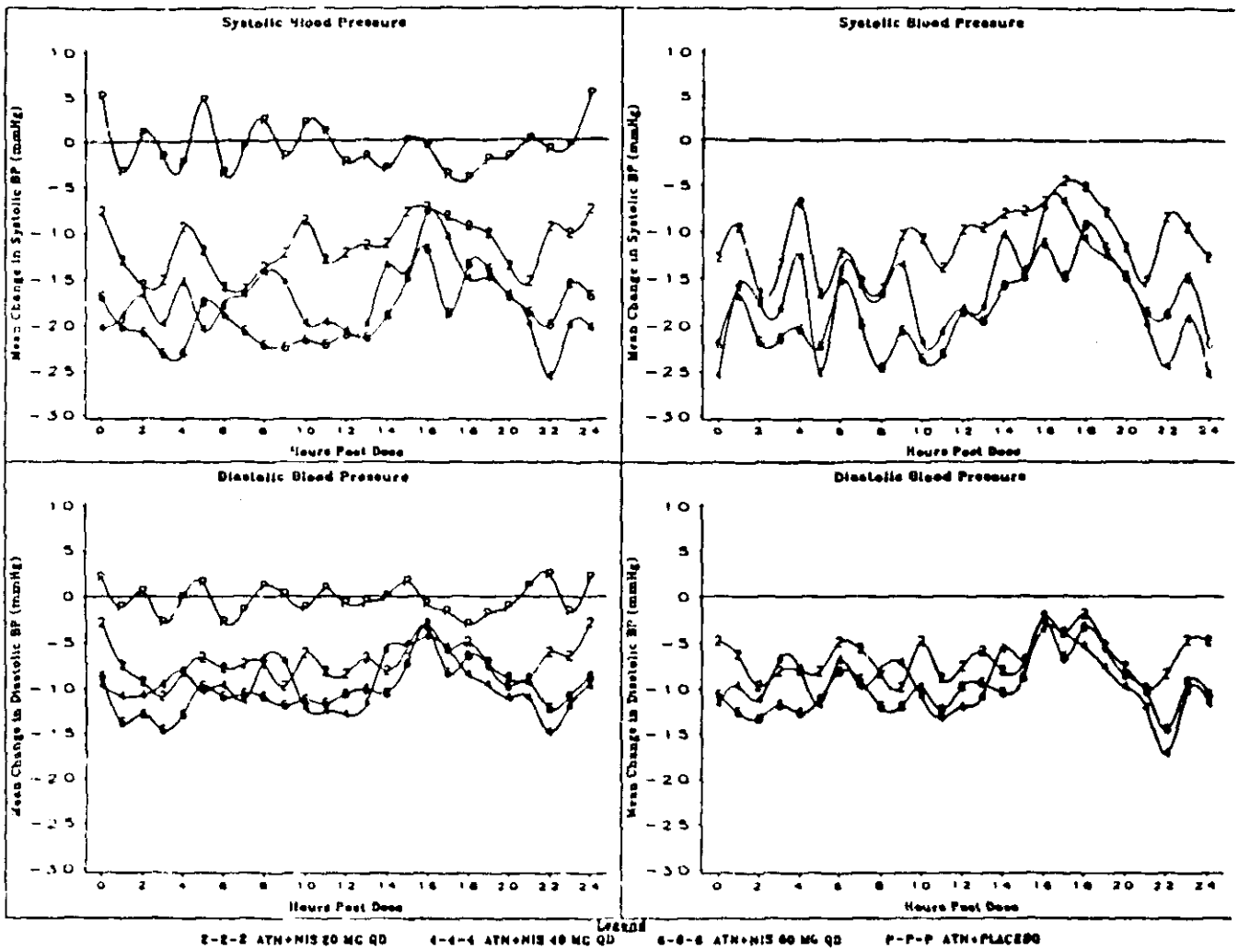
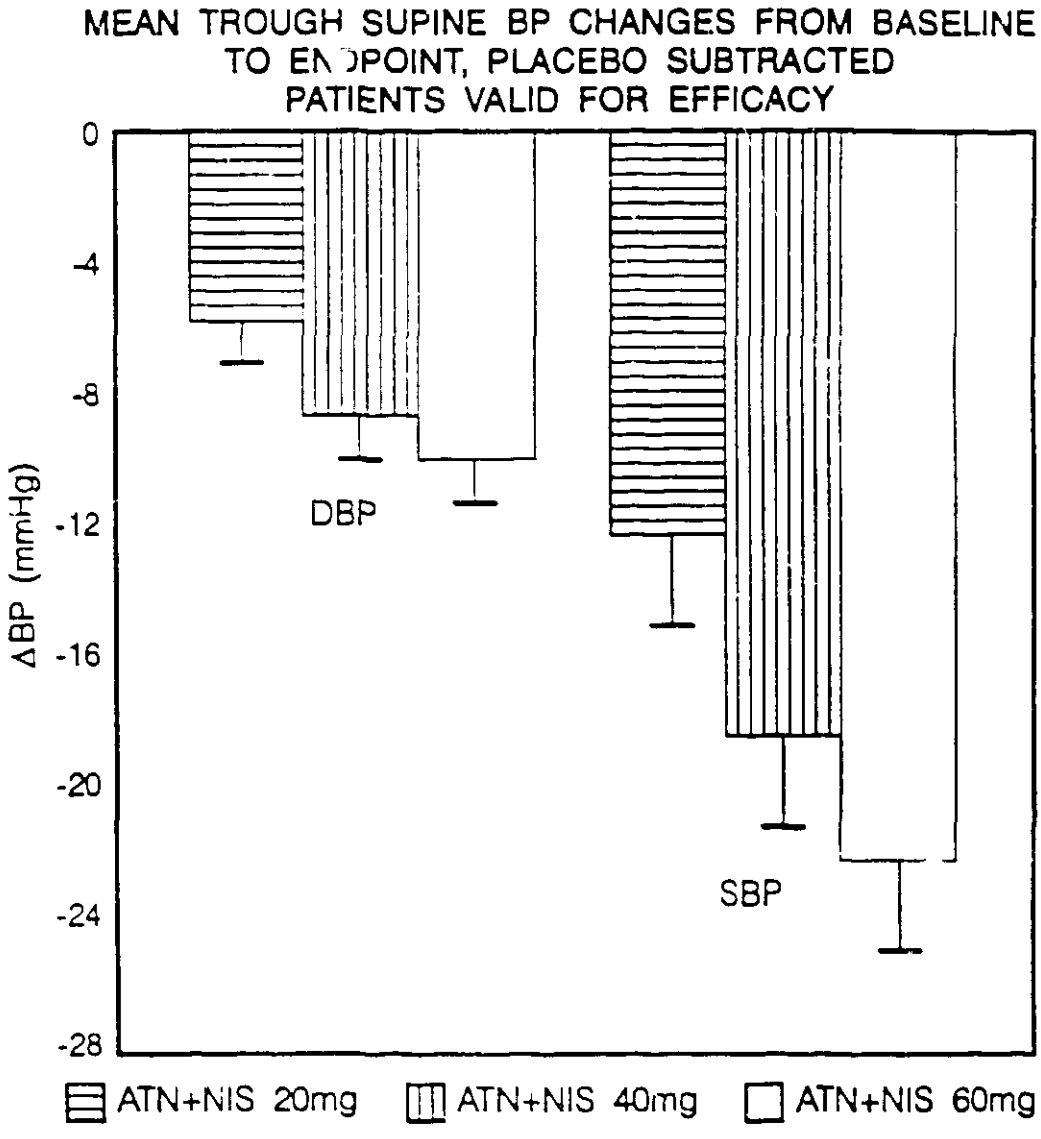


Symbols as in previous graph

The placebo-substracted trough SUDBPs are showed in the following graph:



The trough and peak ambulatory blood pressure changes and trough to peak ratios for patients valid for efficacy analysis are given in the following table :

Smoothed Data - Difference from Placebo Group

		Trough mmHg	Peak mmHg	Hours to Peak	Trough to Peak Ratio
Variable	Drug				
Diastolic	ATN+NIS 20 mg QD	-5.0	-9.4	3	53%
	ATN+NIS 40 mg QD	-12.8	-13.1	23	97%
	ATN+NIS 60 mg QD	-12.9	-13	1	99%
Systolic (at corresponding diastolic peak)	ATN+NIS 20 mg QD	-11.7	-13.6	3	86%
	ATN+NIS 40 mg QD	-22.0	-22.0	23	100%
	ATN+NIS 60 mg	-17.9	-19.0	1	94%
Systolic (actual)	ATN+NIS 20 mg QD	-11.7	-14.0	5	83%
	ATN+NIS 40 mg QD	-22.0	-22.0	24	100%
	ATN +NIS 60 mg QD	-17.9	-22.3	6	80%

Pharmacodynamic Results. Trough plasma samples were drawn at visits 5 and 9. Visit 9 samples for all patients were analyzed for Nisoldipine. The results for patients whose treatment regimens did include Nisoldipine are shown in the following table :

	n	Mean Trough Concentration (ng/ml)	Range of Trough Concentrations (ng/ml)
AT+NIS 20 mg	57	1.6	0-7.41
AT+NIS 40 mg	55	2.5	0-12.0
AT+NIS 60 mg	59	3.3	0-10.40

Conclusions. In this protocol Atenolol was used as a positive control and studies were performed with Atenolol in combination with placebo and with NIS in the concentrations of 20 mg, 40 mg and 60 mg. Atenolo with placebo had not significant effect in blood pressure but in combination with NIS demonstrated significant hypotensive effects in relation with the concentration of NIS. Threfore the possibility of drug interaction should be considered. Measurements of NIS in plasma were performed and they increased as would be expected with increasing concentrations of NIS and in relation to their effects on blood blood pressure but unfortunately concentrations of Atenolol in plasma were not measured. In other studies the sponsor did not find clinically relevant drug interaction between Nisoldipine, and the beta blocker Propanol (study 704, PB#21521, Volume 142, pp. 08-17-0010374). However some studies in the literature do not agree with this conclusion.

Elliott et al studying the interactions between Nisoldipine and Atenolol and Propanolol found that Nisoldipine, in single and multiple doses, significantly increased the peak plasma concentration of Propanolol and Atenolol. There was no evidence that either beta blocker influenced the pharmacokinetics of Nisoldipine. (The interactions between Nisoldipine and two beta-adrenoceptor antagonists : atenolol and propanolol. H.L.Elliott et al. Brit J Clin Pharmacol 1991;32(6):379-85).

Levine et al demonstrated pharacodynamic and pharmacokinetic interactions between Nisoldipine and Propanolol (MAH Levine et al. Pharmacokinetic and pharmacodynamic interactions between Nisoldipine and Propanolol. Clin Pharmacol Ther 1988; 43:39-48).

These contradictions could have been clarified had the sponsor included in this protocol a true placebebo group and groups of Nisoldipine without Atenolol. Plasma levels of Atenolol should have been also measured.

Protocol D90-019

Title of Study : " A Double-Blind Randomized Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine Coat-Core Tablets 30 mg, 60 mg (2X30) and 90 mg (3X30) vs Placebo in Hypertensive Patients ".

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Objectives. The objectives of this study were :

1. To determine the antihypertensive efficacy and safety of NIS tablets in doses of 30 mg. 60 mg and 90 mg daily in patients with mild to moderate hypertension.

2. To assess the time and magnitude of peak blood pressure response and the ratio of trough to peak antihypertensive effect by 24-hour ambulatory blood pressure monitoring.

Inclusion Criteria. Ambulatory patients, male and female, 21 to 75 years of age, with a history of mild to moderate essential hypertension were eligible for the study.

Exclusion Criteria. Patients with the following conditions were excluded from the study : labile hypertension, renovascular or other secondary forms of hypertension, patients whose SUDBP after 3 and 4 weeks of placebo run-in were not ≥100 or ≤ 114 mmHg, previous myocardial infarction or cerebrovascular accident, heart failure, frequent arrhythmias, conduction disturbances, angina pectoris, use of other antihypertensive drugs, and many other drugs.

Also excluded were patients with failure of a major organ system, liver, kidney disease, malignancy or psychosis, patients with previous history of gastrointestinal disease which could result in incomplete absorption of the drug, women with childbearing potential, patients with alcohol or drug abuse, or allergy to dihydropyridines

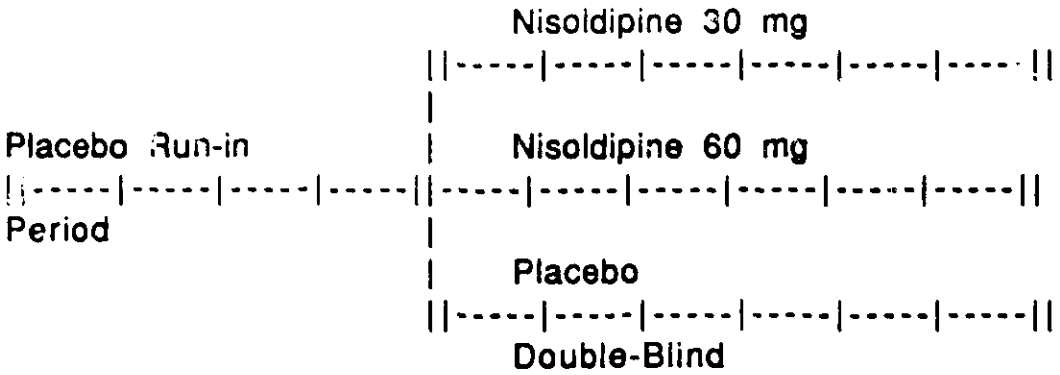
Study Design. . The study consisted of a single-blind placebo run-in period and a treatment period.

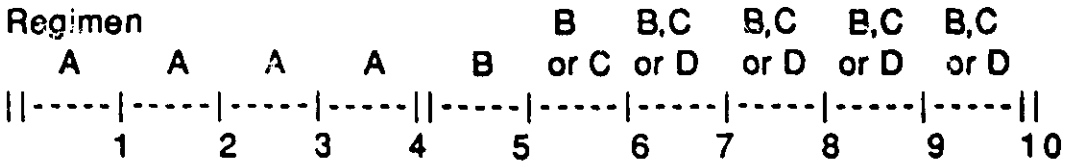
Placebo Run-in Period. During this period of 4 weeks duration pacients discontinued all previous antihypertensive medication and were given a single-blind placebo which consisted of 3 tablets once a day in the morning for a 4-week qualifying run-in period. Then patients with confirmed hypertension, with a trough SUDBP ≥100 to ≤ 114 mmHg after 3 and again after 4 weeks of placebo and whose SUDBP at these 2 visits were within 7 mmHg of each other were admitted into the treatment period.

Treatment Period. After four weeks of single-blind placebo patients with confirmed hypertension were randomized to one of three treatment groups: Placebo, Nisoldipine 30 mg or Nisoldipine 60 mg. Patients randomized to placebo received placebo for the remainder of the study. Placebo randomized to Nisoldipine 30 mg received the same dose for the remainder

of the study. Patients randomized to Nisoldipine 60 mag were given Nisoldipine 30 mg for one or two weeks and the dose was titrated to Nisoldipine 60 mg (2X30) for the final 4 to 5 weeks of the double-blind treatment. A group of patients was to be titrated to 90 mg (3X30) but this arm was discontinued before any patients were randomized because a concurrent high-dose forced-titration clinical pharmacological study showed evidence of symptomatic T wave flattening/or inversion predominantly at doses above Nisoldipine 60 mg.

The study design is shown schematically in the following graph :



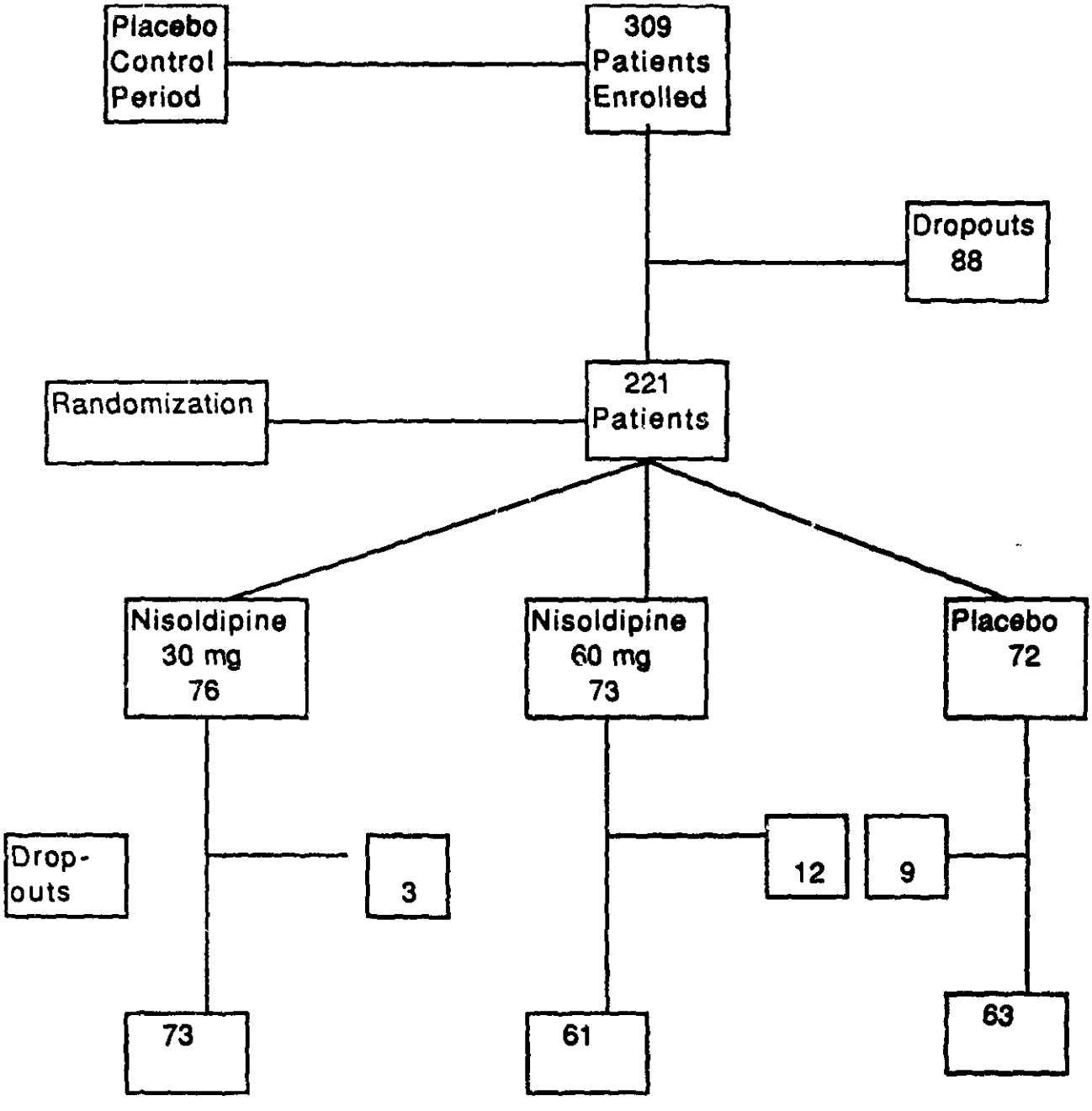


Group	Regimen B	Regimen C	Regimen D
NIS 30 mg	30 mg	30 mg	30 mg
NIS 60 mg	30 mg	60 mg	60 mg
Placebo	Placebo	Placebo	Placebo

Demography. The demography and baseline characteristics in patients valid for analysis of efficacy is given in the following table :

	NIS 30 mg N=76	NIS 60 mg N=66	Placebo N=71
Mean Age (years)	52	52	52
Mean wt (Lbs)	197	197	202
Baseline BP Supine Standing	 157/104 153/104	 158/105 154/104	 155/104 151/103
History of Hypertension (years)	11	9	10
% Male	61	56	52
% Caucasian	58	58	72
% History of Diabetes	9	11	13
% History of Hyperlipidemia	13	12	8
% History of MI	0	2	1
% Mild Hypertensives	62	58	70
% Moderate Hypertensives	38	42	30

The distribution and randomization of patients is seen in the following grapl



The listing of patients who did not qualify for randomization is given in the following table :

Mean supine diastolic blood pressure at visit 4 and at visit 5 did not qualify	45
Adverse events	7
Low diastolic blood pressures	6
Patient request	6
Blood pressure too high	5
Elevated serum transaminase levels	5
Childbearing potential	2
Elevated serum lipids	2
Family considerations	2
Illness not due to study medication	2
Intercurrent medical considerations	2
Administrative problems	1
Change in supine diastolic blood pressure > 7 mmHg from visit 4 to visit 5	1
Low hemoglobin/hematocrit	1
Protocol violation	1
Total	88

The reasons for discontinuation of double-blind therapy population in all randomized patients are given in the following table :

Reason	Nisoldipine		Placebo
	30 mg N=76	60 mg N=73	N=72
Adverse Event	1	11	3
Lack of efficacy	1	0	2
Physician dissatisfied with treatment	0	1	2
Patient dissatisfied with treatment	0	0	2
Lost to follow-up	1	0	0

The listing of dropouts due to adverse events for patients valid for safety analysis is given in the following table :

Drug Group	Adverse Experience Causing Patient to Drop	Day of Onset	Dose/ Duration (Days)	Intensity/ Relation-ship to Drug
Placebo	EKG abnormality	-1	Pla/1	Mild/ Remote
	Cardiac arrest	24	Pla/1	Severe/ Remote
	Frontal headaches	28	Pla/>3	Severe/ Possible
Nisoldipine	Edema lower extremity	24	30 mg/ >7	Moderate/ Probable
	Severe headache	3	30 mg/ >11	Severe/ Possible
	Headache	-1	Pla/5	Severe/ Possible
	Severe headache	0	30 mg/7	Severe/Pos
	2+ ankle edema, bilateral	24	60 mg/ 10	Moderate/ Probable
	3+ pretibial edema, bilateral	24	60 mg/ 10	Severe/ Probable
	Trace edema, bilateral	24	60 mg/ 10	Mild/ Probable
	Atypical chest paln	9	60 mg/5	Mod/Poss
	Headache	12	60 mg/ >1	Severe/ Possible
	3 + ankle edema	33	60 mg/ >3	Severe/ Possible
	Headache,vomiting	7	60 mg/ 1	Severe/ Possible
	Headache	12	60 mg/>1	Sev/Rem
	Confusion,	0	30 mg/>1	Sev/Rem
	Nausea,	0	30 mg/3	Mild/Prob
	Headache	6	30 mg/2	Mod/ Probable

Efficacy

Actual Dosage and Duration of Treatment. Patients were to receive Nisoldipine 30 mg, 60 mg or Placebo over a 6 week double-blind treatment period. Upward titration from Nisoldipine 30 mg to Nisoldipine 60 mg was required in the Nisoldipine 60 mg group after one or two weeks of double-blind medication if trough SUDBP was greater than or equal to 80 mmHg. Placebo and Nisoldipine 30 mg underwent sham titration. The following table shows the number of patients that were given each dose level and had valid visits at each week for the population of patients valid for efficacy :

Double-Blind Visit			6	7	8	9	10	
Week of Double-Blind Therapy			1	2	3	4	6	Endpoint
Group	Regimen	Dose	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Nis 30 mg	B (30 mg qd)		75 (100)	10 (13)	4 (5)	3 (4)	3 (4)	3 (4)
	C (30 mg QD)			66 (87)	71 (95)	71 (96)	70 (96)	73 (96)
NIS 60 mg	B (30 mg QD)		65 (100)	7 (11)	5 (8)	5 (8)	4 (6)	5 (8)
	C (60 mg QD)			59 (89)	59 (92)	59 (92)	58 (94)	61 (92)
Placebo	B (Placebo)		71 (100)	6 (8)	2 (3)	2 (3)	2 (3)	2 (3)
	C (Placebo)			65 (92)	65 (97)	64 (92)	62 (97)	69 (97)

Analysis of Effectiveness. Two hundred thirteen of the 221 enrolled patients had at least one valid blood pressure measurement after randomization and were included in the primary efficacy analysis (endpoint) :76 were randomized to the Nisoldipine 30 mg group, 66 were randomized to the Nisoldipine 60 mg group, and 71 were randomized to the placebo group.

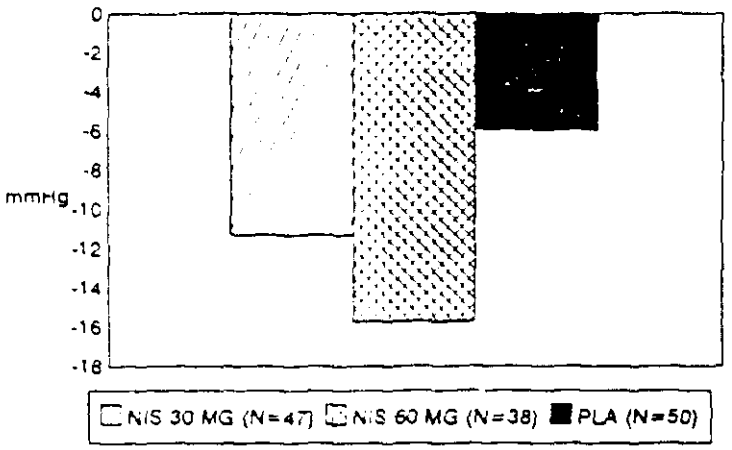
Trough raw means cf blood pressure at each visit as well as at endpoint are given in the following table :

	Supine			Standing		
	NIS 30 mg	NIS 60 mg	Placebo	NIS 30 mg	NIS 60 mg	Placebo
Base-line (N)	158/ 104 (76)	158/ 105 (66)	155/ 104 (71)	154/ 104 (76)	155/ 104 (66)	152/ 103 (71)
Week 1 (N)	148/95 (75)	146/94 (65)	152/98 (71)	144/96 (75)	142/94 (65)	149/ 100 (71)
Week 2	147/93 (76)	141/90 (66)	152/98 (71)	143/95 (76)	139/91 (66)	149/99 (71)
Week 3 (N)	144/92 (75)	139/89 (64)	149/97 (67)	140/93 (75)	136/90 (64)	148/98 (67)
Week 4 (N)	142/92 (75)	139/87 (64)	152/98 (66)	140./93 (75)	135/87 (64)	150/99 (66)
Week 6 (N)	145/92 (73)	140/89 (62)	152/98 (64)	140/94 (73)	135/90 (62)	149/ 100 (64)
End-point (N)	146/93 (76)	141/90 (66)	154/99 (71)	141/94 (76)	137/91 (66)	150/ 101 (71)

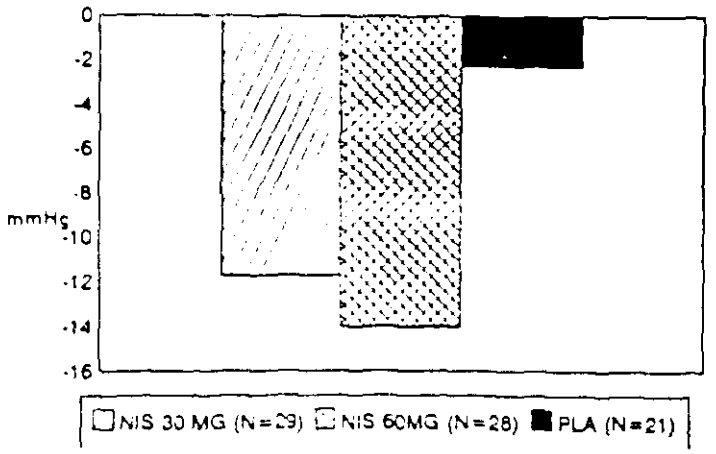
Mean changes (mmHg) in SUDBP at endpoint for patients wit mild (baseline SUDBP ≥ 100 to ≤ 104 mmHg)and moderate (baseline SUDBP ≥ 105 to ≤ 114 mmHg) hypertension are shown in the followings table and figure:

	Nisoldipine 30 mg		Nisoldipine 60 mg		Placebo	
	(N)	Change	(N)	Change	(N)	Change
Mild	47	-11.3	38	-15.7	50	-6.0
Moderate	29	-11.7	28	-14.0	21	-2.3

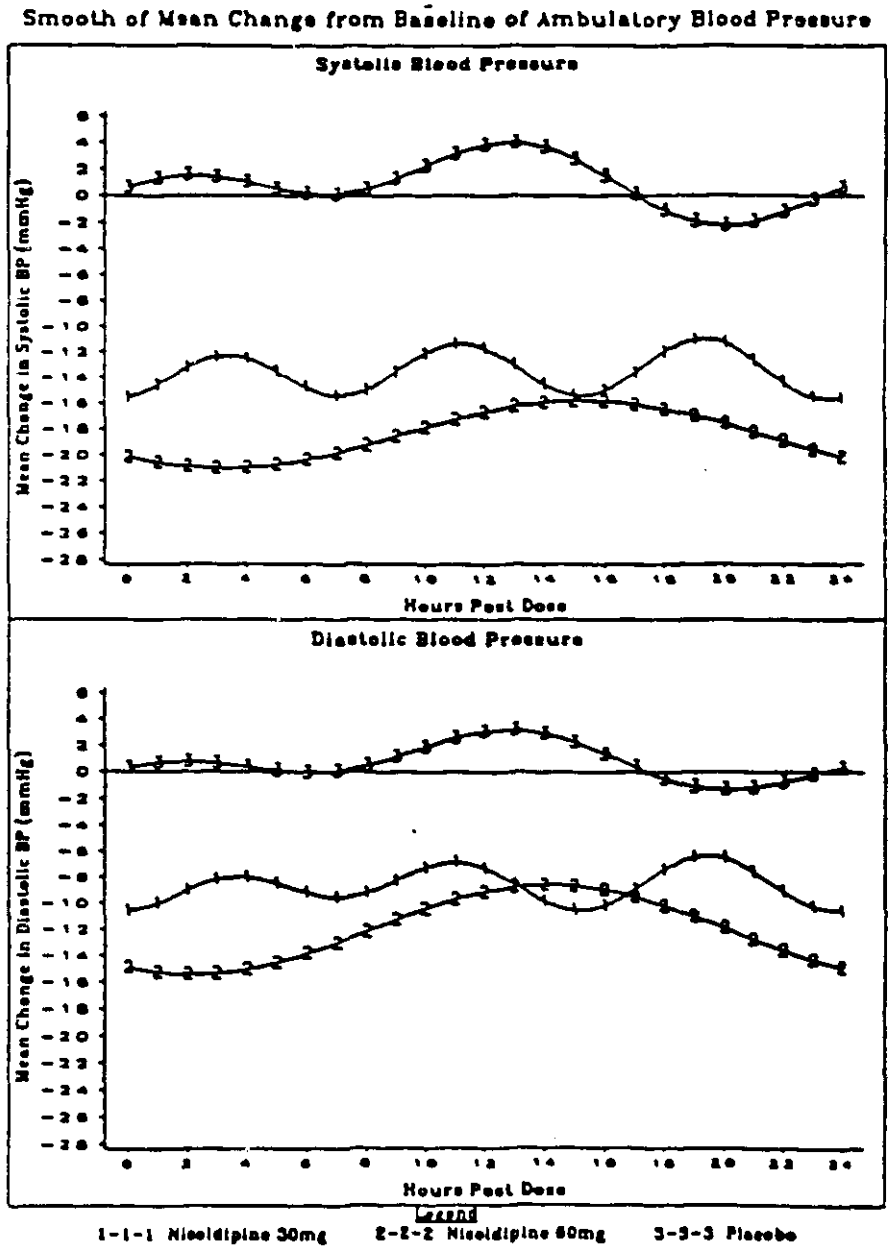
MEAN CHANGE FROM BASELINE (mmHg)
FOR SUPINE DIASTOLIC BP AT ENDPOINT
PATIENTS WITH MILD HYPERTENSION



MEAN CHANGE FROM BASELINE (mmHg)
FOR SUPINE DIASTOLIC BP AT ENDPOINT
PATIENTS WITH MODERATE HYPERTENSION



The 24-hour ambulatory blood pressure profile of mean systolic and diastolic blood pressure responses for the three treatment groups are shown in the following graph :



The ambulatory blood pressure falls and trough to peak ratios change from placebo for patients valid for efficacy analysis are given in the following table :

	N	Peak Hour	Peak value * (mmHg)	Trough* (mmHg)	Trough to Peak ratio
Diastolic BP					
NIS 30 mg	39	13	12.13	9.52	78 %
NIS 60 mg	29	4	15.24	14,22	93 %
Systolic BP (at corresponding time of diastolic peak)					
NIS 30 mg	39	13	17.46	14.52	83 %
NIS 60 mg	29	4	23.13	17.66	76 %
Systolic Blood Pressure (actual)					
NIS 30 mg	39	13	17.46	14.52	83 %
NIS 60 mg	29	5	23.71	17.66	75 %

*Change from placebo, baseline corrected

The mean changes from baseline in diastolic blood pressure over the 24-hour period of ambulatory blood pressure were -8.6 mmHg for Nisoldipine 30 mg, -12.0 mmHg for Nisoldipine 60 mg and +0.7 mmHg for placebo.

Pharmacokinetics Results. Trough blood samples were drawn at all 16 centers at visits 5 an 10. Seven centers also drew blood samples at visit 10.1 at 2 and 12 hours post-dosing. Samples were assayed for Nisoldipine blood levels. Results are presented in the following table :

	N	Mean Concentration (SD) ng/ml	Change in SUBP (Systolic/Diastolic) in mmHg
NIS 30 mg :			
Trough	68	1.5 (1.3)	-12/-11
2 hours post dosing	27	2.3 (1.9)	-17/-15
12 hours post dosing	27	2.1 (1.2)	-19/-17
NIS 60 mg:			
Trough	55	3.2 (2.8)	-17/-16
2 hours post dosing	27	6.0 (5.2)	-20/-19
12 hours post dosing	26	4.9 (2.8)	-21/-22

There was a statistically significant correlation between plasma concentration and change from baseline to endpoint in supine diastolic blood pressure at trough. The greater the correlation, the greater was the decrease in supine diastolic blood pressure. Twenty percent of the variability in the observed change in supine diastolic blood pressure was explained by plasma concentration.

Assessment. The results of this study indicate that Nisoldipine, at the dose of 30 mg and 60 mg daily once daily, is effective in reducing systolic and diastolic blood pressure at trough in patients with mild to moderate hypertension. The reductions in systolic and diastolic blood pressure were greater than 50 percent of peak effect at trough. Furthermore, ambulatory measurements of blood pressure for 24-hours demonstrated that reductions in blood pressure in Nisoldipine-treated patient was maintained through the hours of observation. The effect was more effective with the 60 mg of Nisoldipine than with the 30 mg dose and in the latter more effective than placebo. Pharmacokinetic studies demonstrated that effect on diastolic blood pressure was proportional to the concentration of Nisoldipine in blood.

Side effects were significantly increased by drug administration as compared to control and were greater with the 60 mg Nisoldipine dose than with the 30 mg. Adverse events are to be discussed by another reviewer.

Protocol D89-039

Title of Study : " Comparative Double-Blind Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine 20 mg. 40 mg, 80 mg Coat Core (CC) Tablets vs a Twice Daily Dose of Verapamil SR 240 mg caplets vs Placebo in Hypertensive Patients ".

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Objectives. The objective of this study was to determine the efficacy and safety of once daily doses of Nisoldipine 20 mh, 40 mg and 80 mg to a twice daily dose of Verapamil 240 mg and to Placebo in patients with mild to moderate hypertension.

Inclusion and Exclusion Criteria. Ambulatory male and female patients, 21 years of age or older, with history of mild to moderate hypertension, were eligible for enrollment in this study.

Patients with the following conditions were excluded from this study : recent myocardial infarction or cerebral vascular accident ; herat failure, major arrhythmias, conduction distrurbances, angina pectoris, sinus bradycardia or severe left ventricular dysfunction ; patients with impaired absorption of the drug ; females pregnant or with childbearing potential ; patients with failure of a major organ system such as liver, renal disease, malignancy or psychosis ; alcohol abuse or drug intake ; allergy to dihydropyridines, verapamil or other antagonists ; also excluded were patients who participated in another investigational drug study within the previous 30 days.

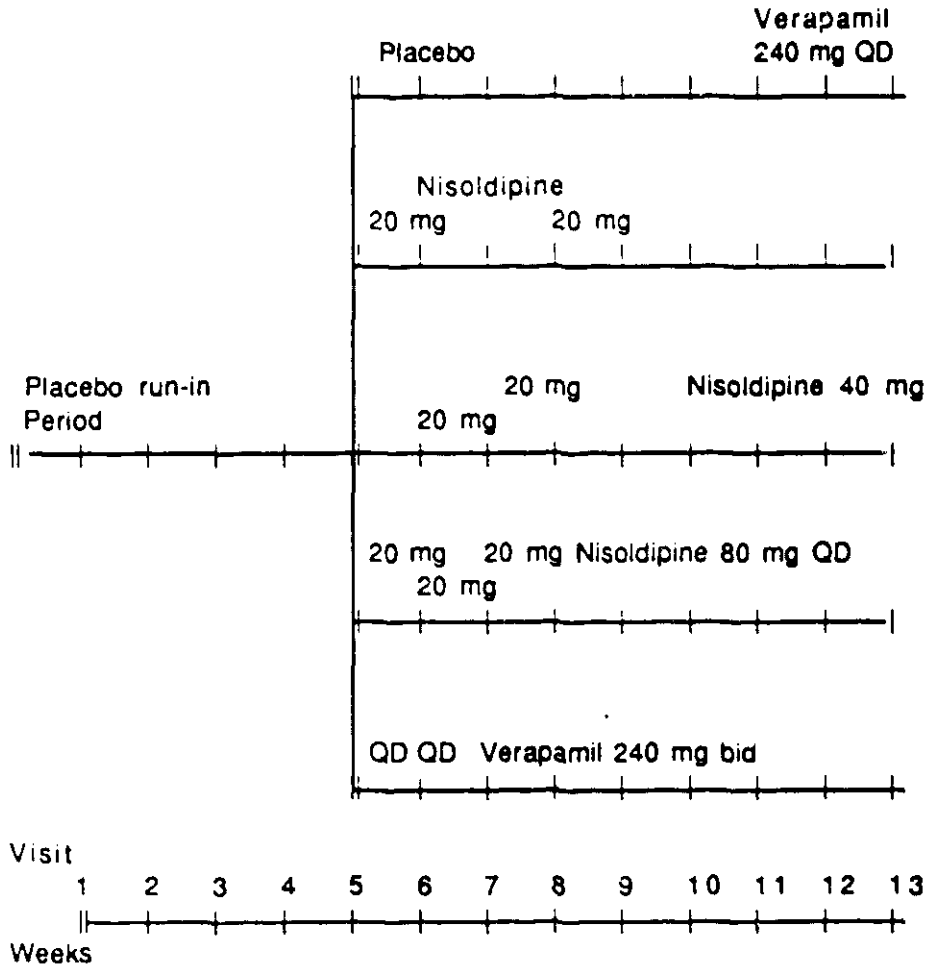
Study Design. The study consisted of a single-blind run-in period and a treatment period.

Single-Blind Run-In Period. Patients were given two placebo tablets and one placebo capsule in the morning and anothe placebo-capsule in the evening each day during a 4-week single-blind-run-in period. Drug for the single-blind placebo run-in period was labeled as Regimen A.

Qualification for Randomization. Patients whose mean SUDBP (the average of 3 readings over a five minute period in the supine position) were 95-114 mmHg after 3 and after 4 weeks on placebo and whose SUDBP after 3 and 4 weeks on placebo were within 7 mmHg of each other were eligible for randomization.

Double-Blind Treatment Period. After the placebo run-in period, a forced titration was designed as follows : Regimen B : Nisoldipine 20 mg, Verapamil 240 mg qd or Placebo which patients took for one week; Regimen C : Nisoldipine 20 mg, Nisoldipine 40 mg, Verapamil 240 mg twice daily or Placebo which patients took for one week ; Regimen D : Nisoldipine 20 mg, Nisoldipine 40 mg, Nisoldipine 80 mg (2 X 40), Verapamil 240 mg twice daily or Placebo which patients took for 6 weeks. After 8 weeks of double-blind drug, patients given Nisoldipine or Verapamil continued on the same drug regimen while patients given Placebo were switched to Verapamil 240 mg qd for the remaining of the 4 weeks of study.

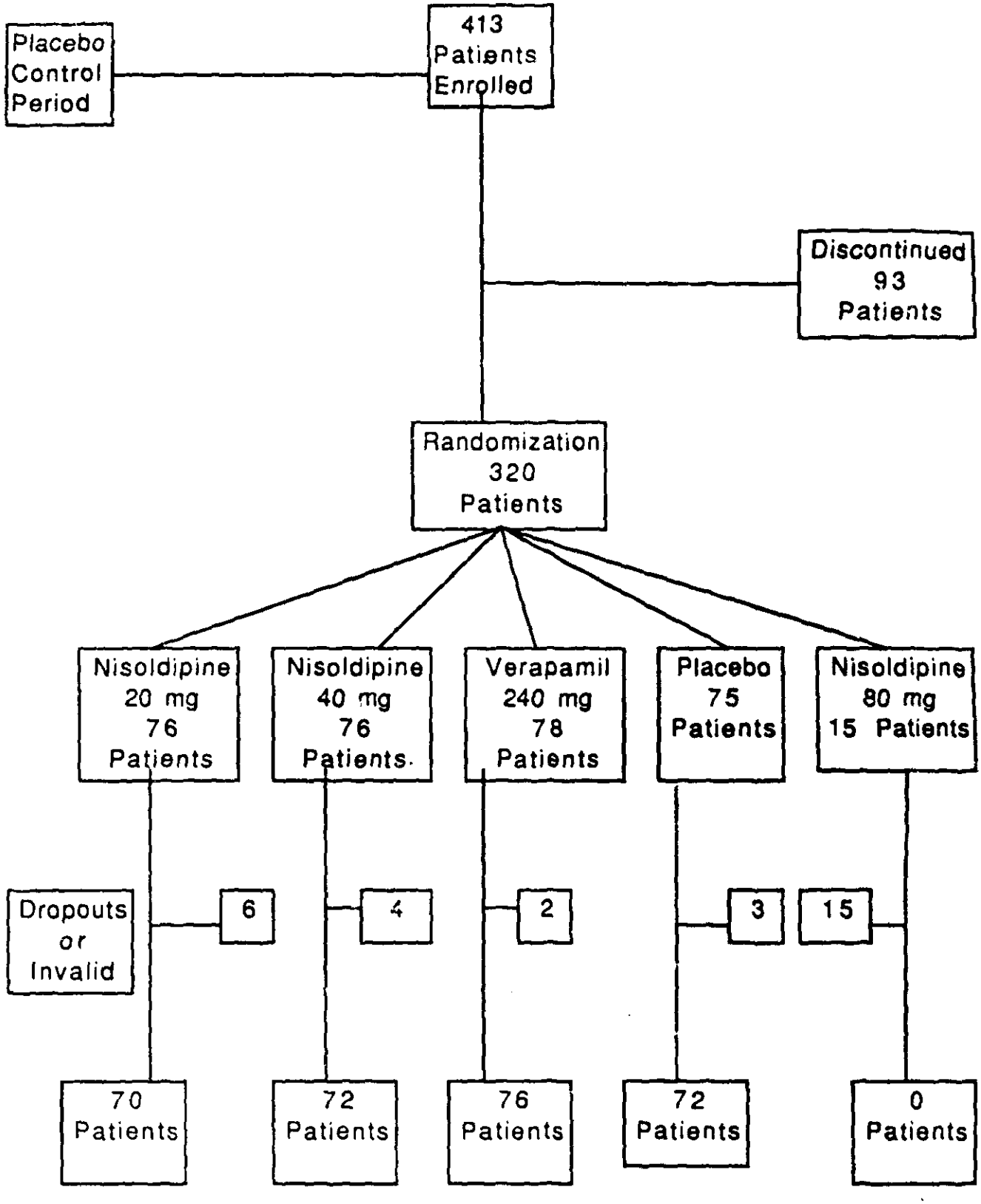
The study design is demonstrated schematically in the following graph :



Demographics. The demographic characteristics are shown in the following table :

	Nisoldipine 20 mg n=70	Nisoldipine 40 mg n=72	Verapamil n=76	Placebo n=72
Mean age (years)	53	54	52	55
Mean wt (lbs)	202	197	198	196
Baseline BP (mmHg)				
Supine	153/100	155/100	151/100	154/100
Standing	151/101	151/101	148/101	151/100
Male	57 %	61 %	55 %	67 %
Black	31 %	24 %	28 %	21 %
History of Diabetes	9 %	1 %	11 %	8 %
History of Hyperlipidemia	3 %	1 %	3 %	10 %
History of MI	3 %	1 %	0 %	1 %
Hypertensives				
Mild	84 %	86 %	83 %	89 %
Moderate	16 %	14 %	17 %	11 %

The distribution of patients and randomization are given in the following graph:



The reasons that disqualified enrolled patients for randomization are given in the following table :

Mean Supine Diastolic Blood Pressure at visit 4 or visit 5 did not qualify for randomization (95 mmHg to 11 mmHg)	47
Adverse events	13
Patient chose to withdraw	9
Other illness/Surgery/Screening abnormality	5
Blood pressure too high off medications for patient's safety	5
Lost to follow-up	4
Elevated transaminases at screening	3
Noncompliance	3
Called to military service	2
Blood pressure too low after in-clinic	1
Inadequate quality control during ambulatory blood pressure	1
Total	93

The number of dropouts during the treatment period and the reasons for elimination from the study are given in the following table :

Nisoldipine 20 mg. N=76

Event	Days on Drug
Palpitations, depression, headache, emesis	2
Headache, shortness of breath, fatigue	3
Headache, flashing, head congestion	4
Headache, flushing, palpitations	6
Peripheral edema	12
Headache, nausea	12
Peripheral edema	24
Peripheral edema	73
Pleural effusion	77
Myocardial infarction	89
Noncompliance	7
Chose to withdraw	54

Nisoldipine 40 mg. N=76

Event	Days on Drug
Headache, rash	1
Headache, nausea	2
Headache, nausea	2
Peripheral edema	10
Peripheral edema	12
Myocardial infarction	13
Headache, tremor, flushing, palpitations hypesthesia, asthenia	14
Peripheral edema	16
Peripheral edema	22
Peripheral edema	40
CVA	41
Chose to withdraw	16
Chose to withdraw	32

Nisoldipine 80 mg. N=15

Headache, flushing, palpitations, chest pain	1
Flushing, palpitation	14
Deep T wave inversion	15
Discontinued	3
Discontinued	4
Discontinued	6
Discontinued	12
Discontinued	13
Discontinued	14
Discontinued	17
Discontinued	20
Discontinued	20
Discontinued	24
Discontinued	28
Discontinued	31

Verapamil 240 mg. N=78

Event	Days on Drug
Headache, dizziness, tachycardia, leg pain, tinnitus	0
Peripheral edema	17
Hypotension	22
Headache, chills, peripheral edema	36
Cholecystitis	7

Placebo. N=75

CVA	6
Fatigue, edema	14
Peripheral edema	32
Lack of efficacy	5
Lack of efficacy	48
Lost to follow-up	21
Lost to follow-up	69
Chose to withdraw	47
Chose to withdraw	62
DBP> 114 mmHg	27
Retinal disorder	55

Efficacy

Criteria for Effectiveness. The change from baseline to endpoint in trough SUDBP (blood pressure measured 24 hours after the previous day's morning dose and 12 hours after the previous day's evening dose) in the Nisoldipine 40 mg group compared to the placebo group was the primary criterion used to determine the effectiveness of the drug.The comparison of Nisoldipine 20 mg to Placebo was of secondary importance.

Secondary efficacy parameters included standing diastolic blood pressure and both standing and supine systolic blood pressure. In addition in eight centers ambulatory blood pressure changes (the difference between measurements made over the 24 hours after 3 weeks of placebo run-in and the 24 hours after 7 weeks of double-blind therapy) were compared among

groups. The 12-hour in-clinic monitoring data were also compared among groups. The peak effect and the time to peak effect were calculated for both the ambulatory and 12-hour in-clinic monitoring. In addition the trough to peak ratio was calculated for ambulatory blood pressure. Plasma samples were drawn at baseline (visit 5) and at visit 11 for analysis of Nisoldipine plasma concentrations.

Statistical Methods. All statistical methods were two-tailed and were conducted at a significance level of 0.05. Pairwise comparisons and within group changes were tested via the least squares means estimated by the model.

Analysis of Effectiveness. The mean blood pressure changes at endpoint (mmHg) for patients valid for efficacy analysis are given in the following table :

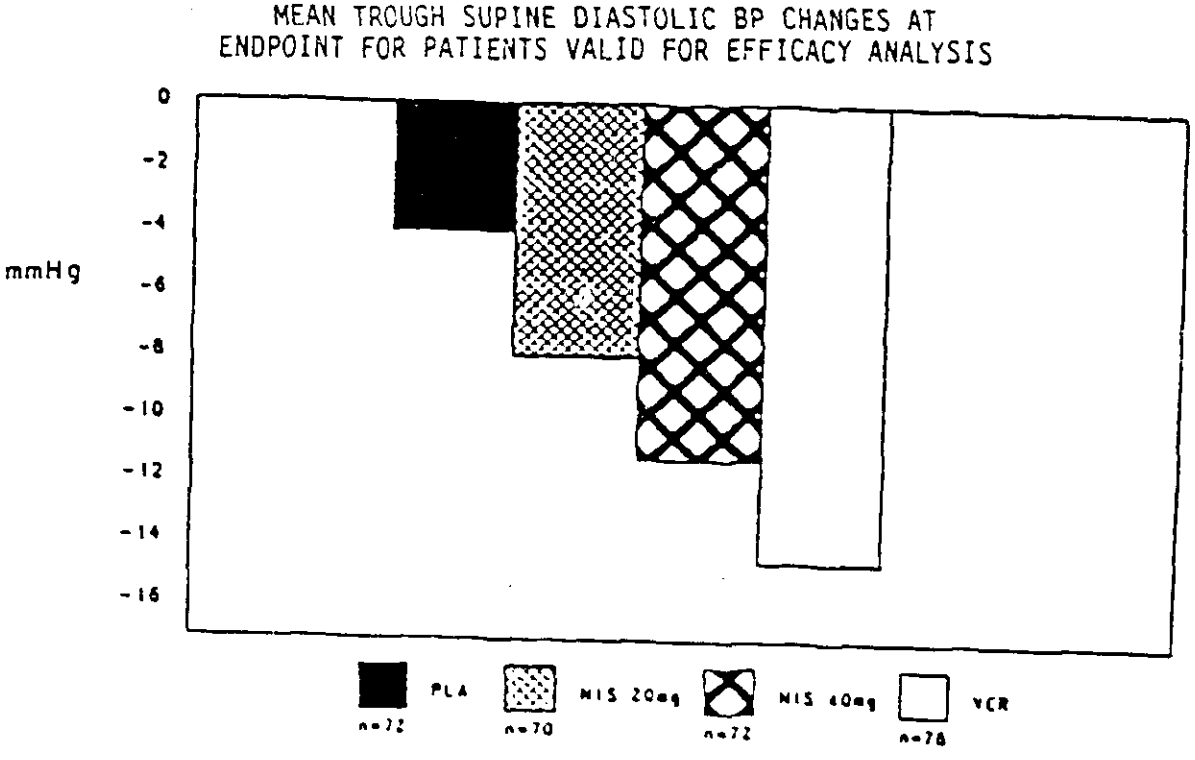
	Nisoldipine 20 mg N=70	Nisoldipine 40 mg N=72	Verapamil N=76	Placebo N=72
Supine				
Diastolic	-8.1 ABP	-11.4 BP	-14.7 P	-4.0
Systolic	-9.6 ABP	-16.2 P	-16.0 P	-2.2
Standing				
Diastolic	-7.1 ABP	-11.8 BP	-13.9 P	-2.0
Systolic	-11.6 BP	-15.4 P	-16.4 P	-2.4

A Significantly different from Nisoldipine 40 mg
B Significantly different from Verapamil
P Significantly different from Placebo

Mean changes (mmHg) in SUDBP at endpoint for patients with mild (baseline SUDBP 95-104 mmHg) and moderate (Baseline SUDBP 105-114 mmHg) are shown in the following table :

	Nisoldipine 20 mg		Nisoldipine 40 mg		Verapamil		Placebo	
	n	Change	n	Change	n	Change	n	Change
Mild	59	-8.4	62	-11.3	63	-14.1	64	-4.1
Moderate	11	-6.5	10	-13.0	13	-18.0	8	-3.5

The effect on SUDBP at endpoint in the Nisoldipine (24 hours after dose), Verapamil group (12 hours after dose) and Placebo group is shown in the figure below :



The results by visit for SUDBP are shown in the following graph :

	Week 1	Week 2	Week 3	Week 4	Week 6	Week 8
NIS 20 mg						
n	69	70	66	65	68	68
Mean Change	-7.8	-7.2	-8.6	-7.5	-7.9	-8.0
NIS 40 mg						
n	72	72	66	65	65	63
Mean Change	-7.4	-9.8	-9.9	-11.2	-11.0	-11.8
Ver						
n	76	75	73	69	73	71
Mean Change	-5.9	-11.0	-12.8	-13.1	-12.6	-14.6
Placebo						
n	72	72	68	67	69	67
Mean Change	-4.0	-4.7	-5.1	-4.4	-5.7	-4.1

During the secon phase of the double-blind period, the differences between the active drugs decreased, while the Placebo group experienced the expected further decrease in blood pressure after switching to Verapamil. The changes from baseline in trough SUDBP at the two visits in thus phase are presented below :

	Week 10	Week 12
NIS 20 mg	-8.8	-10.1
NIS 40 mg	-12.2	-10.3
Verapamil	-13.1	-11.5
Placebo	-7.3	-7.0

Various demographic variables were examined including sex, weight, age, smoking status, race and baseline blood pressure. Of these only age exhibited a marked difference in blood pressure response. Mean changes from baseline in supine blcod pressures for each drug group for patients at least 60 years old vs patients younger than 60 years old are provided in the table below :

	NIS 20 mg		NIS 40 mg		Verapamil		Placebo	
	n	Mean	n	Mean	n	Mean	n	Mean
Diastolic								
Age ≥ 60	28	-10.4	26	-13.6	24	-16.2	23	-4.0
Age < 60	42	- 6.6	46	-10.4	52	-14.1	49	-4.0
Systolic								
Age ≥ 60	28	-14.0	26	-21.2	24	-19.3	23	-2.3
Age < 60	42	-6.8	46	-13.6	52	-14.5	49	-2.2

Responders rate based on trough SUDBP are presented in the following table :

	NIS 20 mg N=70	NIS 40 mg N=69	Verapamil N=76	Placebo N=72
DBP ≤ 90 mmHg	35 (50 %)	50 (69%)	62 (82 %)	19 (26 %)
DBP decrease ≥ 10 mmHg	28 (40 %)	47 (65 %)	59 (78 %)	10 (14 %)

In clinic monitoring was done for 12 hours and 24-hour ambulatory blood pressure monitoring for 24 hours.

The in clinic monitoring, that covered only half of the dosing interval yielded the following results :

Dose	Mean Change Range mmHg/	Hour
Nisoldipine 20 mg	-9.8	12
	-13.5	8
Nisoldipine 40 mg	-10.8	12
	-15.5	4
Verapamil	-11.8	
	-15.1	
Placebo	-2.5	
	-5.5	

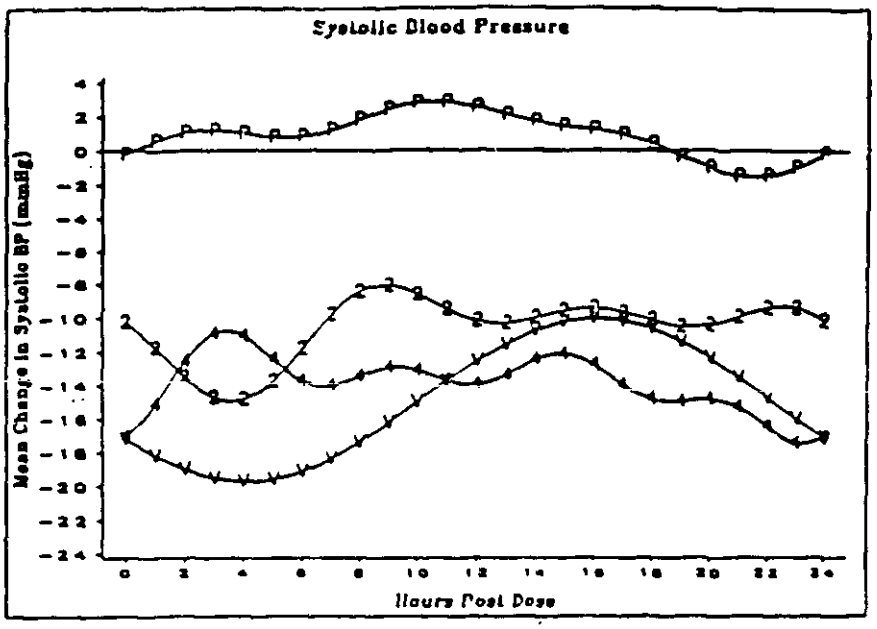
On ambulatory blood pressure monitoring response after 7 weeks of therapy was observed for 24 hours after Nisoldipine 40 mg therapy, 4 hours after Nisoldipine 20 mg therapy, and 4 hours after the morning dose of Verapamil. with blood pressure changes (systolic/diastolic)of -17.5/ -10.7 mmHg, -15.1/10.2 mmHg, and -19.7/-14.3 mmHg respectively. The mean 24-hour systolic and diastolic blood pressure changes during ambulatory blood pressure monitoring were :

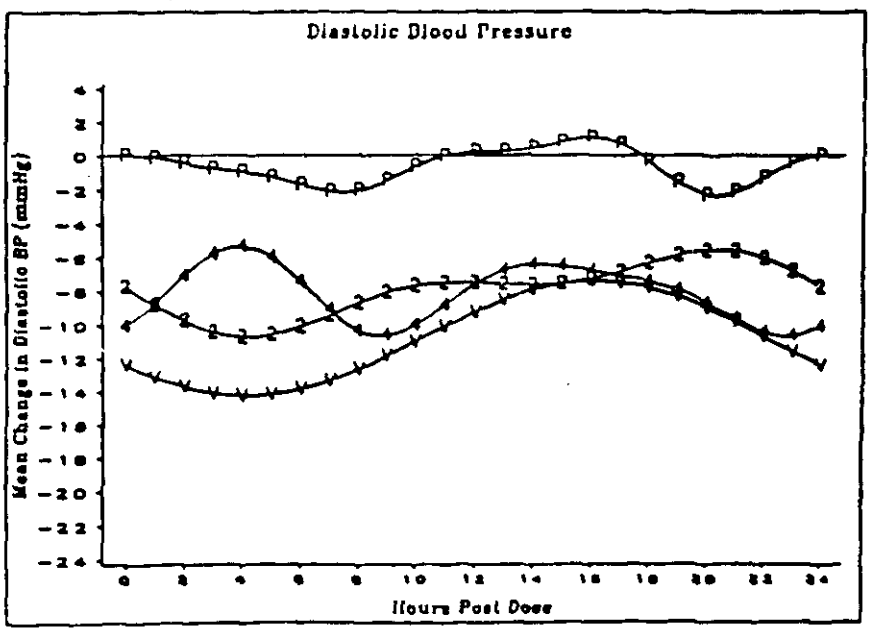
Nisoldipine 40 mg	-13.6/-8.0
Nisoldipine 20 mg	-11.1/-7.9
Verapamil	-14.8/10.8

Based on smoothed ambulatory blood pressure data, the trough/peal ratios for the treatment groups are summarized in the fcllowing table :

	Trough mmHg	Peak mmHg	Trough to peak ratio
Diastolic BP			
NIS 20 mg	-6.7	-9.7	69 %
NIS 40 mg	-11.7	-11.7	100 %
Verapamil	-11.1	-12.9	86 %
Systolic BP			
NIS 20 mg	-9.9	-15	66 %
NIS 40 mg	-14.3	-14.3	100 %
Verapamil	-15.9	-20.9	78 %

The unsmoothed change from baseline ambulatory data in systolic and diastolic blood pressure are shown below

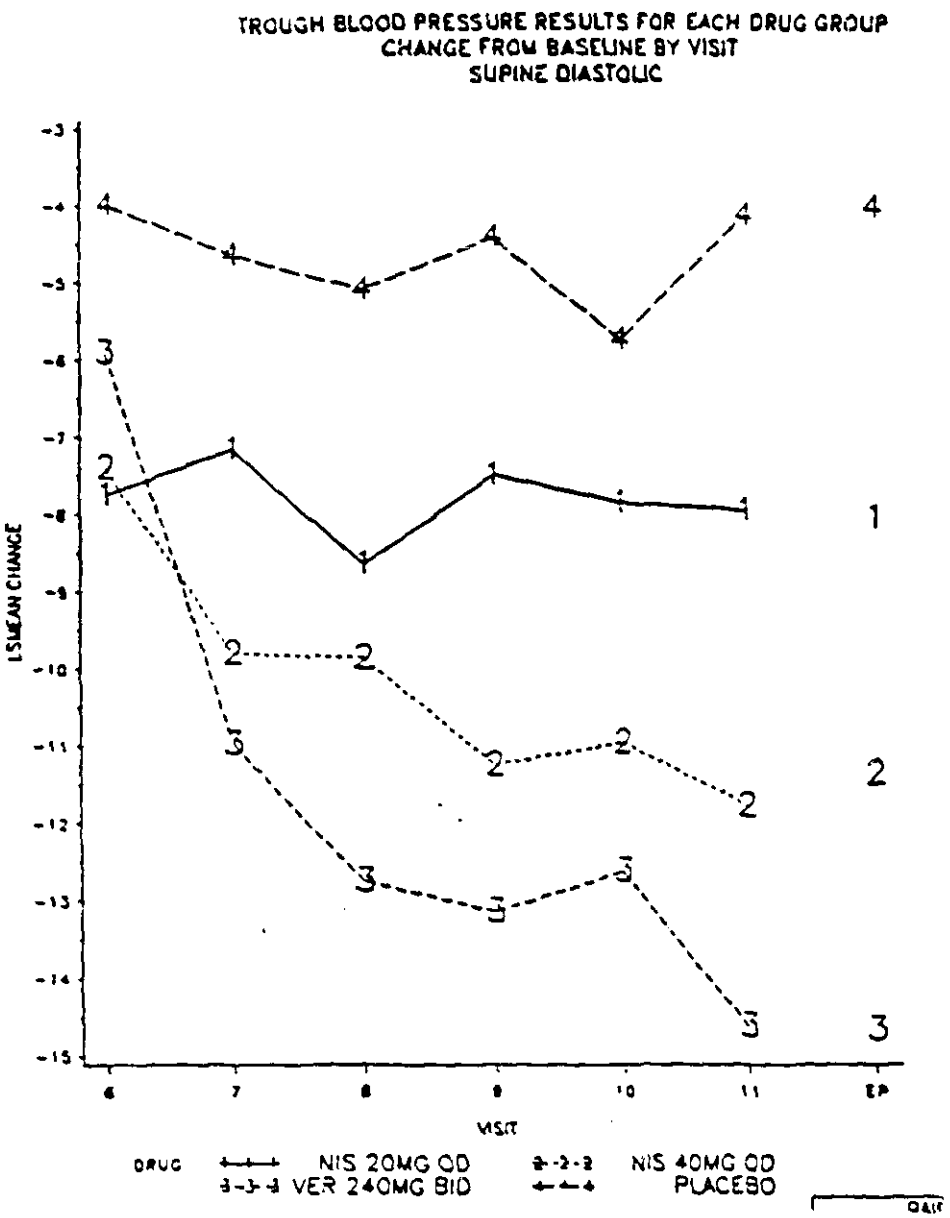




Legend

E-E-E Nis 20mg 4-4-4 Nis 40mg V-V-V Verapamil P-P-P Placebo

The trough blood pressure results for each drug group change from baseline by visist supine diastolic is given in the following graph :



Pharmacokinetic Results. Trough blood samples were drawn at visits 5 and 11. Visit 11 samples were analyzed for Nisoldipine and results are summarized below :

	n	Range of Concentrations (ng/ml)	Mean Concentrations (ng/ml)
NIS 20 mg	66	0-3.19	1.0
NIS 40 mg	61	0-6.83	2.2
NIS 80 mg	3	0-5.24	2.3

Assessment. The study was initially designed to determine the effectiveness of Nisoidipine at doses of 20, 40, 80 mg, Verapamil and placebo. The 80 mg dose of Nisoldipine was dropped when in another study of a high-dose forced-titration study of Nisoldipine 120 mg daily showed asymptomatic T waves flattening and/or inversion on electrocardiogram predominantly at doses above 60 mg daily.

The 20 and 40 concentrations of Nisoldipine demonstrated to be more effective in lowering the blood pressure than placebo, and the 40 mg more effective than the 20 mg. Also the effectiveness was greater in subjects older than 60 years especially in lowering the systolic blood pressure. Verapamil bid was more effective in lowering blood pressure than any of the concentrations of Nisoldipine.

Peak and trough values were determined by ambulatory blood pressure monitoring and the antihypertensive effect was well sustained at 24 hours after dose administration in all concentrations of Nisoldipine evaluated in this study.

By pharmacokinetic studies the concentration of Nisoldipine in blood was determined and was found to be more elevated after the 40 mg administrations of Nisoldipine than after the 20 mg concentration. There was no major difference between the 40 mg and 80 mg dose of Nisoldipine.

Protocol D90-006

Title of Study : " South-African Multicentre Study to Investigate the Anti-Hypertensive Effect of Three Single Oral Daily Doses of Nisoldipine Administered as a Long Acting "Coat-Core" Tablet Formulation."

Principal Investigators and Sites of Investigation.

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Objectives. The objectives of this study were :

1. To compare the anti-hypertensive efficacy and safety of three daily doses of Nisoldipine coat-core formulation, namely 10 mg, 20 mg and 30 mg with placebo.

2. To study a dose-response relationship for Nisoldipine coat-core.

3. To assess the consistency of anti-hypertensive response over 6 weeks.

Additional objectives were :

1. To describe the blood pressure profile of the last day of therapy by continuous automated ambulatory blood pressure monitoring in a group of patients, and hence :

2. To quantify the trough/peak blood pressure relationship for this therapy.

Inclusion Criteria. Patients with newly diagnosed mild to moderate hypertension were eligible to enter the study. In addition patients with mild to moderate hypertension being treated who, in the opinion of the investigator, were no significantly placed at risk by withdrawal of previous anti-hypertensive medication during the 4-week placebo run-in period could also be enrolled in the study.

Exclusion Criteria. Patients were not eligible if they had labile hypertension, clinical evidence of major arrhythmias, angina pectoris, conductio disturbances or heart failure, or recent or impending myocardial infarction, or a cerebral vascular accident in the previous 3 months, history of allergy to dihydropyridines, type 1 diabetes mellitus, impaired renal function, liver disease, elevated transaminases, treatment with antihypertensives or any other drug that may affect the blood pressure or may interact with the effects of calcium antagonists.

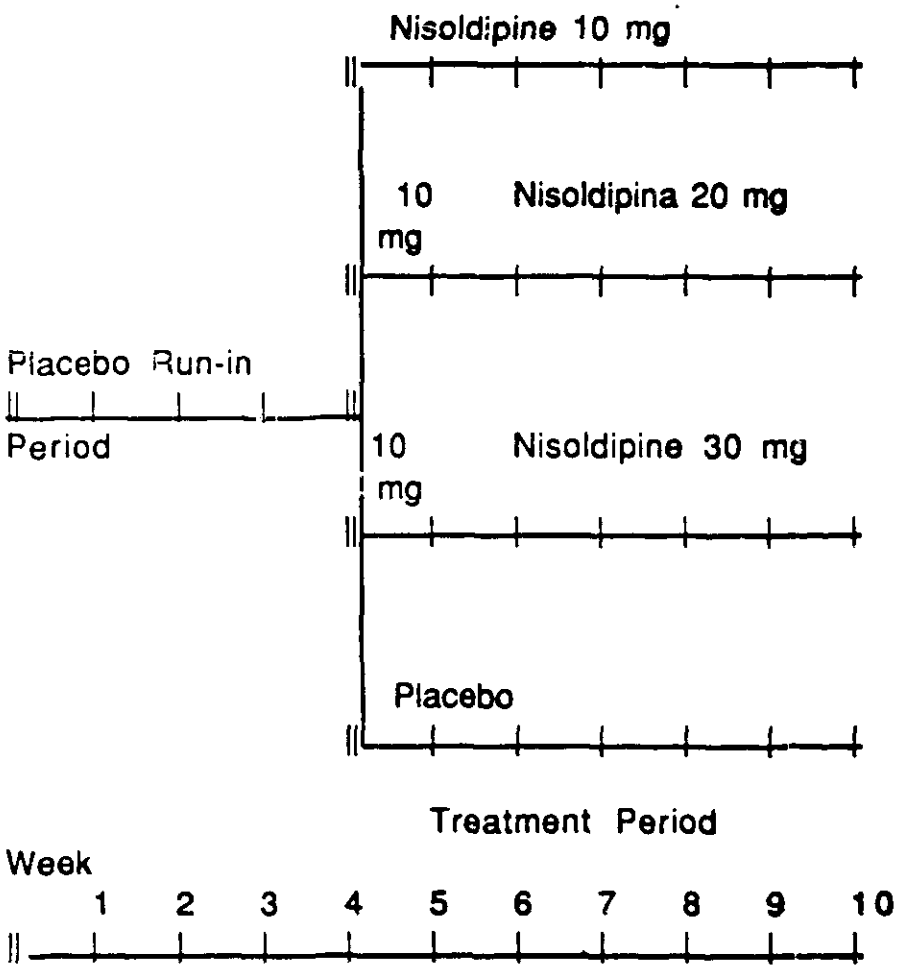
Study Design. This was a 10 week, multi-centre, randomized, placebo controlled, parallel group comparison of Nisoldipine coat-core 10 mg, 20 mg, 30 mg versus placebo. The study consisted of two periods : a single-blind placebo run-in period and a double-blind, randomized, placebo-controlled, group comparison (treatment period.

Placebo run-in Period. During this period of 4 weeks duration all antihypertensive medication was discontinued and one placebo tablet was given to be taken in the morning before breakfast. Patients whose SUDBP was $\geq$ 95 mmHg and $\leq$ 114 mmHg at visits 2 and 3 were eligible for enrollment in the active treatment phase.

Treatment Period. Eligible patients were randomized to one of four arms : placebo, 10 mg Nisoldipine, 20 mg Nisoldipine and 30 mg Nisoldipine.

Patients randomized to placebo or 10 mg Nisoldpine were to receive their treatment for 6 weeks. Patients in the two higher dose groups (Nisoldipine 20 mg or Nisoldipine 30 mg) were to receive 10 mg for the first week following by 5 weeks of their randomized treatment in order to avoid rapid exposure to the higher doses.

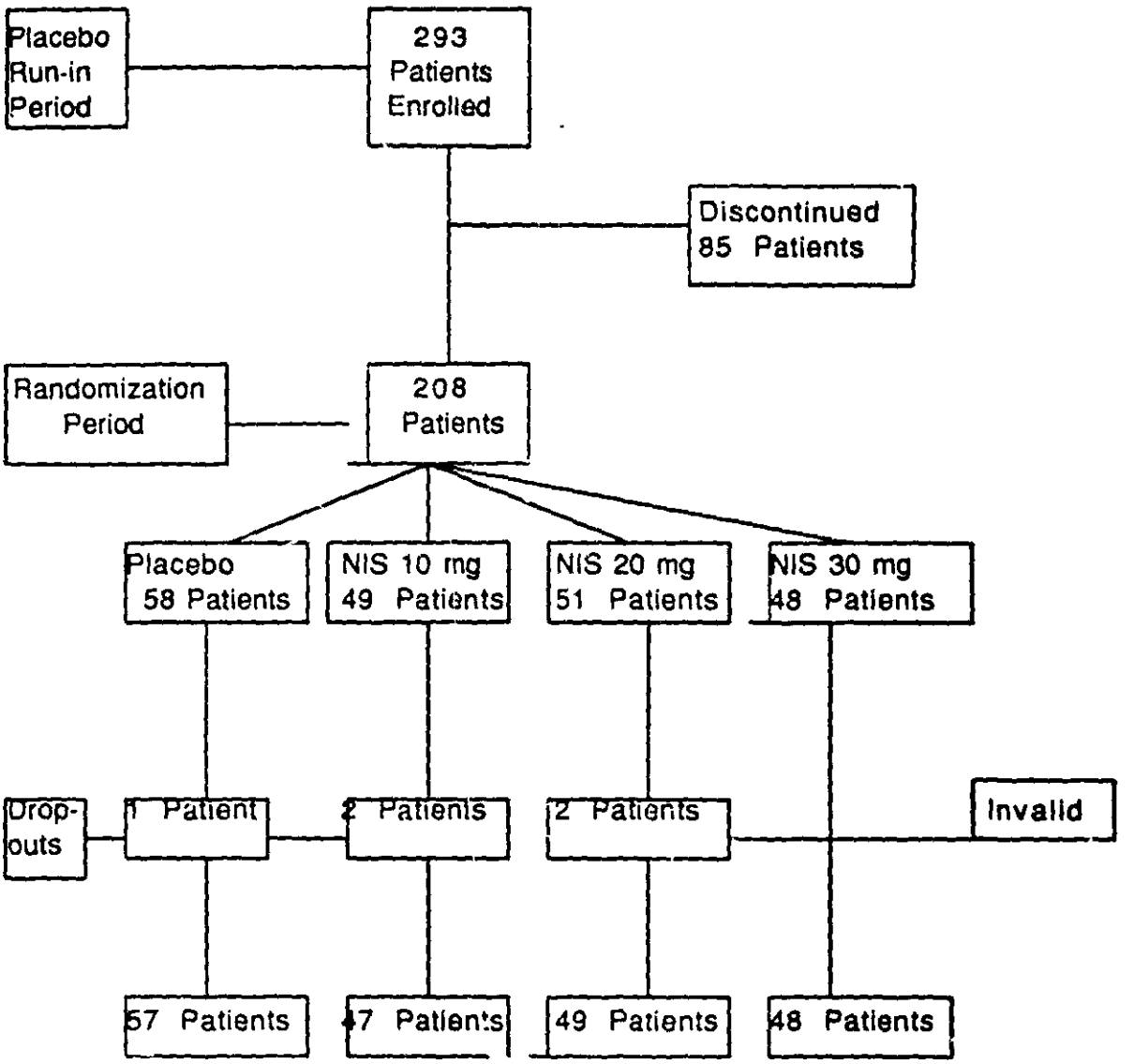
The study design is demonstrated schematically in the following graph :



The demographic information is given in the following table :

		Placebo n=58	NIS 10 mg n=49	NIS 20 mg n=51	NIS 30 mg n=48
Sex (p=0.78)	Male Female	27 (47 %) 31 (53 %)	24 (49 %) 25 (51 %)	20 (39 %) 31 (61 %0	21 (44 %) 27 (56 %)
Race (p=0.98)	Caucasian Black Asian Other	30 (52 %) 27 (29 %) 6 (20 %) 5 (9 %)	24 (53 %) 16 (33 %) 2 (4 %) 5 (10 %)	25 (49 %) 18 (35 %) 6 (12 %0 2 (4 %)	26 (54 %) 14 (29 %) 5 (11 %) 3 (6 %)
Age (years) Mean	Mean Mean=0.2	53	50	55	50
Weight (kg)	Mean (p=0.69)	80.8	77.3	79.7	80.3
Baseline Means BP Supine Systolic Diastolic Standing Systolic Diastolic	 (p=0.17) (p=0.65) (p=0.69) (p=0.09	 163.8 103.5 160.5 105.1	 161.2 104.7 159.7 107.9	 167.2 104.8 163.8 107.4	 164.3 104.4 161.7 107.4
Mild Hypert. n Baseline SDBP Moder. Hypert. N Baseline SDBP		 33 99.7 25 108.5	 25 99.3 24 110.2	 25 99.2 26 110.1	 27 100.1 21 110.1

The distribution and randomization of patients is illustrated in the following graph :



The reasons for patients who did not enter the double-blind treatment period is given in the following table :

Reason	Number of Patients
Supine diastolic blood pressure < 95 mmHg	58
Supine diastolic blood pressure > 114 mmHg	13
Unwilling to continue	5
Patient had raised serum calcium levels	1
Uncontrolled non-insulin dependent diabetes mellitus	1
Raised liver enzymes	3
Left ventricular failure	1
Right ventricular failure when taken off diuretic	1
Major arrhythmias	2
Total	85

Invalid Results and Drop-outs During the Treatment Period. Three patients dropped-out during the treatment period. One patient in the placebo group died after experiencing cerebral hemorrhage 33 days after entering the double-blind treatment period. One patients in the Nisoldipine 10 mg experienced severe tinnitus 30 days after entering the double-blind treatment period. Another patient in the 10 mg Nisoldipine group had a severe headache and dropped 17 days after entering the double-blind treatment period.

Efficacy.

Criteria for Efficacy. The primary variable for assessing efficacy was the trough 24-hour supine diastolic blood pressure (SUDBP), and especifically the change in suDBP from baseline to endpoint (visit 6, week 6 or the last valid visit). The change from baseline in each of the three Nisoldipine treatment groups was compared to the Placebo group. Secondary efficacy variables were supine systolic BP and standing diastolic and systolic blood pressure.

Statistical Analysis. Two types of analysis were followed. The first and primary analysis was the standard endpoint analysis, also referred as the main efficacy analysis. The second was the intent-to treat analysis (ITT).

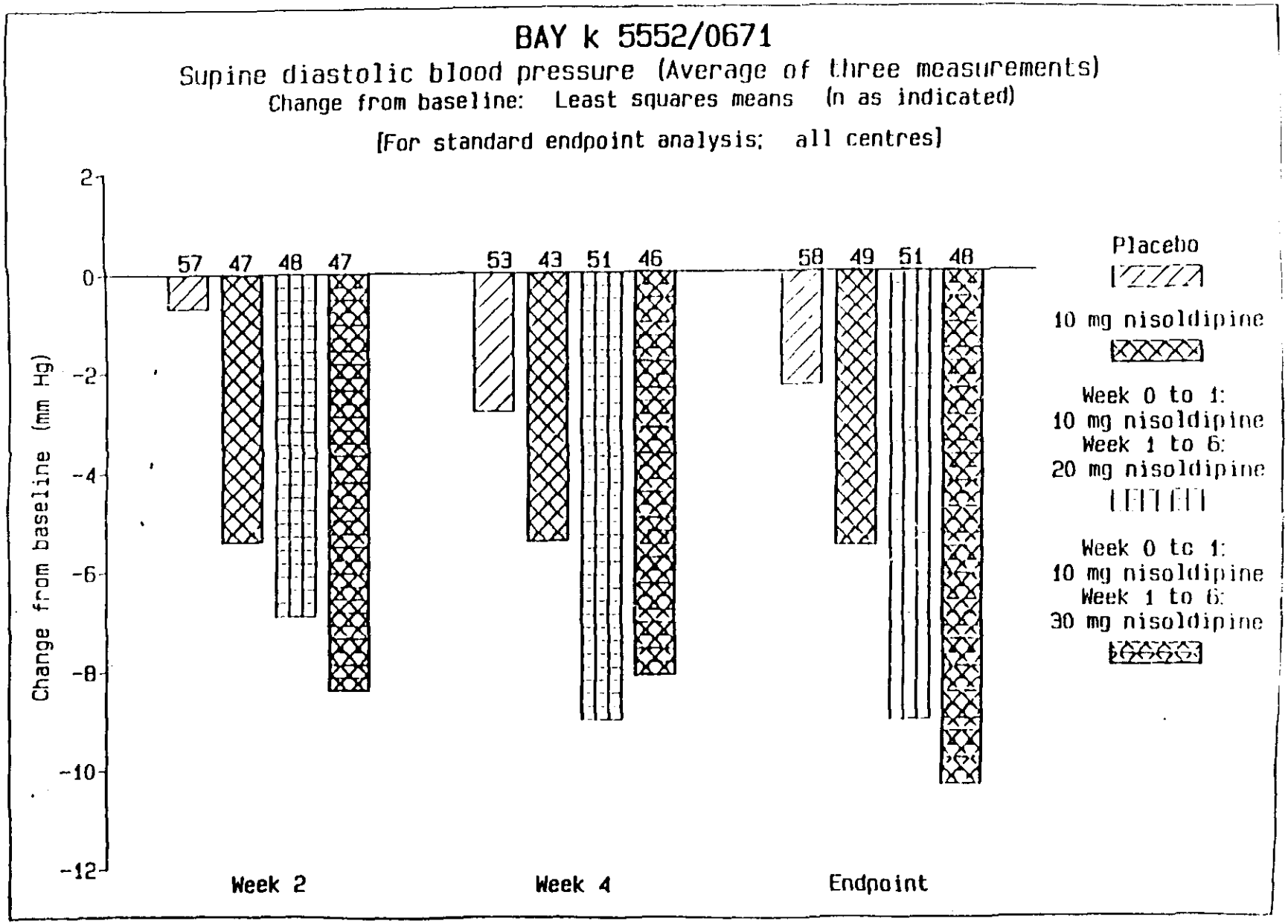
All patients adherent to the protocol with a valid treatment duration of at least 2 weeks on double-blind treatment were included in the main efficacy analysis. These patients completed at least a two-week double-blind treatment period during which they were compliant. and after which the blood pressure was taken between 22.5 h and 25.5 h after the last tablet intake. Patients who discontinued treatment because of lack of efficacy or adverse events were also included. Only 2 patients who received double-blind treatment were considered invalid for the main efficacy

analysis. They were included in the intent to treat analysis. In one patient the baseline measurements were lost
and in another patient only 10 tablets instead of 20 tablets were dispensed.

The results of change from baseline at endpoint in trough blood pressure in all patients valid for the main efficacy analysis (n=206) are given in the following table :

	Placebo n=58	NIS 10 mg n=49	NIS 20 mg n=51	NIS 30 mg n=48
Supine DBP				
Baseline	103.5	104.7	104.8	104.4
Endpoint	101.1	99.3	95.7	94.3
Difference (NIS-Placebo)		-3.2	-6.7	-8.0
Supine SBP				
Baseline	163.8	167.2	167.2	164.3
Endpoint	163.3	149.8	149.8	148.8
Difference (NIS-Placebo)		-8.9	-17.8	-15.9
Standing DBP				
Baseline	105.1	107.9	107.4	107.4
Endpoint	104.6	101.1	98.1	96.9
Difference (NIS-Placebo)		-6.9	-9.2	-10.6
Standing SBP				
Baseline	160.5	159.7	163.8	161.7
Endpoint	160.5	150.6	147.5	145.7
Difference (NIS-Placebo)		-9.5	-15.9	-16.2

The results on SDBP are demonstrated in the following graph :


BAY k 5552/0671
Supine diastolic blood pressure (Average of three measurements)
Change from baseline: Least squares means (n as indicated)
[For standard endpoint analysis; all centres]
Change from baseline (mm Hg)
2
0
-2
-4
-6
-8
-10
-12
57 47 48 47
53 43 51 46
58 49 51 48
Week 2
Week 4
Endpoint
Placebo
10 mg nisoldipine
Week 0 to 1:
10 mg nisoldipine
Week 1 to 6:
20 mg nisoldipine
Week 0 to 1:
10 mg nisoldipine
Week 1 to 6:
30 mg nisoldipine

The mean change from baseline in supine diastolic pressure (mmHg) for each treatment group after stratification for age is shown in the following table : (Main efficacy analysis = 206).

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg	Overall Least Square Means
Age < 45 years	-6.5 (12)*	-4.1 (14)	-10.6 (11)	-8.9 (15)	-7.5
Age≥ 45 and < 65 years	-1.4 (38)	-6.9 (28)	-8.3 (31)	-10.7 (27)	-6.8
Age ≥ 65 years	-1.3 (8)	-2.0 (7)	-10.1 (9)	-10.7 (6)	-6.0

* The number of patients used for calculating the mean values are given in brackets.

Results from ANOVA

Age effect : p=0.62
Treatment Effect : p=0.0001
Treatment by age interaction effect : p=0.12.

These results indicate that there is no association between age and the diastolic blood pressure response.

The mean change from baseline in supine diastolic blood pressure (mmHg) for each treatment group after statification for race is given in the following table : (Main efficacy analysis = 206).

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg	Overall Least Square Means
Caucasian	-1.2 (30)*	-4.6 (26)	-7.5 (25)	-9.2 (26)	-5.6
Black	-2.3 (17)	-5.9 (16)	-10.3 (18)	-10.1 (14)	-7.2
Other	-6.3 (11)	-7.0 (7)	-11.5 (8)	-13.1 (8)	-9.5

Analysis of Response and Normalization Rates. Responders were defined as patients who had SDBP of less than or equal to 90 mmHg or patients who had a drop in SDBP of at least 10 mmHg at endpoint. A patient's blood pressure was said to be normalized when satisfied these two conditions, namely, a drop in supine DBP to 90 mmHg or below, and a drop of at least 10 mmHg.

The following table shows the response rates for each treatment group, odds ratio and relative efficacy of each Nisoldipine treatment relative to Placebo from the main efficacy analysis of the last visit:

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg
Total number of Patients Responders Response Rate	58 10 17 %	49 17 35 %	51 24 47 %	48 30 63 %
Odds Ratio (OR) NIS relative to Placebo 95 % CI for OR		2.4 1.0 ; 5..5	4.6 1.8 ; 12	8.8 3.6 ; 22

Relative Efficacy (RE) NIS relative to Placebo 95 % CI for RE		2.0 1.0 ; 3.9	2.6 1.5 ; 4.8	3.7 2.1 ; 6.2

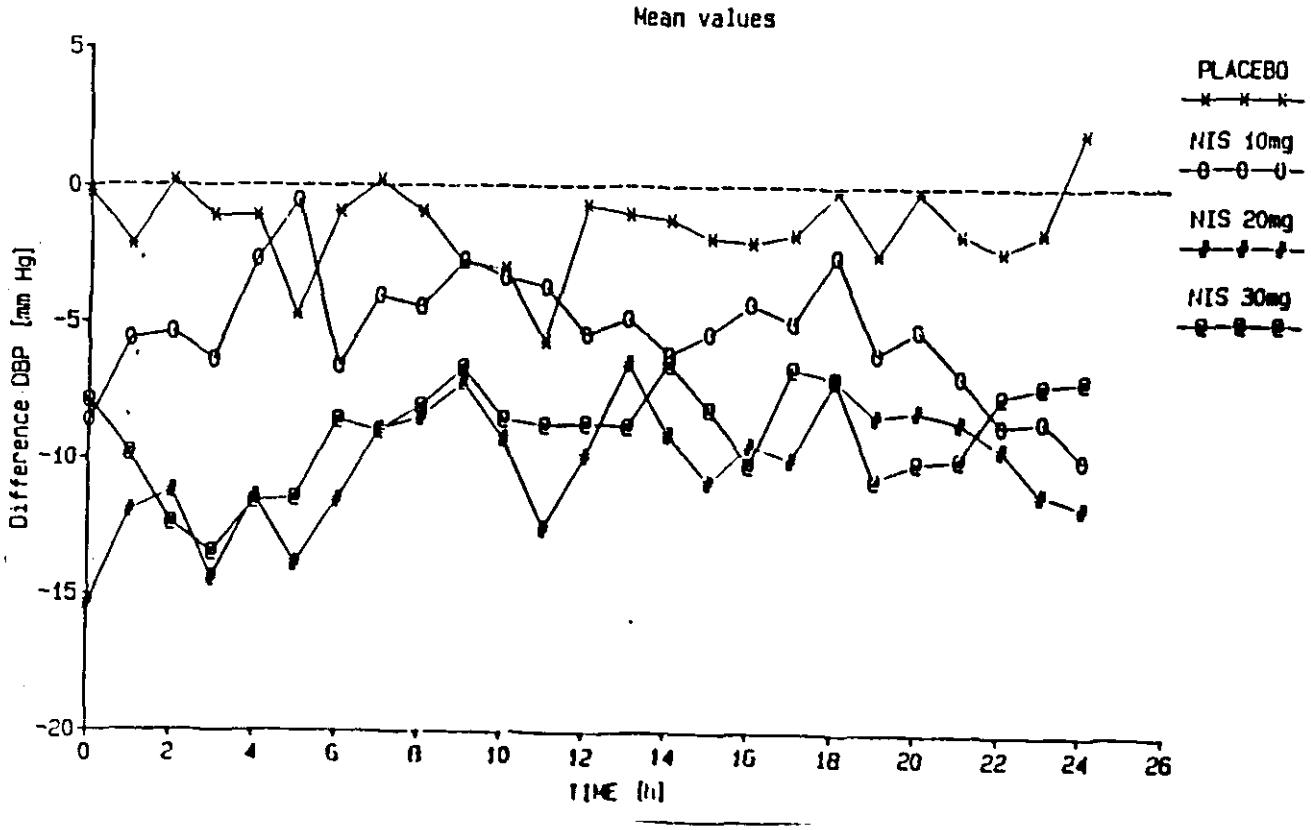
These results can be interpreted as indicating that the response rate for placebo was 17 %, Nisoldipine 10 mg 35 %, Nisoldipine 20 mg 47 % and Nisoldipine 30 mg 63 %. A relative efficacy of 2.6 of Nisoldipine 20 mg vs Placebo means that a positive treatment response is 2.6 times more likely to occur under Nisoldipine 20 mg than placebo. The confidence interval of 1.5 to 4.8 indicates that the true relative efficacy is likely (95% confidence limits) to be at least 1.5 and at most 4.8.

The following table shows the normalization rates for each treatment group, odds ratio, and relative efficacy of each Nisoldipine treatment relative to Placebo from the main efficacy analysis of the last visit :

	Placebo	NIS 10 mg	NIS 20 mg	NIS 30 mg
Total Number of patients	58	49	51	48
Number of Patients Normalization Rate	5 8.6 %	5 10 %	13 25 %	13 27 %
Odds Ratio (OR) NIS relative to Placebo 95% CI for OR		1.2 0.31 ; 4.8	4.3 1.4 ; 1.3	4.3 1.4 ; 13
Relative Efficacy (RE) NIS relative to Placebo 95 % CI for RE		1.2 0.37 ; 3.9	3.1 1.3 ; 7.3	3.3 1.3 ; 8.1

These results can be interpreted in the same manner as described for the response rates.

Analysis of Ambulatory Blood Pressure Monitoring. Of the 165 patients who entered the ambulatory blood pressure monitoring phase of the study 137 patients were evaluable. The means across patients (change from baseline in diastolic blood pressure) are graphically presented in the following figure



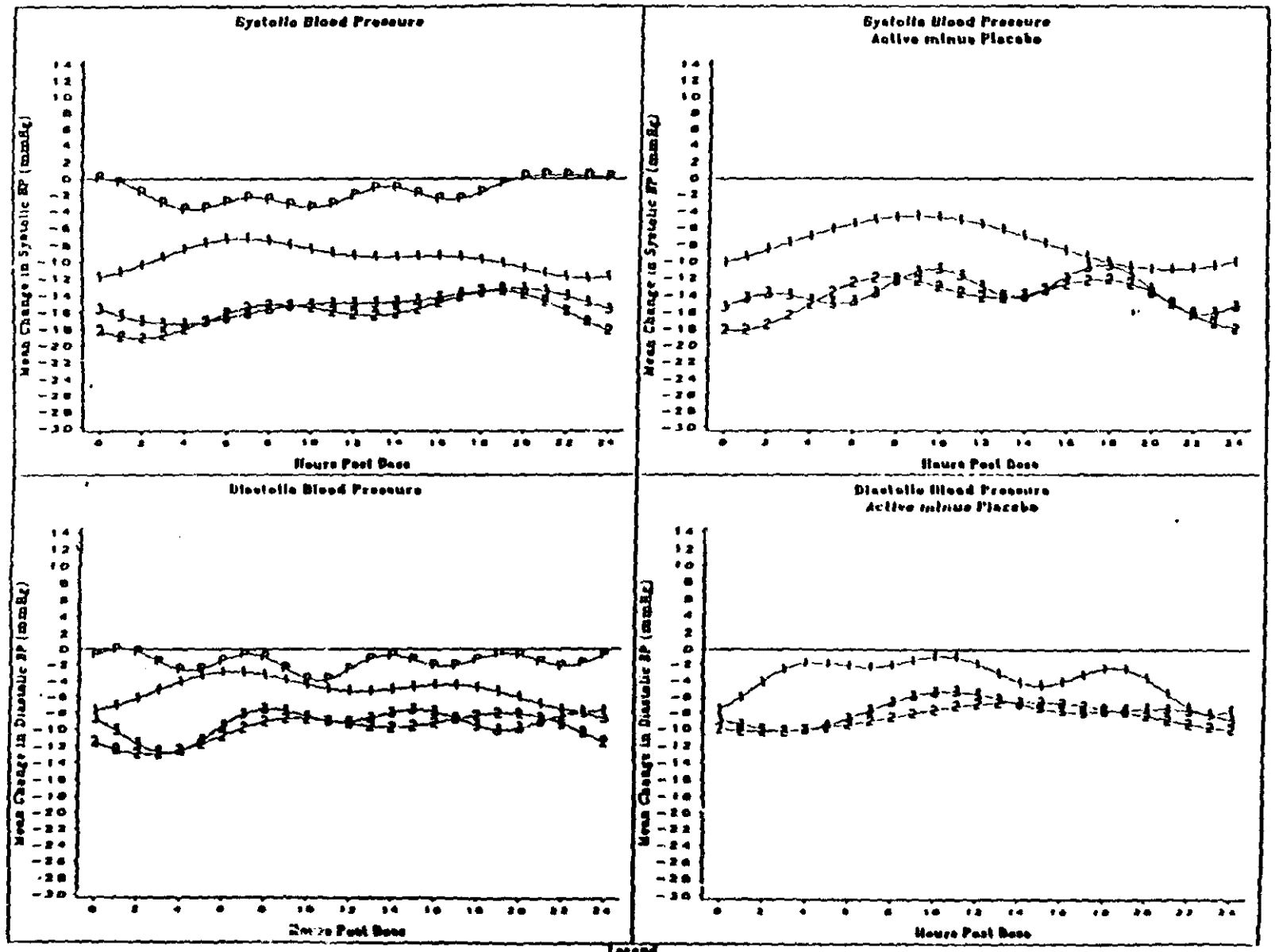
Various clinically meaningful variables could be calculated from the hourly mean diastolic blood pressure profiles The following table shows results of trough/peak ratios calculated from hourly means of ambulatory monitoring data :

	Trough (mmHg)	Peak (mmHg)	Hour of Peak	Trough to peak Ratio
Diastolic BP				
NIS 10 mg.	-11.95	-11.95	24	100 %
NIS 20 mg	-13.70	-13.70	24	100 %
NIS 30 mg	-9.03	-12.57	2	72 %
Systolic BP *				
NIS 10 mg	-15.70	-15.70	24	100 %
NIS 20 mg	-20.72	-20.72	24	100 %
NIS 30 mg	-18.31	-18.31	24	100%
Systolic BP#				
NIS 10 mg	-15.70	-15.70	24	100 %
NIS 20 mg	-20.72	-20.72	24	100%
NIS 30 mg	-18.31	-10.64	2	172%

* Using timepoint of systolic peak.
# Using timepoint of diastolic peak

The results indicate that there was a good dose-response pattern in both systolic and diastolic blood pressure falls from baseline for placebo Nisoldipine 10 and 20 mg while the fall of Nisoldipine 30 mg was very similar to that in the 20 mg group. The effect of the 3 Nisoldipine group was maintained over the entire dosing period. This is also in evidence by observing the following graph of hourly means in a smoothed curve :

Smooth of Mean ange from Baseline of Ambulatory Blood P sure



Assessment. This study demonstrated that Nisoldipine, at concentrations of 10 mg, 20 mg and 30 mg, was more effective than placebo in lowering the blood pressure, but this effect was not potentiated when the dose was increased from 20 to 30 mg. Ambulatory blood pressure measurements were done which demonstrated that the effectiveness of Nisoldipine extended throughout the 24 hours after administration , trough values frequently being equal to peak values.

It is interesting that in this study this calcium channel blocker demonstrated to have a greater effectiveness in blacks, a patients population usually more refractory to antihypertensive treatment, than in caucasians.

In reference to age, this study concluded that Nisoldipine was more effective in individuals 65 years of age or older. (table page 73). This finding is consistent with those of protocol D89-039 in which Nisoldipine was more effective in this age range especially in lowering systolic blood pressure. (table page 60).

Protocol D88-054

Title of Study : " Comparative Double-Blind Pilot Study of the Safety and Efficacy of Once Daily Doses of Nisoldipine 10, 20, 30 mg Core-Coat Tablets vs Placebo in Hypertensive Patients ".

Principal Investigators and Sites of Investigation :

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Objectives. The objectives of this study were :

1. To test whether Nisoldipine core-coat given 10 mg, 20 mg, 30 mg once daily lowers the blood pressure significantly more than placebo at the end of 24-hour dosing interval (trough).

2. To record blood pressure and pulse rates for four hours after the first dose of double-blind drug to monitor patient response to acute administration of the drug.

3. To determine peak response and calculate ratios of trough to peak effect by 24-hour ambulatory blood pressure monitoring.

Inclusion and Exclusion Criteria. Male or female patients, 21 to 70 years of age, with a history of mild to moderate essential hypertension and a mean supine diastolic blood pressure of 95 to 114 mmHg after three and four weeks of placebo were eligible for the study.

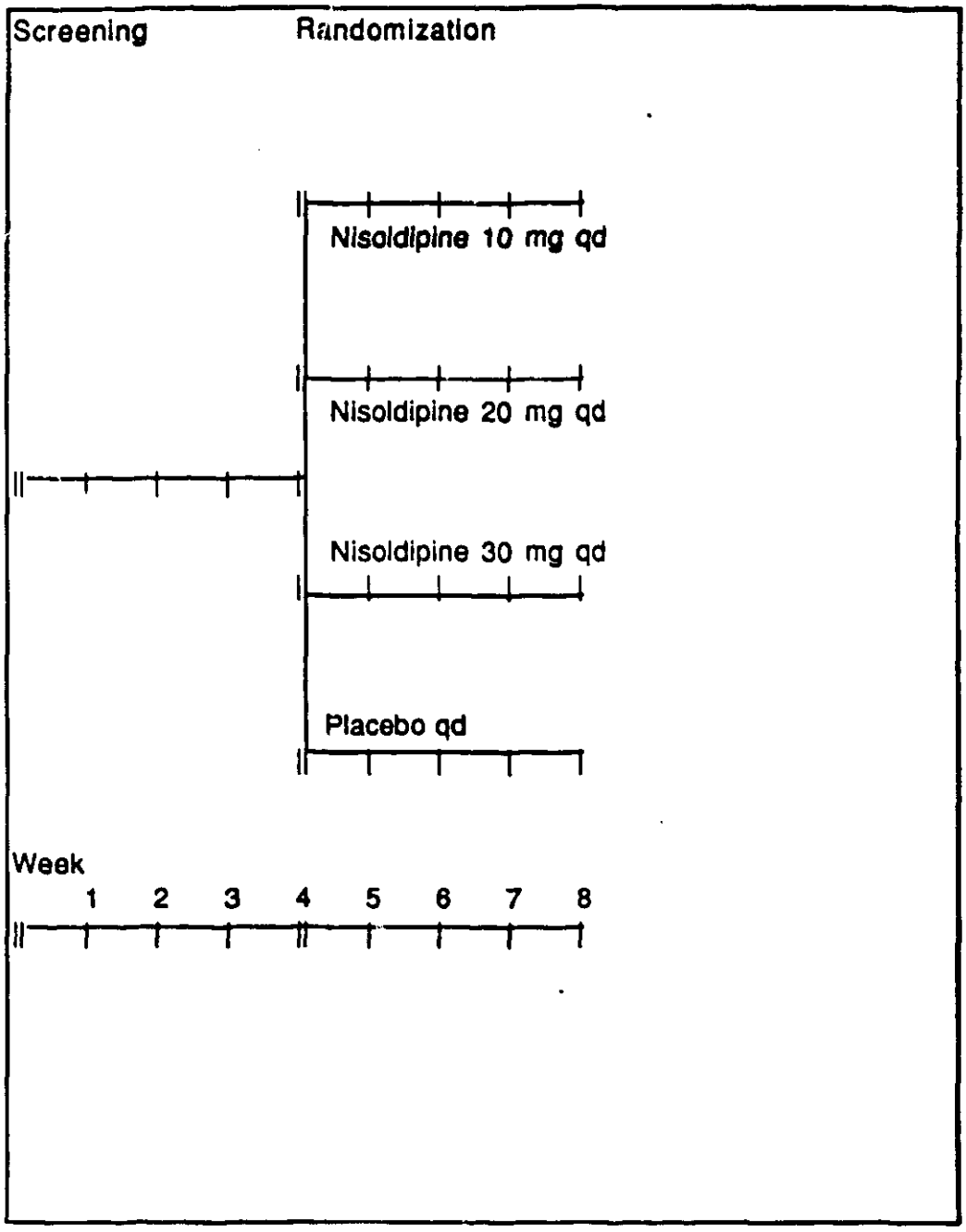
Excluded from the study were patients with labile hypertension, a change in supine diastolic blood pressure greater than 7 mmHg between the last 2 placebo run-in visits, impaired renal or liver function, recent or impending myocardial infarction, or cerebral vascular accident, angina pectoris or intermittent claudication, heart failure, major arrhythmias, conduction disturbance, failure of a major organ system, severe infection, malignancy, psychosis, chronic diarrhea, ulcerative colitis, regional enteritis, diverticulitis, partial or complete gastrectomy or small bowel resection, history of allergy to dihydropiridines, pregnant women or those with childbearing potential and patients known to abuse alcohol or drugs.

Study Design. This was a randomized, double-blind, parallel group, placebo controlled study of eight weeks duration consisting of a screening period and a randomization treatment period.

Screening Period. During this period of 4 weeks duration patients discontinued all previous antihypertensive medication and were given a single-blind placebo once daily. Those patients with a mean supine diastolic pressure ≥ 95 mmHg to ≤ 114 mmHg after three to four weeks of placebo and within 7 mmHg at both visits were transferred to the treatment period.

Randomization Period. Patients were randomized to receive either Nisoldipine 10 mg qd, Nisoldipine 20 mg qd, Nisoldipine 30 mg qd or Placebo qd for four weeks.

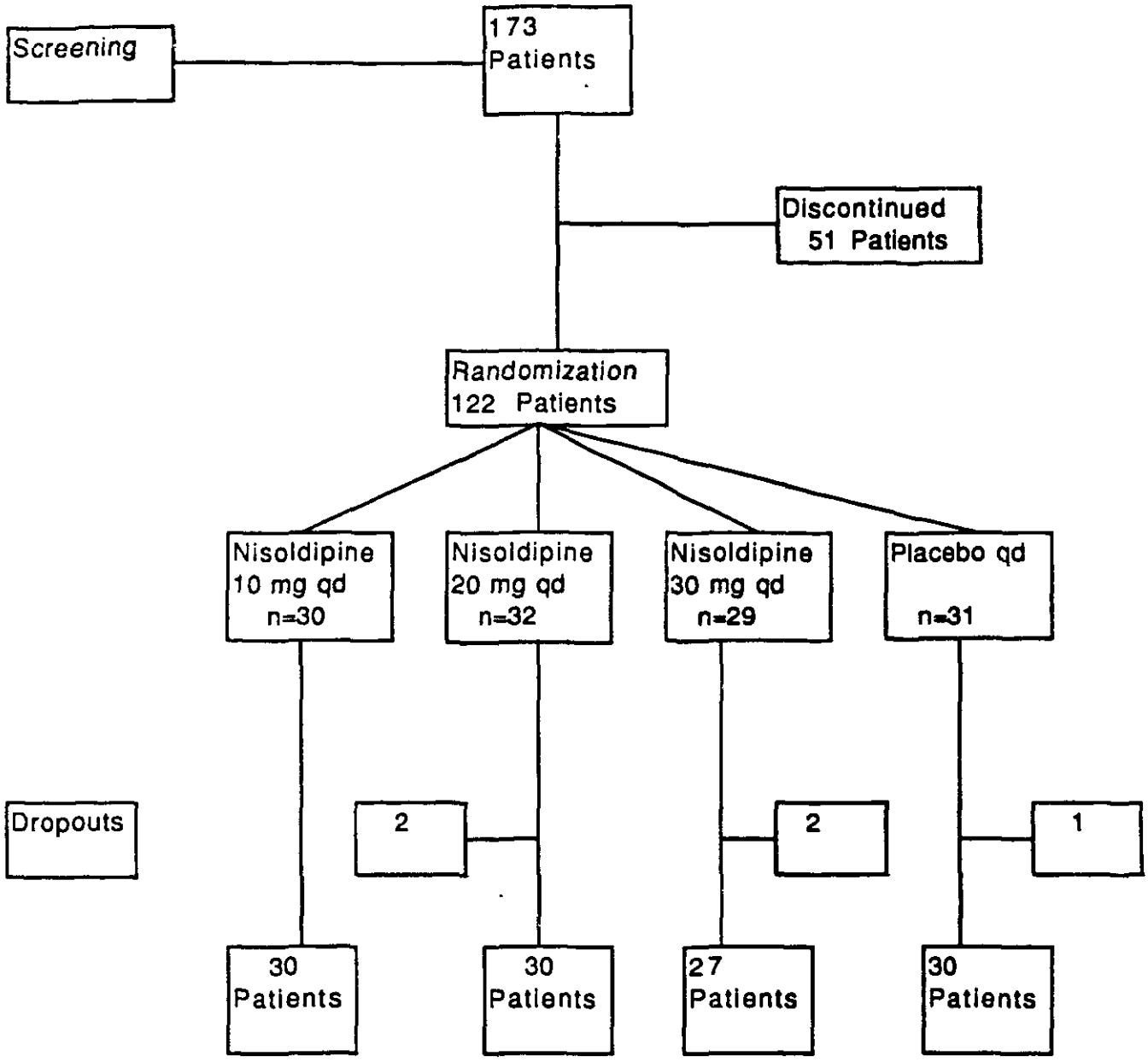
The study design is demonstrated schematically in the following graph :


Screening
Randomization
Nisoldipine 10 mg qd
Nisoldipine 20 mg qd
Nisoldipine 30 mg qd
Placebo qd
Week
1 2 3 4 5 6 7 8

Demography. The demography and baseline characterisitics are given in the following table :

		Nisoldipine 10 mg n=30	Nisoldipine 20 mg n=30	Nisoldipine 30 mg n=29	Placebo n=30
Sex	Male Female	20 (67 %) 10	19 (63 %) 11	19 (66 %) 10	21 (70 %) 9
Race	Caucasian Black Hispanic	23 (77 %) 4 3	23 (77 %) 7 0	20 (69 %) 9 0	23 (77 %) 7 0
Age (years)		56	53	52	51
Weight (lbs)		186	200	207	190
Baseline Blood Pressure mmHg	Supine Standing	146/99 144/100	147/99 144/100	145/99 144/100	148/100 145/101

The distribution of patients and randomization are given in the following graph :


Screening
173 Patients
Discontinued 51 Patients
Randomization 122 Patients
Nisoldipine 10 mg qd n=30
Nisoldipine 20 mg qd n=32
Nisoldipine 30 mg qd n=29
Placebo qd n=31
Dropouts
2
2
1
30 Patients
30 Patients
27 Patients
30 Patients

The reasons that disqualified enrolled patients for randomization are given in the following table :

Reasons for disqualification	Patients
Supine diastolic blood pressure < 95 mmHg	21
+ 7 mmHg difference in supine diastolic blood pressure (visits 4 and 5)	3
Supine diastolic blood pressure >114 mmHg	4
Unable to make scheduled visits	3
Illness not due to study medication	4
Lost to follow-up	2
Abnormal laboratory values	3
Non-compliance	2
Systolic blood pressure above acceptable limit	1
High blood pressure readings during ambulatory monitoring	1
Chest pain at visit 1	1
Chose to withdraw	1
Total	46

The reasons for dropping out during the double-blind randomization period are given in the following table :

Drug Group	Final visit	Days on Drug	Reasons for dropping-out- Severity Drug Relationship
Placebo	7.0	11	Dizziness-Moderate Probable
Nisoldipine 20 mg	6.0	5	Intolerance to all-night visits
	5.5	5	Shortness of breath-Cough Mild-Probable
Nisoldipine 30 mg	8.0	23?	Noncompliance
	6.0	7	Flushing-Severe-Probable

Efficacy

Criteria for Effectiveness. The change in trough supine diastolic blood pressure from baseline to endpoint compared among the groups was the primary efficacy criterion. Endpoint was defined as the last valid visit on the double-blind drug for each valid patient. Patients were to be included in this efficacy analysis if trough blood pressure data was available after at least 7 days on double-blind drug.

Statistical Methods. All tests of significance were performed as two-tailed tests with alpha + 0.05 unless stated otherwise.

Analysis of Efficacy. The mean blood pressures by visit for all patients valid for analysis of efficacy are given in the following table :

Week Post Randomization — Supine Blood Pressure

	Nisoldipine 10 mg Sys/Dia (n)	Nisoldipine 20 mg Sys/Dia (n)	Nisoldipine 30 mg Sys/Dia (n)	Placebo Sys/Dia (n)
Week-1	143/99 (30)	145/99 (30)	144/99 (29)	145/99 (30)
Week 0	145/99 (30)	146/98 (30)	144/98 (29)	147/100 (30)
Week 1	136/91 (30)	136/90 (30)	136/91 (29)	141/95 (30)
Week 2	140/91 (30)	139/92 (29)	133/90 (28)	140/94 (30)
Week 3	138/91 (30)	136/91 (30)	134/89 (28)	142/95 (29)

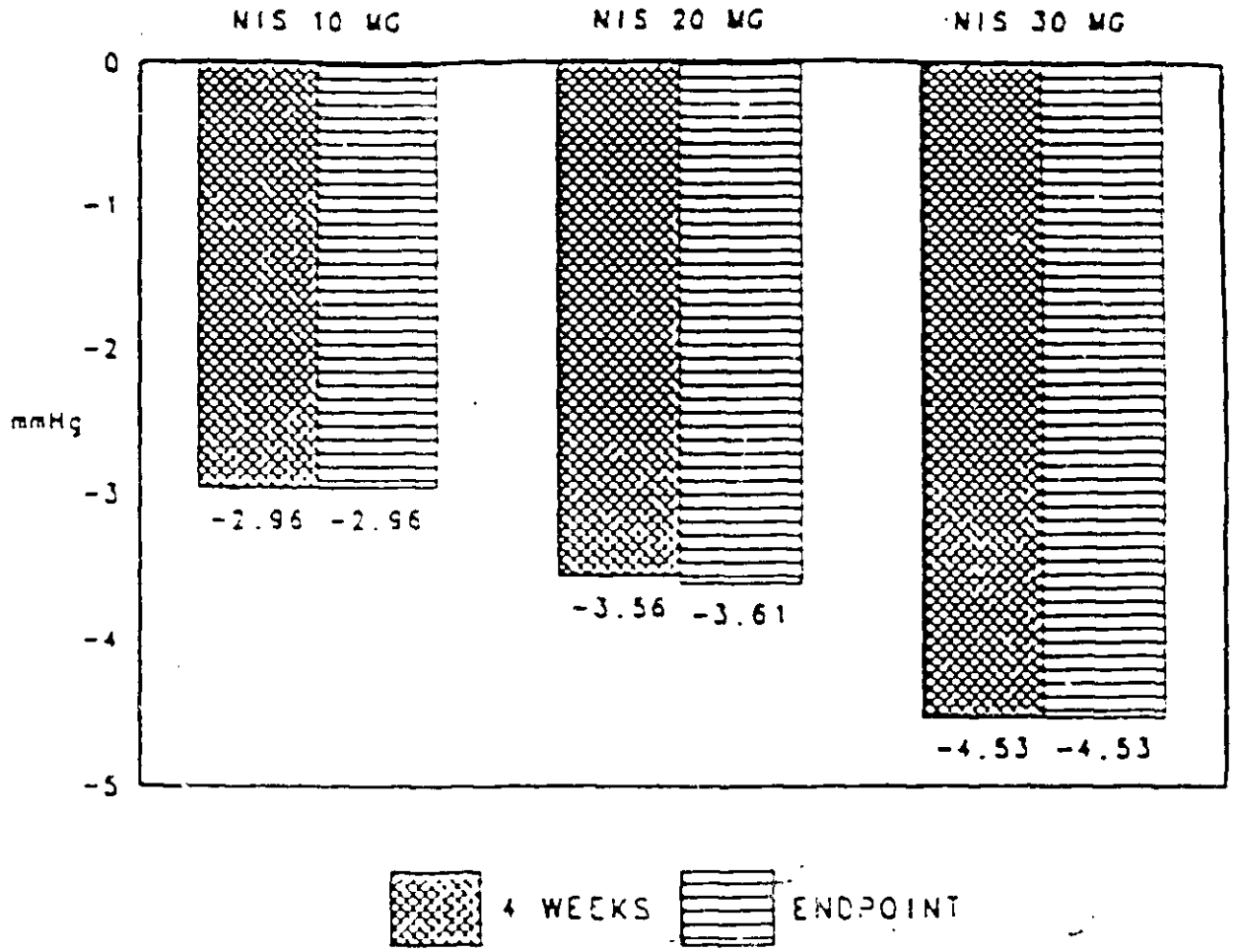
Week 4	137/90 (30)	134/90 (30)	133/89 (27)	144/94 (30)
Endpoint	137/90 (30)	134/90 (30)	133/89 (30)	144/94 (30)

In the following table, the results of the analysis at endpoint are summarized :

	Nisoldipine 10 mg n=30	Nisoldipine 20 mg n=30	Nisoldipine 30 mg n=29	Placebo n=30
Supine				
Systolic	8.4	11.5*	10.7*	3.0
Diastolic	8.3	8.9*	9.9*	5.3
Standing				
Systolic	8.3	11.8*	10.7*	3.4
Diastolic	6.2	7.3	7.0	5.1

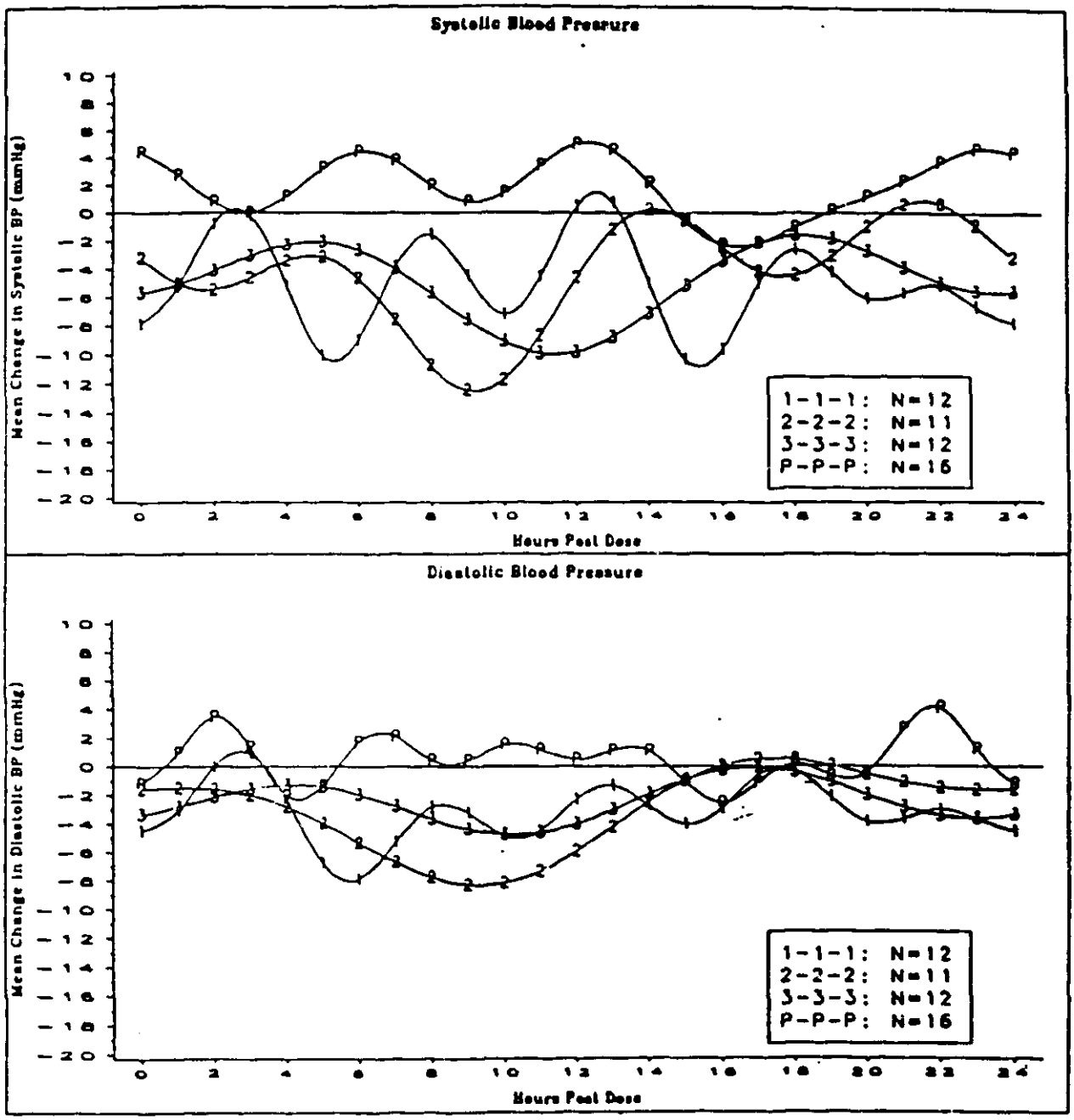
*Significant difference from the placebo group $p<0.05$

The change in trough supine diastolic blood pressure at 4 weeks and endpoint, placebo subtracted, are shown in the following graph :



Ambulatory monitoring and supine in-clinic blood pressures were smoothed and results are demonstrated in the following graph :

SMOOTH OF MEAN CHANGE FROM BASELINE OF AMBULATORY BLOOD PRESSURE



Legend

1-1-1 NIS 10mg
2-2-2 NIS 20mg
3-3-3 NIS 30mg
P-P-P Placebo

The Trough/Peak ratios from smoothed ambulatory monitoring data for valid patients are given in the following table :

	Nisoldipine		
	10 mg (n=12)	20 mg (n=11)	30 mg (n=12)
Diastolic	7 %	35 %	68 %
Systolic	92 %	43 %	108 %
Peak hour Post-dose	6	9	8
Nisoldipine levels at trough ng/ml	0.82	1.04	1.49

Assessment. This is a small pilot studied carried in a relatively small number of subjects consisting mostly of middle-age caucasian male obese patients. Although the results on blood pressure with the 10 mg dose of Nisoldipine was not significantly different from placebo the 20 and 30 mg doses were but the effect of both did not seem to be very different from each other.

Other Studies. Other studies were performed in which Nisoldipine was administered to patients with renal disease, to cirrhotic, elderly and young people. The effect of food on drug absorption was also investigated. The effects of combination with other antihypertensive agents was studied in long term extension studies.

Study in Cirrhosis. Protocol M.M.R.R. # 1118

Title of Study. "The Effect of Cirrhosis on the Steady-State Pharmacokinetics of Nisoldipine Coat-Core Sustained-Release Tablets ".

This was a single center, non-randomized, non-blinded, comparison of single dose and steady-state pharmacokinetics of Nisoldipine coat-core tablets in cirrhotic and healthy subjects.

Sixteen subjects participated in the study : 8 cirrhotic and 8 healthy subjects. There were 4 males and 4 females in each group. In stage 1 a

single 10 mg dose of Nisoldipine was administered and in stage 11 10 mg of Nisoldipine was administered qd for 7 days.

Results. Administration of Nisoldipine to patients with cirrhosis resulted in a 3 to 4-fold increase in peak plasma concentration and $AUC_{(024)}$. Nisoldipine had little effect on blood pressure in either group.

Assessment. These results are indicative of possibility that the dose of Nisoldipine may need to be adjusted in patients with cirrhosis.

Study in Renal Disease. Report 5837 (R).

Title of Study. "Influence of Renal Function on the Pharmacokinetics of Nisoldipine CC Tablets After Single and Multiple Dosing ".

This was a multicenter, non-blinded, non-randomized, comparative study among 4 groups to compare the effects of renal function on the pharmacokinetics of Nisoldipine CC after a single dose as well as after achievement of a steady state.

A total of 40 patients were enrolled in 3 centers.. The following groups of patients were enrolled :

1. Control. Nine subjects with creatinine clearance > 90 ml/min/1.73 m^2

2. Mild Renal Failure. Twenty subjects with creatinine clearance 61 ≤ 90 ml/min/1.73 m^2

3. Moderate Renal Failure. Nine subjects with creatinine clearance 30 to ≤ 60 ml/min/1,73 m^2

4. Severe Renal Failure. Seven subjects with creatinine clearance < 30 ml/min/1.73 m^2.

Results. Although there was not a statistically significant difference in the Nisoldipine AUC_{norm} between the groups with impaired renal function and the normal control, in the former an increase in plasma Nisoldipine of approximately 2-fold could not be excluded.

Assessment. An increase in plasma levels of Nisoldipine in patients with impaired renal function may require the adjustment of the dose. There were only modest effects on blood pressure across all groups.

The Factor Age . Report 5857 (P).

Title of Study : "A Study to Determine the Single Dose and Steady-State Pharmacokinetic Profile of Nisoldipine Coat-Core (CC) Tablet 20 mg in Elderly and Young Volunteers and in Elderly Hypertensive ".

This was an open, multiple-dose, non-randomized study. Nisoldipine CC was administered at the dose of 20 mg qd for 7 days. Plasma samples were collected and blood pressure and heart rate were measured.

The following groups of patients were studied :

Young Volunteers. Twenty healthy young volunteers, 18 to 23 years of age, completed the study.

Elderly Volunteers. Twenty healthy elderly volunteers, 65 to 84 years of age, completed the study.

Hypertensive Elderly. Eleven hypertensive patients, 68 to 77 years of age, completed the study.

Results. The plasma concentrations of Nisoldipine were higher in elderly volunteers and hypertensive patients than in young volunteers. After multiple dose administration the supine diastolic blood pressure remained essentially unchanged in normal young healthy volunteers but a moderate decrease in elderly healthy volunteers and a significant decrease in elderly hypertensive patients was observed.

The Effect of Diet. Study Number D92-045-02.

Title of Study : " The Effect of Food on the Pharmacokinetics of 30 mg and 40 mg Nisoldipine CC Tablets in Healthy Male Volunteers ".

This study was an open-label, randomized, two-way cross over evaluation of the effect of food on the pharmacokinetics of 30 and 40 mg

Nisoldipine. Subjects were randomized to receive a single 30 mg or 40 mg dose of Nisoldipine either in a fasted or a fed state. After one week washout period there was a crossover to the opposite state.

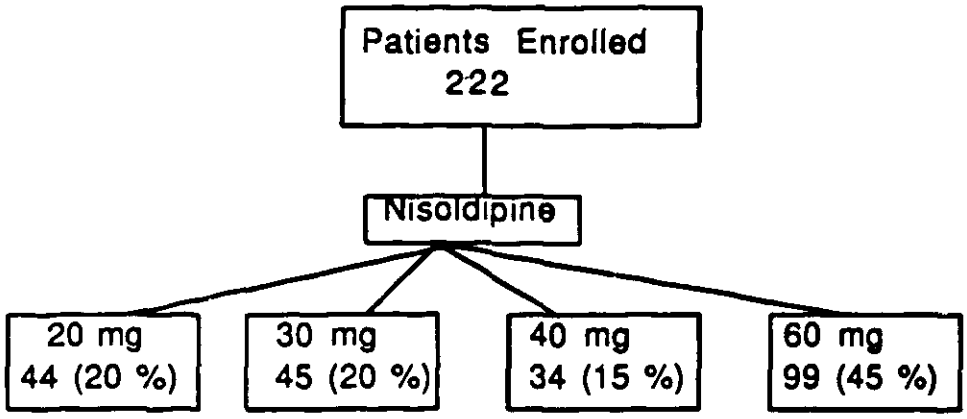
Twenty-eight healthy male subjects between the ages of 18 and 45 years completed the study. There were no significant effects on mean sitting diastolic blood pressures in the fed or fasted states at the 30 or 40 mg. doses.

Long Term Extension Studies. Drug Combination. Protocols X89-039 and X90-019.

These were long term extension studies of the 6-month efficacy studies and safety of Nisoldipine CC in the treatment of mild to moderate hypertension. Patients completing studies D89-039 and D90-019 were given the option of immediately entering an open-label extension protocol.

Patients were initially given Nisoldipine CC 20 mg or 30 mg tablets once a day as initial therapy. Then the dose of Nisoldipine was to be increased sequentially every one or two weeks as tolerated, to 40 mg qd, 60 mg qd and 80 mg qd. or 60 mg qd and 90 mg qd until SUDBP was ≤ 90 mmHg. However the maximum dose of Nisoldipine was in fact limited to 60 mg qd before any patient enrolled. Atenolol 50 mg to 100 mg qd and/or Hydrochlorothiazide 24 to 50 mg qd could be added at the investigator's discretion at any time. Thus tablets used were Nisoldipine CC 20, 40, 2X30 mg for monotherapy with the addition of Atenolol 50 and 100 mg and.or Hydrochlorothiazide 25 and 50 mg for combination therapy.

The distribution of patients is shown in the following graph :



With Atenolol
20 Patients (9 %)
1 Patient 25 mg
15 Patients 50 mg
4 Patients 100 mg

With Hydrochlorothiazide
78 Patients (35 %)
44 Patients 25 mg
34 Patients 50 mg

The results are summarized below :

	Supine		Standing	
	SBP mmHg	DBP mmHg	SBP mmHg	DBP mmHg
Baseline	154.0	101.1	149.7	100.3
Endpoint	135.7	86.0	132,4	86.5
Mean Dif	-18.3	-15.2	-17.3	-13.6

Assessment. These were open-label uncontrolled studies in which results were all pooled together and therefore they should not be valid for evaluation of combined therapy.

Total Assessment of Efficacy

Peak Drug Effect on Blood Pressure. The effect of Nisoldipine on blood pressure at the approximate time of peak drug plasma concentration (i.e. the maximal response between 6-10 hours post-dose) in the supine and standing position is shown below for the systolic and diastolic blood pressure.

	Placebo Substracted Change in Peak Blood Pressure Dose Nisoldipine				
	10 mg	20 mg	30 mg	40 mg	60 mg
Study			SUDBP		
D88-054	-11.6	-9.5	-14.1	NA	NA
D89-039	NA	-8.0	NA	-8.3	NA
D90-019	NA	NA	-6.3	NA	-10.6
			SUSBP		
D88-054	-8.6	-7.6	-12.8	NA	NA
D89-039	NA	-15.2	NA	-15.3	NA
D90-019	NA	NA	-13.0	NA	-11.1
			STDBP		
D88-054	-9.3	-7.8	-11.5	NA	NA
D89-039	NA	-7.6	NA	-8.5	NA
D90-019	NA	NA	-6.6	NA	-13.4
			STSBP		
D88-054	-4.7	-11.6	-11.0	NA	NA
D89-039	NA	-14.4	NA	-17.6	NA
D90-019	NA	NA	-15.5	NA	-19.1

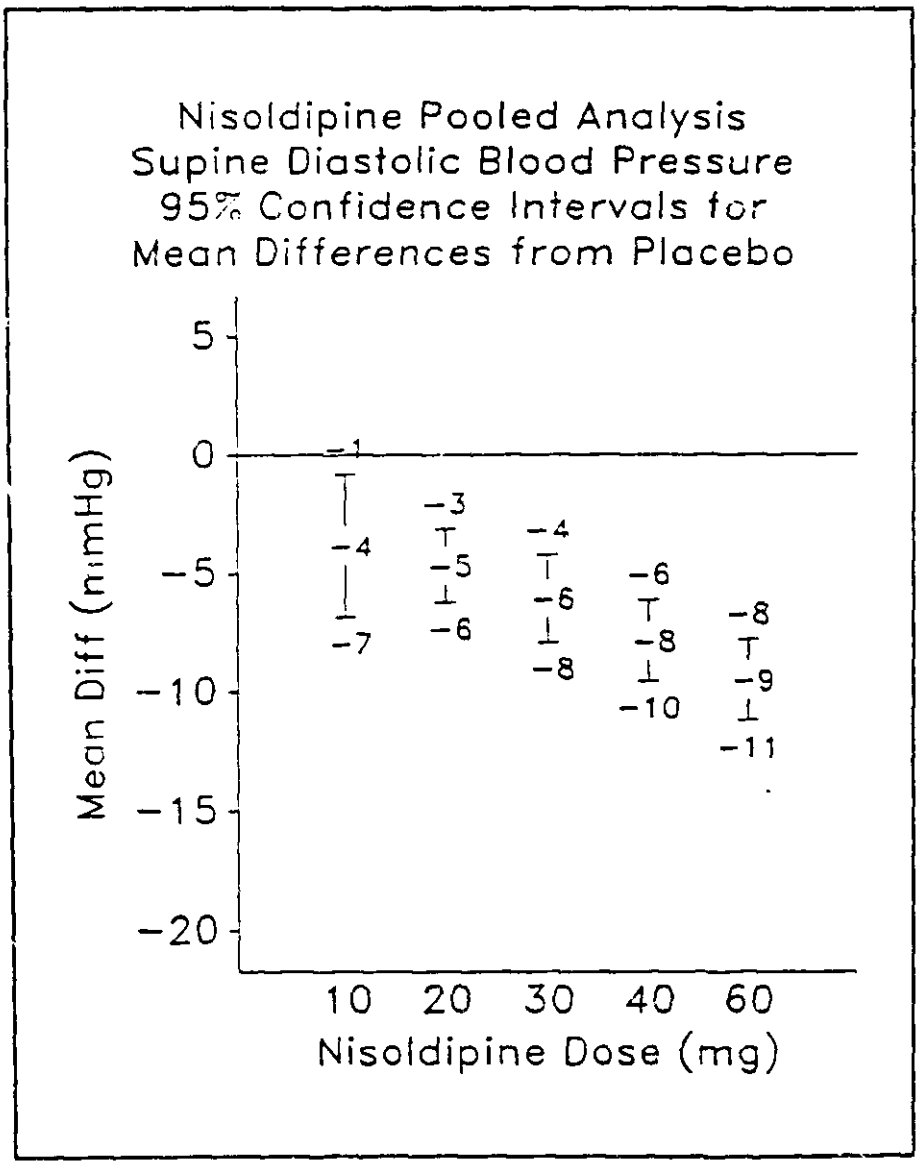
Twenty Four Hour Mean BP Reduction. Ambulatory blood pressure was used in a majority of the clinical trials of Nisoldipine in hypertension. In addition to characterizing the temporal profile of its effect on blood pressure, these data provide an estimate of the time-average reduction in blood pressure for each dosage of the drug. The pooled results of several studies are shown in the following table :

Nisoldipine Dosage (mg)	24 Hour AVG BP Reduction, Mean ± SEM Systolic	Diastolic
Placebo	-0.7±8.7	-0.9±6.3
10	-8.4±11.8	-4.6±7.5
20	-12.7±11.5	-8.4±7.1
30	-12.7±10.8	-7.9±6.9
40	-13.6±12.1	-8.0±6.8
60	-18.4±9.9	-12.0±7.2

The change in trough blood pressure from baseline to endpoint (**Mean±SEM** in mmHg) is given in the following table :

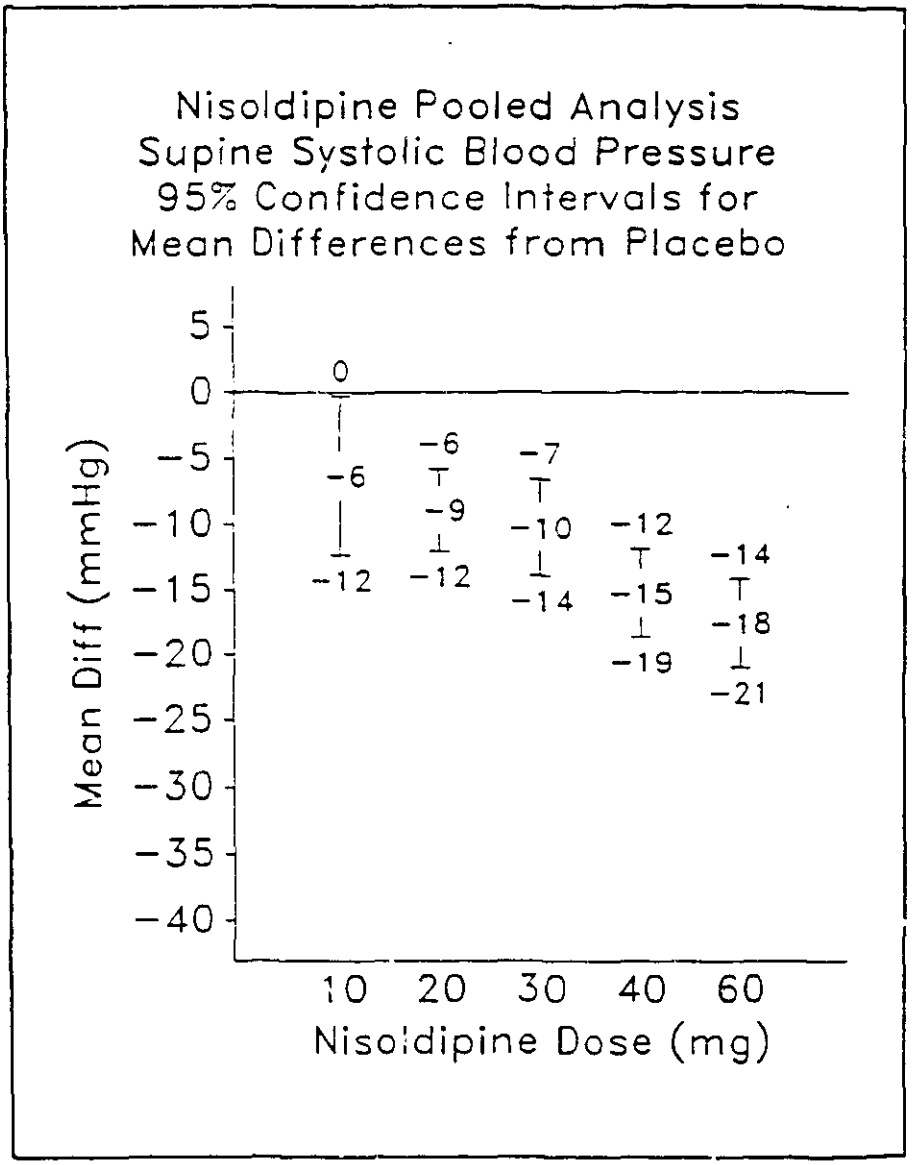
Pooled Dosage	Placebo N=232	Nisoldipine 10 mg N=30	20 mg N=161	30 mg N=105	40 mg N=131	60 mg N=125
SUDBP	-4±0.5	-8.4±1.4	-9.2±0.6	-10.6±0.8	-12.4±0.7	-14.0±0.7
SUSBP	-2.0±0.5	-8.3±2.9	-10.9±1.2	-12.2±1.6	-17.2±1.4	-19.5±1.4
STDBP	-2.7±0.5	-7.0±1.5	-7.9±0.7	-9.0±0.8	-12.6±0.8	-13.6±0.8
STSBP	-1.5±1.0	-8.2±3.0	-11.4±1.3	-12.9±1.6	-18.7±1.5	-19.2±1.5

In the following graph, pooled results of placebo subtracted values for trough SUDBP reduction by dose are demonstrated :



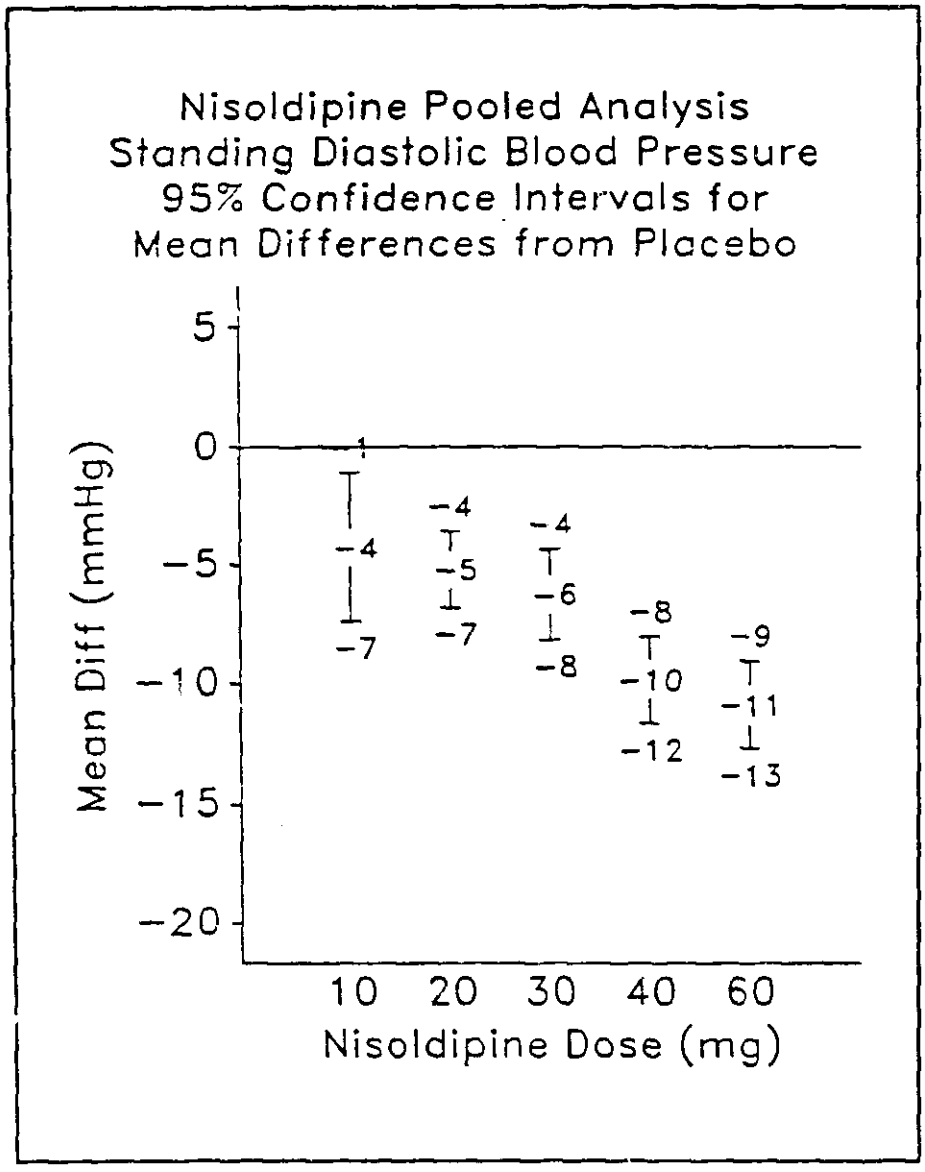
A linear relationship of blood pressure reduction by Nisoldipine in dosages between 10 and 60 mg is apparent without evidence of a plateau.

Similar results for SUSBP are shown in the following figure :



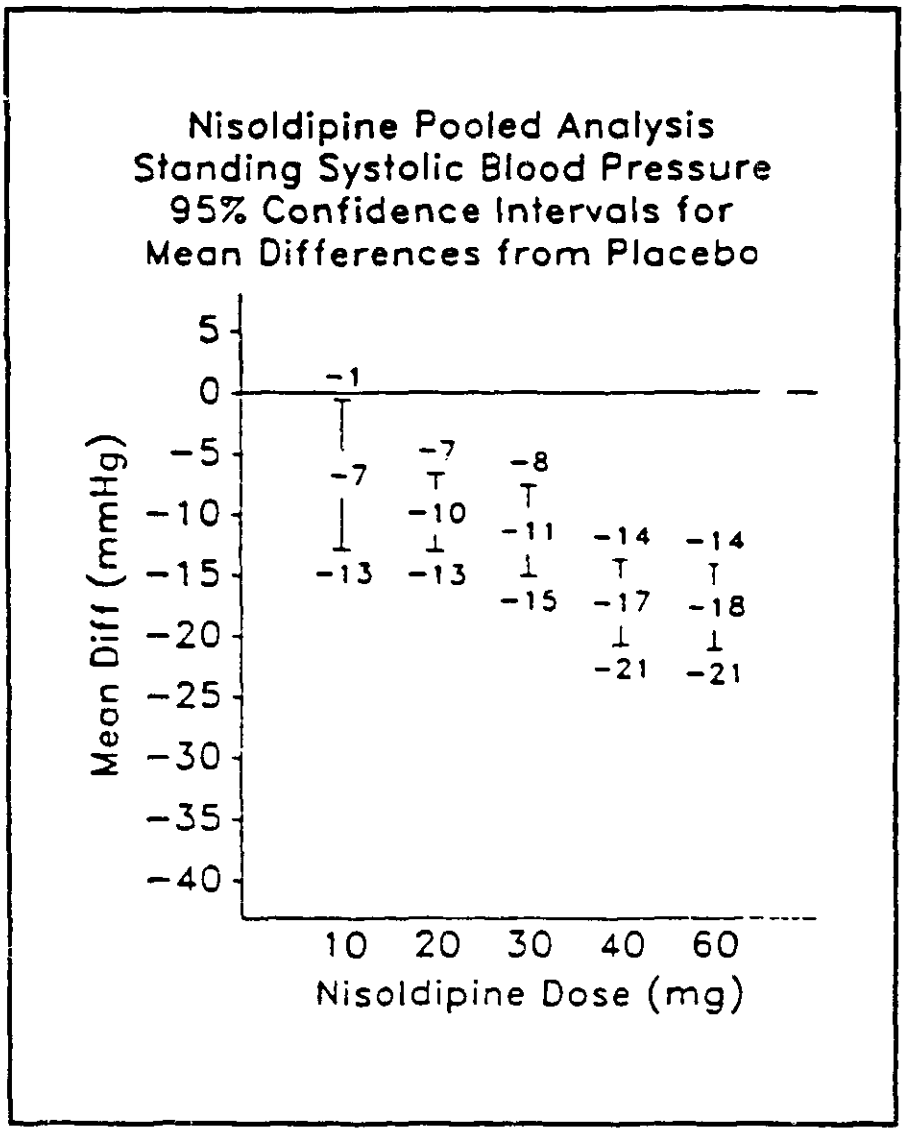
A linear relationship is not as evident as in previous graph but the maximum effect was achieved with 60 mg Nisoldipine dose

Similar results for STDBP are shown in the following figure :



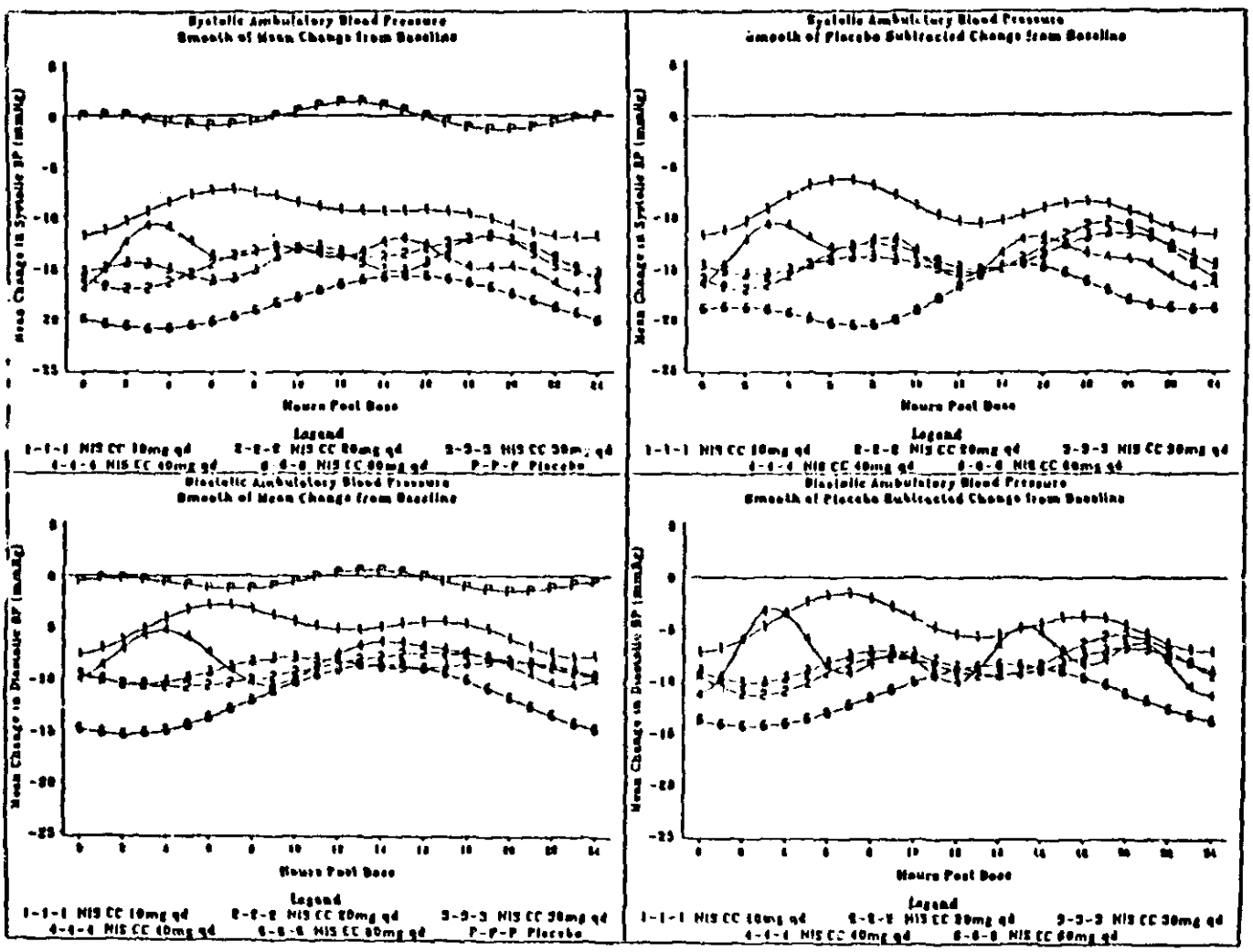
In this case the relationship of blood pressure reduction to dosage is roughly sigmoidal with an apparent plateau at 60 mg.

Similar results for STSBP are shown in the following figure :



The relationship of blood pressure reduction to dosage is sigmoidal with an apparent plateau at 40 mg

A pooled analysis of 24 hour ambulatory blood pressure monitoring is demonstrated in the following 4 graphs :



Through the 24-hour recording there seems to considerable overlapping especially among the higher doses but at trough there is evidence of blood pressure reduction that seems to be dose related.

The effects on diastolic blood pressure at peak and trough and the trough/peak ratios according to dose are given in the following table :

Dosage	Trough/Peak Ratio Diastolic Blood Pressure
10 mg	73 %
20 mg	75 %
30 mg	93 %
40 mg	100 %
60 mg	97 %

Time Course Effect of Nisoldipine. The therapeutic effect of Nisoldipine was achieved early in the course of treatment (approximately 2 weeks) and gradual incremental gain is evident for another 2-4 weeks.

The mean changes in sitting blood pressure from baseline after first dose is given in the following table :

Dose (mg)	N	8 Post-dose Systolic/ Diastolic	24 post-dose Systolic/ Diastolic
Placebo	10	-4.9/-1.9	3.8/-2.2
5	11	-10.4/-4.2	0.3/2.3
10	13	-6.7/-7.1	-0.7/-4.5
20	12	-11.3/-7.8	-5.8/-1.9
30	7	-15.4/-9.6	-13.3/-1.9

Pharmacokinetic and Blood Pressure Results. The mean sitting blood pressure change (mmHg) from baseline at peak and pharmacokinetic parameters (Mean ±SD) at steady state at each dose level is given below :

Dose (mg)	N	8h Post-Dose Sys/Dia	24h Post-Dose Sys/Dia	AUC (0-24h) (ng.h/ ml)	C_{max} (ng/ml)	T_{max} (h)
Placebo	10	-2.5/ -5.9	3.8/ 0.3			
5	11	-9.6/ -4.7	1.9/ -4.3	9.1±5.0	0.7±0.3	9.2±3.0
10	13	-8.9/ -7.1	-5.0/ -4.6	16.2± 3.0	1.1±0.3	6.3±4.8
20	12	-13.2/ -7.6	-5.4/ -4.3	29.4± 11.8	2.3±0.9	4.0±2.4
30	7	-21.7/ -10.5	-11.8/ -5.9	43.2± 23.1	2.9±1.1	5.4±5.0

The mean supine blood pressure change (mmHg) from baseline and pharmacokinetic parameters (mean±SD) at steady state for each dose level is given in the following table :

Dose (mg)	N	8h Post Dose	24 h Post Dose	AUC (0-24h) (ng.h/ ml)	C_{max} (ng/ml)	T_{max} (h)
30	18	-16.4/ -8.4	-14.0/ -10.2	74.28± 7.96	4.79± 0.68	7.22± 0.93
60	18	20.8/ 13.2	16.8/ 15.0	129.76± 12.74	8.48± 0.81	9.08± 1.97
90	9	-22.1± 12.1	-23.0± 13.4	199.31± 16.45	13.02± 1.20	6.78± 2.30
120	3	-30.7/ 25.0	-44.3/ -19.0	226.58± 12.41	14.92± 2.01	4.00± 1.00

To bring up more clearly the relationship between plasma Nisoldipine concentrations and blood pressure decrease, supine diastolic blood pressure changes from baseline at peak (8 h) and trough (24 h) were related to plasma Nisoldipine concentration at this time points using a simple linear regression. Placebo patients were used in this analysis with a plasma Nisoldipine level of Zero. The results for 30 and 60 mg are summarized in the table below :

Timepoint	Nisoldipine Mean Plasma Conc. (ng/ml)	Mean Change in SUDBP (mmHg)	Estimated Slope	Estimated Slope (P-Value)
Day 4, 30 mg (N=18)				
8 hours	3.5	-8.4	-2.55	0.0118
24 hours	2.6	-10.2	-1.42	(0.0689)
Day 8, 60 mg (N=17-18)				
8 hours	6.2	-13.2	-1.14	0.0507
24 hours	5.2	-15.0	-1.39	(0.0027)

Blood Pressure Rebound Upon Withdrawal. Blood pressure rebound was determined 24, 48 and 72 hours after cessation of Nisoldipine 60 mg qd in patients who had reached steady state. There was no evidence of for an exaggerated rebound effect on blood pressure after discontinuance of Nisoldipine at this high dose.

Maintenance of Blood Pressure Reduction in Long Term Studies. There was no evidence of tolerance to the antihypertensive effect of Nisoldipine over 6 months to 1 year of therapy.

Demographic Subgroups. Gender. Trough SUDBP changes from baseline to endpoint for male and female patients are given in the following table :

	Female	Male
Dosage	Nisoldipine-Placebo	Nisoldipine-Placebo
10 mg	-8.85	-2.27
20 mg	-3.21	-5.87
30 mg	-8.47	-6.1
40 mg	-7.82	-8.43
60 mg	-10.31	-10.79

Although dose-response profiles are somewhat erratic the overall effects are similar for men and women.

Race. A comparable analysis of efficacy for race related to dose is demonstrated in the following table :

	White	Black
Dosage	Nisoldipine-Placebo	Nisoldipine-Placebo
10 mg	-3.59	-4.37
20 mg	-4.54	-6.39
30 mg	-7.51	-8.82
40 mg	-6.69	-11.61
60 mg	-11.51	-11.1

Black patients responded with a greater decline in trough SUDBP than did white patients.

Age. In the following table the dose response for patients divided by age less than 65 years and equal or greater than 65 years is demonstrated.

	<65	≥65
Dosage	Nisoldipine - Placebo	Nisoldipine - Placebo
10 mg	-3.69	-6.2
20 mg	-4.98	-5.15
30 mg	-7.31	-5.48
40 mg	-8.21	-8.09
60 mg	-11.08	-8.14

The elderly demonstrated a greater low-dose response and a lesser high-dose response.

Quartile of Baseline Blood Pressure. For Nisoldipine as well as for many other antihypertensive drugs, a higher baseline blood pressure is associated with larger decline on medication. In the table below a dose response according to baseline SUDBP by quartile is demonstrated :

	Q1	Q2
Dosage	Nisoldipine-Placebo	Nisoldipine-Placebo
10 mg	-4.38	-5.97
20 mg	-4.23	-8.18
30 mg	-2.27	-11.49
40 mg	-6.36	-13.81
60 mg	-9.66	-14.16

The relationship of Nisoldipine dosage and decline in blood pressure is least evident in the first quartile and strongest in fourth quartile.

Combination Antihypertensive Therapy. Addition to a background of a beta blocker. One the pivotal studies (D89-029) evaluated the combination of Nisoldipine CC and a beta blocker.To patients who were already receiving Atenolol Nisoldipine was added. The sponsor claims the efficacy of Nisoldipine under these conditions. However there seems to be a drug interaction between these drugs that the sponsor has not recognized (see p. 35 this review).

Long Term Extension Trials. Based on open-label controlled trials and uncontrolled studies of one year duration the sponsor claims that meaningful responses were elicited by the combination of Nisoldipine with diuretics and or/ a beta blocker.

Recommendations. Nisoldipine should be approved as monotherapy for hypertension. The recommended dosage should be 10 mg to 40 mg.

Although the sponsor states that there is no drug interaction between Nisoldipine and beta blockers there are publications stating that such interaction exists (1, 2). This should be stated in the package insert.

Consideration should be given to advising that the dosage may need to be adjusted in patients with renal failure.

There were not well controlled studies of the combination of Nisoldipine with diuretics or other antihypertenive agents. Therefore the claim of efficacy with other drug combination is not well substantiated.

Cristobal G. Duarte, MD - HFD-110

CC.
ORIG. NDA
HFD-110
HFD-110/ CSO/Roeder
HFD-110/CGD/30Jul93

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Table of Contents

NDA 20-356 EA & FONSI

NDA 20356

EA + FONSI

DF

ENVIRONMENTAL ASSESSMENT

AND

FINDING OF NO SIGNIFICANT IMPACT

FOR

Nisocor

(nisoldipine)

Extended Release Tablets

10, 20, 30 and 40 mg

NDA 20-356

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
(HFD-110)

FINDING OF NO SIGNIFICANT IMPACT

NDA 20-356

FEB - 1 1995

Nisocor

(nisoldipine)

Extended Release Tablets

The Food and Drug Administration (FDA) recognizes the National Environmental Policy Act of 1969 (NEPA) as the national charter for protection, restoration, and enhancement of the environment. NEPA establishes policy, sets goals (section 101), and provides procedures (section 102) for carrying out the policy.

Environmental information is to be available to the public and the decisionmaker before decisions are made about actions that may significantly affect the quality of the human environment; FDA actions are to be supported by accurate scientific analyses; and environmental documents are to concentrate on timely and significant issues, not to amass needless detail.

The Food and Drug Administration, Center for Drug Evaluation and Research has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

In support of their new drug application for **Nisocor**, **Miles Inc., Pharmaceutical Division** has conducted a number of environmental studies and prepared an environmental assessment in accordance with 21 CFR 25.31a(a) (attached) which evaluates the potential environmental impacts of the manufacture, use and disposal of the product.

Nisoldipine is a synthetic drug which is administered as an oral tablet in the treatment of hypertension. The drug substance will be manufactured at Bayer AG, Wuppertal, Germany. The drug product is manufactured at Bayer AG, Leverkusen, Germany and packaged at Miles Inc., Pharmaceutical Division, West Haven, CT. The finished drug product will be used in hospitals, clinics and by patients in their homes.

Nisoldipine is completely metabolized to structurally related substances which will be excreted predominantly into publicly owned treatment works (POTW). Chemical and physical test results and information indicate that the major metabolites will most likely be restricted to the aquatic environment and can be classified as pharmacologically inactive based on studies in several mammalian species.

As the major metabolites are expected to persist in the aquatic environment for some time, toxicity studies were conducted. Nisoldipine was used in the studies as it was determined to be more pharmacologically active than the metabolites in mammalian studies. Acute static toxicity studies in water fleas (*Daphnia magna)* and zebra fish (*Brachydanio rerio)* indicate that the drug substance is generally not toxic to aquatic organisms at concentrations of at least 4 orders of magnitude greater than the maximum expected environmental concentration (MEEC).

Microbial inhibition studies indicate that respiration in activated sludge is not inhibited at concentrations of at least 8 orders of magnitude greater than the maximum expected environmental concentration (MEEC).

Disposal of the drug may result from out of specification lots, discarding of unused or expired product, and user disposal of empty or partly used product and packaging. Waste packaging and drug product in the U.S. will be disposed of at a licensed incineration facility. At U.S. hospitals and clinics, empty or partially empty packages will be disposed according to hospital/clinic regulations. From home use, empty or partially empty containers will typically be disposed of by a community's solid waste management system while some unused drug may be disposed of in the sewer system.

The Center for Drug Evaluation and Research has concluded that the product can be manufactured and used without any expected adverse environmental effects. Precautions taken at the sites of manufacture of the bulk product and its final formulation are expected to minimize occupational exposures and environmental release. Bayer AG has received authorization from the appropriate authorities to operate their manufacturing facilities and has provided certification that operation is in accordance with applicable German environmental regulations.

Adverse effects are not anticipated upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places.

1/26/95 Nancy B. Sager
DATE Prepared By
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Review Chemist
Center for Drug Evaluation and Research

1/30/95 Phillip G. Vincent
DATE Approved
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Center for Drug Evaluation and Research

1/31/95 Robert A. Jerussi
DATE Concurred
Robert A. Jerussi, Ph.D.
Associate Director for Chemistry
Center for Drug Evaluation and Research

Attachments:

I: Environmental Assessment and FDA Addendum
II: Material Safety Data Sheet (drug substance)
III: Miles Inc. Regulatory Overview (West Haven, CT)
IV: German Government Certification/Bayer AG
V: Bayer AG Regulatory Overview

CC: Original NDA 20-356/DRoeder copy to NDA/HFD-110
FONSI File 20-356/HFD-102
P. Vincent/HFD-102
Docket File 20-356/HFD-102
FOI Copy/HFD-019

20356.FON
F/T by NBS 01/28/1995

ATTACHMENT I

ENVIRONMENTAL ASSESSMENT

1. Date: January 23, 1995

2. Name of Applicant/Petitioner: Miles Inc.,
Pharmaceutical Division

3. Address: 400 Morgan Lane
West Haven, CT 06516

4. Description of the Proposed Action:

The application proposed is to package Nisoldipine tablets for the purpose of sale to the general public. Some information contained in this assessment is proprietary and is listed in Appendix 1. Nisoldipine is a drug belonging to a class of pharmacological agents known as the calcium channel blockers. Nisoldipine consists of a slow release more rapidly released nisoldipine. It is used as a calcium ion influx inhibitor which inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. This product will be used by patients in the United States for the treatment of hypertension. This product will be used in hospitals, clinics and by patients in their homes.

The product will be packaged in the existing pharmaceutical packaging facilities at the Miles Inc. Pharmaceutical Division's West Haven, CT location. West Haven is an urban setting with a generally flat to slightly hilly terrain and has a temperate climate.

The intermediates in the synthesis of nisoldipine, are not available in the open marketplace. They are manufactured at the Bayer AG facility in Wuppertal.

The production facilities are described in the drug master file for each facility.

The complete addresses of the Bayer production facilities are:

Nisoldipine is synthesized at Wuppertal:

Bayer AG
Friedreich-Ebert-Str. 217
D-42096 Wuppertal
Germany

NISOCOR tablets are manufactured in Leverkusen:

Bayer AG
Bayerwerk
D-51368 Leverkusen
Germany

NISOCOR tablets are packaged in the following configurations:

45 cc	of 30 tablets each
120 cc	of 100 tablets each

All Nisoldipine goods and packaging waste products will be collected for disposal at the West Haven site. Actual disposal will be managed through the office of the manager of environmental and safety affairs located in West Haven. All returned goods and packaging wastes are disposed of by incineration via a manifested isolated disposal program. The main incineration facility for Miles Inc. is Clean Harbors Inc. Clean Harbors is located at 385 Quincy Avenue in Braintree, MA. This is a permitted hazardous waste treatment, transfer and recovery facility with Environmental Protection Agency (EPA) identification number MAD053452637. As a permitted TSDF, the Braintree facility is regularly inspected by the Hazardous Waste personnel from both the State of Massachusetts as well as the Federal EPA.

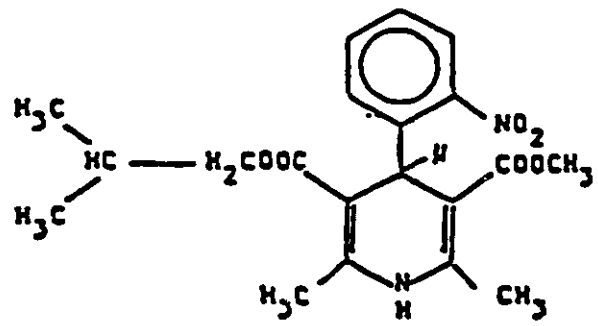
The Clean Harbors incineration process utilized for the destruction of product waste from the West Haven site is a 2-stage incinerator. It's main chamber is of a fixed hearth horizontal design with a ram feeder, that is capable of processing approximately 300 lbs/hr of material at a temperature of approximately 1500° F. The second stage is a fixed hearth chamber where volatile gases are combusted at temperatures in excess of 2000° F. Following the secondary chamber is a wet scrubber designed and managed for volatile and acid gas removal.

Materials that are sent to the Clean Harbors incinerator today typically are not in finished product form. Those that are in finished product form are either in metal tubes or high density polyethylene (HDPE) bottles. Although this application may use blister pack components, the majority of the finished product waste will either be in the form of bulk tablets and/or bulk intermediate, or package in bottles.

5. Nisoldipine is a calcium ion influx inhibitor which inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Clinically it is used to treat hypertension and angina.

Nisoldipine tablets are manufactured by Bayer AG. The production facility for nisoldipine is Elberfeld in Wuppertal in the Federal Republic of Germany. Nisoldipine is synthesized using

The structural formula for nisoldipine is given below.



Chemical Name: Isobutylmethyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate

Description: Yellow, Crystalline Substance.

A material data sheet for Nisoldipine drug can be found in Appendix 3.

No additives are used. The following impurities have been identified, the structural formula for each is given in Appendix 4. Further information on these impurities is given in the nisoldipine drug substance DMF No. (pg 32 - 35).

6. Introduction of substances into the environment:

Nisoldipine tablets, the subject of the NDA, will be packaged at the Miles Inc., Pharmaceutical Division's site in West Haven, CT.

The packaging of Nisoldipine tablets at the West Haven site is a process involving tablet packaging operations only.

Wastes from the packaging of Nisoldipine tablets will be generated in a solid phase only. It will be managed in such a fashion as to have no significant impact upon the production facilities compliance permit status relative to all federal, state and local environmental and safety laws and regulations. Information regarding permits for air, liquid and solid emissions is provided. This information includes permit numbers, issuing agencies and the permit expiration dates, if applicable. A list of all applicable federal, state and local environmental and occupation laws/regulations is provided for Miles Inc. (see Appendix 5).

No significant quantities of chemical substances should be emitted to the environment. Because of the controls exercised during packaging and use of environmental dust collecting systems, it is felt that no significant quantities of chemical substances will be emitted. The only possible emissions from the cleaning of the packaging equipment that will be utilized for this product application, will be the small amounts of dust that may be present on the packaging line during equipment cleaning. These particulates will be vacuumed into a dust collection system for later disposal. It is felt that the collection efficiency of the dust collection system is quite good and no emissions are anticipated.

Dust collection systems in West Haven predominantly utilize pleated filter media of 95% efficiency. Some of the collection systems have media that is simply vibrated to remove contaminant that is then captured into a collection container. Other systems have disposable filter media that is completely removed and disposed of. In either case, materials to be disposed go to the Clean Harbors facility previously described.

As with all of West Haven's current product packaging wastes, Nisoldipine wastes will be containerized for isolated manifested off-site incineration. The incineration of Nisoldipine should be complete resulting in little or no Nisoldipine being contained in incinerator ash.

Two statements regarding environmental certification have been submitted with regard to Nisoldipine On July 16, 1993, Miles submitted a letter from the Administrative District of Köln confirming that the manufacture of nisoldipine coat core tablets is subject to the German Federal Immission Control Act. This act specifies that manufacturing facilities must be constructed and operated to comply with state and local environmental controls. Also, on August 19, 1983 a similar statement was submitted to the nisoldipine drug substance DMF This letter from the President of the Administrative District of Düsseldorf certifies that the manufacture of nisoldipine drug substance in Wuppertal-Elberfeld is in compliance with the German Federal Emission Control Act.(Appendix 6) Also included in Appendix 6 is Miles letter of authorization to DMF . nisoldipine drug substance, a listing of the applicable environmental regulations to which Bayer AG must comply, environmental statements relative to Bayer's environmental compliance status, and descriptions of their environment control facilities.

The following materials are used in the manufacture of Nisoldipine tablets:

- Nisoldipine
- Corn Starch
- Crospovidone
- Microcrystalline Cellulose
- Sodium Lauryl Sulphate
- Povidone 25
- Magnesium Stearate
- Hydroxypropyl Cellulose (high and low viscosity)
- Hydroxypropyl methyl cellulose
- Polyethylene Glycol 4000
- Titanium Dioxide
- Iron Oxide, Yellow
- Iron Oxide, Red
- Lactose

All materials comply with USP/NF the corresponding monographs
In conclusion, the packaging of Nisoldipine at West Haven should have no effect on compliance with existing applicable emission requirements (including occupational) at the federal, state or local level. No modifications of any existing permits will be necessary to package this product.

7. Fate of emitted substances in the environment:

a) Air: No significant concentrations of substances to be emitted, thus no significant impact is expected.

b) Fresh water, esturarine and marine ecosystems: No substances to be emitted directly. Any wash waters from equipment cleaning are discarded to the town operated POTW.

c) Terrestrial ecosystems: Unused packaging, tablets and dusts collected will be incinerated. The small amounts of ingredients remaining in the ash after incineration will pose no threat to a landfill environment. The same is true for the town POTW sludge. The small amount of ingredients remaining will pose no threat to a landfill environment.

Dissociation constant

Log Octanol/Water Partition Coefficient (log Pow)

Water Solubility

Photolysis

Hydrolysis

Nisoldipine is considered stable to hydrolysis.

The MEEC is calculated as follows:

MEEC = (A) (B) (C) (D) (E) (F)

Where A = Production (pounds/year)
B = 1 year / 365 days
C =
D =
E =
F

The test reports on water solubility, partition coefficient and vapor pressure for nisoldipine are included in Appendix 7.

Biological Degradation
[References can be found in Appendix 8.]

Nisoldipine is rapidly and extensively metabolized in man [1]. No unchanged Nisoldipine is found in excreta, demonstrating elimination completely via biotransformation. Nisoldipine is excreted in man only in the form of more polar metabolites of the same backbone chemical structure as the parent compound. Water soluble, inactive metabolites accounting for of the administered dose are excreted in urine. The remainder of the dose is excreted in feces, also solely as metabolites. Of the metabolites excreted in urine, have been identified, comprised o

These structures [1] represent the following biotransformation reactions:

The reported values for log P (4.5) and aqueous solubility (2 mg/L) suggest that the ecological fate studies submitted, having been performed with Nisoldipine itself do represent the worst case scenario in terms of potential bioconcentration and ecotoxicity. We estimate that the major metabolites have higher aqueous solubilities and lower lipophilicities than Nisoldipine. This is based upon the substantially shorter reversed phase chromatographic retention times for) relative to Nisoldipine Log P values and aqueous solubility are known to be primary determinants of reversed phase retention times. In fact, log P values are routinely estimated [2, 3, 4] from variations on the following equations, all based on the linear relationship of the log capacity factor (k) to log P.

log P = a log k + b	where	a and b are constants
k = (tr - to) / to	where	tr =analyte retention time to = void time

The log P values for are reasonably expected to be substantially lower than that of Nisoldipine based on the above relationships. Additionally, both metabolites are (pka estimated 4 - 5) resulting from de-esterification of the parent compound, and should have significantly increased water solubility compared to Nisoldipine itself due to ionization at environmental pH's. Therefore are expected to have a significantly lower potential for ecological effect than Nisoldipine itself.

To the best of our knowledge, we assume that the metabolites do not have any further ecotoxicological properties. A detailed investigation of the metabolites will not be performed since the calculated MEEC and toxicity study data for the active substance demonstrates sufficient safety.

EC50 Daphnia - 48 hours - 33 mg/L

EC50 Fish - 96 hours - between 3.1 mg/L and 7.5 mg/L

A report on the toxicity of nisoldipine to fish and daphnia is included as Appendix 9.

Based on a maximum daily dose of 60 mg nisoldipine per patient per day and an average daily water use of 150 liters, the corresponding quantities of excreted metabolites result in 0.4 ppm per patient per day.

Based upon the above it can be concluded that nisoldipine presents no significant environmental risks and will not accumulate or be widely distributed into the environment.

8. Environmental effects of released substances:
[References in this section can be found in Appendix 8.]

Based on the physicochemical differences between and Nisoldipine outlined in section 7, it is expected that the metabolites will have significantly lower potential for bioconcentration than Nisoldipine. As previously stated, no ecotoxicity studies were performed with either However, preclinical metabolism studies have established that are significant metabolites in rat, dog and monkey, as well as in man. Therefore, in the mammalian toxicity studies performed on Nisoldipine, substantial exposure to can be assumed, and thus their toxicity has been adequately investigated. In addition, safety pharmacology reports have been issued in which independent studies [5 - 8] were performed on both . Both compounds were classified as **pharmacologically inactive** in dog hemodynamic, guinea pig ileum contractility, and rat uterine contractility studies. The IC50 of these metabolites were in each case at least 1000-fold higher than those for Nisoldipine. The combination of an expected lower potential for environmental bioconcentration with the available mammalian toxicity and safety pharmacology data lead us to conclude a significantly lower potential environmental impact for in comparison to Nisoldipine itself.

In all tested concentrations, nisoldipine did not inhibit the respiration of activated sludge. A report on microbial inhibition is included in Appendix 10.

EC50>10,000 mg/L

9. Use of resources and energy:

Besides the use of Nisoldipine plastics and water resources will be used in the packaging of this product.

This product application will not significantly change the use of resources and energy as compared to the existing normal daily activities.

There will be no effects upon the endangered or threatened species or property listed in the National Register or Historic Places.

Nisoldipine packaging will represent less that approximately in terms of total sales, of the production at the West Haven facility. Thus, less that approximately of our total energy consumed within packaging on site will be attributable to Nisoldipine packaging which will result in less than approximately annually.

10. Mitigation measures:

Because the West Haven facility complies with federal and state regulations, there are no significant potential adverse environmental impacts expected associated with the proposed action. Also, if an unplanned release did occur, the West Haven facility's integrated emergency response plan would be implemented to mitigate any release.

11. Alternatives to the proposed action:

The alternative to the proposed action is the non-approval of the NDA by the FDA, in which case Nisoldipine tablets would not be available.

12. List of preparers:

This assessment was prepared by Gary G. Toczylowski, Manager of Environmental and Safety Affairs at Miles Inc., Pharmaceutical Division. He is familiar with the operations to be carried out and knowledgeable of the wastes to be generated.

See Appendix 11 for a listing of Mr. Toczylowski's professional qualifications. In addition to Mr. Toczylowski, the following were involved in the preparation of the environmental assessment for Nisoldipine tablets:

Dr. Karl-Werner Theim, Head of Pharmaceutical Production Group, Environmental and Plant Safety, Bayer AG, Wuppertal, Germany

Dr. Norman C. Franklin, Head of Quality Systems and Documentation in the Production Department, the Pharmaceutical Division and Chairman of the Validation Steering Committee, Bayer AG, Wuppertal, Germany.

13. Certification:

The undersigned official certifies than the information presented is true, accurate and complete to the best of knowledge of the firm or agency responsible for the preparation of the environmental assessment.

Signature of Responsible Official

Manager of Environmental and Safety Affairs
Title

GGT bak

FDA ADDENDUM TO THE ENVIRONMENTAL ASSESSMENT (EA) FOR NDA 20-356

In a separate communication to the Agency, Miles Inc., Pharmaceutical Division provided additional information:

- All values in the EA document for EC_{50} (sludge, bacteria, fish, daphnia) are nominal concentrations;
- The "West Haven Facility" referred to in the document is located at 400 Morgan Lane, West Haven CT 06516;
- The following articles/reports are referenced in the EA:

1. Scherling et al, Arzneim.-Forsch./Drug Res. 38(1), 8, 1105-1110 (1988)
2. Garst and Wilson, J. Pharm Sci., Vol. 73, No. 11, (1984)
3. Garst, J. Pharm Sci., Vol. 73, No. 11, (1984)
4. Serajuddin et al, J. Pharm Sci., Vol. 80, No. 9, (1991)
5. Knorr, A. BAYER Pharma. Report No. 15623 (1987)
6. Knorr, A. BAYER Pharma. Report No. 15622 (1987)
7. Kazda, S. BAYER Pharma. Report No. 15476 (1987)
8. Stasch, J. BAYER Pharma. Report No. 16007 (1987)

References 5-8 are confidential.

ATTACHMENT II

1. Identification of the substance/preparation and the company

Nisoldipin

Bayer AG, PH-P \kologie und Sicherheit
D-42096 Wuppertal, Telephone: (0202) 367557
In case of emergency: (0214) 303030 (Werkfeuerwehr Bayer Leverkusen)

2. Composition/information on ingredients
active drug substance
CAS name: 3,5-Pyridinecarboxylic acid , 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, methyl 2-methylpropyl ester
CAS No.: 63675-72-9
Synonym: Bay K 5552

3. Hazards identification
Hazard warning not required.

4. First-aid measures
Contamination of the eyes must be treated by thorough irrigation with water, with the eyelids held open. A doctor (or eye specialist) should be consulted immediately.
Take off immediately all contaminated clothing.
After contact with skin, wash immediately with plenty of water and soap.
If swallowed, seek medical advice immediately and show this container or label.

5. Fire-fighting measures
Extinguishing media: All extinguishing materials are suitable.

Combustibility: BZ 2 = brief ignition and rapid extinction.
Keep away from naked flame.
In case of fire care must be taken to collect the quenching water.
In case of fire NOx may develop.

6. Accidental release measures
Take up mechanically, fill into labelled, closable containers.
Avoid formation of dust.
Do not let enter into the soil.
If larger product quantities are released it may not be allowed to enter into sewage systems, biological sewage treatment plants, surface waters and/or groundwater.

7. Handling and storage
Transport temperature not more than +50 OC.
Storage temperature not exceeding +50 OC.
Take precautionary measures against static discharges.
Keep away from uninsulated sources of heat.
Keep away from light.
Protect from moisture.
During handling local official regulations must be observed in order to avert impairment of water by the product.
For storage suitable stores with adequate product-reception volume must be used

(to be continued)

7. Handling and storage (Continuation)
In case of fire and/or explosion do not breathe fumes.
Keep away from naked flame.
Do not empty inner sack above vessels containing a mixture of inflammable gases.

8. Exposure controls/Personal protection
Unless the product is entirely enclosed, do not handle it until you have studied the respiratory precautions issued by the appropriate authority or accident prevention association.
Recommended respiratory protection: half-mask with filter type P1.
Eye protection: goggles
Hand protection: gloves of rubber
Other protective equipment: Wear protective clothing.

To clean the floor and all objects contaminated by this material, use water and detergents.
Take off immediately all contaminated clothing.
After contact with skin, wash immediately with plenty of water and soap.
When using do not smoke.
Wash hands before breaks and at end of work.

9. Physical and chemical properties tested in accordance with

Form:	crystalline
Colour:	yellow
Odour:	odourless
Melting range:	147-152 OC
Viscosity:	not applicable
Solubility in water:	0,01 g/l at 20 OC
Solubility in acetone:	245 g/l at 20 OC
Partition coefficient:	log P octanol/water = + 4,46

10. Stability and reactivity
Thermal decomposition: No decomposition when used as directed.
No exothermic reaction without air supply (decomposition) up to 140 OC (Geigy - test)
No exothermic reaction with air supply (spontaneous combustion) up to 115 OC (Geigy - test)
Start of decomposition: 180 OC
(DTA, heating rate 4 OC/min in glass)
Hazardous reactions: No hazardous reaction when used as directed.
Dust explosion class: ST 1.
Geigy test: burning index BZ R 2
Further information: light-sensitive

11. Toxicological information
Acute toxicity:
LD50 oral, rat: >10000 mg/kg

Industrial usage with the usual precautions of industrial hygiene no effects detrimental to health are known.

Ames-test: negative

Chemical-pharmacological effect: antihypertensive

12. Ecological information
Correct handling will produce no environmental problems.
According to the present state of knowledge no disturbance is caused in biological sewage treatment plants if the product is used properly.

Acute bacterial toxicity:
Pseudomonas putida : EC0: >10 mg/l

Toxicity for Daphnia:
before degradation: 5 mg/l
after degradation: 5 mg/l
Toxicity for Daphnia: (nominal concentration)
LC0: 7.8 mg/l
LC50: 127.0 mg/l

Fish toxicity: (nominal concentration)
Zebra barbel (Brachydanio rerio) LC0: 3.9 mg/l 96h
Zebra barbel (Brachydanio rerio) LC50: 5.5 mg/l 96h

COD value: 1771 mg/g
BOD28 value: 0 mg/g
Biological elimination: BOD5/COD: 0 %
Abiotic degradation :
Light stability : Photolytic degradation after 70 sec. 68%

Water pollution class (WGK): 2 - impairment of water quality
(own classification)
WGK = Classification in accordance with the German Water Resources Act

13. Disposal considerations
Transport to suitable incinerator with reduced non-air emission.
Product and material polluted with product must be disposed off according to regulations.

14. Transport information
GGVSee/IMDG Code: -- UN No.: -- MFAG: -- EmS: --
PG: -- MPO: --
GGVE/GGVS: Class -- No. -- RID/ADR: Class -- No. --
Warning sign: Hazard no. 000 Substance no. 0000
ADNR: Class -- No. -- Cat -- ICAO/IATA-DGR: not restr.
Declaration for land shipment: --
Declaration for sea shipment: --
Declaration for shipment by air: --
Other information:
Not dangerous cargo. Keep dry. Avoid heat above +50 DC. Keep separated from foodstuffs.

15. Regulatory information
No labelling is required in accordance with the EEC directives.

16. Other information
All tests to the above mentioned data are based on methods generally used in the FRG.
The instructions given here are valid only for the product as supplied, not for derivatives resulting from its use.

16. Other information (Continuation)
BAYER-Storage class: B
(B if there is a large proportion of readily flammable packing materials)

The data given here is based on current knowledge and experience. The purpose of this Safety Data Sheet is to describe the products in terms of their safety requirements. The data does not signify any warranty with regard to the products' properties.

ATTACHMENT III

WEST HAVEN REGULATORY OVERVIEW

The proposed application to label and package Nisocor Tablets in the exiting Miles Incorporated facility located in West Haven, Connecticut could impact the following federal, state and local environmental and safety laws and regulations that the site is currently in compliance with. However, there are no negative impacts anticipated due to the small size of the proposed activity, as well as internal handling procedures that have been designed to mitigate these potential impacts.

1) State of Connecticut DEP, Regulations for the Abatement of Water Pollution. (Current permit # SP0000141, expires 7/31/95, permit renewal application to be submitted in first quarter of 1995).

2) State of Connecticut DEP, General Permit for the Discharge of Stormwater Associated with Industrial Activity. (No Permit # required only notification. Notification made on 11/20/92).

3) Federal EPA and State DEP, Hazardous and Solid Waste Regulations. (EPA # CTD046418059).

4) Federal EPA and State DEP, BioMedical Waste Disposal and Tracking. (No Permit Required).

5) State DEP, Oil and Chemical Release Reporting Requirements. (No Permit Required).

6) OSHA, Response to Hazardous Waste and Handling of Hazardous Materials Release Emergencies, (HAZWOPER). (No Permit Required).

7) State of Connecticut DEP, Regulations for the Abatement of Air Pollution. (Three permits exist on site. All are associated with our fuel burning equipment on site, i.e., 2 boiler installations and 1 emergency generator). None of the dust collection or equipment utilized in the manufacture of products in West Haven has or requires DEP permits, due to the small size and lack of hazardous materials processed in them.

8) Federal Occupational Safety and Health Administration (OSHA) programs also apply to the West Haven facility. Although permits are not required, compliance with a wide variety of occupational safety programs is. In particular, OSHA regulatory required programs that impact the West Haven location the most include: the laboratory standard, bloodborne pathogens, respiratory protection, lockout/tagout, personal protective equipment, hazard communication and process safety management.

ATTACHMENT IV

Im Dienste der Sicherheit | und einer besseren Umwelt

Neue
Postleitzahl ab 1.7.
Hausadresse: 506
Postfachadresse: 504

Besucheranschrift:
Blumenthalstr. 33, 5000 Köln
Bearbeiter: Herr Odenthal
Zimmer: 371 Durchwahl: 77 40-5

Firma
Bayer AG
Werk Leverkusen
LE W! Genehmigungen
z. Hd. Herrn Dr. Knopf

5090 Leverkusen

Ihr Zeichen	Mein Zeichen	Datum
	2202-Od/Pk	06.05.1993

Betr.: Zulassung von Nisoldipine in den USA

Bezug: Ihr Telefax vom 28.04.93

Sehr geehrte Damen und Herren,

wunschgemäß bestätige ich, daß die Fertigung von Nisoldipine Coat Core Tabletten aus dem Wirkstoff Nisoldipine in Ihrem Werk Leverkusen dem Bundesimmissionsschutzgesetz (BImSchG) unterliegt.

Es handelt sich dabei um eine nach § 22 BImSchG nicht genehmigungsbedürftige Anlage, die so zu errichten und zu betreiben ist, daß

1. schädliche Umwelteinwirkungen verhindert werden, die nach dem Stand der Technik vermeidbar sind,

2. nach dem Stand unvermeidbare schädliche Umwelteinwirkungen auf ein Mindestmaß beschränkt werden und

3. die beim Betrieb der Anlagen entstehenden Abfälle ordnungsgemäß beseitigt werden können.

Die Anlage unterliegt auch anderen öffentlich rechtlichen Vorschriften - insbesondere solchen aus dem Natur-, Landschafts- und Gewässerschutz, deren Einhaltung von staatlichen Umweltbehörden überwacht wird.

Für Belange des Immissionsschutzes ist das Staatlichen Gewerbeaufsichtsamt Köln zuständig.

Erwähnenswerte Beanstandungen im Bereich des Immissionsschutzes liegen nach meiner Kenntnis nicht vor.

Mit freundlichen Grüßen
Im Auftrag

Odenthal

Blumenthalerstrasse 33, 5000 Cologne 1

im Dienste der Sicherheit und einer besseren Umwelt

State Supervisory Office for Trade and Industry Cologne
Mail Box No 140 149, 5000 Cologne 1

Address for visitors:
Blumenthalstr 33, 5000 Cologne 1
Responsible official: Mr Odenthal
Room: 371 Telephone 77 40-525

The company
Bayer AG
Leverkusen Plant
LE WI Concessions
Attn of Dr. Knopf
5090 Leverkusen

Your Reference,	Our Reference	Date
	2202 - Od/Pk	6th May 1993

Concerning: Registration of Nisoldipine in the USA

Reference: Your Telefax of 28th April 1993

Dear Sir or Madam,

As requested I confirm that the manufacture of Nisoldipine Coat Core Tablets from the active ingredient Nisoldipine at your premises in Leverkusen is subject to the Federal Immission Control Act (Bundes-Immissionsschutzgesetz = BImSchG).

In this case, according to § 22 BImSchG (Federal Immission Control Act) the facilities do not require specific approval but must be constructed and operated that

1. based on current state of technology harmful effects upon the environment are prevented,

2. when, according to the current state of the art, unavoidable effects on the environment are present, these must be restricted to a minimum,

3. waste which is generated during the operation of the facilities is lawfully disposed of.

The facilities are subject to other public regulations, in particular for the protection of nature, the landscape and water and compliance with these is controlled by the State Environmental Agency.

Blumenthalerstrasse 33, 5000 Cologne 1

Im Dienste der Sicherheit und einer besseren Umwelt

State Supervisory Office for Trade and Industry Cologne
Mail Box No 140 149, 5000 Cologne 1

The State Supervisory Office for Trade and Industry Cologne is responsible for matters concerned with the Federal Immission Control Act.

To my knowledge no complaints worthy of note are pending.

Yours faithfully

By authority

(Original signed)

(Odenthal)

DER
REGIERUNGSPRÄSIDENT
DÜSSELDORF

Regierungspräsident Düsseldorf, Postfach 300 865, 4000 Düsseldorf 30

Öffentliche Verkehrsmittel ab Hauptbahnhof:
U-Bahn-Linie U 78, U 79 bis Kaiser-Karl-Ring

Telefon: (0211) 475-0
Durchwahl: (0211) 475- 2245
Telefax: (0211) 475-
Auskunft erteilt: Herr Woog
Zimmer: 245

Dienstgebäude
Cecilienallee 2

Bitte in der Antwort mein Zeichen angeben

Ihr Zeichen, Ihre Nachricht vom	Mein Zeichen	Düsseldorf
Betrifft:	55.8851.4.1	7.05.1987

Firma
Bayer AG
z. Hd. Herrn Dr. Thiem
Postfach 10 17 09

5600 Wuppertal 1

FDA - Environmental assessment

Sehr geehrte Damen und Herren!

Wunschgemäß bestätige ich daß ich die Herstellung von

Nisoldipine (Dihydropyridine)

in Ihrem Werk Wuppertal-Elberfeld mit Genehmigungsbescheid

23.8851-8859/3032 vom 13.03.1987

nach dem Bundes-Immissionsschutzgesetz genehmigt habe.

Eine derartige Genehmigung wird nur erteilt, wenn sichergestellt ist, daß Menschen, Tiere und Pflanzen, der Boden, das Wasser, die Atmosphäre sowie Kultur- und sonstige Sachgüter vor schädlichen Umwelteinwirkungen und vor Gefahren, erheblichen Nachteilen und erheblichen Belästigungen geschützt werden und nach dem Stand der Technik Vorsorge gegen schädliche Umwelteinwirkungen getroffen wird.

Gleitende Arbeitszeit
(Kernarbeitszeit montags und donnerstags von 8.30 - 15.00 Uhr, mittwochs bis freitags 8.30 - 14.30 Uhr)
Sprechtage nur montags und donnerstags

Telefax (Zentral)
(0211) 475 - 2671
Telex
85 64 836
rp d

Konto der Regierungshauptkasse
Westdeutsche Landesbank
Girozentrale Düsseldorf
(BLZ 300 500 00) Kto. 4 100 012

Außerdem dürfen andere öffentlich-rechtliche Vorschriften - insbesondere auch aus dem Natur-, Landschafts- und Gewässerschutz - dem Vorhaben nicht entgegenstehen.

Vor Erteilung der Genehmigung sind daher unter Beteiligung der dafür zuständigen Behörden alle relevanten Umweltbelange überprüft worden. Damit ist sichergestellt, daß zum Zeitpunkt der Genehmigung die gültigen Vorschriften eingehalten werden.

Die Anlage unterliegt darüber hinaus der besonderen Überwachung durch staatliche Umweltbehörden, insbesondere durch das für Belange des Immissionsschutzes zuständige Staatliche Gewerbeaufsichtsamt Wuppertal, das wiederum meiner Aufsicht untersteht.

Erwähnenswerte Beanstandungen liegen nach meiner Kenntnis nicht vor.

Mit freundlichen Grüßen
Im Auftrag

PL

(Dr. Pfeifer)

(Original has the Coat of Arms of the Administrative District of Düsseldorf)

THE
PRESIDENT OF THE
ADMINISTRATIVE DISTRICT
DUSSELDORF

Administrative District Düsseldorf,
Mail Box No 300 865, 4000 Düsseldorf 30

Public transport via routes U 79 and U 78
from Main Station to Klever Platz

To

The company
Bayer AG
Mail Office Box 101709
Attn of Dr. Thiem
5600 Wuppertal - 1

Tel Nr (0214) 475 - 0 or
(0214) 475 -2245 Room 245
Telefax 475 2989

Further information will
be given by Mr Woog
Please quote my Reference in your answer

Your Reference, Your communication of

Our Reference
55.8851.4.1

Düsseldorf
7th May 1993

Concerning

FDA - Environmental assessment

Dear Sir or Madam,

As requested I confirm that I have approved the manufacture of

Nisoldipine (Dihydropyridine)

at your premises in Wuppertal - Elberfeld with Certificate

23.8851-8859/ 3032 issued on 13th March 1987

in accordance with the Federal Immission Control Act (Bundes-Immissionsschutzgesetz).

Such approval is only given if it has been ensured that humans, animals and plants, the soil, the water and the atmosphere as well as cultural and other properties are protected from harmful environmental effects and from risks, substantial detriment and substantial inconvience and that measures commensurate with the state of technology have been taken to protect against harmful effects upon the environment.

In addition other public regulations, in particular for the protection of nature, the landscape and water must not bar the intended action.

Prior to approval being issued, all the relevant environmental aspects have therefore been checked with the participation of the responsible authorities. This guarantees that at the time of approval all valid regulations are adhered to.

The facilities are also subject the specific supervision of the state environmental authorities, in particular the State Supervisory Office of Trades and Industry in Wuppertal which is responsible for environmental protection matters, this latter office being under my supervision.

To my knowledge no complaints worthy of note are pending.

Yours faithfully

By authority

(Original signed)

(Dr. Pfeifer)

ATTACHMENT V

Bayer AG is regulated by the following environmental laws and regulations:

1. **"Bendesemissionsschutzgesetz"** (Federal Law for the Protection of the Environment against the Adverse Influences caused by Contamination of the Air by noise, vibration and similar events). Published in Federal Law Gazette, March 15, 1974, amended August 12, 1980.

2. **"Wasserhaushaltsgesetz"** (Federal Law for the Protection of Water) Published in Federal Law Gazette, October 16, 1976, amended March 28, 1980.

3. **"Abfallgesetz"** (Federal Law for Minimization and Disposal of Waste) Published August 27, 1986.

4. **"TA Luft"** (Clean Air Laws) Published in Joint Ministerial Gazette, February 27, 1986.

5. **"TA Lärm"** (Noise Protections Laws) Published in July 16, 1986.

6. **"Chemikaliegesetz"** (Federal Law for Protection Against Dangerous Chemicals), Published in Federal Law Gazette, September 16, 1980.

7. **"Gefahrstoffverordnung"** (Regulations for Dangerous Products)Published in Federal Law Gazette, August 28, 1986.

8. **"Druckbehälterverordnung"** (Regulations for Pressure Vessels for Compressed Gases) Published in Federal Law Gazette, February 27, 1980.

9. **"Störfallverordnung"** (Federal Law for Protection of the Environment) Published in Federal Law Gazette, June 27, 1980.

10. **"Verordnung über Anlagen Zur Lagerung, Abfüllung und Beförderung brennbarer Flüssigkeiten Zu Lande"** (Regulations for Facilities for Storage, Filling and Transport of Inflammable Liquids on Land) Published in Federal Law Gazette, February 27, 1980, amended May 3, 1982.

11. **"Gefahrgutverordnung Straße"** (Regulations for the Transport of Dangerous Products by Road) Published in Federal Law Gazette, July 22, 1985.

12. **"Gefahrgutverordnung Eisenbahn"** (Regulations for the Transport of Dangerous Products by Railway) Published in Federal Law Gazette, July 22, 1985.

13. **"Gefahrgutverordnung See"** (Regulations for the Transport of Dangerous Products by Sea) Published in Federal Law Gazette, July 27, 1985.

14. "Gefahrgutverordnung Binnenschiffahrt" (Regulations for the Transport of Dangerous Products on Waterways within Germany) Published in Federal Law Gazette,March 24, 1983.

15. "IATA - DGR" (Dangerous Goods Regulations, 28th edition.

16. "Verordnung über Trinkwasser und über Wasser für Lebensmittelbetriebe" (Regulations for Drinking Water and Food Handling Factories) Published Federal Law Gazette, may 22, 1986

17. "Futtermittelgesetz" (Federal Law on Feedstuffs) Published in Federal Law Gazette July 2, 1975.

18. "Futtermittelverordnung" (Regulations on Feedstuffs) Published in Federal Law Gazette, April 8, 1981.

19. "Arbeitstättenverordnung" (Regulations for the Working Place) Published Federal Law Gazette, May 20 , 1975

BAYER AG
PHARMA PRODUCTION
5090 LEVERKUSEN

Nisoldipine Tablets (all dosage forms) 8.62 - 1

Environmental Impact Statement
(For public information)

Number of Pages
(without coverpage)

3

Dr. Thiem

Dr. Franklin

valid from 24.03.1993

Environmental Impact Statement
(For public information)

1. Legal Situation

The manufacture of pharmaceutical active ingredients and the manufacture of pharmaceutical dosage forms is regulated by the Act on the Prevention of Harmful Effects on all the compartments of the Environment, e.g. Air Pollution, Water Pollution, Land Pollution, Noise, Vibration, and similar Phenomena (Federal Immission Control Act - BImSchG) of the Federal Republic of Germany, (§§ 22 and 23).

2. Specific Situation

The plant at which the pharmaceutical dosage forms of

Nisoldipine Tablets

or its pharmaceutical intermediates are manufactured is situated in Leverkusen, in the

Administrative District of Köln,

and the installations of this manufacturing site are operated in accordance to the above Act to carry out certain defined pharmaceutical processes, e.g.

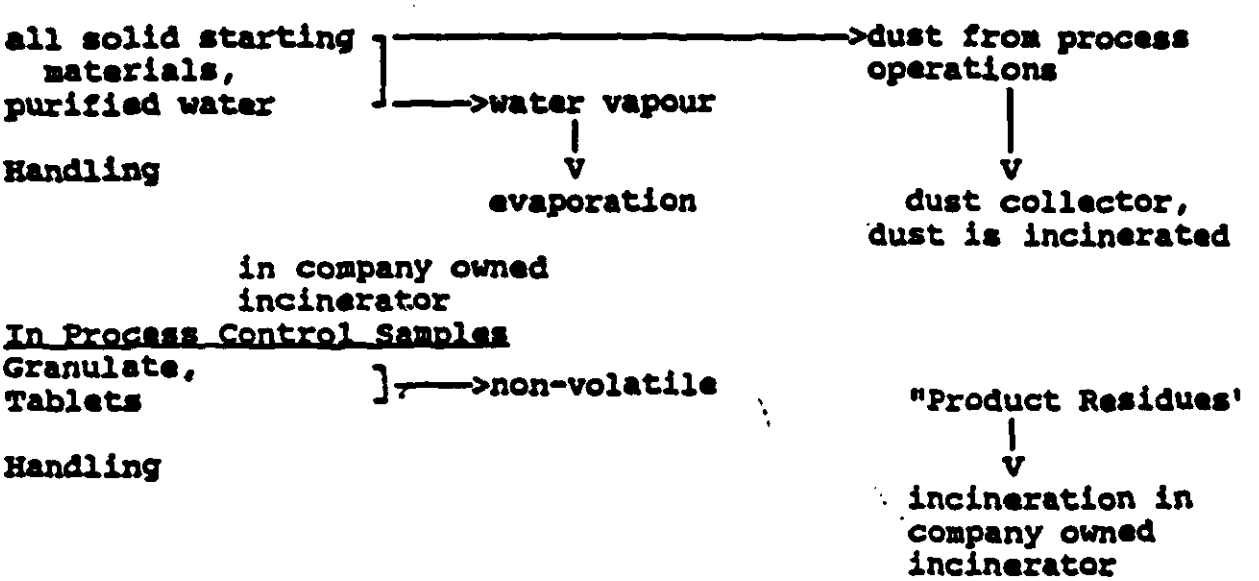
an installation to manufacture solid pharmaceutical dosage forms (Nisoldipine Tablets are "Solid Dosage Forms")

The operations to manufacture Nisoldipine Tablets thus are carried out in accordance to the above Act.

Environmental Impact Statement
(For public information)

3. Process Flow Sheet

The flow sheet for the process is as follows:



The ash from the incinerators is disposed in a company owned landfill operated according to the applicable German Laws on landfill operations.

The possible emission quantities resulting from the manufacturing process are reduced to safe levels.

4. General Requirements

During handling of solids "Total Dust Emission Levels" are to be observed.
Total Dust emitted at a mass flow rate of more than 0,5 kg/h the mass concentration shall not exceed 50 mg/m³

emitted at a mass flow rate of less than 0,5 kg/h the mass concentration shall not exceed 150 mg/m³
The permitted dust emission levels are considered safe towards man and environment. The levels are regulated.

Environmental Impact Statement
(For public information)

5. Specific Additional Requirements

The operation for the installations at which the pharmaceutical dosage forms of NISOLDIPINE TABLETS are manufactured is thus subjected to the following specific requirements:

Waste water must be channeled to a waste water treatment plant, and the water from this waste water treatment plant must meet the requirements for "Treated Water"
(The Decree on the Disposal of Waste Water).

Solid residues resulting from the operation of the installation are to be converted by thermic, chemical, physical, or biological treatment to minimize the volume and to reduce the environmental hazards to safe levels.

6. Compliance with the Law

The design and operation of the installation to manufacture NISOLDIPINE TABLETS is operated in compliance with the applicable Act. Records of the controls exercised during the manufacture of the pharmaceutical dosage forms are maintained. All solid residues resulting from the operation of the installation are to be incinerated in an incineration installation approved for industrial residues.
The ash from the incinerators is disposed in a company owned landfill operated according to the applicable German Laws on landfill operations.

7. Effects of Changing Production Volumes

The production volumes of pharmaceutical dosage forms manufactured may be changed, provided that the total emission levels are not exceeded.

8. Conclusions

Nisoldipine Tablets (all dosage forms) are manufactured with safe emission levels being laid down and regulated.