**AIBMR Life Sciences, Inc.**


June 18, 2020

Susan Carlson, PhD
Division Director
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Department of Health and Human Services
5001 Campus Drive
College Park, MD 20740

Dear Dr. Carlson:

In accordance with regulation 21 CFR Part 170 Subpart E (Generally Recognized as Safe (GRAS) Notice), on behalf of *Shin-Etsu Chemical Co., Ltd.* (the notifier), the undersigned, Timothy Murbach, submits, for FDA review, the enclosed notice that Shin-Etsu AQOAT®, Hypromellose Acetate Succinate (HPMCAS) GRAS for use in foods.

Should you have any questions or concerns regarding this notice, please contact me at 253-286-2888 or kelly@aibmr.com.

Sincerely,

Timothy Murbach, ND, DABT (agent of the notifier)
Senior Scientific & Regulatory Consultant
AIBMR Life Sciences, Inc. ("AIBMR")

# Notice to US Food and Drug Administration of the Conclusion that the Intended Use of Shin-Etsu AQOAT® Hypromellose Acetate Succinate (HPMCAS) is Generally Recognized as Safe

**Submitted by the Notifier:**

Shin-Etsu Chemical Co., Ltd.

**Prepared by the Agent of the Notifier:**

AIBMR Life Sciences, Inc
1425 Broadway, Suite 458
Seattle, WA 98122

**June 18, 2020**

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# Part 1: Signed Statements and Certification

## 1.1 Submission of GRAS Notice

Shin-Etsu Chemical Co., Ltd. (hereinafter called "Shin-Etsu") (the notifier) is submitting a new GRAS notice in accordance with 21 CFR Part 170, Subpart E, regarding the conclusion that Shin-Etsu AQOAT®, hypromellose acetate succinate (HPMCAS) is Generally Recognized as Safe (GRAS) for its intended use, consistent with section 201(s) of the Federal Food, Drug and Cosmetic Act.

## 1.2 Name and Address of the Notifier and Agent of the Notifier

### Notifier

Sakae Obara
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### Agent of the Notifier

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## 1.3 Name of the Substance

Hypromellose acetate succinate (HPMCAS)

Trade name: Shin-Etsu AQOAT®

## 1.4 Intended Conditions of Use

Shin-Etsu AQOAT® HPMCAS, is intended to be used in dietary supplements as an enteric coating agent, carrier for solid dispersions, and an enteric agent in capsule shells at addition levels shown in Table 1 (also reproduced as Table 5) below, typically ranging from 5% to 80% of the final product weight. AQOAT® HPMCAS is not intended for use in foods where standards of identity would preclude such use, infant formula, or any products that would require additional regulatory review by USDA.

**Table 1.** Intended use of Shin-Etsu AQOAT® HPMCAS

| Form | Use | Typical Concentration Range |
|---|---|---|
| Tablets[a] | Enteric Coating | 5–10% (per core weight of tablet) |
| | Delayed Release Agent | 10–80% (per core weight of tablet) |
| | Solid Dispersion (Solubility Enhancement)[b] | 10–80% (per core weight of tablet) |
| Granules | Enteric Coating | 10–50% (per core weight of granule) |
| | Solid Dispersion (Solubility Enhancement) | 10–80% (per core weight of granule) |
| Pellets[c] | Enteric Coating | 10–50% (per core weight of pellet) |
| Capsules[d] | Enteric Coating | 5–30%[e] |
| | Enteric agent in Capsule Shell | 5–30%[e] |

[a] Also includes caplets

[b] 560 mg is the current maximum amount of HPMCAS in one tablet that is listed in FDA's IID (Inactive Ingredient Database). It is presumed that this commercial product is a solid-dispersion tablet formulation, in which the API amount is 240 mg and the weight of one tablet is 870 mg.

[c] Note that no dietary supplement food codes used the term "pellets"

[d] Also includes gel caps, pills, soft gels, and vegi caps.

[e] Concentration calculated using a 165 mg/capsule and assuming a typical 600 mg filled capsule weight.

## 1.5 Statutory Basis for GRAS Conclusion

The conclusion of GRAS status of Shin-Etsu AQOAT® HPMCAS, for its intended conditions of use, stated in Part 1.4 of this notice, has been made based on scientific procedures.

## 1.6 Not Subject to Premarket approval

We have concluded that Shin-Etsu AQOAT® HPMCAS, is GRAS for its intended conditions of use, stated in Part 1.4 of this notice, and, therefore, such use of Shin-

Etsu AQOAT® HPMCAS, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

## 1.7 Data and Information Availability Statement

The data and information that serve as the basis for this GRAS conclusion will be available for review and copying during customary business hours at the office of Shin-Etsu Chemical Co., Ltd. 6-1, Ohtemachi 2-chome, Chiyoda-ku, Tokyo 100-0004, Japan or will be sent to FDA upon request.

## 1.8 Exemption from Disclosure under the Freedom of Information Act

None of the data and information in Parts 2 through 7 of this GRAS notice are considered exempt from disclosure under the Freedom of Information Act (FOIA) as trade secret or commercial or financial information that is privileged or confidential.

## 1.9 Certification of Completion

We hereby certify that, to the best of our knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of Shin-Etsu AQOAT® HPMCAS.

(b) (4)

June 18, 2020

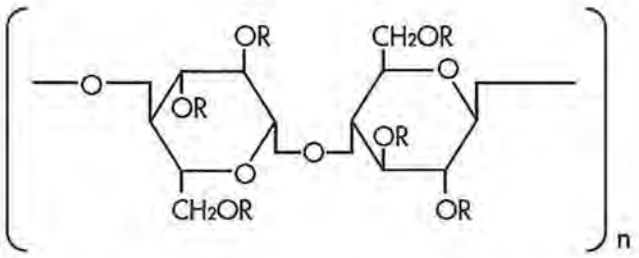
Sakae Obara — Date
General Manager, Cellulose Technical Support Center
Notifier

# Part 2: Identity, Manufacture, Specifications, and Physical or Technical Effect

## 2.1 Identification

Shin-Etsu AQOAT®, a branded form of hypromellose acetate succinate (HPMCAS), is a partially esterified derivative of hydroxypropyl methylcellulose (HPMC), aka hypromellose. HPMC (CAS #9004-65-3) is an odorless and tasteless powder, consisting of a cellulose backbone with ether-linked methyl and hydroxypropyl side groups attached to the cellulose chain hydroxyl groups. The level of methyl and hydroxypropyl substitution influence the physical properties of HPMC.

HPMCAS (CAS #71138-97-1) is a tasteless, white to yellowish powder or granules with a faint acetic acid-like odor. It is manufactured by introducing acetyl and succinyl groups to the hydroxyl groups of HPMC (see Figure 1). As with HPMC, various grades of Shin-Etsu AQOAT® HPMCAS are manufactured, possessing slightly different physical properties (particle size and pH range) due to the level of chemical substitution.




R = -H
-$CH_3$
-$CH_2CH(CH_3)OH$
-$COCH_3$
-$COCH_2CH_2COOH$
-$CH_2CH(CH_3)OCOCH_3$
-$CH_2CH(CH_3)OCOCH_2CH_2COOH$

**Figure 1.** Chemical Structure of Various Grades of Shin-Etsu AQOAT® HPMCAS

## 2.2 Manufacturing

### 2.2.1 Manufacturing Overview

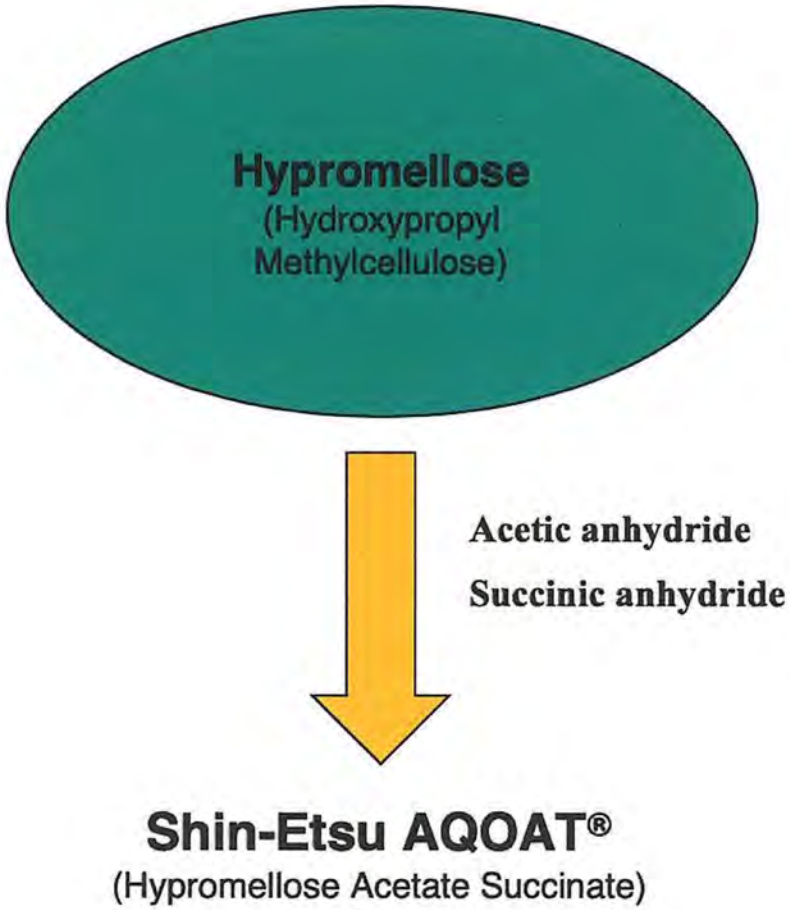



**Figure 2.** Manufacturing Flowchart

To produce Shin-Etsu AQOAT® HPMCAS, acetyl and succinyl groups are introduced to hydroxyl groups of the hypromellose backbone of. The product is purified and, ground to a uniform powder. The manufacturing process is as follows:

1. Dissolve hypromellose in glacial acetic acid in a reactor.

2. Heat and mix the two esterifiers (acetic anhydride and succinic anhydride) and catalyst (sodium acetate) together.
3. After the designated reaction time, the product paste is transferred to water tank and the polymer (HPMCAS) is precipitated by agitation as water is a poor solvent.
4. Wash the polymer using additional water by mixing / decantation, centrifuge to dehydrate and then dry the polymer.
5. Sieve, blend and pack to finalize G (granular) and MP (mid-sized particle) grades. If the F (fine) grade, then mill before sieving, blending and packing.

### 2.2.2 Good Manufacturing Practice

Shin-Etsu AQOAT® HPMCAS, manufactured by Shin-Etsu, is produced under strict adherence to current GMP.

### 2.2.3 Raw Materials

Hypromellose USP, also produced by Shin-Etsu, is used to produce Shin-Etsu AQOAT® HPMCAS. It is a low viscosity grade ranging from 2–15 mPa•s. The materials used to produce hypromellose conform to the Japanese Pharmacopeia (JP), European Pharmacopeia (EP), and US Pharmacopeia (USP) requirements. Shin-Etsu AQOAT® HPMCAS is manufactured from raw materials that are non-GMO, and no irradiation is used in production process. No material of human or animal origin is used, including those derived from Transmissible Spongiform Encephalopathy relevant animal species; seed lots, cell banks, and routine fermentation are not used in the production process.

## 2.3 Specifications

The specifications for the food-grade product Shin-Etsu AQOAT® HPMCAS, along with the specification methods, are listed in Table 2 below.

**Table 2.** Shin-Etsu AQOAT® HPMCAS, Specifications

| Test Items | Specification | | | | | | | | | Method |
|---|---|---|---|---|---|---|---|---|---|---|
| Grade | AS-LG | AS-LMP | AS-LF | AS-MG | AS-MMP | AS-MF | AS-HG | AS-HMP | AS-HF | |
| Acetyl content | 5.0–9.0% | | | 7.0–11.0% | | | 10.0–4.0% | | | JP/NF Monograph |
| Succinyl content | 14.0–18.0% | | | 10.0–14.0% | | | 10.0–14.0% | | | JP/NF Monograph |
| Methoxy content | 20.0–24.0% | | | 21.0–25.0% | | | 22.0–26.0% | | | JP Monograph |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Hydroxypropoxy content** | 5.0–9.0% | | | 5.0–9.0% | | | 6.0–10.0% | | | JP Monograph |
| **Particle size / D50** | - | 70–300 µm | NMT 10 µm | - | 70–300 µm | NMT 10 µm | - | 70–300 µm | NMT 10 µm | Internal (Laser Diffraction) |
| **Particle size / D90** | - | - | NMT 20 µm | - | - | NMT 20 µm | - | - | NMT 20 µm | Internal (Laser Diffraction) |
| **Description** | Conforms | | | | | | | | | JP/NF Monograph |
| **Identification** | Conforms | | | | | | | | | JP <2.25> NF <197A> |
| **Viscosity** | 2.4–3.6 mPa • s | | | | | | | | | JP <2.53> NF <911> |
| **Heavy metals** | NMT 10 ppm | | | | | | | | | JP <1.07> |
| **Limit of free acetic and succinic acids** | NMT 1.0% | | | | | | | | | JP/NF Monograph |
| **Loss on drying** | NMT 5.0% | | | | | | | | | JP <2.41> NF <731> |
| **Residue on ignition** | NMT 0.20% | | | | | | | | | JP <2.44> NF <281> |

Abbreviations: JP, Japanese Pharmacopeia; NF, National Formulary; NMT, Not More Than; AS, Acetate Succinate; L, Low pH; M, Medium pH; H, High pH; F, Fine particle size; MP, Mid-sized particle; G, Granular particle size.

### 2.3.1 Batch Analysis

Production conformity and consistency of Shin-Etsu AQOAT® HPMCAS is tested in production lots. As an example, in Table 3 below, three batches of the AS-MF (Acetate Succinate-Medium pH, Fine particle size) grade were examined and found to be reasonably consistent and compliant with the product specifications. The AS-MF grade is the chemical mid-point and regarded as the representative grade.

**Table 3.** Shin-Etsu AQOAT® HPMCAS Batch Analyses

| **Test Items** | **Specification** | **Lot Number/Date of Manufacture** | | |
|---|---|---|---|---|
| | | **6123236 06/18/2016** | **8123280 12/17/2017** | **9053110 05/15/2019** |
| **Grade** | AS-MF | AS-MF | AS-MF | AS-MF |
| **Description** | Conforms | Conforms | Conforms | Conforms |
| **Identification** | Conforms | Conforms | Conforms | Conforms |
| **Viscosity** | 2.4–3.6 mPa•s | 2.83 mPa•s | 2.94 mPa•s | 2.88 mPa•s |
| **Heavy metals** | NMT 10 ppm | NMT 10 ppm | NMT 10 ppm | NMT 10 ppm |
| **Limit of free acetic and succinic acids** | NMT 1.0% | 0.007% | 0.005% | 0.007% |
| **Loss on drying** | NMT 5.0% | 0.6% | 0.7% | 0.7% |

| | | | | |
|---|---|---|---|---|
| **Residue on ignition** | NMT 0.20% | 0.01% | 0.02% | 0.01% |
| **Acetyl content** | 7.0–11.0% | 8.9% | 9.1% | 9.0% |
| **Succinyl content** | 10.0–14.0% | 10.8% | 11.3% | 10.8% |
| **Methoxy content** | 21.0–25.0% | 23.1% | 23.1% | 23.2% |
| **Hydroxypropoxy content** | 5.0–9.0% | 7.3% | 7.2% | 7.2% |
| **Particle size (for AS-LF, MF and HF only)** | Average (D50): NMT 10 µm 90% cumulation (D90): NMT 20 µm | Average 4.7 µm; 90% cumulation 10.2 µm | Average 4.7 µm; 90% cumulation 10.2 µm | Average 4.8 µm; 90% cumulation 10.1 µm |

Abbreviations: JP, Japanese Pharmacopeia; NF, National Formulary; NMT, Not More Than; AS, Acetate Succinate; L, Low pH; M, Medium pH; H, High pH; F, Fine particle size; MP, Mid-sized particle; G, Granular particle size.

### 2.3.2 Residual Solvent Analysis

Acetic Acid (a Class 3 solvent) is the only solvent used in the manufacture of Shin-Etsu AQOAT® HPMCAS; residual solvent analysis indicates levels to be below 5000 ppm.

### 2.3.3 Heavy Metal Analysis

Heavy metals are not intentionally added or used in the manufacture of Shin-Etsu AQOAT®. Shin-Etsu adheres to ICH Q3D guidelines for elemental impurities. Shin-Etsu defines "not likely present" as the combination of no intentional addition and confirmed values of less than the threshold of oral permitted daily exposure of Option 1, Table A.2.2 of the ICH Q3D guideline. Compliance is tested periodically using an inductively couple plasma analysis.

### 2.3.4 Shelf–Life Stability

A four-year stability study was conducted on Shin-Etsu AQOAT® HPMCAS (AS-MF) at the Shin-Etsu Naoetsu plant in Niigata, Japan from 1993–1997. Shin-Etsu AQOAT® HPMCAS was stored in its typical final packaging, a double-layer polyethylene bag within a 25 kg fiber drum. A control sample was stored in a 500 g polyethylene bag stored in a desiccator-containing silica gel. The temperature ranged from -3 °C in the winter to 43 °C in the summer and relative humidity ranged from 40–100%. The data in the table below demonstrate the stability of Shin-Etsu AQOAT® HPMCAS over 4 years.

**Table 4.** Stability Data Shin-Etsu AQOAT® HPMCAS (AS-MF)

| Package | Year | Appearance | Loss on drying (%) | Viscosity | Acetyl content (%) | Succinyl content (%) | Free succinic acid |
|---|---|---|---|---|---|---|---|
| | | White to yellowish powder | NMT 5.0 | 2.4–3.6 | 7.0–11.0 | 10.0–14.0 | NMT 1.0 |
| **25 kg FD** | 0 | Conforms | 0.5 | 2.72 | 9.4 | 10.9 | 0.05 |
| | 1 | n/c | 1.1 | 2.74 | 9.4 | 11.0 | 0.05 |
| | 2 | n/c | 1.3 | 2.72 | 9.4 | 11.0 | 0.06 |
| | 3 | n/c | 1.5 | 2.73 | 9.3 | 11.0 | 0.08 |
| | 4 | n/c | 1.9 | 2.71 | 9.4 | 11.0 | 0.09 |
| **Control** | 1 | n/c | 0.8 | 2.80 | 9.4 | 11.0 | 0.06 |
| | 2 | n/c | 1.3 | 2.72 | 9.3 | 10.9 | 0.07 |
| | 3 | n/c | 1.5 | 2.72 | 9.3 | 11.0 | 0.07 |
| | 4 | n/c | 1.7 | 2.72 | 9.5 | 11.0 | 0.11 |

NMT; Not More Than, n/c; not changed, FD; Fiber Drum,

## 2.4 Physical or Technical Effect

### 2.4.1 Technical Element

Shin-Etsu AQOAT® HPMCAS is not intended to produce any physical or other technical effects that are relevant to the safety of the ingredient.

# Part 3: Dietary Exposure

## 3.1 Intended Use

Shin-Etsu AQOAT® HPMCAS, manufactured in accordance with current GMP, is intended to be used in dietary supplements as an enteric coating agent, carrier for solid dispersions, and an enteric agent in capsule shells at addition levels shown in Table 5 below, typically ranging from 5% to 80% of the final product weight. Shin-Etsu AQOAT® HPMCAS is not intended for use in foods where standards of identity would preclude such use, infant formula, or any products that would require additional regulatory review by USDA.

**Table 5.** Intended use of Shin-Etsu AQOAT® HPMCAS

| Form | Use | Typical Concentration Range |
|---|---|---|
| Tablets[a] | Enteric Coating | 5–10% (per core weight of tablet) |
| | Delayed Release Agent | 10–80% (per core weight of tablet) |
| | Solid Dispersion (Solubility Enhancement)[b] | 10–80% (per core weight of tablet) |
| Granules | Enteric Coating | 10–50% (per core weight of granule) |
| | Solid Dispersion (Solubility Enhancement) | 10–80% (per core weight of granule) |
| Pellets[c] | Enteric Coating | 10–50% (per core weight of pellet) |
| Capsules[d] | Enteric Coating | 5–30%[e] |
| | Enteric agent in Capsule Shell | 5–30%[e] |

[a] Also includes caplets

[b] 560 mg is the current maximum amount of HPMCAS in one tablet that is listed in FDA's IID (Inactive Ingredient Database). It is presumed that this commercial product is a solid-dispersion tablet formulation, in which the API amount is 240 mg and the weight of one tablet is 870 mg.

[c] Note that no dietary supplement food codes used the term "pellets"

[d] Also includes gel caps, pills, soft gels, and vegi caps.

[e] Concentration calculated using a 165 mg/capsule and assuming a typical 600 mg filled capsule weight.

## 3.2 Estimated Daily Intake (EDI)—Exposure

Exposure to Shin-Etsu AQOAT® HPMCAS from the intended use categories was estimated for the U.S. population using dietary supplement consumption data from the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Surveys (NHANES). The most recent dietary supplement data available at the time of this writing (2011–2012) were analyzed using Creme Food Safety software 3.6 (www.cremeglobal.com). These data were obtained from 7605 individuals who underwent two non-consecutive 24-hour

dietary recall interviews (the first was collected in-person, the second by phone 3–10 days later).

WWEIA food (supplement) codes that were considered most similar to the intended use categories were utilized in the assessment and were assigned the relevant intended use concentrations. In this case, food codes for tablets (including caplets), capsules (including gel caps, pills, soft gels, and vegi caps), and granules were utilized. Note that no food codes for "pellets" were located.

Creme software is a probabilistic modeling tool that uses high-performance computing to predict intake (including total aggregate exposure) of food/supplement groups and/or individual dietary supplement ingredients. Creme Food Safety performs calculations using large-scale food consumption data sets. It bases the calculated estimates on each individual's body weight from the survey, as opposed to averaged body weights. Calculations also incorporated the NHANES assigned sample weights for each individual in the survey, which measure the number of people in the population represented by that specific subject and help to ensure that the results statistically represent the entire U.S. population. Sample weights for NHANES participants incorporate adjustments for unequal selection probabilities and certain types of non-response, as well as an adjustment to independent estimates of population sizes for specific age, sex, and race/ethnicity categories. The data are shown for "consumers" (which includes only data from individuals who reported consuming one or more dietary supplement categories intended to contain the ingredient over the two-day survey period, as opposed to the whole population). Results are given as both absolute exposure (mg/day), as well as exposure relative to body weight (mg/kg bw/day).

The relative standard error (RSE; calculated by dividing the standard error of the estimate by the estimate itself and multiplying by 100) is a statistical criterion that can be used to determine the reliability of estimates as pertains to the population (the larger the RSE the less reliable the estimate).[1] RSE values greater than 25–30% are often considered reasonable cut-offs by which to consider a value unreliable.[1,2] For the purpose of this safety assessment, an RSE value of greater than 25% was used to indicate that the estimated value was unreliable with regard to representing the population. RSE values are shown in the tables below for the 90$^{th}$ percentile values only, as the 90$^{th}$ percentile values are the most pertinent for the exposure estimates.

The Shin-Etsu AQOAT® HPMCAS exposure estimates derived from the Creme assessment based on the intended use categories and concentrations are shown below in Tables 6 and 7.

**Table 6.** Total (aggregate) absolute exposure to Shin-Etsu AQOAT® HPMCAS by proposed use consumers using NHANES 2011–12 data

| Population Group | Age in yrs | N (% of total) | Absolute AQOAT® HPMCAS consumption Daily Average by Consumers (mg/day) | | | | 90th% RSE Value |
|---|---|---|---|---|---|---|---|
| | | | Mean | Mean std err | 90th% | 90th% std err | |
| Children | 2–12 | 321 (21.2) | 681.4 | 37.3 | 800.0 | 225.0 | 28.1* |
| Adolescents | 13–19 | 133 (13.6) | 760.0 | 67.1 | 1586.3 | 375.8 | 23.7 |
| Adults | 20+ | 2073 (51.8) | 1596.8 | 56.9 | 3350.0 | 145.8 | 4.4 |
| Total Population | 2+ | 2527 (43.4) | 1504.0 | 49.9 | 3200.0 | 100.6 | 3.1 |

Creme run #438
*RSE>25; suggests 90th percentile data is not reliable

**Table 7.** Total (aggregate) exposure to Shin-Etsu AQOAT® HPMCAS by proposed use consumers relative to body weight using NHANES 2011–12 data

| Population Group | Age in yrs | N (% of total) | AQOAT® HPMCAS consumption relative to body weight Daily Average by Consumers (mg/kg bw/day) | | | | 90th% RSE Value |
|---|---|---|---|---|---|---|---|
| | | | Mean | Mean std err | 90th% | 90th% std err | |
| Children | 2–12 | 321 (21.2) | 27.8 | 1.5 | 52.6 | 2.3 | 4.4 |
| Adolescents | 13–19 | 133 (13.6) | 11.7 | 1.1 | 18.9 | 5.0 | 26.5* |
| Adults | 20+ | 2073 (51.8) | 21.5 | 0.9 | 46.6 | 2.0 | 4.3 |
| Total Population | 2+ | 2527 (43.4) | 21.7 | 0.8 | 47.1 | 1.8 | 3.8 |

Creme run #438
*RSE>25; suggests 90th percentile data is not reliable

According to the estimates in the tables above, approximately 43.4% of the U.S. total population (ages 2 and above) were identified as potential consumers of Shin-Etsu AQOAT® HPMCAS from one or more of the proposed dietary supplement uses. The 90th percentile estimated exposure to Shin-Etsu AQOAT® HPMCAS in the total population is 3200 mg/day (47.1 mg/kg bw/day). The highest potential consumer population at the 90th percentile on a relative to body weight basis is children (ages 2–12), at an estimated 52.6 mg/kg bw/day, although children also

have the lowest absolute daily estimated exposure at 800 mg/day (although it should be noted that this 90$^{th}$ percentile data had a standard error that was high enough that the RSE calculation was greater than 25%; thus the data is not necessarily considered reliable for this population). The highest absolute daily estimated exposure is by adults ages 20 and older, at 3350 mg/day. These estimates are considered extremely conservative, as they assume that 100% of the intended use dietary supplement products in the market will contain the maximum intended use addition levels of Shin-Etsu AQOAT® HPMCAS. In reality, most dietary supplements do not require enteric coating or solid dispersions.

## Part 4: Self-limiting Levels of Use

There are no known inherent self-limiting levels of use.

## Part 5: Experience Based on Common Use in Food Prior to 1958

The GRAS conclusion for Shin-Etsu AQOAT® HPMCAS is based on scientific procedures, and thus, experience based on common use in food prior to 1958 is not considered pivotal information.

# Part 6: Narrative

## 6.1 Absorption, distribution, metabolism, and excretion (ADME)

### 6.1.1 Toxicokinetics of Hypromellose Acetate Succinate (HPMCAS)

Shin-Etsu sponsored a study (unpublished, 1984) to investigate mass balance and tissue distribution of their HPMCAS in Sprague-Dawley rats (Charles River CD strain). The test material was $^{14}C$ radiolabeled at positions 1 and 4 of the succinyl moiety, which comprised 17% of the mass of the test material and administered to male and female rats by gavage (mass balance = 5 rat/sex, 1.0 g/kg bw, 5 μCi; tissue distribution = 20 rats/sex, 1.5 g/kg bw, 5 μCi). The purity of the test item was ≥95% (<5% impurity as $^{14}C$ succinic acid). In the mass balance experiment, the rats were housed in metabolic cages and urine was collected at 3 and 6 hours, and daily thereafter for 5 days, following administration while feces were collected daily for 5 days (cage washings were also collected and analyzed at the end of the experiment). In the tissue distribution experiment, four animals of each sex were sacrificed at 3, 6, 24, 48, and 120 hours post-administration. Blood was collected under anesthesia just prior to sacrifice for analysis of whole blood and plasma, and brain, pituitary, thyroid, eyes, ovary/testis, salivary glands, thymus, heart, liver, uterus, kidney, lung, spleen, pancreas, adrenals, fat, muscle, and bone marrow were collected, processed, and analyzed.

The total (feces + cage washings + urine) cumulative mean recovery of radioactivity over five days following administration in the mass balance experiment was 96.5% (range, 91.4–104.4%) and 97.3% (range, 91.2–101.9%) of the dose for males and females, respectively. Cumulative mean fecal recovery of radioactivity following the dose was 94.4% (range, 87.5–104.1%) and 96.8% (range, 90.6–101.2%) for males and females, respectively, while cumulative mean urinary recoveries were 1.6% (range, 0.2–3.6%) of the dose in males and 0.4% (range, 0.2–0.5%) of the dose in females. Mean recoveries from terminal cage washings were 0.5% (range, 0.1–1.8%) of the dose in males and 0.1% (range, 0.0–0.2%) of dose in females. While total urinary radioactivity in most animals was ≤ 0.5% of the dose (the amount of succinyl impurity present in the ingredient), in two of five males, urinary excretion was 3.6% of the dose; this may be explained by a small amount of absorption of intact HPMCAS or by absorption of additional radiolabel following intermediate metabolism of HPMCAS by gut bacteria. The latter is more plausible as no more than 0.1 and 0.6% of the dose was recovered within the first 6 and 24 h, respectively, in any individual animal.

Blood and organ tissue samples taken from male and female animals showed no to trace amounts of radioactivity for up to 120 hours. The maximum amount of radioactivity observed was 0.2% of the dose which approximates the content of

0.5% of impurity in radiolabeled HPMCAS. Overall, data from the mass balance and tissue distribution experiments indicates that HPMCAS when administered orally to rats is poorly absorbed and then primarily excreted through the feces within 48 hours.

### 6.1.2 Toxicokinetics of Related Substances

Cellulose is a natural substance present in most diets as it is a major structural carbohydrate of green plants. Chemical processing is necessary to prepare it for use in food products.[3] It is well accepted that modified celluloses in general are minimally absorbed and excreted almost exclusively in the feces after oral administration.[4]

Gorzinski et al. (1986) investigated the fate of $^{14}$C-HPMC in male and female Sprague-Dawley rats.[5] In the first study, the animals (3/sex) were administered a single oral dose (via gavage) of 500 mg/kg bw and monitored for 72 hours in a glass metabolic chamber. Urine, feces, expired $CO_2$ and blood were sampled and analyzed throughout the 72-hour period at the end of which the rats were sacrificed. In a second investigation, the animals (3/sex) received 500 mg/kg bw/day of HPMC (via gavage) for five days. Rats were placed in glass metabolic chambers and urine, feces, expired $CO_2$ and blood were sampled and analyzed over course of 5 days; animals were sacrificed 24-hours after the last dose.

In the single dose and multiple dose studies, the average radioactivity eliminated through the feces was 100–105% and 97–102%, respectively, mostly eliminated within 24 hours. Radioactivity in the plasma was highest at 0.5 hours and decreased quickly over 6 hours. The estimated plasma half-life of $^{14}$C-HPMC was approximately 2 hours for male and female rats. In both studies there was negligible excretion from the urine, expired air and bile and negligible distribution into the carcass and tissues.

Another radiolabeled study conducted by Kitagawa (1974) on a similar compound, hydroxypropyl methylcellulose phthalate (HPMCP), revealed comparable results.[6] $^{14}$C-HPMCP was orally administered (via gavage) to rats at a level of 1.3 g/kg and the rats were then placed in metabolic chambers. Following sacrifice, the concentration of radioactivity was determined in whole blood, plasma, tissues, urine and feces. In whole blood and plasma, the highest amount of radiolabeled HPMCP was found after 6 hours for males and after 2 hours for females. The radioactivity in tissues was low with unappreciable distribution of HPMCP found in all tissues except the liver and kidney. Even in these organs, the levels were extremely low, with the liver having the highest concentration of radioactivity at 0.02% and 0.03 % of the administered dose for male and female rats, respectively. The majority of radioactivity was found in the gastrointestinal tract, with 93.3% and 81.5% found after 6 hours in male and female rats, respectively. After 72 hours, 0.01% of the

radioactivity was found in the gastrointestinal tract. Radioactivity was low in both bile and urine and the majority of radioactivity was found in the feces, up to 95% and 91% for male and female rats, respectively. This study supports the notion that modified cellulose compounds are minimally absorbed and eliminated via feces.

## 6.2 Toxicology Studies Conducted on HPMCAS

A battery of toxicological studies of Shin-Etsu's HPMCAS were conducted and by Hoshi et al., (1985).[7 8 9 10 11] Included were acute (rats and rabbits), subchronic (rats), and chronic (rats) studies, as well as several reproductive and developmental toxicity studies conducted in two species (Sprague-Dawley rats and New Zealand White rabbits) that were carried out in accordance with the "Guidelines for Reproduction Experiments to Evaluate the Safety of Drugs" issued by the Ministry of Health & Welfare, Japanese Government in March 1975". Additionally, a more recent embryo-fetal development study in rats and rabbits was published to further evaluate the findings of Hoshi et al.[12] The studies by Hoshi et al. did not report whether they were conducted according to Good Laboratory Practices (GLP) or, with the exception of the reproductive and developmental studies, whether they followed OECD or other guidelines; some of the study protocols do not meet current internationally harmonized toxicological testing standards. However, they indicate a general lack of safety concern for HPMCAS ingestion at doses up to 2500 mg/kg bw/day, as might be expected from the above summary of unpublished toxicokinetic data for HPMCAS, indicating a lack of significant absorption. Summaries of these studies are reported in the subparts below.

### 6.2.1 Acute Toxicity

New Zealand White rabbits (5 males and 4 females per group) and Sprague-Dawley rats (5 controls/sex/group, 8 treated/sex/group) were administered 0 or 2.5g/kg bw HPMCAS once via gavage in an aqueous suspension of 0.25% carboxymethylcellulose (CMC) solution.[11] The animals were observed for behavioral changes for one week. The animals were then sacrificed, and the absolute weights and relative to body weights of the brain, heart, lungs, liver, submandibular gland (rats only), kidneys, testes, ovaries, spleen, pituitary, thyroid, adrenals, and thymus were recorded (thymus results were not reported).

No behavioral abnormalities were observed, and no deaths occurred. Except for an increased absolute brain weight in treated males, no statistically significant differences in absolute or relative organ weights compared to control were observed in rats. In rabbits, absolute right testes weight was statistically significantly increased in males, and absolute heart weight was significantly increased in females compared to the respective controls. There were no other changes in the absolute or

relative weights organ weighs in rabbits. The $LD_{50}$ was determined to be greater than 2.5 g/kg bw.

### 6.2.2 Subchronic Repeated Dose Oral Toxicity (8-week)

In an 8-week study, male and female Sprague-Dawley rats were divided into 4 groups of 10 rats/sex/group and administered HPMCAS by gavage at 0 (control; 0.25% CMC solution only), 0.63, 1.25, and 2.5 g/kg bw/day six days per week.[11] Observations and examinations were conducted as follows in Table 8:

**Table 8.** Subchronic Oral Toxicity Study in Rats: Observations

| **Observations/Examinations** | |
|---|---|
| General observations/Mortality | Daily |
| Body weights | Daily for 1st month, then twice weekly |
| Food consumption and water intake | Twice weekly |
| Hematological and biochemical analyses | Total protein, A/G ratio, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total cholesterol, urea nitrogen, glucose, white blood cell count, red blood cell count, hematocrit, hemoglobin, platelets |
| Urinalysis | Urine was collected using metabolic cages—evaluations consisted of urobilinogen, occult blood, ketone bodies, bilirubin, glucose, protein, and pH. |
| Absolute and relative organ weights | Brain, heart, lung, liver, submandibular gland, kidney, testis, ovaries, spleen, pituitary, thyroid, adrenal, thymus |
| Histopathological examination | Brain, heart, liver, intestines, kidney, spleen, lung, pancreas, adrenal glands, thyroid, testis, ovary, bone marrow. |

No mortality or behavioral changes were observed throughout the study. The authors reported a non-significant trend towards decreased body weight gain in both sexes; no statistically significant differences were observed in food consumption (water intake results were not reported).

Various statistically significant changes were also found in hematological evaluations as shown in Table 9 below. Clinical chemistry results revealed a statistically significant decrease in total protein in mid-dose males, a statistically significant decrease in alkaline phosphatase high-dose males, and a statistically significant increase in urea nitrogen in mid-dose females. No statistically significant differences compared to controls were observed in urinalysis parameters.

**Table 9.** Statistically Significant Hematological Changes

| Dose | WBC (/mm³) | RBC (x10³/mm³) | HCT (%) | HGB (g/dL) | PLT (10/ml) |
|---|---|---|---|---|---|
| Males | | | | | |
| C | 8540.0 ± 686.00 | 498.0 ± 33.27 | 44.0 ± 1.00 | 15.82 ± 0.26 | 53.78 ± 7.27 |
| LD | 7660.0 ± 835.82 | 529.6 ± 24.21 | 46.4 ± 1.33 | 16.74 ± 0.37 | 47.38 ± 7.19 |
| MD | 7640.0 ± 864.64 | 491.6 ± 26.09 | 44.4 ± 1.03 | 15.76 ± 0.18 | 47.78 ± 7.90 |
| HD | 5580.0 ± 597.83* | 552.0 ± 31.13* | 46.0 ± 1.41 | 16.16 ± 0.40 | 30.12 ± 6.10* |
| Females | | | | | |
| C | 9640.0 ± 816.46 | 594.2 ± 43.14 | 49.0 ± 1.67 | 18.44 ±0.44 | 38.36 ±4.76 |
| LD | 7100 ± 561.25* | 573.2 ± 24.66 | 46.6 ± 0.75* | 16.92 ± 0.42* | 51.88 ± 5.59 |
| MD | 9300 ± 251.00 | 473.0 ± 17.26* | 47.4 ± 0.68 | 17.24 ± 0.19 | 46.42 ± 7.55 |
| HD | 7320.0 ± 517.11* | 522.2 ± 41.77 | 47.3 ± 0.95 | 17.90 ± 0.60 | 35.50 ±4.50 |

C, control group; LD, low dose group; MD, middle dose group; HD, high dose group; WBC, white blood cell count; RBC, red blood cell count; HCT, hematocrit; HGB, hemoglobin; PLT, platelet count.
*p<0.05

Various statistically significant changes were found without a dose response in absolute and relative organ weights in both sexes as shown in the tables below.

**Table 10.** Statistically Significant Absolute Organ Weight Changes

| Dose | | Lung (g) | L. Kidney (g) | Thyroid (mg) | Dose | | Thyroids (mg) |
|---|---|---|---|---|---|---|---|
| Males | C | 2.03 ± 0.32 | 1.52 ± 0.03 | 191.3 ± 6.0 | Females | C | 16.75 ± 0.74 |
| | LD | 1.56 ± 0.16 | 1.43 ± 0.06 | 172.9 ± 6.4* | | LD | 22.66 ± 1.73* |
| | MD | 1.26 ± 0.05* | 1.54 ± 0.03 | 186.3 ± 7.9 | | MD | 16.93 ± 1.23 |
| | HD | 1.38 ± 0.10 | 1.37 ± 0.04* | 197.2 ± 7.6 | | HD | 19.42 ± 1.68 |

C, control group; LD, low dose group; MD, middle dose group; HD, high dose group; L, left.
*p<0.05

**Table 11.** Statistically Significant Organ Weight Relative to Body Weight Ratios

| Dose | | Lung (%) | Liver (%) | Thyroids (x10$^{-30/0}$)† | Dose | | Adrenal (L) (x10$^{-30/0}$)† | Thyroids (x10$^{-30/0}$)† |
|---|---|---|---|---|---|---|---|---|
| Males | C | 0.52 ± 0.08 | 3.77 ± 0.12 | 6.36 ± 0.31 | Females | C | 12.91 ± 0.63 | 7.39 ± 0.36 |
| | LD | 0.41 ± 0.04 | 4.00 ± 0.07 | 5.04 ± 0.39* | | LD | 13.72 ± 0.81 | 10.14 ± 0.75* |
| | MD | 0.33 ± 0.01* | 4.06 ± 0.04* | 5.84 ± 0.34 | | MD | 10.85 ± 0.54* | 7.43 ± 0.54 |
| | HD | 0.37 ± 0.03 | 3.83 ± 0.08 | 6.18 ± 0.33 | | HD | 12.57 ± 0.61 | 8.78 ± 0.75 |

C, control group; LD, low dose group; MD, middle dose group; HD, high dose group; L,left.
*p<0.05; †units not explained (numbers divided by zero are not defined).

All of the above statistically significant changes were considered relatively small in magnitude and/or were not dose-dependent and were without correlating histopathology with the possible exception of the decreased WBC count in low- and high-dose females which may be correlated with splenic hemosiderosis observed in these animals. As statistically significant decreases were not observed in RBC counts or platelet counts in female animals of these dose groups and the authors did not perform WBC differentials, it is difficult to confirm this correlation.

Nonetheless, while the incidence of '+' splenic hemosiderosis was greatest in the low- and high-dose females, the overall incidence ('±' and '+' combined) of hemosiderosis was similar in control group females and splenic hemosiderosis also occurred in all male groups with lower incidence. Slight lymphocytic cell infiltration of Glisson's capsule was observed histologically in 3 of 10 high-dose animals of both sexes. As this change was of a low degree and frequency and was also observed with similar frequency in control and high-dose animals in the chronic study described below, it was considered to be a spontaneous finding without toxicological relevance in this study. All other lesions observed during the histological examination occurred with similar frequencies among the groups, including the control group.

The observed changes in organ weights and clinical pathology were not considered toxicologically relevant effects of HPMCAS. No histopathological findings considered related to the test item were observed in the examined organs. In conclusion, while not determined by the authors, based on the evidence presented, the NOAEL in this study can be considered 2.5 g/kg bw/day of HPMCAS.

### 6.2.3 Chronic Repeated Dose Oral Toxicity (26-week)

In a 26-week study, male and female rats were divided into 3 groups of 10 rats/sex/group and administered 0 (control; 0.25% CMC solution), 1.25, or 2.5 g/kg bw/day of HPMCAS by gavage six days per week.[11] Observations and examinations were conducted as follows:

**Table 12.** Chronic Oral Toxicity Study in Rats: Observations

| Observations/Examinations | |
|---|---|
| General observations/Mortality | Daily |
| Body weights | Daily for 1st month, then once weekly |
| Food consumption and water intake | Twice weekly |
| Hematological and biochemical analyses | 13th and 26th weeks—Total protein, A/G ratio, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase total cholesterol, urea nitrogen, glucose, white blood cell count, red blood cell count, hematocrit, hemoglobin, platelets. |
| Urine analyses | Urine was collected using metabolic cages—evaluations consisted of urobilinogen, occult blood, ketone bodies, bilirubin, glucose, protein, and pH. |
| Absolute and relative organ weights | Brain, heart, lungs, liver, submandibular gland, kidneys, testis, ovaries, spleen, pituitary, thyroid, adrenal glands. |
| Histopathological examination | Heart, liver, kidney, spleen, lung, pancreas, adrenal gland, thyroid, stomach, testis, ovary, cerebrum, cerebellum, bone marrow. |

The authors reported that "some of the rats died of pneumonia" although how many and in which groups was not reported, and there were there conflicting and

incomplete reporting of related results as follows: the discussion section states that "no deaths ... were seen," and the histopathology table reports findings of pneumonia in the lungs of only 2 of 10 control males but also shows that only nine high-dose males were examined histologically, yet no mention is made of the missing high-dose animal anywhere in the article.

No behavioral abnormalities were observed. A statistically significant decrease in body weight gain was reported in males of the high-dose group at 24 weeks; however, the change was transient and was not observed during previous weeks or at week 26. No statistically significant changes were found in food consumption or water intake.

Results of hematological and clinical chemistry examinations at week 13 were not reported; the following results relate to the week 26 examination. No statistically significant differences were observed in hematological or urinalysis parameters when compared to controls. Clinical chemistry analyses indicated a statistically significant increase in alkaline phosphatase in the low-dose group males and a statistically significant increase in the albumin/globulin ratio in the low- and high-dose group in males. In female rats, findings were statistically significant decreases in lactose dehydrogenase and alkaline phosphatase in the high-dose group; note, decreases in these enzymes are generally in the direction opposite of concern. The statistically significant biochemical indices at week 26 were small and were not dose-related, and no correlating findings were noted upon microscopic examination of tissues; therefore, they were not considered toxicologically relevant.

A statistically significant increase in absolute left thyroid weight in the males in the low-dose group and a statistically significant increase in absolute right thyroid weight in the males in the high-dose group were observed. Absolute right adrenal weights in males in the low- and high-dose groups were also statistically significantly increased, although not dose-dependently. In males, various statistically significant changes in the mean organ weight relative to body weight ratios were recorded (see table below), but no correlating histopathology was observed in these organs. No statistically significant changes were found in absolute or relative organ weights in the female animals.

**Table 13.** Statistically Significant organ weight relative to bodyweight ratios in male rats.

| Dose | Kidney(R) (g) | Kidney(L) (g) | Spleen (g) | Thyroid(R) (mg) | Thyroid(L) (mg) | Adrenal(R) (mg) |
|---|---|---|---|---|---|---|
| C | 0.28 ± 0.007 | 0.28 ± 0.007 | 0.17 ± 0.006 | 1.59 ± 0.127 | 1.61 ± 0.132 | 4.09 ± 0.173 |
| LD | 0.30 ± 0.008 | 0.30 ± 0.009* | 0.15 ± 0.005* | 2.09 ± 0.190* | 2.12 ± 0.146* | 5.41 ± 0.296* |
| HD | 0.32 ± 0.010* | 0.31 ± 0.011* | 0.17 ± 0.008 | 2.33 ± 0.143 | 2.07 ± 0.167 | 5.04 ± 0.247* |

C, control group; LD, low dose group; MD, middle dose group; HD, high dose group; L, left; R, right.
*p<0.05

Histological lesions observed were similar to those observed in the subchronic study and occurred with similar frequencies among the groups, including cellular infiltration of Glisson's capsule (2 of 10 control females, 2 of 10 low-dose females, and 1 of 9 control males). HPMCAS was determined to be non-toxic at these doses. Based on the reported evidence, we consider the NOAEL to be 2.5 g/kg bw/day.

### 6.2.4 Fertility Study in Rats

In a study focused on the effects of HPMCAS on fertility, reproductive function, and early embryonic development, male Sprague-Dawley rats (25/group) were administered HPMCAS daily beginning 60 days before pairing and continuing until completion of mating, and female rats (25/group) were administered HPMCAS from 14 days prior to mating up to day 7 of gestation.[9] Both sexes were administered HPMCAS via gavage at 625, 1250 or 2500 mg/kg bw/day suspended in 0.25% CMC solution. Control animals were given CMC solution alone. Males were sacrificed and subjected to necropsy at completion of mating; dams were sacrificed on day 21 of gestation, and all fetuses were examined. Observations and examinations were conducted as follows:

**Table 14.** Fertility Study in Rats: Observations

| **Observations/Examinations** | |
|---|---|
| General observations/Mortality | During premating, mating, and gestation periods. |
| Body weights | Twice weekly during pre-mating and mating; dams daily during gestation period |
| Food consumption and water intake | Twice weekly |
| Necropsy of males | Gross pathological examinations were conducted, and testes weights were obtained. |
| Necropsy of dams | Gross pathological examinations were conducted. |
| Fertility parameters | Number of corpora lutea, implantation sites, viable and dead fetuses, and resorbed embryos were counted, and positions of implantations were observed. Fetal body weights and placenta weights were measured, and fetal sex was recorded. Pre- and post-implantation loss was calculated. |
| Fetal examination* | All fetuses were subjected to external examination. Approximately two-thirds of viable fetuses per litter were randomly selected for skeletal examination, and visceral examinations were conducted on the remaining fetuses. |

*Because dosing did not cover the period of organogenesis, susceptibility to test item effects on fetal development is expected to low.

No abnormalities were noted in the general condition of animals, and no deaths occurred during the study in either sex. A few slight, but statistically significant changes, in body weight, food consumption, or water consumption were observed transiently in male or female animals but overall body weight development was not

affected. No gross pathological changes related to treatment were observed in male or female animals at the respective necropsies. A single neoplasm (not described) was detected in one dam from the high-dose group and was considered an individual finding. The absolute left testes weight was slightly, but statistically significantly, increased in the high-dose males; however, total testicular weight compared to controls was unaffected.

A few statistically significant changes in fertility parameters (see Table 15) were observed and considered to be without toxicological or biological significance. The number of pregnant females, copulation and pregnancy rates, pre- and post-implantation losses, and sex ratios were similar among the groups.

**Table 15.** Statistically significant reproductive changes

| Dose | Mean corpora lutea (total number) | Mean live fetuses (total number) | Mean litter weight (g) | Mean placental weight (g) |
|---|---|---|---|---|
| C | 15.2 ± 0.3 | 13.6 ± 0.4 | 68.20 ± 1.92 | 0.51 ± 0.01 |
| LD | 16.1 ± 0.3* | 14.4 ± 0.4 | 72.22 ± 1.98 | 0.54 ± 0.01* |
| MD | 15.7 ± 0.3 | 14.0 ± 0.5 | 70.42 ± 2.31 | 0.55 ± 0.01** |
| HD | 15.5 ± 0.3 | 14.8 ± 0.3* | 74.14 ± 1.19** | 0.54 ± 0.01* |

C, control group; LD, low dose group; MD, middle dose group; HD, high dose group; L, left; R, right.
*p<0.05, **p<0.01

The authors also conducted and reported results of fetal external, skeletal, and visceral examinations. Clubfoot was observed in one low-dose and four high-dose fetuses, and a short tail was observed in one of the high-dose cases. Skeletal variations (i.e., supernumerary ribs, asymmetry of sternebrae, deformations of the vertebral body) were observed at low frequencies and without statistically significant differences in treated versus control groups while skeletal malformations were observed in one fetus each of the control (shortening of ribs), low- (shortening of ribs), and mid- (fission of thoracic vertebral centra) dose groups and in eight fetuses of the high-dose group (fission of thoracic vertebral centra x 4, lack of thoracic, lumbar, sacral, and caudal vertebra x 1, lack of ribs x 1, deformation of ribs x 1, and shortening of ribs x 3). It is unclear from the reporting whether the high-dose changes were statistically significant. Visceral malformations observed were pyelectasis in one control and three mid-dose fetuses, and dilatation of the cerebral ventricle was observed in two mid-dose fetuses. The authors considered the external, skeletal, and visceral observations as "physiological variation" common to the rat strain used but did not present historical control data. Additionally, the dams were not dosed during the period of organogenesis; thus, it is unlikely that development of the limbs, skeleton, kidneys, and brain were susceptible to a teratogenic effect during this study.

The NOAEL was determined as 2500 mg/kg bw/day HPMCAS for paternal and maternal toxicity and reproductive performance.

### 6.2.5 Embryo-fetal Developmental Toxicity Study with Extended Evaluation in Rats

In a another study, pregnant female rats were administered HPMCAS from gestation day (GD) 7 to GD 17 in order to investigate the potential for developmental toxicity of the test item during the period of organogenesis.[7] The animals (40+/group) were administrated HPMCAS in 0.25% CMC at 0, 625, 1250 and 2500 mg/kg bw/day by gavage. On GD 21, two-thirds of the pregnant females (27 to 30/group) were anesthetized and fetuses were delivered by cesarean section. The remaining one-third of dams were allowed to deliver spontaneously, and the growth, development, and reproductive ability of the F1 offspring were evaluated. F1 pups were culled to 10 pups/litter on postnatal day (PND) 7. Approximately, 12–15 (based on 'n's reported in data tables) F1 pups/sex/group were sacrificed and subjected to necropsy at weaning (interim sacrifice). Of the remaining pups, one male and one non-litter mate female from each litter were mated within groups at 11 weeks of age, and F2 fetuses were examined at the scheduled sacrifice on GD 21. Examinations were conducted as follows:

**Table 16.** EFD Study in Rats: Observations, Hoshi et al., 1985

| **EFD Observations/Examinations** | |
|---|---|
| Maternal Parameters | General condition and behavior, food and water consumption, body weight, number of corpora lutea, implantation sites, placental weights, gross pathological examination (including mammary gland development). |
| Embryo-fetal survival | Viable and dead fetuses, resorptions, implantation losses. |
| Fetal growth | Individual fetal body weights and sex. |
| Fetal morphology | External (all viable fetuses), skeletal (2/3 of viable fetuses), and visceral (remaining 1/3) examinations. |
| **Extended F1 Generation Examinations** | |
| Body weight | PND 0 (birth), 3, 7, 10, 14, 17 and 21 and once weekly thereafter. |
| Food consumption and water intake | Weekly. |
| Developmental parameters | Separation of ear auricular, emerged abdominal hair, eruption of lower incisors, separation of eyelids, descent of testes, and vaginal opening. |
| Interim sacrifice at weaning | Absolute and relative (to bw) organ weights (brain, thymus, heart, lung, liver, spleen, kidney, testis, ovary, uterus) and skeletal development. |
| Functional development | Righting reflex, corneal reflex, auditory function, pain response, swimming ability, and chinning test at 3 weeks; open-field test and learning test/avoidance reflex at 8 to 10 weeks. |
| Sex cycle | Estrous cycle (diestrus, proestrus, estrus I and II) determined at 9-11 weeks. |
| Reproductive ability | Copulation and pregnancy rates, number of corpora lutea, implantation sites, placental weights, litter weights, viable and dead fetuses, resorptions, implantation losses. |
| **Extended F2 Generation Examinations** | |

| | |
|---|---|
| Fetal growth and morphology | Viable F2 fetuses weighed, sexed, and examined for external (all viable fetuses), skeletal (2/3 of viable fetuses), and visceral (remaining 1/3) abnormalities. |

No deaths occurred and no abnormal behavior was observed in F0 dams. There were no statistically significant effects on body weight development of the F0 dams, and no abnormalities were observed before or during parturition in the dams that were carried through to spontaneous delivery. In the F0 dams sacrificed on GD 21, no statistically significant differences were observed in numbers of corpora lutea or implantation sites.

No statistically significant differences among the groups were observed in dead and live fetuses, early or late resorptions, pre- or post-implantation losses, mean litter weights, sex ratios, or mean body weights of the fetuses. Mean placental weight was statistically significantly decreased compared to controls in the low- and high-dose groups. In the F1 fetuses, clubfoot was observed externally in all groups and increased in incidence dose-dependently (controls, 3/377; low-dose, 8/376; mid-dose, 9/347; high-dose, 16/386); however, the increases were not statistically significant compared to the concurrent control group. Short tail was observed in two mid-dose fetuses. Pyelectasis was observed with similar frequency in all groups during the visceral examination. Various skeletal variations were observed with similar frequencies among the groups although on evaluation of rate of ossification, a statistically significant decrease in the mean number of sternal vertebral centra was observed in fetuses of the high-dose group dams compared to controls. No skeletal malformations were observed in the high-dose group fetuses while in the control and lower dose groups several malformations were observed in individual fetuses as follows: fission of the thoracic vertebral centra (1 low-dose), synostosis of sternebrae (1 low-dose), and shortening of ribs (1 control and 1 mid-dose).

The mean gestation period of F0 dams that delivered spontaneously was statistically significantly longer in the low dose group (22.1 days) compared to controls (21.6 days). The authors reported that the increase was "within a normal range of gestation period," which also agrees with general literature values.[13] No statistically significant differences compared to controls in delivery rate, viability index, sex ratios, or nursing rate were observed.

No external alterations or statistically significant differences in mean body weight or body weight gain, food consumption and water intake, developmental markers, or functional development were observed during the lactation period in the F1 pups. At the interim sacrifice, a few statistically significant changes were observed in absolute and relative organ weights but were not dose related. Except for a single control female, no skeletal malformations were observed in the F1 pups, and with respect to the state of ossification, no significant differences in number of caudal vertebrae were observed.

No significant differences in estrous cycle lengths were observed in F1 females. The copulation rate was 100% in all mated groups, and pregnancy rates were 95, 90, 100, and 85% in the control, low-, mid-, and high-dose group females, respectively. Food consumption and water intake were statistically significantly decreased compared to controls in F1 dams during the gestation period, but the decreases were not dose related, and body weight gain was not affected.

The numbers of corpora lutea and implantation sites and mean litter weights of the high-dose F1 dams were statistically significantly increased compared to controls. No significant differences in treated groups compared to controls were observed in early or late resorptions, pre- or post-implantation losses, total live and dead fetuses, sex ratios, or mean fetal weights. Mean placental weights of the low- and mid-dose dams were statistically significantly increased compared to controls.

Clubfoot was observed in one control and one high-dose F2 fetus on external examination, and pyelectasis was observed in two mid-dose fetuses during the visceral examination. Incidence of fetuses exhibiting skeletal variations did not differ statistically between the groups, and a skeletal malformation (fusion of thoracic vertebral centra) was observed in one low- and one-high dose fetus.

The authors concluded that the dose-dependent increase in incidence of clubfoot observed in F1 fetuses was not related to the test item due to lack of statistical significance and the results of the unpublished toxicokinetic study reported in Subpart 6.1.1 above demonstrating that HPMCAS is not significantly absorbed. While the authors did not report historical data, the toxicokinetic data support the conclusion, and it is further supported by presence of the alteration in control animals (albeit at lower incidence), the lack of an associated underlying skeletal defect in fetuses subjected to skeletal examination, the lack of occurrence of clubfoot in F1 pups delivered spontaneously, and the high incidence compared to the background occurrence rates reported in the literature (see further discussion at Subpart 6.2.8). Therefore, the NOAELs for maternal and developmental toxicity of HPMCAS in Sprague-Dawley rats were determined as 2500 mg/kg bw/day.

### 6.2.6 Embryo-fetal Developmental Toxicity Study in Rabbits

Four groups of 12–13 pregnant New Zealand White rabbits were administered 0, 625, 1250 or 2500 mg/kg bw/day of HPMCAS via gavage on GDs 6–18.[8] The vehicle-control was 0.25% CMC. All dams were sacrificed on GD 29 by air embolism, and fetuses were extracted by cesarean section and examined. Detailed examinations were conducted as follows:

**Table 17.** EFD Study in Rabbits: Observations, Hoshi et al., 1985

| Observations/Examinations | |
|---|---|
| Maternal parameters | Behavior; food and water consumption; body weight; gross pathological examination; placenta weight; and number of corpora lutea, resorption sites, and implantation sites. |
| Fetal survival | Number and distribution of viable and dead fetuses; live fetuses were placed in temperature- and humidity-controlled box for examining the survival ability at 6 and 24 h. |
| Fetal growth | Individual fetal body weights and sex. |
| Fetal morphology | Thoracic and abdominal organs examined for position, color, and form. 1/3 of viable fetuses processed for visceral examination and 2/3 processed for skeletal examination. |

No deaths, abortions, or abnormal behaviors were observed. Slight variations in maternal body weights were observed during the dosing period but were not reported as statistically significant, and weight gain from the end of treatment to termination was reported as steadily increasing although, graphically, body weights in the mid- and high-dose groups were slightly lower than those of the control and low-dose groups from GD 13 through 29, but body weights were well within ± 10% of the control group in all treated groups throughout the study. The authors reported that mean food consumption and water intake of the treated groups were similar to those of the control group, except between GD 12 and 13 in the low-dose group. Based on the body weight graph, the variation in food consumption and water intake in the low-dose group did not adversely affect body weight development, and, in fact, the mean body weight of the low-dose group, which had varied slightly below mean control body weight from GD 7–12, caught up to the control group on GD 13 and remained very close throughout the remainder of the study.

No statistically significant differences were observed between groups in numbers of corpora lutea or implantations, early or late resorptions, pre-and post-implantation losses, live or dead fetuses, fetal viability at 6 or 24 h, sex ratio, or mean litter, placental, or individual male and female fetal weights. The single dead fetus observed occurred in the control group. No external or visceral malformations were observed in any groups. Skeletal variations (asymmetry of sternebrae) and malformations (synorstosis of ribs and bifurcation of ribs) were observed in only one fetus of the high-dose group, and no statistically significant differences were noted in the state of ossifications among the groups. The NOAELs for maternal and developmental toxicity were determined as 2500 mg/kg bw/day HPMCAS in New Zealand White rabbits.

### 6.2.7 Peri- and Postnatal Developmental Toxicity Study with Extended Evaluation in Rats

Lastly, Hoshi et al., conducted a peri- and postnatal study in which 20+ pregnant female Sprague-Dawley rats/group were administered 0, 625, 1250, and 2500 mg/kg bw/day HPMCAS by gavage from GD 17 through PND 21; the vehicle-control was 0.25% CMC.[10] All dams were allowed to deliver spontaneously and rear their young, and litters were culled to 10 pups on PND 7. Dams were sacrificed and necropsied following weaning on PND 21. Evaluations were extended in order to examine the effects of exposure during the perinatal period (and possibly via nursing during the postnatal period) on reproductive ability of the F1 generation. Male and female offspring of each F0 dam dose group were evaluated as follows: Forty pups/sex/group (F1a) were subjected to sacrifice and necropsy following weaning (PND 21) while an additional 10 pups/sex/group (F1b) were followed through postnatal week (PNW) 11, and 25 pups/sex/group (F1c) were mated, and five dams of each group were sacrificed and their fetuses examined on GD21 while the remaining dams were allowed to go through gestation, deliver spontaneously, and rear their pups. The development of the F2 pups was observed until weaning.

**Table 18.** Pre- and Postnatal Development Study in Rats: Observations

| **Observations/Examinations** | |
|---|---|
| *Maternal (F0)* | |
| General condition and behavior | Throughout study. |
| Body weight | Daily during gestation; twice weekly after parturition. |
| Food consumption and water intake | Twice weekly during gestation and nursing period. |
| Frequency of nursing | PND 7 to 21. |
| Necropsy | Number of implantations. |
| *Offspring (F1)* | |
| Observations at birth | Stillbirths, body weighs, sex, and external abnormalities. |
| Body weight | Twice weekly up to age 3 weeks. |
| Developmental | Separation of ear auricular, emergence of abdominal tear, eruption of lower incisors, separation of eyelids, decent of testes, and vaginal opening. |
| F1a necropsy | Absolutely and relative organ weights (brain, hypophysis, thymus, heart, lung, liver, spleen, kidney, ovary, and testes); skeletal examination. |
| Body weight development (F1b) | Body weight, food consumption, and water intake measurements weekly from weaning through 11 weeks of age. |
| Physical/locomotor development (F1b) | Swimming test, chinning test, auditory function test, and reflex of eyelid (PND 21) and open-field test (PNW 8) and learning ability test (PNW 8–10). |
| F1c parameters | Body weight, food consumption, and water intake weekly during premating and mating (stated as performed, but results were not reported); body weight, food consumption, and water intake during gestation; reproductive ability, number of corpora lutea, resorption sites, implantation sites, and pre- and post-implantation loss. |

| *Offspring (F2)* | |
|---|---|
| Observations at spontaneous delivery | Still and live births, mean litter weights, mean individual male and female body weighs, and sex ratios. |
| Fetal morphology | External, visceral, and skeletal examinations. |
| Development and locomotor ability | F2 born from spontaneous delivery; specific parameters and result data not reported. |

No deaths, remarkable effects, or abnormal behavior were observed in the F0 dams. No statistically significant changes in body weight were observed between the control and treated group dams. A statistically significant decrease in food consumption was noted in late pregnancy in the mid- and high-dose dams, but no effect was observed on body weight gain; no other effects on food consumption or water intake were observed during the gestation or nursing periods.

Administration of HPMCAS to F0 dams during the perinatal period did not result in statistically significant differences in gestation length, implantations, numbers of still and live born pups, sex ratio, delivery rate, or viability index, and no external abnormalities were observed in any F1 pups at birth. Administration of HPMCAS to F0 dams during the perinatal and lactation periods did not affect nursing rate or body weight development of the F1 pups. Separation of the pinna and eruption of lower incisors was statistically significantly delayed in F1 pups compared to controls; the authors reported that the delays were "within a normal range." No other statistically significant differences in developmental parameters were observed in the F1 pups.

Following necropsy of the F1a pups, observed incidences of skeletal variations and malformations were similar among the groups. With respect to state of ossification, a slight, statistically significant decrease in number of caudal vertebrae was observed dose-dependently in male F1a pups of the high-dose F0 dams; no effect was observed when considering number of caudal vertebrae in male and female pups combined.

A statistically significant dose-related increase compared to controls in absolute liver weight was observed in male F1a pups of the high-dose group F0 dams, and increased liver weight relative to body weight was also dose-dependently statistically significantly increased in the mid- and high-dose male pups and high-dose female pups. In the F1a females, absolute liver weights were not statistically increased compared to controls, but a trend towards a dose relationship was noted. Left and total kidney weights relative to bodyweight were also statistically significantly dose-dependently increased in the high-dose F1a males and left kidney weight relative to bodyweight was statistically significantly increased in F1a females. Statistically significant dose-related decreases in absolute and relative right ovary weights were observed in high-dose female F1a pups. Thymus weight relative to bodyweight was statistically significantly dose-dependently increased in high-dose females. Other statistically significant alterations in absolute and relative organ

weights compared to controls were decreased absolute left and total testes weights and relative left, right, and total testes weights in low-dose males; decreased absolute and relative heart weights in mid-dose females; and increased relative lung weight in mid- and high-dose females. The authors reported, without further explanation, that the observed changes in organ weights were without toxicological concern. Because the authors did not comment on or provide historical control data or conduct histological examinations of the weighted organs, it is difficult to comment on the observed organ weight alterations based purely on the objective data. The short dosing period during the gestation phase in the F0 dams did not cover the period of organogenesis but did cover histogenesis. Due to the negligible oral absorption of HPMCAS observed in the unpublished toxicokinetic study described in Subpart 6.1.1, it is further unlikely that fetuses would have been exposed in utero or that the pups would have been exposed via nursing; for both of which to occur, additional membrane transport would have been necessary. For this reason, and in the absence of maternal toxicity, we find it unlikely that these changes were attributable to the test item. The statistically significant changes in absolute and relative organ weights in the F1a pups are shown in Tables 19–21 below.

**Table 19.** Statistically significant absolute organ weight changes in F1a Pups

| Dose | | Liver (g) | Testes (L) (mg) | Testes (Total) (mg) | Dose | | Heart (mg) | Ovary (R) (mg) |
|---|---|---|---|---|---|---|---|---|
| Males | C | 1.616 ± 0.058 | 96.0 ± 3.0 | 191.3 ± 6.0 | Females | C | 199.0 ± 4.6 | 9.4 ± 0.31 |
| | L | 1.684 ± 0.054 | 86.5 ± 3.1* | 172.9 ± 6.4* | | L | 194.4 ± 5.5 | 9.25 ± 0.45 |
| | M | 1.717 ± 0.063 | 93.2 ± 3.9 | 186.3 ± 7.9 | | M | 180.9 ± 4.7** | 8.37 ± 0.44 |
| | H | 1.810 ± 0.065* | 99.2 ± 3.8 | 197.2 ± 7.6 | | H | 190.3 ± 5.0 | 8.31 ± 0.31* |

*p<0.05, **p<0.01

**Table 20.** Statistically significant organ weight relative to bodyweight changes (F1a males)

| Dose | | Liver (g) | Kidney (L) (mg) | Kidney (Total) (mg) | Testes (R) (mg) | Testes (L) (mg) | Testes (Total) (mg) |
|---|---|---|---|---|---|---|---|
| Males | C | 4.202 ± 0.105 | 608.0 ± 13.9 | 1245.5 ± 25.6 | 249.6 ± 7.4 | 251.6 ± 7.1 | 501.2 ± 14.3 |
| | L | 4.387 ± 0.088 | 619.0 ± 16.6 | 1249.5 ± 28.0 | 224.9 ± 6.7* | 225.9 ± 6.4** | 450.8 ± 12.8* |
| | M | 4.527 ± 0.102* | 622.6 ± 15.1 | 1270.9 ± 26.1 | 251.9 ± 8.1 | 245.8 ± 7.9 | 497.7 ± 15.7 |
| | H | 4.648 ± 0.102** | 657.6 ± 12.9* | 1331.1 ± 22.8* | 252.0 ± 7.0 | 255.0 ± 6.4 | 507.0 ± 13.2 |

*p<0.05, **p<0.01

**Table 21.** Statistically significant organ weight relative to bodyweight changes (F1a females)

| Dose | | Thymus (mg) | Heart (mg) | Liver (g) | Lung (mg) | Kidney (L) (mg) | Ovary (R) (mg) |
|---|---|---|---|---|---|---|---|
| Females | C | 411.3 ± 17.6 | 534.2 ± 11.2 | 4.255 ± 0.082 | 1012.2 ± 16.8 | 639.5 ± 11.0 | 25.30 ± 0.79 |
| | L | 430.1 ± 17.9 | 545.7 ± 13.5 | 4.374 ± 0.080 | 1009.4 ± 20.0 | 628.1 ± 11.9 | 25.72 ± 1.03 |
| | M | 436.0 ± 15.9 | 502.9 ± 10.8* | 4.461 ± 0.084 | 1087.1 ± 20.1** | 655.5 ± 14.1 | 22.95 ± 1.06 |
| | H | 458.3 ± 14.0* | 526.8 ± 11.6 | 4.708 ± 0.107** | 1064.7 ± 18.9* | 693.5 ± 12.3** | 22.83 ± 0.74* |

*p<0.05, **p<0.01

Following weaning, there were no statistically significant differences in body weights or body weight gains of F1b pups from PNW 4 through 11 although the authors reported food consumption and water intake were statistically significantly decreased in groups of pups of the treated F0 dams compared to controls over the period. The authors also reported that the deviations were "considered within a normal ranges." Based on the graphs it is unclear whether the authors' statement concerning food and water consumption was with respect to all dose groups, both sexes, and/or over which specific weeks. For example, only in the graph for water intake in male F1b pups was the control curve clearly above the other dose groups from PNW 5–11 while in females, the control curve was clearly below the dose group control curves from PNW 8–11. In the food consumption graphs all group curves are very close together throughout the entire period, and the resolution of the graph impedes discerning exactly when the various dose curves crossed one another; in both sexes, the control curves appear to be slightly above or slightly below some or all of the other dose curves at various time points. Despite the above confusion, the slight alterations in food and water consumption did not affect body weight development and, therefore, we consider them to be without toxicological relevance. With the exception of an increased bar holding time in the chinning test in high-dose female F1b pups, no statistically significant differences were observed in functional developmental parameters in the F1b pups.

No statistically significant differences in bodyweight gain, food consumption, or water intake during gestation or in copulation or pregnancy rates of the F1c dams were observed. There were no statistically significant differences in frequency of external, visceral, or skeletal malformations or skeletal variations in F2 fetuses delivered by cesarean section although a trend towards a dose-dependent increase was observed for pyelectasis. With respect to the state of ossification, statistically significant increases were observed in the mid- and high-dose groups in only one (number of proximal phalanges in the hindpaw) of the 11 markers evaluated. The authors reported that no developmental abnormalities or effects on locomotor ability were observed from birth through weaning in the F2 pups of F1 dams allowed to deliver spontaneously; however, the authors did not report any data or describe specific evaluations conducted on these pups.

Due to inadequate or incomplete reporting of methods and data, it is difficult to draw definitive conclusions with respect to the several statistically significant and dose related alterations observed in this study. Indeed, the authors themselves were less than definitive in their concluding remarks. Nonetheless, based on the totality of evidence from the three additional developmental and reproductive toxicity studies conducted by Hoshi et al. and the study by Cappon et al. described below (albeit, these additional studies were conducted during different dosing windows), the short period of in utero dosing in the current study, and the likelihood, based on toxicokinetic data, that exposure of dams and pups to HPMCAS in the current study was negligible, the results observed are considered unlikely to represent a toxicological concern.

### 6.2.8 Embryo-fetal Developmental Toxicity Study in Rats and Rabbits

In order to further evaluate the potential developmental toxicity of HPMCAS, Cappon et al. (2003) administered doses of 0, 50, 150, 625, and 2500 mg/kg bw/day HPMCAS to groups of approximately 20 pregnant Sprague-Dawley rats and New Zealand White rabbits during the respective periods of organogenisis.[12] The lower doses were prepared as suspensions in 0.5% methylcellulose, which was also used as the vehicle-control, while the high-dose was prepared as a suspension in 1% methylcellulose. The test solutions were administered by gavage to rats (19–20/group) once daily from GD 6–17 and to rabbits (18–20/group) twice daily, at a 6 h interval, from GD 7–19. The study was conducted in accordance with International Conference on Harmonization (1994): Guideline on detection of toxicity to reproduction for medicinal products (Federal Register 59:48746–48752) in order to 1) further investigate the dose-related increase in frequency of clubfoot observed in external fetal examinations in the rat embryo-fetal developmental toxicity (EFD) study by Hoshi et al. (1985) and 2) because the number of litters investigated in the rabbit EFD study by Hoshi et al. (1985) "was not consistent with current regulatory requirement." The rat and rabbit dams were sacrificed on GD 21 and 29, respectively, and maternal and fetal examinations were carried out. Observations and examinations were conducted as follows:

**Table 22.** EFD Study: Observations in Rats and Rabbits, Cappon et. al., 2003

| **Observations/Examinations** | |
|---|---|
| *Maternal* | |
| Morbidity and mortality | At least twice daily. |
| Body weight and food consumption | Daily beginning on gestation day 5 or 6. |
| Necropsy | Gross pathology, gravid uterine weight, number of corpora lutea, and number, type and location of implantation sites. |
| *Fetuses* | |
| C-section | Number of viable fetuses, sex ratio, and fetal weight. |
| External and visceral development | All rats and rabbits. |
| Skeletal development | All rabbits and 50% rats. |

With the exception of a transient increase in bodyweight gain in low-mid-dose rabbit dams between GD 7 and 13, no statistically significant changes were observed in maternal bodyweights, bodyweight gains or food consumption in treated rats or rabbits compared to controls. No statistically significant differences were observed in numbers of pregnant animals, gravid uterine weights, numbers of corpora lutea or implantation sites, early or late resorptions, pre- or post-implantation losses, or number of viable fetuses in either rats or rabbits. Rat fetal weight was statistically significantly increased (mostly due to the male contribution according to the authors) in the high-dose group compared to controls; the increase was dose-related but remained within the normal range of published historical control data of the breeder (Charles River Laboratories), and was, therefore, considered due to normal biological variation. Additionally, in rabbits, a statistically significant increase in fetal weight was noted in the low-dose group and was also considered normal biological variation. No statistically significant differences compared to controls were observed in external, visceral, or skeletal malformations and variations in rat or rabbit fetuses. In the rat fetuses no skeletal malformations or skeletal limb variations were observed, and in the rabbit fetuses none of the observed skeletal malformations involved limbs. The incidences of observed skeletal malformations and variations in rats and rabbits were all within the historical normal ranges of the breeder.

The authors concluded that the dose-related increase in clubfoot observed in rat fetuses by Hoshi et al. (1985) was not related to treatment with HPMCAS. The authors further suggested that the findings observed by Hoshi et al. may have been due to misdiagnosis or an artifact of the cesarean section or examination procedures citing the following evidence: 1) the incidence of clubfoot in Sprague-Dawley rat historical control data published by Charles River Laboratories is 0.001% and the incidence of clubfoot in Sprague-Dawley rat historical control data from Japan published by Morita et al. (1987) is 0.006% while the incidence in the EFD study in rats by Hoshi et al. was much higher, even in control animals (0.80%), 2) in the EFD study in rats by Hoshi et al. clubfoot was observed only in fetuses harvested on GD 21 and was not observed in the pups that were spontaneously delivered by dams of the same dose groups, 3) the external diagnosis of clubfoot by Hoshi et al. in the EFD study in rats did not have an underlying skeletal finding, 4) clubfoot was observed in low- and high-dose fetuses in the rat fertility study by Hoshi et al. (1985) in which dosing was terminated on GD 7 (prior to the period of limb development), 5) not all presentations of clubfoot in rats are malformations, and the term is no longer preferred in such cases. Based on the reported results, we agree with the authors' conclusion that HPMCAS was not teratogenic in rats or rabbits and further conclude that the NOAELs for maternal and developmental toxicity were 2500 mg/kg bw/day in Sprague-Dawley rats and New Zealand White rabbits.

## 6.3 Toxicological Studies on HPMC

In 2007, Burdock published a review that covered older studies, as well as more recent toxicological data on HPMC, including acute (rats), subacute (rats, rabbits and dogs), chronic (2-year) (rats) and teratogenicity/reproduction toxicological studies (rats).[4] The majority of these studies were devoid of any adverse effects. However, administration of extremely high doses of HPMC (10–30 g/kg bw/day; up to 30% of diet) resulted in growth retardation and anemia in a few of the studies. Because there was no tissue-specific toxicity noted, these findings were attributed to the anti-nutrient effects of HPMC and undernourishment of the animals rather than any direct toxic effects of the test item. HPMC was concluded to be "extremely low in chronic oral toxicity".

Most recently, Thackaberry et al. (2010) investigated the safety and tolerability of HPMC along with other compounds, for use as a vehicle in general toxicology studies.[14] HPMC was administered via gavage at 20 mg/kg bw/day to mice, rats, dogs, and cynomolgus monkeys for approximately 90 days. Clinical observations, body weights, food consumption parameters, clinical pathology, and histopathological examinations were performed. The suitability of HPMC for use as a vehicle substance (e.g., a compound without biological effects) was confirmed with a lack of toxicological effects on all parameters examined.

## 6.4 Additional Scientific Studies

### 6.4.1 Studies on HPMCAS in Laboratory Animals

As summarized in the table below, Hoshi et al., conducted additional investigations using different methodologies in various species (mice, rats, guinea pigs, dogs, and frogs) to evaluate the effects of Shin-Etsu HPMCAS when dosed intraperitoneally and orally.[15] There were no significant adverse effects on the central and autonomic nervous systems, cardiovascular (respiration, blood pressure, and heart rate) or digestive systems, and Shin-Etsu HPMCAS also had no effect on hemolysis, coagulation, or urine parameters (see Table 23). All investigations were included in one publication with minimal details regarding methods.

**Table 23.** Additional evaluations of Shin-Etsu HPMCAS

| Animal Species and number of HPMCAS treated animals | Body System | Test item dose (single dose unless otherwise specified) | Examinations | Results |
|---|---|---|---|---|
| ***Central Nervous System*** | | | | |
| Dogs, Beagle, male (CSK Co. Ltd.) (n=6; control and treated not specified) | General behavior | 250 mg/kg bw orally | Behavior was observed for 6 hours after administration and also at 24 hours. | No significant changes in behavior were observed. |
| Rats, Sprague-Dawley male (Charles River Japan Inc.) (n=26) | Rectal temperature | 125 and 250 mg/kg bw orally | Rectal temperature was measured before administration, and at 20, 60, 120 and 240 minutes after the administration using an electric thermistor. | Test item significantly increased the rectal temperature of rats 20 minutes after administration of 250 mg/kg bw and at 125 mg/kg bw from hours 1–4. No clear dose-dependent response was found. |
| Rats, Sprague-Dawley male (Charles River Japan Inc.) (n=16) | Motor coordination (rotarod method) | 250 mg/kg bw orally | Motor coordination was measured at 30 and 60 minutes after oral administration of test item. Falling down from the rotarod within 5 minutes was judged as loss of motor coordination. | No significant change was observed in motor coordination. |
| ***Autonomic Nervous system*** | | | | |
| Mice, male, ICR strain (CLEA Japan Co. Ltd.) (n=20) | Effect on pupillary diameter | 0.75 and 1.5 g/kg injected subcutaneously | Test item was injected subcutaneously and the right and left pupillary diameters were measured 5, 15, 30 and 60 minutes later. | No mydriatic action was observed. |
| Guinea pigs, male, Hartley, (Ichikawaya) (n=10) | Anti-sialagogic action | 125 and 250 mg/kg bw orally | Test item was administered 30 minutes prior to the subcutaneous administration of pilocarpine (10 mg/kg bw). Immediately after the administration of pilocarpine, the animals were restrained in Bowman cages in a prone position and the volume of saliva collected was determined during 2 hours. | Test item tended to suppress sialagogic action in the first hour at oral doses of 125 and 250 mg/kg. However, both dosages increased the volume of saliva over a 2-hour period. |
| Guinea pigs, male, Hartley, (Ichikawaya) | Effect on isolated smooth muscle | N/A | The ileum of the deceased animals was isolated and suspended in an organ bath | No significant effect on the contraction of isolated ileum. |

| | | | | |
|---|---|---|---|---|
| (n=not clear, "groups of 4–5") | | | filled with Tyrode's solution. Contractions of the preparations were isotonically recorded on a kymograph. | |
| Rats, female, Sprague-Dawley (Charles River Japan Inc.) (n=not clear, "groups of 4–5") | Effect on contraction of isolated uterus | N/A | The uterus horn was isolated from deceased animals and suspended in an organ bath filled with Tyrode's solution. Contractions of the preparation were isotonically recorded on a kymograph. | No significant effect on the contraction of isolated uterus. |
| ***Cardiovascular system*** | | | | |
| Dogs, Beagle, male (CSK Co. Ltd.) (n=not clear, "groups of 4–6") | Effect on respiration, blood pressure, heart rate and ECG | N/A | Animals were anesthetized and respiration via a tracheal cannula, blood pressure via the carotid artery and heart rate were simultaneously recorded on a cardiograph. Blood flow in the femoral artery was measured with a blood flow meter. The test item was injected into the femoral artery at a constant speed of 0.4 mL/10 sec. | No significant changes were observed in blood pressure or respiration rate immediately or after 30 or 60 minutes after test item administration. No significant effect of HPMCAS on actions of histamine, noradrenaline, serotonin, adrenaline, isoproterenol or acetylcholine. No changes were found in the ECG pattern of dogs and no influence on femoral artery blood flow was noted. |
| ***Digestive organs*** | | | | |
| Mice, male, ICR strain (CLEA Japan Co. Ltd.) (n=29) | Effect on transportation of $BaSO_4$ in small intestine | 1.25 and 2.5 g/kg bw orally | Test item was administered and 20 minutes later 0.2 mL of 50% BaSO4 was administered and after another 20 minutes the animals were sacrificed and the whole intestines were removed. Mobility ratio (%) was calculated as distance of $BaSO_4$ transportation divided by the whole length of small intestine x 100 | Oral administration of HPMCAS to mice tended to suppress the transportation of $BaSO_4$, but this was non-significant. |
| Rats, Sprague-Dawley male (Charles River Japan Inc.) (n=12) | Effect on gastric secretion | N/A | Animals were anesthetized and the abdomen was opened and pylorus ligated. The test compound was injected into the duodenum at doses of 62.5 and 125 mg/kg bw. The abdomen was closed and then four hours later, the stomach | Intra-duodenal administration of HPMCAS suppressed the volume and slightly acidified the gastric juice and output of pepsin per 1 hour was slightly |

| | | | | |
|---|---|---|---|---|
| | | | was removed and gastric juice collected. | increased; however, none of these changes were significant and a clear dose-dependency was not found. |
| Rats, Sprague-Dawley male (Charles River Japan Inc.) (n=8) | Effect on bile excretion | 1.25 and 2.5 g/kg bw orally | Animals were anesthetized, abdomen was opened and a plastic cannula was inserted into the bile duct. The volume and weight of bile collected 1 and 3 hours after the test item administration was measured. | No significant changes in bile excretion. |
| ***Others*** | | | | |
| Rats, Sprague-Dawley male (Charles River Japan Inc.) (n=9) | Effect on urinary volume | 875 and 1750 mg/kg bw orally | Animals were placed in metabolic cages for 5 hours and urine was collected hourly. | No significant effect on urinary volume. |
| Rats, Sprague-Dawley male (Charles River Japan Inc.) (n=16) | Electrolyte excretion | 0.3 mL/100 g bw orally | Animals were placed in metabolic cages for 5 hours and urine was collected and analyzed for sodium and potassium. | No significant effect on electrolyte excretion. |
| Rabbits, male, New Zealand White strain (Ichikawaya) (n=9) | Hemolytic action | N/A | The washed red blood cells of the animal were exposed to 1.25 and 2.5% of the test item and hemolysis was analyzed. | No significant hemolytic action was observed. |
| Rabbits, male, New Zealand White strain (Ichikawaya) (n=3) | Plasma re-calcification time | 250 mg/kg i.p. injection | Whole blood from ear artery was analyzed 0, 1, 15, 30 and 60 min after i.p. injection of test item to measure plasma recalcification time. | No significant effect was observed. |
| Rats, Sprague-Dawley male (Charles River Japan Inc.) (single dose n=15; consecutive doses n=10) | Blood biochemical analyses | 125 and 250 mg/kg bw orally for 10 consecutive days | One hour after the single administration and after the end of 10 successive administrations, blood was collected from jugular vein and serum glucose, total cholesterol, triglycerides, NEFA levels; lactate and insulin levels were investigated. | Neither single oral administration nor 10 successive daily administrations produced dose-related changes in blood biochemistry. |
| Guinea pigs, male, Hartley, (Ichikawaya) (n=12) | Vascular permeability | 250 and 500 mg/kg bw orally | Skin of the back was depilated, and animals were given test item. One hour later they received an i.v. injection of pontamine sky blue and 0.1 mL of histamine solution intracutaneously. Animals were sacrificed 30 minutes later and skin on back was removed. Ratio of vascular permeability was calculated. | No significant influence on histamine-induced vascular permeability. |

### 6.4.2 Human Studies

Three clinical trials, two of which included HPMCAS as a suspension aid and dispersant for drug formulations at a concentration of 1% by weight[16, 17] and one in which a suspension of HPMCAS powder was used as a placebo (dose not specified)[18] were located on clinicaltrials.gov. The use of HPMCAS in this manner supports general recognition of its safety for ingestion by humans.

## 6.5 Authoritative Safety Opinions

### 6.5.1 European Food Safety Authority

The European Food Safety Authority (EFSA) re-evaluated many types of modified celluloses (including microcrystalline cellulose, powdered cellulose, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, ethyl methyl cellulose, sodium carboxy methyl cellulose and enzymatically hydrolyzed carboxy methyl cellulose) as food additives (note, the substance that is the subject of this GRAS notice—HPMCAS–is not on this list).[19] An acceptable daily intake (ADI) was not specified for unmodified and modified celluloses as they are not absorbed and are excreted intact in the feces. They can as well be fermented by intestinal flora in animals and humans. Because of structural, physicochemical and biological similarities, the panel considered it possible to read-across between all the celluloses. Acute toxicity is low and there is no geno-toxic concern. Short-term and subchronic dietary toxicity studies performed at levels up to 10% did not indicate specific treatment-related adverse effects. Chronic studies resulted in NOAELs reported up to 9,000 mg/kg bw/day. No carcinogenic properties were detected, and there were no observed effects on reproductive performance or developmental effects at doses greater than 1,000 mg/kg bw by gavage. Combined exposure to celluloses at the 95$^{th}$ percentile was up to 506 mg/kg bw daily. The panel concluded there was no need for an ADI, and there was no safety concern for reported uses and use levels.

### 6.5.2 World Health Organization

In 1989, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) re-evaluated their previously established (1974) group ADI for modified celluloses of up to 25 mg/kg bw/day (as a sum of total modified celluloses), which was based on the highest "no-effect level" (NEL) obtainable in lifespan studies without causing nutritional effects and using a safety factor of 100.[20] It was suggested by the Committee that this ADI could be exceeded for "dietetic purposes" (e.g., a situation where use is primarily intended to take advantage of the non-caloric property of these additives). Seven modified celluloses, ethyl cellulose, hydroxypropyl cellulose, HPMC, methyl ethyl cellulose, sodium carboxymethyl cellulose, ethyl

cellulose and ethyl hydroxyethylcellulose were re-evaluated in order to include the "most recent information on technology and toxicology". After a review of available data on modified celluloses as a group, including human data indicating no adverse effects (specifically no significant clinical laxative effects) upon ingestion of up to 30 g/day/person, the Committee concluded that "the establishment of an acceptable daily intake (ADI) expressed in numerical form is not deemed necessary" and, therefore, allocated an ADI "not specified" to the seven modified celluloses.[20]

## 6.6 Allergenicity

Shin-Etsu AQOAT® HPMCAS does not contain or have added, and is manufactured in a facility free of, all eight major allergens (milk, egg, fish, Crustacean shellfish, tree nuts, wheat, peanuts, and soybeans) identified, and required to be disclosed in labeling, in the Food Allergen Labeling and Consumer Protection Act (FALCPA). Additionally, Shin-Etsu AQOAT® HPMCAS does not contain gluten, celery, mustard, sesame seeds, sulfur dioxide and sulfites or any derivatives or products of the aforementioned.

## 6.7 History of Consumption

HPMC has been utilized as a food additive, specifically as an emulsifier, stabilizer, thickener, and gelling agent, as well as an ingredient for film coatings for pharmaceutical tablets for over 50 years, and its pharmacological and biochemical effects have been studied extensively.[21] [4] Due to its chemical nature as a high-viscosity, non-digestible polysaccharide, it has also been studied and used as a dietary fiber. HPMCAS is known to have a pH-dependent solubility and an inhibitory effect on re-crystallization of drugs from super-saturated drug solution, positioning it as a superior enteric film coating ingredient and solid dispersion carrier. Both HPMC and HPMCAS are listed in the FDA inactive ingredient database and are subjects of USP/NF and JP monographs.

Modified celluloses, aka "cellulose derivatives", or "cellulosics" are vastly important to the pharmaceutical industry. In one report, over 600 prescription drug products were found that included modified cellulose ingredients, approximately 71% in tablet form, 20% in capsules and oral liquids, and the remaining found in injectable, nasal, ophthalmic, topical, transdermal, implant and buccal forms. HPMC was found to be the most frequently used modified cellulose, found in approximately 62% of all dosage forms, while HPMCAS was found primarily in enteric coatings and delayed release dosage forms.[22]

**Table 24.** Inactive Ingredient Applications for HPMCAS*

| Route | Dosage Form | CAS Number | UNII | Potency Amount | Potency Unit |
|---|---|---|---|---|---|
| Oral | Capsule | 71138971 | N/A | 44.6 | mg |
| Oral | Capsule, delayed release | 71138971 | N/A | 51.8 | mg |
| Oral | Capsule, extended release | 71138971 | N/A | 66.78 | mg |
| Oral | Granule | 71138971 | N/A | 45.8 | mg |
| Oral | Tablet | 71138971 | N/A | 560 | mg |
| Oral | Tablet, delayed release | 71138971 | N/A | 325 | mg |
| Oral | Tablet, extended release | 71138971 | N/A | 29 | mg |
| Oral | Tablet | 71138971 | 6N003M473W | 19.83 | mg |
| Oral | Tablet, delayed release | 71138971 | 6N003M473W | 29.7 | mg |
| Oral | Tablet, extended release | 71138971 | 6N003M473W | 9.92 | mg |

*Table derived from FDA's Inactive Ingredient Database, updated June 12, 2020

## 6.8 Adverse Events

Shin-Etsu states that no adverse event reports associated with the consumption of this ingredient to date have been received by the company.

No FDA letters regarding concern for safety to companies that market products containing HPMCAS were located. A search of FDA's Recalls, Market Withdrawals, & Safety Alerts search engine, and FDA's Center for Food Safety and Applied Nutrition Adverse Event Reporting System did not uncover any mention of HPMCAS products. All databases were accessed on June 15, 2020.

## 6.9 Current Regulatory Status

A thorough search for the current regulatory status of HPMCAS, relevant to its use in food in the United States, was conducted and no information was located. The starting raw material used in the production of HPMCAS by Shin-Etsu, HPMC, is an approved food additive pursuant to 21 CFR 172.874.

## 6.10 Basis for the GRAS Conclusion

Shin-Etsu's Shin-Etsu AQOAT® HPMCAS has been the subject of a thorough safety assessment as described above. The totality of evidence supporting safety is comprised of data and information that establish the safety of Shin-Etsu AQOAT® HPMCAS under the conditions of its intended use and data and information that is corroborative of safety. The general availability and general acceptance, throughout the scientific community of qualified experts, of the data and information that establish the safety of Shin-Etsu AQOAT® HPMCAS under its intended conditions of use establish the general recognition of this data and information. Together, the establishment of safety based on scientific procedures and its general recognition

form the basis for Shin-Etsu's conclusion of GRAS status of Shin-Etsu AQOAT® HPMCAS for its intended use.

### 6.10.1 Data and Information that Establish Safety

The scientific data, information, and methods forming the basis of this conclusion are:

- The establishment of identity, demonstrating Shin-Etsu AQOAT® HPMCAS is a pure compound classified as "modified cellulose";
- The method of manufacture and specifications, demonstrating the safe production and the robust quality control standards of Shin-Etsu AQOAT® HPMCAS;
- The pharmacokinetic studies demonstrating related modified cellulose substances, including the starting material, HPMC, for manufacture of Shin-Etsu AQOAT® HPMCAS, are minimally absorbed and excreted almost exclusively in the feces following oral administration;
- The 8-week and 26-week repeated-dose oral toxicity studies in rats on Shin-Etsu AQOAT® HPMCAS demonstrating no evidence of histopathological changes or other toxicological effects and, thereby, establishing a lack of adverse health effects or target organs from repeated exposure to Shin-Etsu AQOAT® HPMCAS in rats;
- Several reproductive and developmental toxicity studies in rats and rabbits demonstrating no evidence of concerns for toxic effects of Shin-Etsu AQOAT® HPMCAS on fertility and reproductive performance in rats or growth and development in rats and rabbits; and
- The conservative exposure estimates for the intended conditions of use.

Data indicates that modified celluloses are not absorbed to any significant extent by rats or humans, and the reports of EFSA and JECFA summarized in Subpart 6.5 of this GRAS notice demonstrate the general acceptance of this data. As such, the imposition of limits on exposure in humans are not necessary in order to establish a reasonable certainty of safety of the intended use of Shin-Etsu AQOAT® HPMCAS based on the totality of data presented in this GRAS notice. In the 8-week and 26-week oral toxicity studies and the reproductive and developmental toxicity studies the NOAELs were 2500 mg/kg bw/day in male and female Sprague Dawley rats; these were the maximum feasible doses that could be administered to the animals, and therefore, the highest doses tested. NOAELs for developmental toxicity in New Zealand White rabbits were also 2500 mg/kg bw/day, the highest dose tested. Based on the intended use of the ingredient as an enteric coating agent, carrier for solid

dispersions, and capsule shell, the maximum EDI at the 90th percentile of consumers in the total population is approximately 47.1 mg/kg bw/day, and the highest maximum EDI at the 90th percentile of consumers in the consumer subgroups (children ages 2–12 y) is 52.6 mg/kg bw/day. The evidence presented in this report supports a conclusion that the intended use of Shin-Etsu AQOAT® HPMCAS is reasonably certain to be safe.

### 6.10.2 Data and Information that are Corroborative of Safety

The safety of Shin-Etsu AQOAT® HPMCAS is corroborated by:

- Multiple experiments in mice, rats, guinea pigs, dogs, and frogs to evaluate effects of intraperitoneal and oral doses of HPMCAS on central and autonomic nervous systems and cardiovascular and digestive systems that showed no significant adverse effects;
- The acute toxicity studies in rabbits and rats demonstrating the $LD_{50} > 2.5$ g/kg bw HPMCAS;
- The approval of HPMCAS as an inactive ingredient in pharmaceutical preparations;
- The sales of approximately 3000 metric tons of its Shin-Etsu AQOAT® HPMCAS without any post-market adverse event reports;
- Data and information establishing the safety of HPMC, a related modified cellulose and starting material for the manufacture of Shin-Etsu AQOAT® HPMCAS, including the review of multiple toxicological studies in rats, rabbits, and dogs, the published 90-day oral toxicity studies in mice, rats, dogs, and nonhuman primates, and the approved food additive and GRAS statuses of the ingredient;
- The general scientific recognition of the safety of modified celluloses, including the substantial history of use of the similar substance HPMC as a food additive for over 50 years, the 2007 FDA 'no questions' letter for HPMC GRAS use up to 20 g/person/day, the generally accepted knowledge that this class of ingredients has a non-absorbable nature within the human gastrointestinal tract, and the human data reviewed by JECFA on modified celluloses in general indicating no adverse effects (specifically no significant clinical laxative effects) upon ingestion of up to 30 g/day/person;
- The unpublished toxicokinetic study on Shin-Etsu AQOAT® HPMCAS supporting the general consensus that modified celluloses are not significantly absorbed and excreted mainly in the feces and also supporting the interpretation of effects observed in the oral toxicological studies as

without toxicological relevance and unrelated to administration of the test item.

### 6.10.3 General Recognition

The scientific data, information, and methods herein reported, that provide the basis of this GRAS conclusion by scientific procedures are published and available in the public domain. Part 7 of this GRAS notice contains the citations for the published studies. These publicly available data and information fulfill the requirement of the GRAS standard for general availability of the scientific data, information, and methods relied on to establish the safety of Shin-Etsu AQOAT® HPMCAS for its intended conditions of use. The peer-review of the published studies and lack of Letters to the Editor or other dissenting opinions, as well as the opinions of EFSA and JECFA and the FDA 'no questions' response letter to a GRAS notice with respect to similar modified celluloses provide ample evidence of general recognition among qualified experts that there is reasonable certainty that consumption of Shin-Etsu AQOAT® HPMCAS for its intended use is not harmful. The general availability and acceptance of these scientific data, information, and methods satisfy the criterion of the GRAS standard that general recognition of safety requires common knowledge throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food that there is reasonable certainty that the substance is not harmful under the conditions of its intended use.

### 6.10.4 Data and Information that are Inconsistent with the GRAS Conclusion

In the peri- and postnatal developmental toxicity study in rats by Hoshi et al. (1985), reported in Subpart 6.2.7, there were some statistically significant dose-related findings that were not adequately explained by the data presented in the study.[10] Nonetheless, as discussed in Subpart 6.2.7, we believe the totality of evidence presented in this GRAS notice with respect to developmental and reproductive toxicity and toxicokinetics of Shin-Etsu AQOAT® HPMCAS sufficiently lay to rest any concerns that this data may present.

We have reviewed the available data and information and are not aware of any additional data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status.

### 6.10.5 Information that is Exempt from Disclosure under FOIA

There are no data or information in this GRAS notice that are considered exempt from disclosure under FOIA as trade secret or commercial or financial information that is privileged or confidential.

# Part 7: Supporting Data and Information

Initial literature searches for the safety assessment described in Part 6 of this GRAS notice were conducted between May 2015 and January 2016. Additional literature searches were conducted from December 2018 through August 2019 and on June 15, 2020.

## 7.1 Data and Information that are *not* Generally Available

The following information described in this GRAS notice is not generally available:

- The unpublished toxicokinetic study in rats on Shin-Etsu AQOAT® HPMCAS summarized in Subpart 6.1.1 of this report.

This study corroborates the general recognition that modified celluloses are not significantly absorbed and are excreted intact in the feces and also corroborates the interpretation of the toxicological data on Shin-Etsu AQOAT® HPMCAS. Therefore, we believe the safety conclusion can still be made even if qualified experts throughout the scientific community do not generally have access to this information.

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