METHYLENE BLUE- methylene blue injection, solution
Amneal Pharmaceuticals LLC

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use METHYLENE BLUE INJECTION safely and effectively. See full prescribing information for METHYLENE BLUE INJECTION.
METHYLENE BLUE INJECTION, USP for intravenous use
Initial U.S. Approval: 2016

WARNING: SEROTONIN SYNDROME WITH CONCOMITANT USE OF SEROTONERGIC DRUGS AND OPIOIDS

See full prescribing information for complete boxed warning.

Methylene blue injection may cause serious or fatal serotonergic syndrome when used in combination with serotonergic drugs and opioids. Avoid concomitant use of METHYLENE BLUE INJECTION with selective serotonin reuptake inhibitors (SSRIs), serotonin norepinephrine reuptake inhibitors (SNRIs), monoamine oxidase inhibitors (MAOIs) and opioids *(5.1, 7)*

RECENT MAJOR CHANGES

Boxed Warning	11/2023
Indications and Usage (1)	01/2024
Warnings and Precautions (5)	11/2023

INDICATIONS AND USAGE

Methylene blue injection is an oxidation-reduction agent indicated for the treatment of pediatric and adult patients with acquired methemoglobinemia. (1)

DOSAGE AND ADMINISTRATION

- Administer 1 mg/kg intravenously over 5-30 minutes. (2.1)
- If methemoglobin level remains above 30% or if clinical symptoms persist, give a repeat dose of up to 1 mg/kg one hour after the first dose. (2.1)
- Administer a single dose of 1 mg/kg in patients with moderate or severe renal impairment. (2.2)

DOSAGE FORMS AND STRENGTHS

50 mg/10 mL (5 mg/mL) (0.5%) single-dose ampule. (3)

CONTRAINDICATIONS

Methylene blue injection is contraindicated in the following conditions (4):

- Severe hypersensitivity to methylene blue
- Patients with glucose-6-phosphate dehydrogenase deficiency (G6PD) due to the risk of hemolytic anemia

WARNINGS AND PRECAUTIONS

- Hypersensitivity: If severe or life threatening allergic reaction occurs, discontinue methylene blue injection, treat the allergic reaction, and monitor until signs and symptoms resolve (5.2)
- Lack of Effectiveness: Consider alternative treatments if there is no resolution of methemoglobinemia after 2 doses (2.1, 5.3)
- Hemolytic Anemia: Discontinue methylene blue injection and transfuse (5.4)
- Interference with *In-Vivo* Monitoring Devices: Use methods other than pulse oximetry to assess oxygen saturation (5.5)
- Effects on Ability to Drive and Operate Machinery: Advise patients to refrain from these activities until neurologic and visual symptoms have resolved (5.6)

ADVERSE REACTIONS

The most commonly reported adverse reactions (≥2%) included headache, hypokalemia, diarrhea, hypomagnesemia, myoclonus, nausea, and seizure-like phenomena. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Amneal Pharmaceuticals at 1-877-835-

5472 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

USE IN SPECIFIC POPULATIONS

- Pregnancy: Only use during pregnancy if the potential benefit justifies the potential risk to the fetus. (8.1)
- Lactation: Discontinue breast-feeding for up to 8 days after treatment. (8.2).
- Hepatic Impairment: Monitor patients longer for toxicity and drug interactions due to delayed clearance. (8.7)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 11/2024

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

WARNING: SEROTONIN SYNDROME WITH CONCOMITANT USE OF SEROTONERGIC DRUGS AND OPIOIDS

Methylene Blue Injection may cause serious or fatal serotonergic syndrome when used in combination with serotonergic drugs and opioids. Avoid concomitant use of methylene blue injection with selective serotonin reuptake inhibitors (SSRIs), serotonin norepinephrine reuptake inhibitors (SNRIs), monoamine oxidase inhibitors (MAOIs) and opioids *[see Warnings and Precautions (5.1)* and *Drug Interactions (7)].*

1 INDICATIONS AND USAGE

Methylene blue injection, USP is indicated for the treatment of pediatric and adult patients with acquired methemoglobinemia.

2 DOSAGE AND ADMINISTRATION

2.1 Dosage and Administration

- Ensure patent venous access prior to administration of methylene blue injection. Do not administer methylene blue injection subcutaneously.
- Administer methylene blue injection 1 mg/kg intravenously over 5 to 30 minutes.
- If the methemoglobin level remains greater than 30% or if clinical signs and symptoms persist, a repeat dose of methylene blue injection 1 mg/kg may be given one hour after the first dose.
- If methemoglobinemia does not resolve after 2 doses of methylene blue injection, consider initiating alternative interventions for treatment of methemoglobinemia.

2.2 Recommended Dosage for Renal Impairment

- The recommended dosage of methylene blue injection in patients with moderate or severe renal impairment (eGFR 15 to 59 mL/min/1.73 m^2) is a single dose of 1 mg/kg.
- If the methemoglobin level remains greater than 30% or if the clinical symptoms persist 1 hour after dosing, consider initiating alternative interventions for the treatment of methemoglobinemia.

2.3 Preparation

Methylene blue injection is hypotonic and may be diluted before use in a solution of 50 mL 5% Dextrose Injection in order to avoid local pain, particularly in the pediatric population. Use the diluted solution immediately after preparation.

Avoid diluting with sodium chloride solutions, because it has been demonstrated that chloride reduces the solubility of methylene blue.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

Discard unused portion.

3 DOSAGE FORMS AND STRENGTHS

Methylene blue injection, USP: 50 mg/10 mL (5 mg/mL) (0.5%) clear dark blue solution in single-dose ampules.

4 CONTRAINDICATIONS

Methylene blue injection is contraindicated in the following conditions:

- Severe hypersensitivity reactions to methylene blue or any other thiazine dye *[see Warnings and Precautions (5.2)]*.
- Patients with glucose-6-phosphate dehydrogenase deficiency (G6PD) due to the risk of hemolytic anemia *[see Warnings and Precautions (5.3, 5.4)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Serotonin Syndrome with Concomitant Use of Serotonergic Drugs and Opioids

The development of serotonin syndrome has been reported with use of methylene blue class products. Most reports have been associated with concomitant use of serotonergic drugs (e.g., selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), monoamine oxidase inhibitors (MAOIs). Opioids and dextromethorphan may increase the risk of developing serotonin syndrome. Some of the reported cases were fatal. Symptoms associated with serotonin syndrome may include the following combination of signs and symptoms: mental status changes (e.g., agitation, hallucinations, delirium, and coma), autonomic instability (e.g., tachycardia, labile blood pressure, dizziness, diaphoresis, flushing, and hyperthermia), neuromuscular symptoms (e.g., tremor, rigidity, myoclonus, hyperreflexia, and incoordination), seizures, and/or gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea). Avoid concomitant use of methylene blue injection with serotonergic drugs and opioids.

Patients treated with methylene blue injection should be monitored for the emergence of serotonin syndrome. If symptoms of serotonin syndrome occur, discontinue use of methylene blue injection, and initiate supportive treatment. Inform patients of the increased risk of serotonin syndrome and advise them to not to take serotonergic drugs within 72 hours after the last dose of methylene blue injection *[see Drug Interactions (7)* and*Patient Counseling Information (17)]*.

5.2 Hypersensitivity

Anaphylactic reactions to methylene blue injection class products have been reported. Patients treated with methylene blue injection should be monitored for anaphylaxis. If anaphylaxis or other severe hypersensitivity reactions (e.g., angioedema, urticaria, bronchospasm) should occur, discontinue use of methylene blue injection and initiate supportive treatment. Methylene blue injection is contraindicated in patients who have experienced anaphylaxis or other severe hypersensitivity reactions to a methylene blue injection class product in the past.

5.3 Lack of Effectiveness

Methemoglobinemia may not resolve or may rebound after response to treatment with methylene blue injection in patients with methemoglobinemia due to aryl amines such as aniline or sulfa drugs such as dapsone. Monitor response to therapy with methylene blue injection through resolution of methemoglobinemia. If methemoglobinemia does not respond to 2 doses of methylene blue injection or if methemoglobinemia rebounds after a response, consider additional treatment options *[see Dosage and Administration (2.2)]*.

Patients with glucose-6-phosphate dehydrogenase deficiency may not reduce methylene blue injection to its active form *in vivo*. Methylene blue injection may not be effective in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency.

5.4 Hemolytic Anemia

Hemolysis can occur during treatment of methemoglobinemia with methylene blue injection. Laboratory testing may show Heinz bodies, elevated indirect bilirubin and low haptoglobin, but the Coombs test is negative. The onset of anemia may be delayed 1 or more days after treatment with methylene blue injection. The anemia may require red blood cell transfusions *[see Adverse Reactions (6.1)]*. Use the lowest effective number of doses of methylene blue injection to treat methemoglobinemia. Discontinue methylene blue injection and consider alternative treatments of methemoglobinemia if severe hemolysis occurs.

Treatment of patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency with methylene blue injection may result in severe hemolysis and severe anemia. Methylene blue injection is contraindicated for use in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency *[see Contraindications (4)]*.

5.5 Interference with In Vivo Monitoring Devices

- Inaccurate Pulse Oximeter Readings

The presence of methylene blue in the blood may result in an underestimation of the oxygen saturation reading by pulse oximetry. If a measure of oxygen saturation is required during or shortly after infusion of methylene blue injection, it is advisable to obtain an arterial blood sample for testing by an alternative method.

- Bispectral index monitor

A fall in the Bispectral Index (BIS) has been reported following administration of methylene blue class products. If methylene blue injection is administered during surgery, alternative methods for assessing the depth of anesthesia should be employed.

5.6 Effects on Ability to Drive and Operate Machinery

Treatment with methylene blue injection may cause confusion, dizziness and disturbances in vision *[see Adverse Reactions (6)]*. Advise patients to refrain from driving or engaging in hazardous occupations or activities such as operating heavy or potentially dangerous machinery until such adverse reactions to methylene blue injection have resolved.

5.7 Interference with Laboratory Tests

Methylene blue injection is a blue dye which passes freely into the urine and may interfere with the interpretation of any urine test which relies on a blue indicator, such as the dipstick test for leucocyte esterase.

6 ADVERSE REACTIONS

The following adverse reactions are discussed in greater detail in other sections of the labeling:

- Serotonin Syndrome with Concomitant Use of Serotonergic Drugs *[see Warnings and Precautions (5.1)]*
- Anaphylaxis *[see Warnings and Precautions (5.2)]*
- Lack of Effectiveness *[see Warnings and Precautions (5.3)]*
- Hemolytic Anemia *[see Warnings and Precautions (5.4)]*
- Interference with In-Vivo Monitoring Devices *[see Warnings and Precautions (5.5)]*
- Effects on Ability to Drive and Operate Machinery *[see Warnings and Precautions (5.6)]*
- Interference with Laboratory Tests *[see Warnings and Precautions (5.7)]*

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The safety of methylene blue injection in adults with acquired methemoglobinemia was assessed in 24 patients who received at least 1 dose of methylene blue injection *[see Clinical Studies (14)].* Most doses administered were 1 mg/kg (88.5%), but doses from 1 mg/kg to 2 mg/kg were administered. All patients received at least one dose of methylene blue injection; two received two doses. Serious adverse reactions occurred in 4.2% of patients who received methylene blue injection. A serious adverse reaction of seizure-like phenomenon was reported in one patient. Adverse reactions (≥2%) included headache, hypokalemia, diarrhea, hypomagnesemia, myoclonus, nausea, and seizure-like phenomena.

The safety of methylene blue injection in pediatric patients with acquired methemoglobinemia was assessed in two retrospective case series that included two pediatric patients treated with methylene blue injection and 12 treated with another methylene blue product. The case series included patients in the following age groups: 3 neonates (<1 month), 4 infants (1 month to <2 years), 4 children (2 years to <12 years), and 3 adolescents (12 years to <17 years). The safety profile in pediatric patients was similar to that in adult patients.

Other adverse reactions reported to occur following administration of methylene blue class products include the following:

Blood and lymphatic system disorders: hemolytic anemia, hemolysis, hyperbilirubinemia

Cardiac disorders: palpitations, tachycardia

Eye disorders: eye pruritus, ocular hyperemia, vision blurred

Gastrointestinal disorders: abdominal pain lower, dry mouth, flatulence, glossodynia, tongue eruption

General disorders and administration site conditions: death, infusion site extravasation, infusion site induration, infusion site pruritus, infusion site swelling, infusion site urticaria, peripheral swelling, thirst

Investigations: elevated liver enzymes

Musculoskeletal and connective tissue disorders: myalgia

Renal and urinary disorders: dysuria

Respiratory, thoracic and mediastinal disorders: nasal congestion, oropharyngeal pain, rhinorrhea, sneezing

Skin and subcutaneous tissue disorders: necrotic ulcer, papule, phototoxicity

Vascular disorders: hypertension

7 DRUG INTERACTIONS

Clinically significant drug interactions with methylene blue injection are described below:

The concomitant use of methylene blue injection with other drugs that affect the serotonergic neurotransmitter system has resulted in serotonin syndrome. Although the mechanism is not clearly understood, literature reports suggest methylene blue injection is a potent reversible inhibitor of monoamine oxidase. Avoid concomitant use of methylene blue injection with medicinal products that enhance serotonergic transmission including antidepressants like SSRIs (selective serotonin reuptake inhibitors), SNRIs (serotonin and norepinephrine reuptake inhibitors), MAOIs (monoamine oxidase inhibitors), bupropion, buspirone, clomipramine, mirtazapine, linezolid, opioids, and dextromethorphan because of the potential for serious CNS reactions, including potentially fatal serotonin syndrome. If the intravenous use of methylene blue injection cannot be avoided in patients treated with serotonergic medicinal products, choose the lowest possible dose and observe closely the patient for CNS effects for up to 4 hours after administration *[see Warnings and Precautions (5.1)* and *Clinical Pharmacology (12.3)]*.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

Methylene blue injection may cause fetal harm when administered to a pregnant woman.

Intra-amniotic injection of pregnant women with a methylene blue class product during the second trimester was associated with neonatal intestinal atresia and fetal death. Methylene blue produced adverse developmental outcomes in rats and rabbits when administered orally during organogenesis at doses at least 32 and 16 times, respectively, the clinical dose of 1 mg/kg *[see Data]*. Advise pregnant women of the potential risk to a fetus.

In the U.S. general population, the estimated background risks of major birth defects and miscarriage in clinically recognized pregnancies are 2% to 4% and 15% to 20%, respectively.

Clinical Considerations

Fetal/neonatal adverse reactions

Intra-amniotic injection of a methylene blue class product hours to days prior to birth can result hyperbilirubinemia, hemolytic anemia, skin staining, methemoglobinemia, respiratory distress and photosensitivity in the newborn. Following administration of methylene blue injection to a pregnant woman at term, observe the newborn for these adverse reactions and institute supportive care.

Data

Animal Data

Methylene blue was administered orally to pregnant rats at doses of 50 to 350 mg/kg/day, during the period of organogenesis. Maternal and embryofetal toxicities were observed at all doses of methylene blue and were most evident at the 200 and 350 mg/kg/day doses. Maternal toxicity consisted of increased spleen weight. Embryo-fetal toxicities included reduced fetal weight, post-implantation loss, edema, and malformations including enlarged lateral ventricles. The dose of 200 mg/kg (1200 mg/m^2) in rats is approximately 32 times a clinical dose of 1 mg/kg based on body surface area.

Methylene blue was administered orally to pregnant rabbits at doses of 50, 100, or 150 mg/kg/day, during the period of organogenesis. Maternal death was observed at the methylene blue dose of 100 mg/kg. Embryofetal toxicities included spontaneous abortion at all dose levels and a malformation (umbilical hernia) at the 100 and 150 mg/kg/day doses. The dose of 50 mg/kg (600 mg/m^2) in rabbits is approximately 16 times a clinical dose of 1 mg/kg based on body surface area.

8.2 Lactation

Risk Summary

There is no information regarding the presence of methylene blue in human milk, the effects on the breastfed infant, or the effects on milk production. Because of the potential for serious adverse reactions, including genotoxicity discontinue breast-feeding during and for up to 8 days after treatment with methylene blue injection *[see Clinical*

Pharmacology (12.3)].

8.4 Pediatric Use

The safety and effectiveness of methylene blue injection for the treatment of acquired methemoglobinemia have been established in pediatric patients. Use of methylene blue injection is supported by two retrospective case series that included 2 pediatric patients treated with methylene blue injection and 12 treated with another methylene blue class product. The case series included pediatric patients in the following age groups: 3 neonates (less than 1 month), 4 infants (1 month up to less than 2 years), 4 children (2 years up to less than 12 years), and 3 adolescents (12 years to less than 17 years). The efficacy outcomes were consistent across pediatric and adult patients in both case series *[see Clinical Studies (14)]*.

8.5 Geriatric Use

Clinical studies of methylene blue injection did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects. Other reported clinical experience has not identified differences in responses between the elderly and younger patients. methylene blue injection is known to be substantially excreted by the kidney, so the risk of adverse reactions to this drug may be greater in patients with impaired renal function. Because elderly patients are more likely to have decreased renal function, treatment of methemoglobinemia in these patients should use the lowest number of doses needed to achieve a response *[see Dosage and Administration (2)]*.

8.6 Renal Impairment

Methylene blue concentrations increased in subjects with renal impairment (eGFR 15 to 89 mL/min/1.73m^2) significantly *[see Clinical Pharmacology (12.3)]*. Adjust methylene blue injection dosage in patients with moderate or severe renal impairment (eGFR 15 to 59 mL/min/1.73 m^2) *[see Dosage and Administration (2.2)]*. No dose adjustment is recommended in patients with mild renal impairment (eGFR 60 to 89 mL/min/1.73 m^2).

8.7 Hepatic Impairment

Methylene blue is extensively metabolized in the liver. Monitor patients with any hepatic impairment for toxicities and potential drug interactions for an extended period of time following treatment with methylene blue injection.

10 OVERDOSAGE

Hypotension, wheezing and reduced oxygenation have been reported in patients who received methylene blue class products in single doses of 3 mg/kg or more.

Administration of large intravenous doses (cumulative dose ≥ 7 mg/kg) of a methylene blue class product caused nausea, vomiting, precordial pain, dyspnea, tachypnea, chest tightness, tachycardia, apprehension, tremor, mydriasis, blue staining of the urine, the skin and mucous membranes, abdominal pain, dizziness, paresthesia, headache, confusion, mild methemoglobinemia (up to 7%) and electrocardiogram changes (T-wave flattening or inversion). These effects lasted 2 to 12 hours following administration.

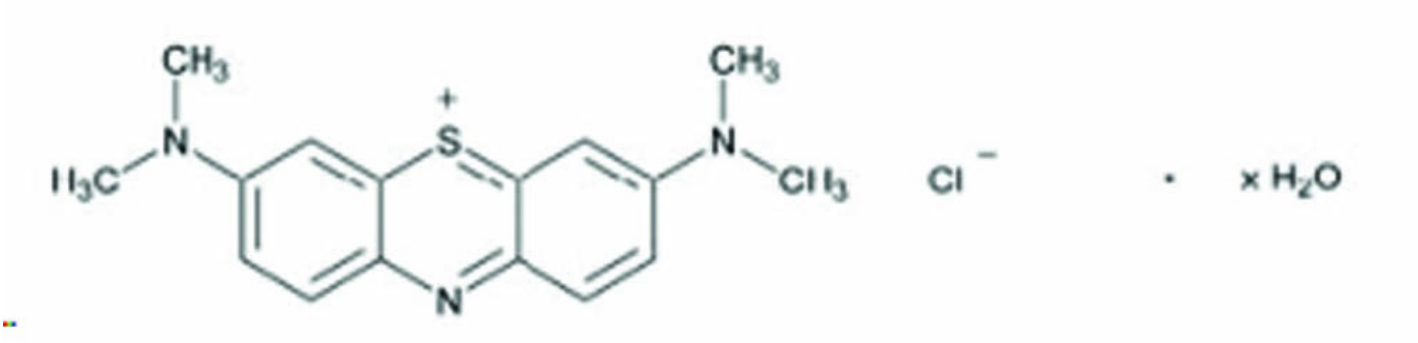
A severe overdosage (single dose of 20 mg/kg or more) of a methylene blue class product caused severe intravascular hemolysis, hyperbilirubinemia and death.

In case of overdose of methylene blue injection, maintain the patient under observation until signs and symptoms have resolved, monitor for cardiopulmonary, hematologic and neurologic toxicities, and institute supportive measures as necessary.

11 DESCRIPTION

Methylene blue is an oxidation-reduction agent.

Its chemical name is 3,7-bis(dimethylamino)phenothiazin-5-ium, chloride hydrate. The molecular formula of methylene blue injection, USP is $C_{16}H_{18}ClN_3S.xH_2O$ and its molecular weight of 319.86 g/mol for the anhydrous form. The structural formula of Methylene blue is:



Methylene blue injection, USP is a sterile solution intended for intravenous administration. Each mL of solution contains 5 mg methylene blue and water for injection. Methylene blue injection, USP is a clear dark blue solution with a pH value between 3.0 and 4.5. The osmolality is between 10 and 15 mOsm/kg. Methylene blue injection, USP strength is expressed in terms of trihydrate.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Methylene blue is a water-soluble thiazine dye that promotes a non-enzymatic redox conversion of metHb to hemoglobin. In situ, methylene blue is first converted to leucomethylene blue (LMB) via NADPH reductase. It is the LMB molecule which then reduces the ferric iron of metHb to the ferrous state of normal hemoglobin.

12.2 Pharmacodynamics

Low concentrations of methylene blue speeds up the *in vivo* conversion of methemoglobin to hemoglobin. Methylene blue has been observed to stain tissues selectively. The exposure-response or –safety relationship for methylene is unknown.

Cardiac Electrophysiology

The results of a thorough QT study demonstrated methylene blue injection at an intravenous dose of 2 mg/kg as a 5-minute intravenous infusion had no effect on the QT, PR or QRS intervals.

12.3 Pharmacokinetics

The mean (CV%) Cmax and AUC of methylene blue 2,917 ng/mL (39%) and 13,977 ng.hr/mL (21%) following a 2 mg/kg dose administered as a 5-minute intravenous infusion.

Distribution

The mean± standard deviation steady state volume of distribution of a 2 mg/kg dose of methylene blue injection was 255 L ± 58. The mean plasma protein binding of methylene blue is approximately 94% *in vitro*. Methylene blue exhibits concentration-dependent partitioning into blood cells *in vitro*. The blood-to-plasma ratio was 5.1±2.8 at 5 minutes from the start of a 2 mg/kg dose administered as a 5-minute intravenous infusion and reached a plateau of 0.6 at 4 hours in a clinical study. Methylene Blue is a substrate for the P-glycoprotein (P-gp, ABCB1) transporter, but not for BCRP or OCT2 *in vitro*.

Elimination

Methylene blue has a half-life of approximately 24 hours in humans.

Metabolism

Methylene blue is metabolized by CYPs 1A2, 2C19 and 2D6 *in vitro*; however, the predominant *in vitro* pathway appears to be UGT-mediated conjugation by multiple UGT enzymes, including UGT1A4 and UGT1A9.

Azure B, which is a minor impurity in methylene blue, is also formed in humans as a metabolite of methylene blue, with an overall drug/metabolite AUC ratio of greater than 6:1. Azure B has 8-fold lower potency than methylene blue.

Excretion

Approximately 40% of methylene blue is excreted into the urine unchanged.

Specific Populations

Renal Impairment

After a single 1 mg/kg dose of methylene blue injection, $AUC_{0\text{-}96h}$ increased by 52%, 116%, and 192% in subjects with mild (estimated glomerular filtration rate (eGFR) 60 to 89 mL/min/1.73 m^2), moderate (eGFR 30 to 59 mL/min/1.73m^2), and severe (eGFR 15 to 29 mL/min/1.732m^2) renal impairment, respectively. C_{max} increased by 42%, 34%, and 15% in subjects with mild, moderate, and severe renal impairment respectively *[see*

Dosage and Administration (2.2) and*Use in Specific Populations (8.6)]*. The half-life was unchanged in patients with mild to moderate renal impairment.

The $AUC_{0\text{-}96h}$ of Azure B after a single 1 mg/kg dose increased by 29%, 94%, and 339% in subjects with mild (estimated glomerular filtration rate (eGFR) 60 to 89 mL/min/1.73 m^2), moderate (eGFR 30 to 59 mL/min/1.73m^2), and severe (eGFR 15 to 29 mL/min/1.732m^2) renal impairment, respectively. C_{max} increased by 23%, 13%, and 65% in subjects with mild, moderate, and severe renal impairment, respectively *[see Dosage and Administration (2.2)* and*Use in Specific Populations (8.6)]*

Drug Interactions Studies

Clinical Studies:

The coadministration of 2 mg/kg dose of methylene blue injection with midazolam (a CYP3A4 substrate), caffeine (a CYP1A2 substrate), warfarin (a CYP2C9 substrate), and dextromethorphan (a CYP2D6 substrate) in a cocktail study did not affect the exposure of these substrates compared to their exposure without methylene blue injection administration.

In Vitro Studies:

Cytochrome P450 (CYP450) Enzymes:

Methylene blue inhibits CYP isozymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4/5. Possible time-dependent inhibition of CYP2C9, CYP2D6 and CYP3A4/5 (testosterone as substrate) was also observed. Methylene blue induces CYP1A2 but does not induce CYP2B6 or CYP3A4.

UDP-Glucuronosyltransferase (UGT):

Methylene blue inhibits UGT1A9 and UGT1A4, but did not significantly inhibit UGTs 1A1, 1A3, 1A6, 2B7 or 2B15.

Transporter:

Methylene blue is both a substrate for and an inhibitor of P-gp but is not a substrate for BCRP or OCT2 *in vitro*. Methylene blue is not a significant inhibitor of BCRP, OAT1, OAT3, OAT1B1 or OAT1B3. Methylene blue inhibits OCT2, MATE1 and MATE2-K.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis and Mutagenesis, Impairment of Fertility

In a two-year carcinogenicity study, rats were administered oral doses of methylene blue at 5, 25, or 50 mg/kg. Methylene blue caused pancreatic islet adenomas or carcinomas (combined) in male rats. In a two-year carcinogenicity study, mice were administered oral doses of methylene blue at 2.5, 12.5, or 25 mg/kg. There were no drug-related neoplastic findings in mice.

Methylene blue was genotoxic in gene mutation assays in bacteria (Ames test), and in an *in vitro* sister chromatid exchange test and an *in vitro* chromosomal aberration test in Chinese hamster ovary (CHO) cells. Methylene blue was negative for micronucleus induction in bone marrow or peripheral blood collected from mice treated with methylene blue.

Fertility studies with methylene blue have not been conducted. *In vitro*, methylene blue reduced motility of human sperm in a concentration dependent manner.

14 CLINICAL STUDIES

14.1 Treatment of Acquired Methemoglobinemia

The efficacy of methylene blue injection in the treatment of patients with methemoglobinemia was evaluated in 24 adult patients with acquired methemoglobinemia: in study NCT03542760, a prospective, multicenter, observational registry. Of the 24 subjects enrolled 92% were white, 8% were black, 67% were female, and 33% were male. Hispanic or Latino was 12.5%; non-Hispanic or Latino was 87.5%, and ethnicity data were missing for 0%. The mean age was 46 years, and the ages ranged from 19 to 72 years. Each individual received at least 1 intravenous dose of methylene blue injection; two received 2 doses. Most doses administered were 1 mg/kg (88.5%), but doses from 1 mg/kg to 2 mg/kg were administered. The recommended methylene blue injection dose is 1 mg/kg; lower or greater doses are not recommended. The maximum recommended number of doses is two *[see Dosage and Administration (2.1)]*.

In total, 22 of the 24 (91.7%) subjects had post treatment methemoglobin (metHb) assessment; 22 of the 22 subjects had baseline metHb with a mean concentration of 12.3% and a range of 4.1% to 30.0%. 21 of 22 (95.5%) subjects who had baseline metHb had at least a 50% reduction in metHB from baseline in their first assessment post baseline. This first post dosing assessment occurred from 0.7 to 27.3 hours from the end of first methylene blue injection infusion with a median time of 2.9 hours. There were 9 subjects that had baseline metHb and had metHb assessed within 2 hours of the end of the first methylene blue injection treatment; 6 of the 9 (67%; 95% CI (30.9%, 91.0%)) had at least a 50% reduction in metHb at 1 hour postdosing.

Available vital sign data including blood pressure, heart rate and respiratory rate were reviewed at baseline and compared to data collected within 2 hours post methylene blue injection infusion. Prior to treatment with methylene blue injection, 12 of the 18 (67%) of patients had a respiratory rate exceeding the upper limit of normal (≥ 20 bpm). Of these, 8 of the 12 (67%) experienced a normalization of respiratory rate within 2 hours post methylene blue injection infusion. There was minimal impact on other vital signs.

At baseline, the most common prespecified signs and symptoms of methemoglobinemia (reported by ≥2 subjects [8.3%] overall) were fatigue (33.3%), dyspnea (29.2%), cyanosis (12.5%), depressed CNS (12.5%), dizziness (8.3%), headache (8.3%), and

weakness (8.3%). Following treatment with methylene blue injection, signs and symptoms of methemoglobinemia improved.

The efficacy of methylene blue injection in the treatment of methemoglobinemia in pediatric patients was assessed in 14 patients in two retrospective case series (2 patients received methylene blue injection and 12 who received another methylene blue product). The ages ranged from 6 days to 16 years. The efficacy outcomes were consistent across the pediatric and adult populations.

16 HOW SUPPLIED

Methylene blue injection, USP is supplied in 10 mL single-dose ampules. Each 10 mL ampule contains 50 mg of Methylene blue as a clear dark blue solution. A box contains ten ampules.

Box of 10 ampules of 50 mg/10 mL (0.5%): NDC 70121-2715-2

Storage:

Store at 20°C to 25°C (68°F to 77°F); excursions permitted to 15°C to 30°C (59°F to 86°F) [See USP Controlled Room Temperature].

Any unused product or waste material should be disposed of in accordance with local practice.

Do not refrigerate or freeze.

Keep the ampule in the original package to protect from light.

17 PATIENT COUNSELING INFORMATION

Serotonin Syndrome

Advise patients of the possibility of serotonin syndrome, especially with concomitant use of serotonergic agents such as medications to treat depression and migraines. Advise patients to seek immediate medical attention if the following symptoms occur after treatment with methylene blue injection: changes in mental status, autonomic instability, or neuromuscular symptoms with or without gastrointestinal symptoms *[see Warnings and Precautions (5.1)]*.

Pregnancy

Advise pregnant women of the potential risk to the fetus with the use of methylene blue injection during pregnancy *[see Use in Specific populations (8.1)]*.

Breastfeeding

Advise patients to discontinue breast-feeding for up to 8 days after treatment with methylene blue injection *[see Use in Specific populations (8.2)]*.

Driving and Using Machines

Advise patients to avoid driving and use of machines during treatment with methylene blue injection. Driving can be affected as a result of a confusional state, dizziness and possible eye disturbances *[see Warnings and Precautions (5.6)]*.

Phototoxicity

Advise patients to take protective measures against exposure to light, because phototoxicity may occur after administration of methylene blue *[see Adverse Reactions (6.1)]*.

Skin and Body Fluid Blue Discoloration

Advise patients that methylene blue injection may cause a blue discoloration of the skin and body fluids *[see Adverse Reactions (6.1)]*.

Manufactured by:

Steriscience Sp. z o.o.

No. 10, Daniszewska Street,

Warsaw, Poland - 03-230

Distributed by:

Amneal Pharmaceuticals LLC

Bridgewater, NJ 08807

Rev. 11-2024-00

PRINCIPAL DISPLAY PANEL

NDC 70121-**2715**-2

Rx only

Methylene Blue Injection, USP

50 mg/10 mL

(5 mg/mL) (0.5%)

Intravenous Use only

For slow intravenous injection

Use 5% Dextrose Injection When Diluting

10 Single-Dose Ampules

Discard unused portion

amneal®

NO VARNISH ZONE
W110.5 x H55 mm

F.P.O
XXXX

XXXXXXXXX

NDC 70121-**2715**-2

Rx only

Methylene Blue Injection, USP

50 mg/10 mL

(5 mg/mL) (0.5%)

Intravenous Use only

For slow intravenous injection
Use 5% Dextrose Injection When Diluting

XXXX

Each 10 mL ampule contains 50 mg methylene blue dissolved in Water for injection, USP – **Intravenous use only.**

See package insert for full prescribing information.

Store at 20°C to 25°C (68°F to 77°F); excursions permitted to 15°C to 30°C (59°F to 86°F). [See USP Controlled room Temperature].

Do not refrigerate or freeze.

Keep the ampule in the original package. Protect from light.

For Single-Dose only

Any solution remaining in the opened ampule must be discarded.

Manufactured by:
Steriscience Sp. z o.o.
No. 10, Daniszewska Street,
Warsaw, Poland - 03-230

Distributed by:
Amneal Pharmaceuticals LLC
Bridgewater, NJ 08807

Rev. 11-2024-00

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METHYLENE BLUE

methylene blue injection, solution

Product Information			
Product Type	HUMAN PRESCRIPTION DRUG	**Item Code (Source)**	NDC:70121-2715
Route of Administration	INTRAVENOUS		

Active Ingredient/Active Moiety		
Ingredient Name	**Basis of Strength**	**Strength**
METHYLENE BLUE (UNII: T42P99266K) (METHYLENE BLUE CATION - UNII:ZMZ79891ZH)	METHYLENE BLUE	5 mg in 1 mL

Inactive Ingredients	
Ingredient Name	**Strength**
WATER (UNII: 059QF0KO0R)	

Product Characteristics			
Color	blue (clear dark blue)	**Score**	
Shape		**Size**	
Flavor		**Imprint Code**	
Contains			

Packaging

#	Item Code	Package Description	Marketing Start Date	Marketing End Date
1	NDC:70121-2715-2	10 in 1 BOX	03/03/2025	
1	NDC:70121-2715-3	10 mL in 1 AMPULE; Type 0: Not a Combination Product		

Marketing Information

Marketing Category	Application Number or Monograph Citation	Marketing Start Date	Marketing End Date
ANDA	ANDA216955	03/03/2025	

Labeler - Amneal Pharmaceuticals LLC (827748190)

Establishment

Name	Address	ID/FEI	Business Operations
STERISCIENCE SP		[redacted]	analysis(70121-2715) , label(70121-2715) , manufacture(70121-2715) ,

Z O O		522218056	pack(70121-2715) , sterilize(70121-2715)