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April 8, 2011

Via Federal Express

Food and Drug Administration
Division of Animal Feeds (HFV-224)
Office of Surveillance and Compliance
Center for Veterinary Medicine
7519 Standish Place
Rockville, Maryland 20855

**Re: CVM GRAS Notification for Polyoxyethylene (20) Sorbitan
Monostearate; Our File No. EM13458-01**

Dear Sir or Madam:

On behalf of our client, Emerald Carolina Chemicals, LLC (the Notifier), we hereby respectfully request to participate in the pilot program for Generally Recognized as Safe (GRAS) determinations¹ for the safe use of polyoxyethylene (20) sorbitan monostearate (CAS Reg. No. 9005-67-8) as a component of the Notifier's FoamBlast[®] FMT defoamer, which is used as a processing aid in the production of distillers grains used in animal feed for food-producing animals. As discussed in detail in the enclosed dossier of information, the defoamer product is added to the condensed distillers solubles (*i.e.*, thin stillage concentrate) to assist in separating out corn oil during processing of grain from ethanol distillation. Accordingly, the polyoxyethylene (20) sorbitan monostearate defoamer component may be present at minute levels as an impurity in distillers grains fed to the food-producing animals.

A submission is provided, in triplicate, for the polyoxyethylene (20) sorbitan monostearate component of the defoamer. The submission includes a determination, based on scientific procedures, that polyoxyethylene (20) sorbitan monostearate is GRAS based on its presence as an impurity in animal feed as a result of its use in the processing of distillers grains.

¹ See Substances Generally Recognized as Safe Added to Food for Animals; Notice of Pilot Program, 75 Fed. Reg. 31800 (June 4, 2010).

KELLER AND HECKMAN LLP

Food and Drug Administration
April 8, 2011
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We trust that this submission satisfies the Agency's needs, and will be deemed accepted and complete. Should any questions arise, please contact us, preferably by telephone or e-mail, so that we can promptly respond.



Sincerely,

A handwritten signature in blue ink, appearing to read "Devon Wm. Hill". The signature is fluid and cursive, written over the printed name.

Devon Wm. Hill

Enclosure

**Generally Recognized As Safe (GRAS)
Notification
for
Polyoxyethylene (20) Sorbitan Monostearate
(CAS Reg. No. 9005-67-8)**

Prepared for:

U.S. Food and Drug Administration
Center for Veterinary Medicine
Division of Animal Feeds (HFV-224)
7519 Standish Place
Rockville, MD 20855

Notifier:

Emerald Carolina Chemicals, LLC
8309 Wilkinson Boulevard
Charlotte, NC 28214-9052

April 8, 2011

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APPENDICES

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APPENDIX 2 – Certificates of Analysis

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APPENDIX 4 – Stability Certification Letter

I. Introduction

On behalf of Emerald Carolina Chemicals, LLC (Emerald or the Notifier), Keller and Heckman LLP submits the enclosed dossier of information in support of this notification that polyoxyethylene (20) sorbitan monostearate (CAS Reg. No. 9005-67-8), a component of the Notifier's FoamBlast® FMT defoamer, is Generally Recognized as Safe (GRAS) when present as an impurity in feed for the food-producing target animals, as a result of the defoamer's use as a processing aid in the production of dried and wet distillers grains (DG) with added solubles. More specifically, the 'whole' stillage produced during ethanol distillation is filtered by a mechanical centrifuge to remove water-soluble solids) to produce a 'thin stillage.' The 'thin stillage' is then condensed from 5-10% solids to up to 40% solids into 'condensed distillers solubles' (CDS), which contains corn syrup.

After the defoamer is added, the CDS is processed in a mechanical centrifuge to separate out the corn oil. CDS is a liquid byproduct that contains corn oil, as well as fermentation byproducts, spent yeast cells, and other nutrients which remain after corn grain has been fermented to produce ethanol. The Notifier's defoamer product is added to the CDS at levels up to 100 parts per million (ppm) to assist in separating the corn oil from the CDS. Polyoxyethylene (20) sorbitan monostearate makes up 20% of the Notifier's defoamer; thus the substance is added at levels up to 20 ppm to the CDS. Once the corn oil has been separated from the CDS, the resulting "de-oiled" CDS is then added to dried and wet DG to produce either wet distillers grains with solubles (WDGS) or dried distillers grains with solubles (DDGS). The WDGS and DDGS may then be used as a component of animal feed and fed to food-producing animals in accordance with normal feeding practice. In addition, the separated corn oil may be used in the production of biodiesel fuel, or added back into certain grades of DG fed to food-producing animals as a source of fat.

The defoamer and its components, including the polyoxyethylene (20) sorbitan monostearate, serve no technical purpose in the animal feed itself. Accordingly, the GRAS substance that is the subject of this notification is only present as a potential impurity animal feed containing DG processed with the defoamer.

The determination of GRAS status is on the basis of scientific procedures, in accordance with 21 CFR § 170.30(b) and conforms to the guidance issued by the Food and Drug Administration (FDA) under *proposed* 21 CFR § 170.36, 62 Fed. Reg. 18938 (Apr. 17, 1997) and FDA's Notice of Pilot Program: Substances Generally Recognized as Safe Added to Food for Animals, 75 Fed. Reg. 31806 (June 4, 2010).

We submit information in the following areas:

- identity of the notified substance;
- intended conditions of use and technical effect;
- manufacturing specifications and stability certification;
- description of the ethanol production process and DDGS and WDGS manufacture method of the notified substance;
- toxicology summary;
- dietary exposure assessment for the food-producing target animal species;
- dietary exposure assessment for humans;
- estimation of daily intake for the notified substance; and
- GRAS determination for the notified substance, as a proposed conclusion determined by scientific procedures for use as a component of a processing aid (defoamer) in the production of DDGS and WDGS used in animal feed for food-producing target animals.

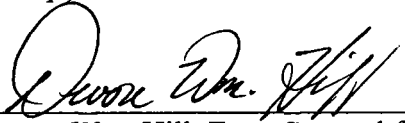
It is the Notifier's expectation that FDA will concur that the information presented fully supports the determination that the Notifier's polyoxyethylene (20) sorbitan monostearate is GRAS when present as an impurity in animal feed as a result of its use as a component of a processing aid (*i.e.*, the Notifier's defoamer product) in the production of WDGS and DDGS. This notification does not attempt to assess use in conjunction with DG as a component of food administered to companion or non-food producing animals.

II. Administrative Information

A. Claim Regarding GRAS Status

Polyoxyethylene (20) sorbitan monostearate is GRAS based on scientific procedures, when present as an impurity, at levels up to 20 ppm, in animal feed for the food-producing target animal species as a result of its use as an emulsifier in the production of wet and dried distillers grain with added solubles (WDGS and DDGS, respectively). The WDGS and DDGS may be used as components of animal feed for the food-producing target animals in accordance with normal feeding practice. Polyoxyethylene (20) sorbitan monostearate serves no technical purpose in the animal feed itself. Accordingly, the GRAS substance that is the subject of this notification is only present as a potential impurity in the WDGS and DDGS due to its use in the processing of the CDS.

The use of polyoxyethylene (20) sorbitan monostearate in this manner as a component of the Notifier's FoamBlast® FMT defoamer has been determined to be exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 301 *et. seq.*).



Devon Wm. Hill, Esq., Counsel for the Notifier

4-8-11
Date

B. Name and Address of the Notifier

Notifier	Acknowledgement of Receipt of Notification and Inquiries to be directed to:
Mr. Barry Ferguson Sales/Export Manager Emerald Carolina Chemicals, LLC 8309 Wilkinson Boulevard Charlotte, NC 28214-9052 barry.ferguson@emeraldmaterials.com Office: 704-391-6419 Cell: 336-250-8533	Keller and Heckman LLP 1001 G Street N.W. Suite 500 West Washington, DC 20001 Mr. Devon Wm. Hill hill@khlaw.com Office: 202-434-4279 Fax: 202-434-4646

A letter authorizing Keller and Heckman to serve as agent for the Notifier is provided as **Appendix 1**.

C. Basis for GRAS Determination

This GRAS determination is based upon the publicly available scientific literature pertaining to the safety of the substance, and a dietary exposure assessment, as demonstrated herein. Additionally, as described in more detail below, polyoxyethylene (20) sorbitan monostearate (and similar substances) is permitted for direct use in food for humans or animal feed.

D. Availability of Information

Much of the data and information that are the basis for the GRAS determination are enclosed with the notification. The Notifier also will retain copies of all of the data and information that form the basis for the GRAS determination, which are available for FDA's review at reasonable times, and copies will be sent to FDA upon request. Requests for copies and arrangements for review of materials cited herein may be directed to:

Keller and Heckman LLP
1001 G Street, N.W.
Suite 500 West
Washington, DC 20001
ATTN: Devon Wm. Hill, Esq.
hill@khlaw.com
202-434-4279 (tel.)
202-434-4646 (fax)

III. Detailed Information about the Identity of the Notified Substance

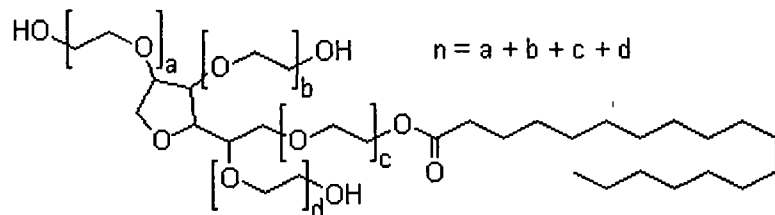
A. Names and Other Identities of the Notified Substance

Chemical Name: Polyoxyethylene (20) sorbitan monostearate

CAS Reg. No.: 9005-67-8

Formula: C₆₄ H₁₂₆ O₂₆

Structural Formula for polyoxyethylene (20) sorbitan monostearate:



where $n = 20$.

B. Common or Usual Name of the Notified Substance

- Polyoxyethylene (POE) 20 sorbitan monostearate
- Synonym: Polysorbate 60

C. Intended Conditions of Use and Technical Effect of the Notified Substance

Polyoxyethylene (20) sorbitan monostearate will be used as a component (emulsifier constituent) of a processing aid (the Notifier's defoamer product) used in the production of WDGS and DDGS, respectively. As noted above, the defoamer is added to the CDS at levels up to 100 ppm; the polyoxyethylene (20) sorbitan monostearate comprises 20% of the defoamer and thus is used at level of 20 ppm in the CDS. With respect to the intended technical effect, the defoamer is used as a chemical processing aid to assist in separating the corn oil from the CDS to produce "de-oiled" CDS¹, which is then added to the DDG and WDG to produce WDGS and DDGS, respectively. The WDGS and DDGS may then be used as components of animal feed for the food-producing target animals in accordance with normal feeding practice. The defoamer and its components, including the polyoxyethylene (20) sorbitan monostearate, serve no technical purpose in the animal feed itself. Accordingly, the GRAS substance that is the subject of this notification is only present as a potential impurity in the WDGS and DDGS due to its use in the processing of the CDS.

¹ The CDS is put through a mechanical centrifuge to separate out the corn oil.

D. Manufacturing Specifications for the Notified Substance

(b) (4)



The Certificates of Analysis for each of the 4 lots are provided in **Appendix 2** and a Food-Grade Assurance Letter from the Notifier's supplier is provided in **Appendix 3**.

E. Stability Certification for the Notified Substance

The polyoxyethylene (20) sorbitan monostearate used by the Notifier has been certified by the manufacturer as being stable for one year in an unopened drum and stored inside under normal conditions. See **Appendix 4** for the Certification letter provided by the Notifier's supplier.

F. Manufacturing Method of the Notified Substance

(b) (4)



G. Detailed Description of Ethanol Distillation Process

Ethanol is distilled during the production of non-food grade and food grade ethanol. After distillation is performed to remove the ethanol from the fermentation mash, the remaining distillation residue, known as stillage (or whole stillage), is pumped from the bottom of the distilling column into centrifuges that separate the wet DG without solubles (WDG) from the stillage. The 'thin' stillage that remains after the WDG is removed from the whole stillage is a liquid that contains approximately 5-10% solids. The thin stillage is then routed to the fermentation tanks as make-up water, or sent to an evaporation system, which concentrates the thin stillage into CDS (which contains up to 40% solids. CDS, or concentrated thin stillage (which is also known as corn syrup), is high in protein and fat, and contains corn oil as well as fermentation byproducts, spent yeast cells, and other nutrients.

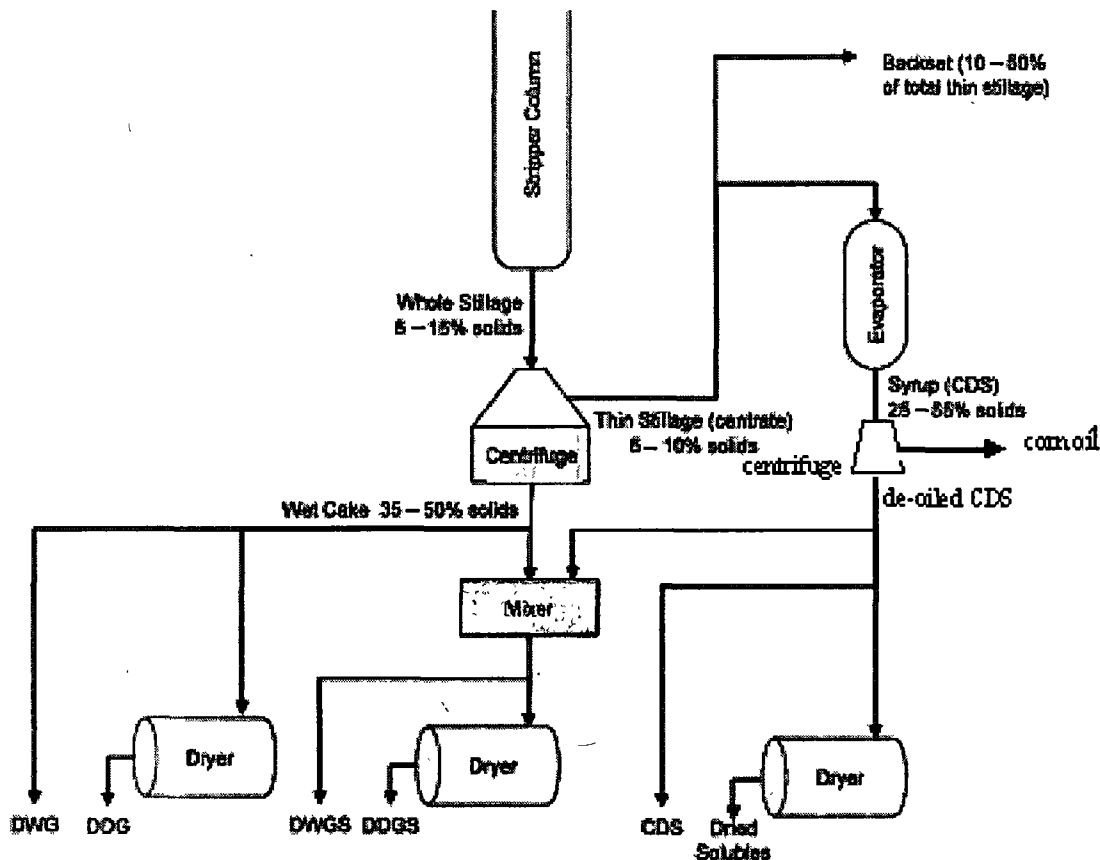
The Notifier's FoamBlast® FMT defoamer is then added at levels up to 100 ppm to the CDS to assist in separating out the corn oil from the corn syrup. Polyoxyethylene (20) sorbitan monostearate comprises 20% of the defoamer and thus is used at level of 20 ppm in the CDS. After the defoamer is added, the CDS enters a mechanical centrifuge that separates out the corn oil. The polyoxyethylene (20) sorbitan monostearate is a component in a defoamer used as a chemical additive in the separation of corn oil from the CDS. Once the corn oil has been separated from the CDS and recovered, the resulting solubles-rich "de-oiled" CDS is then mixed back in with the wet DG (without solubles) and/or dried DG (without solubles), creating DDGS and WDGS, respectively. The separated corn oil may be used in the production of biodiesel fuel, sold into the industrial or specialty chemicals market, or added back into certain grades of DG and fed to food-producing animals as a source of fat.

The DDGS and WDGS, which include the reintroduced solubles from the CDS syrup, may be used as a component of feed for food-producing animals in accordance with normal feeding practice.

This GRAS notification is for DG collectively, including at least four non-fermentable residue byproducts of ethanol fermentation including wet distillers grains without solubles (WDG), dried-distillers grains without solubles (DDG), CDS, WDGS and DDGS. (We include WDG and DDG in this notification although they do not *per se* include any de-oiled CDS because they may include re-added corn oil; our calculations will provide for dietary exposure from any polyoxyethylene (20) sorbitan monostearate that may be present in the corn oil.) For this purpose, data is provided on DDGS to represent the “worst-case” for potential residues.² The reintroduction of the solubles into the grains (by adding the “de-oiled” CDS to the DDG or WDG) will bring any residual polyoxyethylene (20) sorbitan monostearate that may be in the solubles into the DDGS or WDGS, while subsequent drying of the grains will concentrate any residual polyoxyethylene (20) sorbitan monostearate in the DDGS or WDGS. Therefore, we consider as the “worst-case” that the residual polyoxyethylene (20) sorbitan monostearate will be highest in DDGS. See **Figure 1** below.

² Although CDS can be sold separately as a feed supplement when it is used to control dust and condition dry feed ratios, because de-oiled CDS has a much lower fat content and thus cannot provide a sizeable boost in energy level to animal feed, we expect that all de-oiled CDS will be added back to the distillers grains to produce wet and dry distillers grains with solubles. Therefore, the use of DDGS will provide the maximum dietary exposure to the defoamer components.

Figure 1: Ethanol production process.³



H. Calculated Residual Levels in Distillers Grains

As discussed above, to assist in separating the corn oil from the CDS grains, the defoamer is added to the CDS at levels up to 100 ppm; the polyoxyethylene (20) sorbitan monostearate comprises 20% of the defoamer and thus is used at level of 20 ppm in the CDS. To determine the “worst-case” residual level of the polyoxyethylene (20) sorbitan monostearate present in the DDGS and WDGS, we conservatively assume that all of the Notifier’s defoamer product added to the CDS will remain in the de-oiled CDS (and thus, all of the polyoxyethylene

³ Ethanol Coproducts for Ruminant Livestock Diets. Kenneth Kalscheur and Alvaro Garcia, Dairy Science Department, SDSU, Kurt Rosentrater, USDA - Agriculture Research Service and Cody Wright, Department of Animal and Range Science, SDSU. August 2008. See <http://www.thebeefsite.com/articles/1632/ethanol-coproducts-for-ruminant-livestock-diets>.

(20) sorbitan monostearate present in the defoamer remains in the corn oil-free CDS). This is a conservative assumption because polyoxyethylene (20) sorbitan monostearate is both hydrophobic and hydrophilic in nature, and thus has no particular affinity for either the corn oil or the de-oiled CDS.⁴ Because CDS has a maximum fat content of 10%⁵, the maximum worst-case residual level of polyoxyethylene (20) sorbitan monostearate in the de-oiled CDS is 22.2 ppm ($20 \text{ ppm} \div 0.9 = 22.2 \text{ ppm}$).

IV. Detailed Summary of the Basis for Notifier's GRAS Determination

a. Safety Evaluations and Toxicology Summary

Polyoxyethylene (20) sorbitan monostearate (*i.e.*, Polysorbate 60) is one of a class of polysorbates which are substances prepared by the reaction of sorbitan fatty acid esters with ethylene oxide. Other polysorbates include Polysorbate 20, CAS Reg. No. 9005-64-5 ("Polyoxyethylene (20) sorbitan monolaurate"), Polysorbate 40 (CAS Reg. No. 9005-66-7) ("Polyoxyethylene (20) sorbitan monopalmitate"), ("Polyoxyethylene (20) sorbitan monostearate"), Polysorbate 65 (CAS Reg. No. 9005-71-4) ("Polyoxyethylene (20) sorbitan tristearate"), and Polysorbate 80 (CAS Reg. No. 9005-65-6) ("Polyoxyethylene (20) sorbitan monooleate").

⁴ This conservative assumption also ensures that all potential sources of dietary exposure to the Polyoxyethylene (20) sorbitan monostearate are covered because, as noted above, the corn oil recovered from the CDS may, in some cases, be used as a component of animal feed (fat source) for food-producing animals.

⁵ CDS typically has a dry matter content of 25-30%, and a fat content (on a dry matter basis) of 20% (Using Distillers Grains in the U.S. and International Livestock and Poultry Industries, B.A. Babcock, D.J. Haynes, and J.D. Lawrence eds, The Midwest Agribusiness Trade Research and Information Center, 2008, *see* http://www.card.iastate.edu/books/distillers_grains). In some cases, CDS can have dry matter content as high as 45% (*see* <http://beef.osu.edu/bee/beefAgst29.html>); in that situation, the fat content can be as high as $20\% \times 45\% = 9\%$ in the CDS. We therefore conservatively assume that the entire 10% fat content in the CDS is attributable to the corn oil.

Polyoxyethylene (20) sorbitan monostearate is listed in the Food Chemicals Codex, 5th Ed. (2004), p. 347 (“Polysorbate 60”) and is permitted for direct addition to food for human consumption in 21 C.F.R. §§ 172.515 (“Synthetic flavoring substances and adjuvants”), 172.836 (“Polysorbate 60”), 172.838 (“Polysorbate 65”), 172.840 (“Polysorbate 80”) as an emulsifier for cakes and cake mixes, whipped edible oil toppings, vegetable fat-water emulsions intended for use as substitutes for milk or cream in coffee beverages, an emulsifier in cake icings and cake fillings, and use in confectionary coatings and cocoa products. Furthermore, polyoxyethylene (20) sorbitan monostearate is listed as a food additive permitted in feed and drinking water of animals in Section 573.960 (“Sorbitan monostearate”) in combination with sorbitan monostearate as an emulsifier in mineral premixes and dietary supplements for animal feeds, and as a diluent that may be safely used in drug color additive mixtures exempt from certification in Section 73.1001 (“Diluents in color additive mixtures for drug use exempt from certification”). Based on the above clearances, we can conclude in parallel that the use of this substance in the Notifier’s defoamer product is GRAS.

As part of an assessment for this GRAS notification, we have evaluated the toxicology associated with polyethoxylated (20) sorbitan monostearate. In our review of the public toxicology literature, we note that toxicity studies on polyethoxylated sorbitan monooleate (Polysorbate 80) (CASRN 9005-65-6) have also been used to support the toxicity profile for polyethoxylated (20) sorbitan monostearate. Both compounds are polyethoxylated sorbitan fatty esters and contain a C-18 fatty carbon chain length. Both polyethoxylated (20) sorbitan monostearate, which is also known as Polysorbate 60, and Polysorbate 80 contain 20 ethylene oxide units. The only difference between the two polysorbates is that the oleate analog contains a site of unsaturation within the carbon chain, which is absent in the stearate compound. As such, toxicity studies performed on polyethoxylated sorbitan monooleate can be applied to polyethoxylated (20) sorbitan monostearate. A range of limited genotoxicity studies has generated no convincing evidence of activity in polyethoxylated (20) sorbitan monostearate. Because inadequate information regarding carcinogenicity or ADME studies is available on polyethoxylated (20) sorbitan monostearate, similar studies conducted on Polysorbate 80 are applied to polyethoxylated (20) sorbitan monostearate. We also reviewed evaluations of the toxicological database for the individual and collective polysorbates that were conducted by the

Joint FAO/WHO Expert Committee on Food Additives (JECFA),⁶ the European Food Safety Authority (EFSA),⁷ the Cosmetic Ingredients Review (CIR) Expert Panel,⁸ and the Japan Food Safety Commission.⁹

1. Absorption, Distribution, Metabolism and Excretion

The metabolism of polysorbates in rats has been studied in detail with¹⁴C-label tracer techniques. When administered orally, the ester link of the polysorbate molecule is hydrolyzed by pancreatic lipase, and the fatty acid moiety is released, to be absorbed and metabolized as any other dietary fatty acid. The efficiencies with which rats hydrolyzed and absorbed the labeled fatty acid portions of Polysorbates 60 and 80 when fed at a dietary level of 10 percent, were 98 percent and 100 percent, respectively.¹⁰ The polyoxyethylene sorbitan moiety left after hydrolysis of the ester is poorly absorbed from the rat's gastrointestinal tract. When the sorbitol moiety of Polysorbate 80 was labeled, 91 percent of the radioactivity was recovered in the feces, 2.1 percent in the urine, 1.6 percent in the carcass, and none in expired CO₂, liver, kidney,

⁶ JECFA, Toxicological Evaluation of Some Food Additives Including Anticaking agents, Antimicrobials, Antioxidants, Emulsifiers and Thickening Agents, WHO Food Additives Series No. 5 (1974).

⁷ The reviews actually were conducted by the Scientific Committee on Food (SCF), EFSA's predecessor. See Evaluation of Polysorbates 20, 40, 60, 65, 80 (paragraph 12), Reports of the Scientific Committee for Food (Fifteenth Series) (1985); Opinion on Polyoxyethylene (20) Sorbitan Mono-Oleate (Polysorbate 80), Reports of the Scientific Committee for Food (Thirty-fourth Series) (September 17, 1993).

⁸ The Cosmetic Ingredient Review (CIR) was established in 1976 by the Cosmetic, Toiletry, and Fragrance Association (CTFA) with the support of the Food and Drug Administration and Consumer Federation of America. To review and assess the safety of cosmetic ingredients openly and without bias, an Expert Panel was established. Results of the CIR Expert Panel's reviews are published in scientific, peer-reviewed literature.

⁹ Evaluation Report of Food Additives: Polysorbates (Polysorbates 20, 60, 65, and 80), Food Safety Commission (June 2007).

¹⁰ Oser BL and Oser M (1957). Nutritional studies on rats on diets containing high levels of partial ester emulsifiers. Clinical and metabolic observations. *J. Nutr.* 61,149-66; A.N. Wick and L. Joseph (1956). The Fate of Ingested Polyoxyethylene (20) Sorbitan Monostearate. *J of Food Science*, 21, 250-253.

spleen, adrenals, brain, gonads, or fat.¹¹ Polysorbate 80 is most likely hydrolyzed by pancreatic lipase, with the liberated oleic acid following the normal metabolic pathways of unsaturated fatty acids. (In the case of polyoxyethylene (20) sorbitan monostearate, stearic acid is expected to be liberated on hydrolysis and metabolized by the normal metabolic pathways for saturated fatty acids.) The source of the polyoxyethylene in the urine was that portion absorbed from the upper intestinal tract following hydrolysis of the ester bonds. Since there was no carryover of the polyoxyethylene sorbitan in the urine during the post-medication control periods, there was no storage of this moiety in the body.¹² The doses tolerated by rodents in these studies show each of the polysorbates, including polyethoxylated (20) sorbitan monostearate, to be relatively harmless by acute oral administration.

2. Acute Studies

a. Oral Studies

An oral LD₅₀ value of 20-38 g/kg of polyethoxylated (20) sorbitan monostearate has been reported in rats.^{13,14, 15,16}

¹¹ Treon JF, Goncwer LE, Nelson MF and Kirschman JC. (1967). In: *Chemistry, Physics, and Application of Surface Active Substances*, Vol. 111, p. 381, New York: Gordon and Breach.

¹² Culver PJ, Wilcox CS, Jones CM, and Rose, RS. (1951). Intermediary metabolism of certain Polyoxyethylene derivatives in man. I. Recovery of the Polyoxyethylene moiety from urine and feces following ingestion of Polyoxyethylene (20) and sorbitan monooleate and of Polyoxyethylene (40) monostearate. *J. Pharmacol. Exp. Ther.* 103, 377-81.

¹³ Atlas Toxicology Lab (ATL) (1970-1971). Series of unpublished studies, 1970-1971. In: Summary of published and unpublished safety data on Tween (Polysorbate) products, submission of unpublished data by CTFA.

¹⁴ Brandner JD. (1974). Unpublished report submitted by ICI America, 1973. In: Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. *WHO Food Additive Series*, No. 5, pp. 254-63.

¹⁵ Krantz JC (1970-1971). Series of unpublished studies, 1943-1947. In: Summary of published and unpublished safety data on Tween (Polysorbate) products, submission of unpublished data by CTFA.

¹⁶ Marszall L (1973). Toxicological aspects of the use of Span and Tween products in pharmaceuticals, cosmetics, and the food industry. *Bromatol. Chern. Toksykol.* 6(2), 187-95.

b. Mutagenesis

Based on the Evaluation Report of Food Additives for polysorbates (20, 60, 65 and 80), polyethoxylated (20) sorbitan monostearate tested negative results in the Ames test.^{17,18} Ames studies using two strains of *Salmonella typhimurium* (TA98 and TA100) were performed in 3 independent assays and the results were negative both with and without S9 mix.^{19,20,21} In addition it also did not induce in vitro transformation of hamster embryo cells.²²

A *Bacillus subtilis* rec-assay was performed as a DNA repair study for polyethoxylated (20) sorbitan monostearate, and while Kada *et al.* found the assay to be positive for reverse mutation, Kawachi *et al.* and Morita *et al.* obtained negative results.^{23,24,25} Several other studies, including clastogenicity studies, were conducted, but results were negative.²⁶

¹⁷ Evaluation Report of Food Additives (Polysorbates 20, 60, 65 and 80). 2007. Japanese Food Safety Commission, 2007. www.fsc.go.jp/english/.../foodadditive/polysorbate_report.pdf

¹⁸ Kada, T, Hirano K and Shirasu Y. 1980. Screening of environmental chemical mutagens by the rec-assay system with *Bacillus Subtilis*. *Chem. Mutag.* 6:149-173.

¹⁹ Kawachi T, Yahagi T, Kada T, Tazima Y, Ishidate M, Sasaki M, Sugiyama T. 1981. Cooperative program on short-term assays for carcinogenicity in Japan. *IARC Sci. Pub.* 27: 323-330.

²⁰ Morita K, Ishigaki I, Abe T. 1981. Mutagenicity of cosmetic-related substances. *J. Soc. Cosmet. Chem. Japan.* 15: 243-253.

²¹ Inoue K, Sunakawa T, Takayama S. 1980. Studies of in vitro cell transformation and mutagenicity by surfactants and other compounds. *Fd. Cosmet. Toxicol.* 18: 289-296.

²² *Id*

²³ Kada T, Hirano K, Shirasu Y. Screening of environmental chemical mutagens by the Rec-assay system with *Bacillus Subtilis*. 149-373, BIBRA

²⁴ Kawachi T, Yahagi T, Kada T, Tazima Y, Ishidate M, Sasaki M, Sugiyama T. 1981. Cooperative program on short-term assays for carcinogenicity in Japan. *IARC Sci. Pub.* (1981) 27: 323-330.

²⁵ Morita K, Ishigaki I, Abe T. Mutagenicity of cosmetic-related substances. *J. Soc. Cosmet. Chem. Japan.* (1981) 15: 243-253.

²⁶ Kawachi T, Yahagi T, Kada T, Tazima Y, Ishidate M, Sasaki M, Sugiyama T. 1981. Cooperative program on short-term assays for carcinogenicity in Japan. *IARC Sci. Pub.* (1981) 27: 323-330.

Mutagenicity tests involving Polysorbate 80 included a reverse mutation assay using *S. typhimurium* strains TA98, TA100, TA1535 and TA1537. All negative results were obtained with or without S9 mix in this test.^{27,28} In a chromosome aberration study using mammalian cells, the results were negative, with or without a metabolic activation system.^{29,30} In addition, two micronucleus assays using rodents were conducted, and the results were negative in both assays.^{31,32} Negative results were also obtained in a dominant lethal study to examine effects on mammalian germ cells.³³ In rec-assays of Polysorbate 80 using *B. subtilis* and *E. coli*, the results were also negative.^{34,35}

Although positive results were reported for polyethoxylated (20) sorbitan monostearate in one of three *Bacillus subtilis* rec-assays, polyethoxylated (20) sorbitan monostearate cannot be categorized as genotoxic in the rec- assay due to the conflicting data obtained by different

²⁷ Kawachi T, Yahagi T, Kada T, Tazima Y, Ishidate M, Sasaki M, Sugiyama T. 1981. Cooperative program on short-term assays for carcinogenicity in Japan. IARC Sci. Pub. 27: 323-330.

²⁸ Morita K, Ishigaki I, Abe T. 1981. Mutagenicity of cosmetic-related substances. J. Soc. Cosmet. Chem. Japan. 15: 243-253.

²⁹ Kawachi T, Yahagi T, Kada T, Tazima Y, Ishidate M, Sasaki M, Sugiyama T. 1981. Cooperative program on short-term assays for carcinogenicity in Japan. IARC Sci. Pub. 27: 323-330.

³⁰ Ishidate M, Odashima S. 1977. Chromosome studies with 134 compounds on Chinese hamster cells in vitro-a screening for chemical carcinogens. Mut. Res. 48: 337-353.

³¹ Jenssen G, Ramel C. 1980. The micronucleus test as part of a short-term mutagenicity test program for the prediction of carcinogenicity evaluated by 143 agents tested. Mut. Res. 75: 191-203

³² Scott K, Topham JC. Assay of 4CMB, 4HMB and BC by the micronucleus studies subcutaneous administration. Mut. Res. (1982) 100: 365-371.

³³ Anderson D, McGregor DB, Purchase IFH, Hodge MCE, Cuthbert JA. 1977. Dominant-lethal test results with known mutagens in two laboratories. Mut. Res. 43: 231-246.

³⁴ Kawachi T, Yahagi T, Kada T, Tazima Y, Ishidate M, Sasaki M, Sugiyama T. 1981. Cooperative program on short-term assays for carcinogenicity in Japan. IARC Sci. Pub. (1981) 27: 323-330.

³⁵ Sugimura T. et al. 1976. Fundamentals in cancer prevention. Ed. Magee PN. et al. University of Tokyo p.191.

investigators. Genotoxicity assessment for polyethoxylated (20) sorbitan monostearate showed clear negative results in Ames test performed in three independent assays. In addition it also did not induce *in vitro* transformation of hamster embryo cells. Therefore, based on the weight of evidence of these results, as well as the negative genotoxicity found in Polysorbate 80, polyethoxylated (20) sorbitan monostearate is not considered a genotoxic compound.

3. Chronic Studies

Numerous long-term feeding studies have been carried out on polysorbates using a variety of animal species. In these studies, animals were fed polysorbates at dietary levels of up to 25 percent, for periods of up to two years and, in some cases, over multiple generations. Most of these studies included detailed clinical, gross pathologic and histopathological observations. After reviewing many of these studies, the FAO/WHO Committee on Food Additives concluded that the polysorbates cause no toxicological effects at a level of five percent in the daily diet of test animals. Indeed, many species tolerated much greater quantities for extended periods of time.³⁶ Oser and Oser (1957) determined the effects of Polysorbate 60 and 80 at dosage levels of five and ten percent in the diet of rats, and were observed for two years and over for four successive generations.³⁷ The rats were evaluated by various criteria, which can be summarized under the headings of growth, feeding efficiency, clinical observations, reproductive efficiency, hematology, urology, and histopathology. The 20 percent dosage level was chosen as one that “was expected to induce an adverse response.” The most notable effect at this level was diarrhea. There were also some effects on post-natal survival, lactation efficiency, breeding activity, growth rate, and longevity. The 10 percent dosage level produced only diarrhea. Diarrhea and reproduction at high dosage levels were alleviated by the addition of fat to the diet. The five percent level was chosen as a “substantial multiple of the maximum conceivable human level,” and no adverse effects were noted at this level. Even at the highest dosage levels, the

³⁶ FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES. (1974). Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. *WHO Food Additives Series*, No. 5, pp. 254-63.

³⁷ Oser BL and Oser M (1957). Nutritional studies on rats on diets containing high levels of partial ester emulsifiers. 111. Clinical and metabolic observations. *J. Nutr.* 61, 149-66.

polysorbates gave no evidence of cumulative toxicity or of a progressively changing, physiologic response through the four consecutive generations. A purified casein diet that contained five percent polyethoxylated (20) sorbitan monostearate caused diarrhea and growth retardation in rats, whereas a soybean meal diet with up to 15 percent polyethoxylated (20) sorbitan monostearate caused neither diarrhea nor any other adverse reactions. More recent studies have confirmed this protective effect against toxicity by certain diets and have attributed it to dietary fiber.^{38,39, 40}

Oral bioassays for carcinogenesis have been conducted on polyethoxylated (20) sorbitan monostearate using dogs, hamsters, and mice in a single study, at a dose level comprising one to ten percent of diet and lasting from four months to one year in duration.⁴¹ These studies showed no evidence for carcinogenicity via oral route. However, when applied topically to the skin, the polyethoxylated (20) sorbitan monostearate produced skin tumors in some studies – mostly benign dermal tumors with a tendency toward regression. After reviewing many of these studies and conducting multiple experiments, Setala (1960) concluded that the polysorbates, including polyethoxylated (20) sorbitan monostearate, are not carcinogenic when applied to the skin.⁴² In addition, a more comprehensive carcinogenicity study was conducted by the National Toxicology Program (NTP) on rats and mice for up to 103 weeks, containing 0, 25,000, or 50,000 ppm Polysorbate 80. There was no evidence of carcinogenic activity in female F344/N rats or in male or female B6C3F1 mice. However, in male rats at the high dose (5%), the

³⁸ Ershoff BH. (1960). Beneficial effects of alfalfa meal and other bulk-containing or bulk-forming materials on the toxicity of nonionic surface-active agents in the rat. *J. Nutr.* 70, 484-90.

³⁹ Ershoff BH. (1976). Synergistic toxicity of food additives in rats fed a diet low in dietary fiber. 1. *Food Sci.* 41(4), 949-51.

⁴⁰ Ershoff BH and Marshall WE (1975). Protective effects of dietary fiber in rats fed toxic doses of sodium cyclamate and Polyoxyethylene sorbitan mono-stearate (Tween 60). *J. Food Sci.* 40(2), 357-61.

⁴¹ Brush MK, Mccoy JR, Rosenthal HL, Stanber, LA and Allison JB (1957). The addition of non-ionic surface-active agents of the polyoxyethylene type to the diet of the hamster, mouse, and dog. *J. Nutr.* 62, 601.

⁴² Setala K. (1960). Progress in carcinogenesis, tumor-enhancing factors. A bio-assay of skin tumor formation. *Progr. Exp. Tumor Res.* 1, 225-78.

incidence of adrenal medullary pheochromocytomas was marginally increased. Inflammation of the forestomach in male and female mice at the highest dose was also observed. The NOEL from this study was 2.5% or 25,000 ppm. NTP concluded that there was only “equivocal evidence” of carcinogenicity in rats. NTP’s definition of “equivocal evidence” states that such studies depict a “marginal increase of neoplasms that may be chemically related”.

It should be noted that no dose related increase in the incidence of adrenal pheochromocytomas was observed in the NTP study. The marginal increase was only observed at a very high dose level of 5%. In addition, a strong association has been observed between the severity of chronic progressive glomerulonephropathy (CPN) and the incidence of adrenal pheochromocytoma in selected studies involving male Fischer 344 rats at the NTP.⁴³ It was concluded that the possible correlation between the severity of CPN and the incidence of pheochromocytoma may influence interpretation of carcinogenic effects in male rats and any observed increase in these tumors may not be relevant to humans if the animals have CPN. Therefore, based on these considerations it cannot be stated that the compound is a carcinogen. It is not clear whether the NTP report signifies a carcinogenic effect for this compound since the compound was not carcinogenic in female F344 rats and male and female B6C3F1 mice and the results of the bioassay were inconclusive in the male F344/N rats. NTP also concluded on the basis of a mutagenic battery that Polysorbate 80 was not mutagenic.⁴⁴

In another study, PEG-20 sorbitan laurate (Polysorbate 20) was used to conduct a reproductive/developmental toxicology study. This study is relevant in the case of polyethoxylated (20) sorbitan monostearate due to structural similarity between the compounds. Polysorbate 20 was given by gavage in doses of 0, 500, or 5000 mg/kg/day to time-mated SD rats on gestational days 6 through 15 with termination on gestation day 20. The maternal

⁴³ Nyska, A., Haseman, J.K., Hailey, J.R., Smetana, S., Maronpot, R.R. 1999. The association between severe nephropathy and pheochromocytoma in the male F344 rat -The National Toxicology Program Experience. *Toxicol. Path.* 27(4):456-462.

⁴⁴ NTP (1992) Technical Report Series No. 415. Toxicology and carcinogenesis studies of Polysorbate 80 in F344/N rats and B6C3F₁ mice.

LOAEL was 5000 mg/kg/day which was based upon a 14% decrease in weight gain during treatment and the maternal NOAEL was 500 mg/kg/day. No adverse effects upon prenatal development were noted, therefore, the developmental NOAEL was >5000 mg/kg/day. In addition, there was no harmful effects on the prenatal development in a teratology study of polyethoxylated (20) sorbitan monostearate which was fed to pregnant rats at 99 mg/kg/day (0.1%) from gestational day 7 to 14.⁴⁵

4. Organizational Reviews and Establishment of ADI for Polysorbates Based on Available Data

In 1973, JECFA evaluated the effects of Polysorbates 20, 40, 60, 65, and 80 when used as food additives. Based on their findings, JECFA determined that the no adverse effect level (NOAEL) for the class was equivalent to an intake level of 2,500 mg/kg body weight/day, and assigned an acceptable daily intake (ADI) for the entire class of polysorbates of 0-25 mg/kg bw/day, after application of a 100-fold safety factor to the NOAEL.⁴⁶ (This is also the safety factor FDA traditionally applies to the NOAEL established in a 2-year study.)

In 1978, the European Union's Scientific Committee on Food evaluated the safety of this class of polysorbates, mainly based on the chronic toxicity study of polyethoxylated (20) sorbitan monostearate.⁴⁷ Following its assessment, the SCF established an interim ADI of 0-25 mg/kg bw/day for the group of polysorbates, but requested data from a 90-day oral feeding study and a metabolism study in one animal species, which SCF deemed necessary for a final evaluation and issuance of a permanent ADI.⁴⁸ In 1983, SCF conducted a reevaluation based on a 13-week oral

⁴⁵ EPA. 2005. Action Memorandum: Inert Reassessment –Members of the Sorbitan Fatty Acid Esters and the Polysorbates.

⁴⁶ See 17th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (1973).

⁴⁷ Wick, A.N., Joseph L., The Fate of Ingested Polyoxyethylene (20) Sorbitan Monostearate in Rats, *Food Res.* 21: 250-53 (1956).

⁴⁸ See Evaluation of Polysorbates 20, 40, 60, 65, 80 Reports of the Scientific Committee for Food (Fifteenth Series).

feeding study in rats that had then been submitted in response to the 1978 request. In this further study, polyethoxylated (20) sorbitan monostearate was fed to rats at levels of 1%, 2%, and 5% of the diet (equivalent to 500, 1,000, and 2,500 mg/kg bw/day, respectively).⁴⁹ With regard to polysorbate metabolism, the SCF accepted a review of the existing data in lieu of the metabolic study requested in 1978.⁵⁰ The review submitted to the SCF indicated that the existing data demonstrated intestinal hydrolysis of the ester group, followed by metabolism of the fatty acid thus released by the normal pathways. Following reevaluation, SCF established a permanent group ADI for the polysorbates of 0-10 mg/kg bw/day, after application of a 100-fold safety factor to the lowest NOAEL of 2% in the diet reported in the 90-day study.⁵¹ In 1992, SCF again reevaluated the ADI, based on the NTP bioassay on Polysorbate 80.⁵² As noted above, upon considering the relevance of the bioassay results to the safety of polysorbates in the diet, the SCF reaffirmed the ADI of 0-10 mg/kg bw/day.⁵³

Turning to FDA, the Agency has established a group ADI for the cleared Polysorbates of 1,500 mg/person/day (0-25 mg/kg bw/day).⁵⁴ FDA used the NOAEL for diarrhea in a repeated-dose toxicity study as a basis for setting the ADI. FDA also emphasized that hamsters apparently are more sensitive than rats or dogs (by comparison, hamsters developed marked

⁴⁹ See BIBRA (1981), A Short-term (13 week) Study in Rats with Polyoxyethylene (20) Sorbitan Monostearate. These data are unpublished, so we have not independently reviewed the BIBRA Report.

⁵⁰ See Evaluation of Polysorbates 20, 40, 60, 65, 80, Paragraph 12, Reports of the Scientific Committee for Food (Fifteenth Series).

⁵¹ *Id.* See BIBRA (1983), A Review of the Status of the Polysorbates Prepared for the Ad Hoc Polysorbate Group, April 1983. These data are unpublished, so we have not independently reviewed the BIBRA Report.

⁵² See Opinion on Polyoxyethylene (20) Sorbitan Mono-Oleate (Polysorbate 80), Reports of the Scientific Committee for Food (Thirty-fourth Series) (September 17, 1993).

⁵³ *Id.*

⁵⁴ See "Food Additives Permitted for Direct Addition to Food for Human Consumption; Polysorbate 60," 64 Fed. Reg. 57974, 57975 (October 28, 1999).

diarrhea at a 5% dose level in the feed, while the same dose level produced no such similar effects in rats or dogs).⁵⁵

Based on explicit statements from EFSA, JECFA, and the Japan Food Safety Commission as described above, we can reasonably conclude that there is no basis to view the toxicity of polyoxyethylene (20) sorbitan monostearate any differently from other polysorbates in the class.⁵⁶ Thus, the current ADI of 10 mg/kg bw/day may be applied broadly to all polysorbates, including polyoxyethylene (20) sorbitan monostearate. Furthermore, because EFSA's reevaluation of polysorbates following its review of the NTP carcinogenicity study on Polysorbate 80 did not result in a lowering of the ADI for the class of polysorbates from 10 mg/kg bw/day, we can conclude that the NTP carcinogenicity study conducted on Polysorbate 80 in rats and mice is not applicable to exposure in mammals to Polysorbate 80 (or other polysorbates) through their diets. Also, the fact that as recently as 1999, which post-dates the NTP carcinogenicity study, FDA cleared a new food additive use for polyethoxylated (20) sorbitan monostearate in the face of the equivocal carcinogenicity results in male rats on Polysorbate 80 suggests that FDA, likewise, does not consider the results of that study to be relevant to mammalian intake of polysorbates.⁵⁷ In light of the foregoing, the use of an ADI of 10 mg/kg bw-animal/day for polyoxyethylene (20) sorbitan monostearate is appropriate in this analysis.

⁵⁵ January 28, 1960 Memorandum From Division of Pharmacology to Mr. Alan T. Spiher (cited in Japan Food Safety Commission Report on Polysorbates, Footnote 84). Note that while the date of the FDA review memorandum (1960) is from many years ago, FDA reaffirmed the ADI as recently as 1999, in the Federal Register notice cited in footnote 49 above.

⁵⁶ FDA also states that the current ADI of 1,500 mg/p/day applies to "all regulated polysorbates." See 64 Fed. Reg. at 57975.

⁵⁷ In 1999, FDA cleared for the use of Polysorbate 60, alone or in combination with Polysorbate 65 and/or Polysorbate 80 as an emulsifier in ice cream, frozen custard, fruit sherbet, and nonstandardized frozen desserts, provided the maximum amount of the additive alone or in combination does not exceed 0.1% of the finished dessert. See 64 Fed. Reg. 57974-76 (October 28, 1999).

5. Human Ingestion Studies

a. Acute Oral Toxicity

Polyethoxylated (20) sorbitan monostearate has been given a toxicity rating of practically nontoxic, with a probable oral lethal dose in humans greater than 15 g/kg.⁵⁸ Chusid and Diamond (1955) reported an accidental overdose of Polysorbate 80 administered to a four month old male infant weighing less than eight pounds. In that case, 19.2 g of Polysorbate 80 was ingested daily for two consecutive days with no other food. The infant passed six loose stools but showed no other evidence of intoxication.⁵⁹

In an attempt to determine the effect of large doses of polyethoxylated (20) sorbitan monostearate on the alimentary tract of man, Steigmann et al. (1953) fed a single 20 g-dose to each of 11 subjects of both sexes and various ages. There were no significant changes in gastric motility or gastric acidity, and no subjective reports of adverse symptoms.⁶⁰

b. Long Term Feeding

Waldstein et al. (1954) evaluated the pharmacologic effect of polyethoxylated (20) sorbitan monostearate administered by mouth in patients and normal subjects. A group of 34 elderly patients in chronic disease infirmary, and a group of 10 normal hospital personnel were fed 6 g of polyethoxylated (20) sorbitan monostearate daily for 28 days. Clinical and laboratory tests produced no evidence of adverse effects in either group.⁶¹

⁵⁸ Cosselin RE, Hodge HC, Smith RP and Gleason MN (1976). *Clinical Toxicology of Commercial Products: Acute Poisoning*, 4th ed. Baltimore, MD: Williams & Wilkins.

⁵⁹ Chusid E and Diamond J (1955). Accidental massive overdose of monitan in an infant. *J. Pediat.* 46, 222.

⁶⁰ Steigmann F, Goldberg EM, and Schoolman HM (1953). The effect of emulsifying agents (Tween 60 and Span 60) on the gastrointestinal tract. *Am. J. Dig. Dis.* 20, 380-4.

⁶¹ Waldstein SS, Schoolman HM, and Popper H (1954). The effect of feeding large amounts of emulsifiers polyoxyethylene (20) sorbitan monostearate (Tween 60) and sorbitan monostearate (Span 60) to humans. *Am. J. Dig. Dis.* 21, 181-5.

Steigmann et al. (1953) fed 6 g of polyethoxylated (20) sorbitan monostearate per day for 28 days to each of 10 subjects. No significant effects were found on the physiologic activity of the gastrointestinal tract in any of the subjects.⁶²

Preston et al. (1953) fed daily 1 g doses of polyethoxylated (20) sorbitan monostearate to three normal children; one child received treatment for 13 days, another for 31 days, and the third for 34 days. No harmful effects were observed in any of the patients as reflected by careful clinical examinations, including tests for duodenal enzymes, fecal fat, and nitrogen.⁶³

Page (1949) studied 20 normal adults who were fed 4 g/day for 28 days of an emulsifier mixture containing 20 percent polyethoxylated (20) sorbitan monostearate as a supplement to their regular diet. An additional 20 subjects were each fed 8 g/day for 28 days of a mixture consisting of 80 percent Polysorbate 61 and 20 percent polyethoxylated (20) sorbitan monostearate (Polysorbate 60). A third group of 20 subjects was fed 4 g/day for 28 days of an emulsifier mixture containing 6 percent polyethoxylated (20) sorbitan monostearate. The test doses were administered in three equal portions daily in conjunction with chocolate syrup. No significant variations were observed in any of the subjects as evidenced by physical examination, hematology, and urinalysis.⁶⁴

Jeans and Stearns (1970-1971) studied the effects of adding emulsifier mixtures containing Polysorbates 60 and 80 to the daily diets of nine infants ranging in ages from one week to seven months. Daily administration of approximately 0.2 g polyethoxylated (20) sorbitan monostearate with 0.04 g Polysorbate 80 was continued for periods of 1.5 to five months, with three of the infants receiving approximately 0.4 g polyethoxylated (20) sorbitan

⁶² Steigmann F, Goldberg EM, and Schoolman HM (1953). The effect of emulsifying agents (Tween 60 and Span 60) on the gastrointestinal tract. *Am. J. Dig. Dis.* 20, 380-4.

⁶³ Preston E, Hunt AD, Scott TF and Spur B. (1953). Short-term feeding studies in infants and children with certain surface-active agents used in food technology: Absence of evidence of toxic effects. *J. Clin. Nutr.* 1(7), 539-50.

⁶⁴ Page SC. Jr. (1949). Experimental safety of sorbitan monostearate and its Polyoxyethylene derivatives. *Fed. Proc.* 8, 323.

monostearate with 0.04 g Polysorbate 80 per day for an additional one to two months. Careful observation of the patients, including comparative growth curves and nutritional balance studies, indicated no adverse effects as a result of feeding the emulsifiers.⁶⁵

6. Summary of Toxicological Effects

The polysorbates are a series of polyoxyethylene sorbitan esters that differ with respect to the number of polymerized ethylene oxide subunits and the number and type of fatty acid moieties present. They are used as general purpose, hydrophilic, nonionic surfactants in a variety of cosmetic products. Some of the polysorbates are also approved by FDA for use in various pharmaceuticals and food products.^{66,67,68}

Studies employing radioactive tracer techniques show that the polysorbates are hydrolyzed by pancreatic and blood lipases; the fatty acid moiety is released to be absorbed and metabolized, whereas the polyoxyethylene sorbitan moiety is very poorly absorbed and is excreted unchanged. Most or all of these effects can most likely be related to the surface active properties of the intact polysorbate molecule.

⁶⁵ Jeans PC and Stearns G (1970-1971). Infant feeding of Span 60 and Tween 60, unpublished report, 1951. In: Summary of published and unpublished safety data on Tween (Polysorbate) products, submission of unpublished data by CTFA.

⁶⁶ Polysorbates 60 (polyethoxylated (20) sorbitan monostearate) and 80 are approved for direct use in all food types as synthetic flavorings (21 CFR 172.515)

⁶⁷ Polysorbates 60 (polyethoxylated (20) sorbitan monostearate), 65, and 80 are approved for direct use in a wide variety of specified food types as emulsifiers; solubilizers, dispersing agents, surfactants, wetting agents, opacifiers, defoaming agents, dough conditioners, and/or adjuvants. Usage limits range from 10 ppm to 4.5 percent of the finished product; limits for vitamin mineral preparations range from 175 to 475 mg/day, based on the recommended daily dose (21 CFR 172.836, 172.383, 172.840 and as amended 9/5/80).

⁶⁸ Polysorbates 60 (polyethoxylated (20) sorbitan monostearate) and 80 are approved for indirect addition to all food types as components of adhesives (21 CFR 175.105). Polysorbates 60 and 80 are approved for indirect addition to all food types as emulsifiers and/or surfactants (21 CFR 178.340). The FDA has also approved Polysorbates 60 and 80 for various uses in animal feeds (21 CFR 573.840-.860).

The above toxicology studies have demonstrated that polyoxyethylene sorbitan fatty acids in general are hydrolyzed to their respective fatty acids and polyoxyethylene sorbitan moieties in the gastrointestinal tract. The resultant fatty acids are common components of a wide variety of foods, are readily absorbed, and are primary components of lipid metabolism. Polyoxyethylene sorbitans remaining after hydrolysis are poorly absorbed from the gastrointestinal tract, as well as any unhydrolyzed polyoxyethylene sorbitan fatty acids, and will be excreted in the feces.

In conclusion, polysorbates are not known to be mutagenic, and the weight of evidence suggests that the substances are not carcinogenic. Furthermore, as discussed above, the studies described herein support an ADI of 10 mg/kg bw/day for polyoxyethylene (20) sorbitan monostearate, which we believe to be extremely conservative. Both FDA and JECFA have affirmed an ADI of 25 mg/kg bw/day for polysorbates such as Polysorbate 60; thus, we believe a higher ADI would be supportable. Nonetheless, as a conservatism, we have chosen a target ADI of 10 mg/kg bw/day. With regards to establishing an ADI for polysorbates, the most sensitive toxicological endpoint based on oral feeding studies is diarrhea. Collectively, the data demonstrate the onset of diarrhea to occur primarily at polysorbate levels of 10% of the diet or greater. Further, as described previously, diarrhea was only seen at levels of 5% of the diet for hamsters, which FDA concluded were much more sensitive than rats or dogs. In this instance, the 5% level corresponds to a NOEL of 2500 mg/kg bw/day, and an ADI of 25 mg/kg bw/day after application of a 100-fold safety factor. An ADI of 10 mg/kg bw/day corresponds to a 2% feeding level, which is clearly supported by the data presented above.

IV. Correlation of Data to Target Animal Species

Although the animal species tested were predominantly rodents and dogs, and the target species are livestock animals consisting of both poultry and ruminants, we believe the toxicology data presented above is equally applicable to the target animals. When consumed, the polyoxyethylene (20) sorbitan monostearate will be hydrolyzed (in the digestive tracts of both the animals tested and the target animals) to stearic acid and polyoxyethylene (20) sorbitan. The stearic acid will be readily absorbed and metabolized by both types of animals as a fatty acid. The polyoxyethylene (20) sorbitan will not be absorbed, but will pass through both types of

animals' digestive tracts; any trace amounts of polyoxyethylene (20) sorbitan absorbed through the intestinal tract will be eliminated from the body (in unmetabolized form) in the urine. Any unhydrolyzed polyoxyethylene (20) sorbitan will not be absorbed into the bodies of either type of animal, and will readily pass through their digestive tracts. As polyoxyethylene sorbitan with its ether linkages is not expected to be affected by the action of microorganisms that may be present in ruminal fluids of certain target animals,⁶⁹ we expect that there will be no breakdown of the polyoxyethylene components when consumed by ruminants. Thus, the ADI presented above is equally applicable to all target animals.

V. Dietary Exposure Assessment for Target Animals

As discussed above, the Notifier intends to use the defoamer at a maximum use level of 100 ppm in the CDS; the polyoxyethylene (20) sorbitan monostearate comprises 20% of the defoamer and thus is used at level of 20 ppm in the CDS.⁷⁰ Once the defoamer has been added to the CDS, and the corn-oil separated out, the de-oiled CDS is then added to either dried DG to create DDGS or wet DG to create WDGS; which can then be used as components of animal feed for the food-producing target animals. As indicated above, the worst-case residual level of the polyoxyethylene (20) sorbitan monostearate in the de-oiled CDS is approximately 22.2 ppm, conservatively assuming the entire 10% fat content in CDS is attributable to the removed corn oil. Once de-oiled, CDS syrup is then incorporated into the distillers grains at a level of 25% on a solids weight basis;⁷¹ the resulting solubles-enriched DG (either as WDGS or DDGS) product

⁶⁹ Report of the Scientific Committee for Animal Nutrition on the Use of Polyethylene Glycol 6000 and of a Polyoxypropylene-polyoxyethylene polymer in Feeding Stuffs, January 15, 1980, see http://ec.europa.eu/food/fs/sc/oldcomm6/other/21_en.pdf.

⁷⁰ $(100 \text{ ppm})(0.20) = 20 \text{ ppm}$.

⁷¹ Whole stillage with an 85-90% water content (10%-15% solids) is separated into a wet DG stream with a water content of 65-70% (*i.e.*, 30-35% solids) and a thin stillage stream with a water content of 90-95% (*i.e.*, 5 -10% solids). The thin stillage stream is condensed in an evaporator into CDS with a water content of 60% (*i.e.*, 40% solids). While the water and solids contents noted above vary depending on the production plant and processing techniques, and although a portion of the thin stillage is recycled back to the fermentation vessel, we can use the approximate water and solid contents to conservatively determine the maximum amount of CDS solids that are added to wet or dry DG to make WDGS or DDGS, respectively. In this regard,

Continued on next page

may be added to animal feed as WDGS or DDGS at a maximum level of 30% on a solids basis.⁷² Although the de-oiled CDS can be sold separately as a feed supplement when it is used to control dust and condition dry feed ratios, because of its much lower fat content, the de-oiled CDS cannot provide a sizeable boost in energy level when added directly into animal feed. Accordingly, we expect that all de-oiled CDS will be added back to the DG to produce WDGS and DDGS. Therefore, the use of DDGS will provide the maximum dietary exposure to the defoamer components.

De-oiled CDS typically has a solids content of 40% with a polyoxyethylene (20) sorbitan monostearate concentration of 22.2 ppm. Polyoxyethylene (20) sorbitan monostearate has a concentration of 55.5 ppm based on CDS solids.⁷³

Because the de-oiled CDS is added to the DG at 25% on a solids basis, the maximum potential concentration of polyoxyethylene (20) sorbitan monostearate in animal feed is: $(55.5 \text{ ppm}) (0.25) = 13.9 \text{ ppm}$ on a solids basis.

Distiller's grains are typically fed as a portion of daily feed to target animals such as cattle, dairy cows, sheep, swine, turkeys, and broiler chickens. The recommended daily feed diets for cattle, dairy cows, sheep, turkeys and swine include up to 30% distillers grains on a dry

we note that a whole stillage stream with 1 kg of DG contains approximately 7.3 kg of water. The whole stillage stream is then separated into wet DG with a maximum solids content of 35% (which we assume contains the bulk of the 1kg of DG), and into a thin stillage stream with a solids content of about 5% (consisting of 5.5 kg of water and 0.33 kg of solids). The thin stillage is then condensed to 40% solids, but still contains 0.33 kg of solids which is then added back to the 1 kg of solids in the wet DG prior to drying. Therefore, the "addition rate" of the CDS to DG is $0.33/1.33 \text{ kg}$ or 25% on a solids basis. In an actual process, the ratio of solids in the condensed thin stillage stream is expected to be much less than 25%, so this provides a worst-case addition of CDS containing polyoxyethylene (20) sorbitan monostearate to the DDGS.

⁷² "Using Distillers Grains in the U.S. and International Livestock and Poultry Industries," B.A. Babcock, D.J. Haynes, and J.D. Lawrence eds, The Midwest Agribusiness Trade Research and Information Center, 2008, see http://www.card.iastate.edu/books/distillers_grains.

⁷³ $22.2 \text{ ppm} \div 0.40 = 55.5 \text{ ppm}$.

weight basis. The daily feed intake of broiler chickens may include up to 15% by weight dry distillers grains.⁷⁴

The Distillers Grain Technology Council has stated that DG can be used in daily feed for the food-producing target animals as presented in Table 1 below.⁷⁵ Weights and intakes of feed are nominal, meaning that they are representative of populations of animals generally, and may not be specific to particular categories of food-producing animals raised under specific conditions.⁷⁶ The quantity of food consumed per day per animal may not be representative of food intakes for a specific period of time during growth, but rather reflect an average that approximates intakes over an expected lifetime.

TABLE 1. Feeding Data for Food-Producing Target Animals

Target Animal Species	Weight (kg)	Food Consumed (g/day)	Distillers Grains (dry weight basis) Consumed per Day		
			(%)	(g/day)	g/kg bw/day
Beef Cattle	500	10,000	30%	3,000	6
Dairy Cattle	500	10,000	30%	3,000	6
Poultry ⁷⁷ (broiler)	2.5	232.5	15%	34.9	14
Sheep	60	2,400	30%	720	12
Swine	60	2,400	30%	720	12

⁷⁴ Using Distillers Grains in the U.S. and International Livestock and Poultry Industries, see http://www.matric.iastate.edu/DGbook/distillers_grain_book.pdf.

⁷⁵ Distillers Grains Technology Council, University of Louisville, Lutz Hall Room 435, Louisville, Kentucky 40292: www.distillersgrains.org.

⁷⁶ SAX'S Dangerous Properties of Industrial Materials. Ninth Edition (1996). Table 2. Van Nostrand Reinhold Company. New York.

⁷⁷ The feed consumption for broiler chickens is reported to be 93 mg/kg bw/day – Predicting Feed Intake of Food-Producing Animals, Subcommittee on Feed Intake, Committee on Animal Nutrition, Board on Agriculture, National Research Council, National Academy Press, Washington, D.C., 1987.

The amount of distillers grains consumed on a dry basis for each animal is calculated as follows for cattle:

$$(10,000 \text{ g-food}/500 \text{ kg bw}) \times (0.3 \text{ g-distillers grains/g-food}) \\ = 6 \text{ g-distillers grains/kg bw}$$

The maximum distillers grains consumed by beef cattle, on a dry weight basis, is 6 g/kg bw/day. With a maximum residual level of 13.9 mg/kg of polyoxyethylene (20) sorbitan monostearate in distiller's grains on a dry weight basis, a maximum dietary intake for beef cattle is calculated as follows:

$$6 \text{ g-distillers grain/kg bw} \times (13.9 \text{ mg- POESMS/kg-distillers grains}) \times (\text{kg}/1000 \text{ g}) \\ = 0.08 \text{ mg POESM/kg bw/day}$$

The dietary intake of polyoxyethylene (20) sorbitan monostearate by other food-producing target animals is similarly calculated and presented in the table below:

TABLE 2. EDIs for Target animals

Target Animal Species	EDI (mg/kg-bw/day) for Polyoxyethylene (20) Sorbitan Monostearate
Beef Cattle	0.08
Dairy Cattle	0.08
Poultry (Broiler)	0.2 ⁷⁸
Sheep	0.17 ⁷⁹
Swine	0.17 ⁸⁰

Poultry consume the highest amount of DG per body weight per day among all the food-producing target animals, thus providing a worst-case dietary intake of 0.2 mg/kg bw/day for polyoxyethylene (20) sorbitan monostearate for all food-producing target animals. As shown above, a very conservative ADI of 10 mg/kg-bw/day has been established for polyoxyethylene (20) sorbitan monostearate for the target animals. Accordingly, we conclude that the residual polyoxyethylene (20) sorbitan monostearate that may be present in the animal feed as an impurity, as a result of the use of polyoxyethylene (20) sorbitan monostearate in the Notifier's defoamer product, as described above, is safe for the target animals.

⁷⁸ 14 g-distillers grain/kg bw x (13.9 mg-PEGDO/kg-distillers grains) x (kg/1000 g) = 0.2 mg PEGDO/kg bw/day.

⁷⁹ 12 g-distillers grain/kg bw x (13.9 mg-PEGDO/kg-distillers grains) x (kg/1000 g) = 0.17 mg PEGDO/kg bw/day.

⁸⁰ 12 g-distillers grain/kg bw x (13.9 mg-PEGDO/kg-distillers grains) x (kg/1000 g) = 0.17 mg PEGDO/kg bw/day.

VI. Dietary Exposure Assessment for Humans of Polyoxyethylene (20) Sorbitan Monostearate

Table 3. EDI Summary for Polyoxyethylene (20) sorbitan monostearate

Dietary Exposure	EDI (mg/kg bw/day)
Animal Dietary Exposure to Polyoxyethylene (20) Sorbitan Monostearate	0.2 mg/kg bw/day
Human Dietary Exposure to Polyoxyethylene (20) Sorbitan Monostearate	0.007 mg/kg bw/day

As described above, polysorbates such as polyoxyethylene (20) sorbitan monostearate are readily hydrolyzed to their respective fatty acid, stearic acid, as well as polyoxyethylene sorbitan moieties upon ingestion. Fatty acids are naturally present in the diet from a wide variety of foods, are naturally present in the body, and are readily absorbed and metabolized. The polyoxyethylene sorbitan moieties are not well-absorbed by the body, and, if any are absorbed through the intestinal wall and excreted via urine. Therefore polyoxyethylene (20) sorbitan monostearate does not become a component of edible animal fats, tissues, and organs intended for human consumption. Furthermore, the radiolabeling studies also described above have shown that the polyoxyethylene sorbitan moiety is not retained by the body. Since polysorbates such as polyoxyethylene (20) sorbitan monostearate do not remain polysorbates *per se* upon ingestion by the animal, there is zero potential exposure to such polysorbates in humans, based on the consumption of any edible components of the animal, including tissues, fats, organs, eggs and milk. Moreover, there is no exposure in humans to the polyoxyethylene sorbitan moiety, since it is not retained in the animal. Nevertheless, for the sake of conservatism, we will assume, as worst-case, that at slaughter, polyoxyethylene (20) sorbitan monostearate may be present in the edible portions of the carcass at levels equal to the amount of the compound consumed on

that day.⁸¹ We will also conservatively assume that the compound is equally distributed throughout the carcass and in any milk or eggs that may be produced by the target animals.

To determine the dietary intake of polyoxyethylene (20) sorbitan monostearate by the consumption of edible parts of a species of target animals, FDA assigns consumption values for different edible products of each species, based on the relative amount of each organ or tissue that is consumed by individuals.⁸² Specifically, according to FDA's Guidance for Industry: *General Principles for Evaluating the Safety of Compounds used in Food-Producing Animals*, FDA assumes that these consumption values (*i.e.*, grams consumed per person per day) are applied to all species of the target animals, as it is assumed that when an individual consumes a full portion⁸³ of a meat product from one species, that individual will not also consume a full portion of a meat product from another species. Additionally, FDA assumes that on a daily basis an individual consumes a full portion of milk in addition to a full portion of eggs⁸⁴ in addition to the full portion of edible muscle and organ tissue (from one animal species). These values are used to determine the exposure of polyoxyethylene (20) sorbitan monostearate, based on the level of polyoxyethylene (20) sorbitan monostearate in each edible portion of the target animal. The consumption values and the polyoxyethylene (20) sorbitan monostearate levels are summarized in the table below, based on the assumptions that (1) the maximum daily intake of polyethylene glycol (400) dioleate of 0.2 mg/kg bw/day is evenly distributed throughout the

⁸¹ This is a conservative assumption in that the polyoxyethylene (20) sorbitan monostearate is not readily absorbed through the intestinal tract and any fatty acids and polyoxyethylene components that may be absorbed are readily metabolized or directly excreted, respectively and not stored in animal tissues and organs. As the majority of the polyoxyethylene (20) sorbitan monostearate will pass directly through the digestive system, this clearly provides a worst-case for human dietary exposure.

⁸² As described in *FDA's Guidance for Industry: General Principles for Evaluating the Safety of Compounds used in Food-Producing Animals*; <http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm052180.pdf>.

⁸³ According to FDA's guidance on *General Principles for Evaluating the Safety of Compounds used in Food-Producing Animals*, a full portion of meat consists of 300 g of muscle tissue, 100 g of liver, 50 g of kidney, and 50 g of fat.

⁸⁴ According to FDA, the estimated daily intake is 1.5 liters for milk and 100 grams for eggs.

muscle tissues, organs, milk, and eggs of the food-producing target animals and (2) the polyethylene glycol (400) dioleate is metabolized on a daily basis:

TABLE 4. Consumption Values for Polyoxyethylene (20) sorbitan monostearate

Edible Product	Consumption (g food/day)	Polyoxyethylene (20) Sorbitan Monostearate Level (µg/g tissue)
Muscle	300 g	0.2
Liver	100 g	0.2
Kidney	50 g	0.2
Fat	50 g	0.2
Milk	1.5 L	0.2
Eggs	100 g	0.2

To estimate the dietary exposure of polyoxyethylene (20) sorbitan monostearate, the Notifier considered each edible portion of cattle. In addition, based on FDA’s assumptions discussed above, the Notifier assumed that a full portion of milk and eggs are consumed in addition to a full portion of edible muscle or organ tissues. Based on this, the Notifier calculated the relative level of polyoxyethylene (20) sorbitan monostearate in each edible product to obtain, in essence, a dietary exposure for individual human consumers. The exposures due to milk and eggs, as well as the sum of all the exposure values (to obtain a cumulative dietary exposure level) are calculated as follows:

Muscle:

$$(0.2 \mu\text{g POESM}/1 \text{ g muscle}) \times (300 \text{ g muscle}/\text{person}/\text{day})$$

$$= 60 \mu\text{g POESM}/\text{person}/\text{day}$$

Liver:

$$(0.2 \mu\text{g POESM}/1 \text{ g liver}) \times (100 \text{ g liver}/\text{person}/\text{day})$$

$$= 60 \mu\text{g POESM}/\text{person}/\text{day}$$

Kidney:

$$(0.2 \mu\text{g POESM}/1 \text{ g kidney}) \times (50 \text{ g kidney}/\text{person}/\text{day})$$

$$= 10 \mu\text{g POESM}/\text{person}/\text{day}$$

Fat:

$$(0.2 \mu\text{g POESM}/1 \text{ g fat}) \times (50 \text{ g fat}/\text{person}/\text{day}) \\ = 10 \mu\text{g POESM}/\text{person}/\text{day}$$

The total dietary exposure to polyoxyethylene (20) sorbitan monostearate for a individual consumer not consuming eggs and milk is calculated as follows:

$$60 \mu\text{g POESM}/\text{person}/\text{day} \text{ (muscle)} + 20 \mu\text{g POESM}/\text{person}/\text{day} \text{ (liver)} + 10 \mu\text{g} \\ \text{POESM}/\text{person}/\text{day} \text{ (kidney)} + 10 \mu\text{g POESM}/\text{person}/\text{day} \text{ (fat)} \\ = 100 \mu\text{g POESM}/\text{person}/\text{day}$$

The dietary exposure to polyoxyethylene (20) sorbitan monostearate for a individual consumer who does consume eggs and milk is calculated as follows:

Milk:

$$(0.2 \text{ mg POESM}/1.0 \text{ L milk}) \times (1.5 \text{ L milk}/\text{person}/\text{day}) \\ = 0.3 \text{ mg POESM}/\text{person}/\text{day}$$

Eggs:

$$(0.2 \mu\text{g POESM}/1 \text{ g egg}) \times (100 \text{ g egg}/\text{person}/\text{day}) \\ = 20 \mu\text{g POESM}/\text{person}/\text{day}$$

Thus, the cumulative exposure to polyoxyethylene (20) sorbitan monostearate from the consumption of all animal (cattle) products (*i.e.*, muscle tissue, organ tissue (liver and kidney), and fat), and milk and eggs (poultry) provides us with the estimated daily intake (EDI) for the GRAS substance as follows:

$$0.1 \text{ mg} + 0.3 \text{ mg} + 0.02 \text{ mg} = 0.42 \text{ mg POESM}/\text{person}/\text{day}$$

Assuming an individual consumes 3 kg of food per day, this results a dietary concentration of $0.42 \text{ mg} \div 3 \text{ kg} = 0.14 \text{ ppm}$ per day. The estimated daily intake (EDI) for polyoxyethylene (20) sorbitan monostearate is calculated as follows:

$$\text{EDI (POESM)} = 0.14 \text{ mg}/\text{kg} \times 3 \text{ kg-food}/\text{p}/\text{d} = 0.42 \text{ mg}/\text{p}/\text{d}.$$

Assuming that an average individual weighs 60 kg, the EDI also may be expressed as

$$0.42 \text{ mg}/\text{p}/\text{d} \div 60 \text{ kg bw} = 0.007 \text{ mg}/\text{kg bw}/\text{d}.$$

VII. Conclusion

Based on the dossier of information provided in this GRAS notification, and on the scientific procedures discussed herein, the Notifier has concluded that polyoxyethylene (20) sorbitan monostearate (CAS Reg. No. 9005-67-8), a component of the Notifier's FoamBlast® FMT defoamer, is Generally Recognized as Safe (GRAS) when present as an impurity, at levels up to 20 ppm, in the feed for the food-producing target animals, as a result of the use of the defoamer as a processing aid in the production of dried and wet distillers grains with added solubles. Furthermore, the Notifier has concluded that the publicly available information and relevant data on polysorbates as a class is directly relevant and fully support the Notifier's conclusion.

APPENDIX 1



February 28, 2011

U.S. Food and Drug Administration
Center for Veterinary Medicine
Division of Animal Feeds (HFV-224)
7519 Standish Place
Rockville, Maryland 20855

Re: Authorization to Act as Agent for Carolina Chemical LLC

Dear Sir or Madam:

This is to advise that the law firm of Keller and Heckman LLP, its employees, associates, and agents, specifically including, but not limited to Devon Wm. Hill, are authorized to act as agents on behalf of Carolina Chemical LLC (a subsidiary of Emerald Performance Materials, LLC) with regard to its Generally Recognized as Safe (GRAS) Notification for Polyoxyethylene (20) Sorbitan Monostearate (CAS Reg. No. 9005-67-8), submitted to the U.S. Food and Drug Administration, Center for Veterinary Medicine.

This letter is our authorization to you to permit said firm to undertake appropriate communications relevant to making submissions or inquiring as to the status of the above referenced GRAS Notification filed by or on behalf of Carolina Chemical LLC, including examination of all relevant information including confidential business, proprietary, and trade secret information submitted or developed under the Federal Food, Drug and Cosmetic Act.

Sincerely,

A handwritten signature in cursive script that reads "Barry Ferguson".

Barry Ferguson
Sales/Export Manager

Emerald Carolina Chemical, LLC

8309 Wilkinson Blvd. / Charlotte, NC, 28214 / Phone. 704-393-0089 / Fax: 704-391-7340

www.emeraldmaterials.com

APPENDIX 2

(b) (4)

Certificate of Analysis

(b) (4)

Customer:
Emerald Carolina
8309 WILKINSON BLVD.
CHARLOTTE, NC 28214

POEGASORB 60		Number of Containers	3
Lot Number:	(b) (4)	Pounds Per Container:	470
Test Number	Test Name	Limits	Result
102334	Expiration Date (Retest after)	24 Months	August, 2012
102333	Mfg Date	Report	August, 2010
103753	Ethylene Oxide (PPM)	5 Max	< 5
111116	1/4 Dioxane (PPM)	5 Max	0
101700	Heavy Metals, ppm	10 PPM Max	< 10
100200	Gardner Color	6 Max	3.0
100100	Appearance@25°C	Off White Paste	PASS
100400	Acid Value (mg KOH/g)	2 Max	0.186
100600	Saponification Value (mg KOH/g)	45-55	49.61
100500	Hydroxyl Number (mg KOH/g)	81-96	87.33
100900	KF Moisture, %	3 Max	2.12

(b) (4)

10-22-10
-P8

(b) (4)

Certificate of Analysis

(b) (4)

Customer:

Emerald Carolina
8309 WILKINSON BLVD.
CHARLOTTE, NC 28214

POEGASORB 60		Number of Containers	4
Lot Number:	(b) (4)	Pounds Per Container:	470
Test Number	Test Name	Limits	Result
102334	Expiration Date (Retest after)	24 Months	June, 2012
102333	Mfg Date	Report	June, 2010
103753	Ethylene Oxide (PPM)	5 Max	< 5
111116	1/4 Dioxane (PPM)	5 Max	0
101700	Heavy Metals, ppm	10 PPM Max	< 10
100200	Gardner Color	6 Max	5 20
100100	Apparance@25°C	Off White Paste	PASS
100400	Acid Value (mg KOH/g)	2 Max	0 3
100600	Saponification Value (mg KOH/g)	45-55	50 4
100500	Hydroxyl Number (mg KOH/g)	81-96	89 0
100900	KF Moisture, %	3 Max	0 10

(b) (4)

8-4-10
PJS

(b) (4)

Certificate of Analysis

(b) (4)

Customer:

Emerald Carolina
8309 WILKINSON BLVD.
CHARLOTTE, NC 28214

POEGASORB 60		Number of Containers	2
Lot Number:	(b) (4)	Pounds Per Container:	470
Test Number	Test Name	Limits	Result
102334	Expiration Date (Retest after)	24 Months	February, 2012
102333	Mfg Date	Report	February, 2010
103753	Ethylene Oxide (PPM)	5 Max	< 5
111116	1/4 Dioxane (PPM)	5 Max	0
101700	Heavy Metals, ppm	10 PPM Max	< 10
100200	Gardner Color	6 Max	2 70
100100	Appearance@25°C	Off White Paste	PASS
100400	Acid Value (mg KOH/g)	2 Max	0 5
100600	Saponification Value (mg KOH/g)	45-55	51 3
100500	Hydroxyl Number (mg KOH/g)	81-96	91.3
100900	KF Moisture, %	3 Max	0 07

(b) (4)

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of

(b) (4)

Certificate of Analysis

(b) (4)

Customer:

Emerald Carolina
8309 WILKINSON BLVD.
CHARLOTTE, NC 28214

POEGASORB 60		Number of Containers	1
Lot Number:	(b) (4)	Pounds Per Container:	470
Test Number	Test Name	Limits	Result
102334	Expiration Date (Retest after)	24 Months	February, 2012
102333	Mfg Date	Report	February, 2010
103753	Ethylene Oxide (PPM)	5 Max	< 5
111116	1/4 Dioxane (PPM)	5 Max	0
101700	Heavy Metals, ppm	10 PPM Max	< 10
100200	Gardner Color	6 Max	3.5
100100	Appearance@25°C	Off White Paste	PASS
100400	Acid Value (mg KOH/g)	2 Max	0.4
100600	Saponification Value (mg KOH/g)	45-55	51.1
100500	Hydroxyl Number (mg KOH/g)	81-96	88.8
100900	KF Moisture, %	3 Max	0.07

(b) (4)

5.20.10
PS

APPENDIX 3

(b) (4)



October 1, 2010

To Whom It May Concern:

(b) (4) POEGASORB 60K and PEGASORB 60 are food grade and meets the criteria under 21 CFR §173.340.

POEGASORB 60K and POEGASORB 60 also meets the criteria under 21 CFR §582.1 as related to substances generally recognized as safe in animal feeds.

Regards,

(b) (4)



(b) (4)



APPENDIX 4

(b) (4)



March 23, 2010

Barry Ferguson
Emerald Performance Materials
8309 Wilkerson Blvd.
Charlotte, NC 28214

Dear Mr. Ferguson,

Per your request from March 22, 2011 for shelf life of POEGASORB 60 and POEGASORB 60K please find the following. POEGASORB 60 and POEGASORB 60K should be considered to have a one year shelf life in an unopened drum and stored under normal inside storage conditions. After 12 months, POEGASORB 60 and POEGASORB 60K can be requalified for another year by rechecking against qualifying specifications.

Thank you for your interest in (b) (4) and if you have any additional needs, please let your representative, (b) (4) or me, at (b) (4) know and we will be happy to see address those needs.

Sincerely,

(b) (4)



(b) (4)



Krause, Andrea

From: Krause, Andrea
Sent: Friday, December 16, 2011 2:28 PM
To: 'hill@khlaw.com'
Cc: Wong, Geoffrey K
Subject: Reference Request: AGRN 000-005 and 000-007

Attachments: References_AGRN 000-007.pdf; References_AGRN 000-005.pdf

Mr. Hill,
Attached are the lists of references that we were unable to locate. Please let me know if you have any questions.

Regards,

Andrea Krause, Ph.D.
Staff Fellow Chemist
FDA, Center for Veterinary Medicine
Division of Animal Feeds, HFV-224
5219 Standish Place
Rockville, MD 20855
Phone: (240) 276-9768
Fax: (240) 453-6882
email: andrea.krause@fda.hhs.gov

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References_AReferences_A
000-007.pdf 000-005.pdf

Polyoxyethylene (20) Sorbitan Monostearate (AGRN 000-007)

7.

The reviews actually were conducted by the Scientific Committee on Food (SCF), EFSA's predecessor. See Evaluation of Polysorbates 20,40,60,65,80 (paragraph 12), Reports of the Scientific Committee for Food (Fifteenth Series) (1985); Opinion on Polyoxyethylene (20) Sorbitan Mono-Oleate (Polysorbate 80), Reports of the Scientific Committee for Food (Thirtyfourth Series) (September 17,1993).

8.

The Cosmetic Ingredient Review (CIR) was established in 1976 by the Cosmetic, Toiletry, and Fragrance Association (CTFA) with the support of the Food and Drug Administration and Consumer Federation of America. To review and assess the safety of cosmetic ingredients openly and without bias, an Expert Panel was established. Results of the CIR Expert Panel's reviews are published in scientific, peer-reviewed literature.

13.

Atlas Toxicology Lab (ATL) (1970-1971). Series of unpublished studies, 1970-1971. In: Summary of published and unpublished safety data on Tween (Polysorbate) products, submission of unpublished data by CTFA.

15.

Krantz JC (1970-1971). Series of unpublished studies, 1943-1947. In: Summary of published and unpublished safety data on Tween (Polysorbate) products, submission of unpublished data by CTFA.

16.

Marszall L (1973). Toxicological aspects of the use of Span and Tween products in pharmaceuticals, cosmetics, and the food industry. Bromatol. Chern. Toksykol. 6(2), 187-95.

19.

Kawachi T, Yahagi T, Kada T, Tazima Y, Ishidate M, Sasaki M, Sugiyama T. 1981. Cooperative program on short-term assays for carcinogenicity in Japan. IARC Sci. Pub. 27: 323-330.

23.

Kada T, Hirano K, Shirasu Y. Screening of environmental chemical mutagens by the Rec-assay system with Bacillus Subtilis. 149-373, BIBRA

35.

Sugimura T. et al. 1976. Fundamentals in cancer prevention. Ed. Magee PN. et al. University of Tokyo p.191.

36.

FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES. (1974). Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. WHO Food Additives Series, No. 5, pp. 254-63.

46.

See 17th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (1973).

48.

See Evaluation of Polysorbates 20, 40, 60, 65, 80 Reports of the Scientific Committee for Food (Fifteenth Series).

49.

BIBRA (1981), A Short-term (13 week) Study in Rats with Polyoxyethylene (20) Sorbitan Monostearate. These data are unpublished, so we have not independently reviewed the BIBRA Report.

51.

Id. See BIBRA (1983), A Review of the Status of the Polysorbates Prepared for the Ad Hoc Polysorbate Group, April 1983. These data are unpublished, so we have not independently reviewed the BIBRA Report.

52.

Opinion on Polyoxyethylene (20) Sorbitan Mono-Oleate (Polysorbate 80), Reports of the Scientific Committee for Food (Thirty-fourth Series) (September 17, 1993).

55.

January 28, 1960 Memorandum From Division of Pharmacology to Mr. Alan T. Spiher (cited in Japan Food Safety Commission Report on Polysorbates, Footnote 84).

64.

Page SC. Jr. (1949). Experimental safety of sorbitan monostearate and its Polyoxyethylene derivatives. Fed. Proc. 8, 323.

Krause, Andrea

From: Hill, Devon W. [Hill@khlaw.com]
Sent: Wednesday, December 21, 2011 4:15 PM
To: Krause, Andrea; Wong, Geoffrey K
Cc: Chowdhury, Azim
Subject: Reference Request: AGRN 000-005 and 000-007
Attachments: KH.zip

Dear Dr. Krause,

With the Holidays fast approaching, we wanted to give you an update on where things stand with respect to the requested reports referenced in AGRN 000-005 and AGRN 000-007. We have experienced a bit more difficulty in pulling these documents than we initially expected, in part because our staff toxicologist who worked on these GRAS notifications last year has since left the firm and some of his files became dispersed. As a result, we've had to re-order several of the reports which we were not able to locate in our files.

Attached please find the following with respect to **AGRN 000-005**:

- Footnote 11: Seventeenth Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Tech. Report Series; 1974, No. 539; FAO Nutrition Meetings Report Series, 1974, No. 53; and
- Footnote 12: FASEB (1979). Evaluation of the Health Aspects of Certain Silicates as Food Ingredients. U.S. Department of Commerce, National Technical Information Service PB-301 402.

The report cited in footnote 15 of AGRN 000-005, "BIBRA Working Group (1991). Polydimethylsiloxane, Toxicity Profile, BIBRA Toxicology International" has been ordered. We expect to receive a copy next week.

Attached please find the following with respect to **AGRN 000-007**:

- Footnote 7: Evaluation of Polysorbates 20,40,60,65,80 (paragraph 12), Reports of the Scientific Committee for Food (Fifteenth Series) (1985);
- Footnote 7: Opinion on Polyoxyethylene (20) Sorbitan Mono-Oleate (Polysorbate 80), Reports of the Scientific Committee for Food (Thirtyfourth Series) (September 17, 1993);
- Footnote 8: Cosmetic Ingredient Review (CIR) - Final Report on Safety Assessment of Cosmetic Ingredients; → *unable to open file*
- Footnote 16: Marszall L, et. al - Toxicological aspects of the use of span and tween products;
- Footnote 19: Kawachi T, et. al - Cooperative Program on Short-term assays for carcinogenicity in Japan;
- Footnote 23: Kada T, et. al - Screening of Environmental Chemical Mutagens;
- Footnote 36: FAO-WHO Expert Committee on Food Additives (1974);
- Footnote 46: 17th JECFA (1973) - POE 20 Sorbitan Monooleate;
- Footnote 48: [Same as Footnote 7];
- Footnote 52: [Same as Footnote 7]; and
- Footnote 64: Page SC, Jr. (1949). Experimental safety of sorbitan monostearate and its Polyoxyethylene derivatives. Fed. Proc. 8, 323.

The report cited in footnote 35, "Sugimura T. et al. 1976. Fundamentals in cancer prevention. Ed. Magee PN. et al. University of Tokyo p.191" has been ordered. We expect to receive a copy next week.

Regarding footnotes 13 and 15 in AGRN 000-007, we have not been able to locate the actual reports cited (they

are unpublished). However, we have located the attached "Final Report on the Safety Assessment of Polysorbates 20, 21, 40, 60, 61, 65, 80, 81 and 85" from the International Journal of Toxicology (1984), which similarly references those reports (see footnotes 233 and 235 therein). Rather than citing to the unpublished reports in the GRAS notification, our toxicologist should have instead cited the attached Final Report (see page 41 therein) regarding the acute oral toxicity of the polysorbates. We apologize for this oversight.

Finally, regarding the BIBRA reports in footnotes 49 and 51 of AGRN 000-007, we have ordered these and expect to receive copies next week. We are still also searching our files for the report cited in Footnote 55 "January 28, 1960 Memorandum From Division of Pharmacology to Mr. Alan T. Spilher." We will let you know as soon as we are able to locate this memorandum.

If you have any further questions or concerns, please do not hesitate to let us know.

Best regards and Happy Holidays,

Devon Hill

Devon Wm. Hill
Partner
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From: Krause, Andrea [mailto:Andrea.Krause@fda.hhs.gov]
Sent: Friday, December 16, 2011 2:28 PM
To: Hill, Devon W.
Cc: Wong, Geoffrey K
Subject: Reference Request: AGRN 000-005 and 000-007

Mr. Hill,
Attached are the lists of references that we were unable to locate. Please let me know if you have any questions.

Regards,

Andrea Krause, Ph.D.
Staff Fellow Chemist
FDA, Center for Veterinary Medicine
Division of Animal Feeds, HFV-224
5219 Standish Place
Rockville, MD 20855
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Krause, Andrea

From: Chowdhury, Azim [chowdhury@khlaw.com]
Sent: Friday, December 23, 2011 2:24 PM
To: Krause, Andrea
Cc: Hill, Devon W.
Subject: RE: Reference Request: AGRN 000-005 and 000-007
Attachments: Cosmetic Ingredient Review - Final Report on Safety Assessment of cosmetic ingredients.pdf; Evaluation of the Health Aspects of Certain Silicates as Food.pdf, Sugimura T. et al. 1976. Fundamentals in cancer prevention. Ed. Magee PN. et al. University of Tokyo p.191.pdf

Dear Dr. Krause,

Devon Hill asked me to respond to your below request. Please find attached PDFs of "Evaluation of the Health Aspects of Certain Silicates as Food" and "Cosmetic Ingredient Review-Final Report on Safety Assessment of Cosmetic Ingredients." I've also attached the missing footnote 35 from AGRN 000-007, "Sugimura T. et al. 1976. Fundamentals in cancer prevention. Ed. Magee PN. et al. University of Tokyo p.191." If you have any problems opening these electronic files, please let us know.

We are trying to locate an English translation of the Marszall article, and will get back to you as soon as possible.

If there is anything else you need, please do not hesitate to let us know.

Best regards and Happy Holidays,

Azim

Azim Chowdhury
Associate
tel: 202.434.4230 fax 202.434.4646 | chowdhury@khlaw.com
1001 G Street, N.W., Suite 500 West | Washington, D.C. 20001

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From: "Krause, Andrea" <Andrea.Krause@fda.hhs.gov>
Date: December 23, 2011 11:51:58 AM EST
To: "Hill, Devon W." <Hill@khlaw.com>
Subject: RE: Reference Request: AGRN 000-005 and 000-007

1/12/2012

Mr. Hill,

Thank-you for your prompt reply. We are unable to open two of the files you sent ("Evaluation of the Health Aspects of Certain Silicates as Food" and "Cosmetic Ingredient Review-Final Report on Safety Assessment of Cosmetic Ingredients"). If you could resend those two files at your convenience, it would be much appreciated. Also, the article by Marszall L, et al. (Tox aspects of the use of span and tween products) is in another language. If you have a translation of that in your possession, could you send it as well? If you don't have it, there's no need to get it--we can make do without--just thought I'd check. Thanks again.

Regards,
Andrea

From: Hill, Devon W. [mailto:Hill@khlaw.com]
Sent: Wednesday, December 21, 2011 4:15 PM
To: Krause, Andrea; Wong, Geoffrey K
Cc: Chowdhury, Azim
Subject: Reference Request: AGRN 000-005 and 000-007

Dear Dr. Krause,

With the Holidays fast approaching, we wanted to give you an update on where things stand with respect to the requested reports referenced in AGRN 000-005 and AGRN 000-007. We have experienced a bit more difficulty in pulling these documents than we initially expected, in part because our staff toxicologist who worked on these GRAS notifications last year has since left the firm and some of his files became dispersed. As a result, we've had to re-order several of the reports which we were not able to locate in our files.

Attached please find the following with respect to **AGRN 000-005**:

- Footnote 11: Seventeenth Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Tech. Report Series; 1974, No. 539; FAO Nutrition Meetings Report Series, 1974, No. 53; and
- Footnote 12: FASEB (1979). Evaluation of the Health Aspects of Certain Silicates as Food Ingredients. U.S. Department of Commerce, National Technical Information Service PB-301 402.

The report cited in footnote 15 of AGRN 000-005, "BIBRA Working Group (1991). Polydimethylsiloxane, Toxicity Profile, BIBRA Toxicology International" has been ordered. We expect to receive a copy next week.

Attached please find the following with respect to **AGRN 000-007**:

- Footnote 7: Evaluation of Polysorbates 20,40,60,65,80 (paragraph 12), Reports of the Scientific Committee for Food (Fifteenth Series) (1985);
- Footnote 7: Opinion on Polyoxyethylene (20) Sorbitan Mono-Oleate (Polysorbate 80), Reports of the Scientific Committee for Food (Thirtyfourth Series) (September 17, 1993);
- Footnote 8: Cosmetic Ingredient Review (CIR) - Final Report on Safety Assessment of Cosmetic Ingredients;
- Footnote 16: Marszall L, et. al - Toxicological aspects of the use of span and tween products;
- Footnote 19: Kawachi T, et. al - Cooperative Program on Short-term assays for carcinogenicity in Japan;
- Footnote 23: Kada T, et. al - Screening of Environmental Chemical Mutagens;

- Footnote 36: FAO-WHO Expert Committee on Food Additives (1974);
- Footnote 46: 17th JECFA (1973) - POE 20 Sorbitan Monooleate;
- Footnote 48: [Same as Footnote 7];
- Footnote 52: [Same as Footnote 7]; and
- Footnote 64: Page SC. Jr. (1949). Experimental safety of sorbitan monostearate and its Polyoxyethylene derivatives. Fed. Proc. 8, 323.

The report cited in footnote 35, "Sugimura T. et al. 1976. Fundamentals in cancer prevention. Ed. Magee PN. et al. University of Tokyo p.191" has been ordered. We expect to receive a copy next week.

Regarding footnotes 13 and 15 in AGRN 000-007, we have not been able to locate the actual reports cited (they are unpublished). However, we have located the attached "Final Report on the Safety Assessment of Polysorbates 20, 21, 40, 60, 61, 65, 80, 81 and 85" from the International Journal of Toxicology (1984), which similarly references those reports (see footnotes 233 and 235 therein). Rather than citing to the unpublished reports in the GRAS notification, our toxicologist should have instead cited the attached Final Report (see page 41 therein) regarding the acute oral toxicity of the polysorbates. We apologize for this oversight.

Finally, regarding the BIBRA reports in footnotes 49 and 51 of AGRN 000-007, we have ordered these and expect to receive copies next week. We are still also searching our files for the report cited in Footnote 55 "January 28, 1960 Memorandum From Division of Pharmacology to Mr. Alan T. Spiher." We will let you know as soon as we are able to locate this memorandum.

If you have any further questions or concerns, please do not hesitate to let us know.

Best regards and Happy Holidays,

Devon Hill

Devon Wm. Hill
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From: Krause, Andrea [<mailto:Andrea.Krause@fda.hhs.gov>]
Sent: Friday, December 16, 2011 2:28 PM
To: Hill, Devon W.
Cc: Wong, Geoffrey K
Subject: Reference Request: AGRN 000-005 and 000-007

Mr. Hill,
Attached are the lists of references that we were unable to locate. Please let me know if you have any questions.

Regards,

Andrea Krause, Ph.D.
Staff Fellow Chemist
FDA, Center for Veterinary Medicine
Division of Animal Feeds, HFV-224
5219 Standish Place
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Krause, Andrea

From: Hill, Devon W. [Hill@khlaw.com]
Sent: Tuesday, January 10, 2012 11:00 AM
To: Krause, Andrea; Wong, Geoffrey K
Cc: Chowdhury, Azim
Subject: Reference Request: AGRN 000-005 and 000-007
Attachments: JC Krantz - 1951 36 BullSchMedUnivMD 48.pdf; Reports of the Scientific Committee for Food (Fifteenth Series) (1985);(2).pdf

Dear Dr. Krause,

I apologize that I missed your call this morning as I was in a meeting. I will plan to call you this afternoon, but first I wanted to provide you with a substantive response regarding our efforts to respond to FDA's request for certain references mentioned in our filing.

Following up on your request for the references cited in AGRN 000-005 and AGRN 000-007, the purpose of this e-mail is to provide you with an update on the status of our search. Unfortunately, we were unable to locate an English translation of the Marszall article in our files; if you would like us to have the article translated, please let us know.

Additionally, we were unable to locate the unpublished data cited in Footnote 15 of AGRN 000-007 by Krantz JC. However, in lieu of that unpublished information, please see the attached article by the same author (Krantz), "Sugar Alcohols -- XXVII. Toxicological, Pharmacodynamic and Clinical Observations on Tween 80." We believe this article summarizes the safety data on Tween 80, polyoxyethylene (20) sorbitan oleate, and the C18 oleate analog of polyoxyethylene (20) sorbitan stearate. This article cites to studies and presents data from studies conducted in the time period of 1943-1947 on the Tween (Polysorbate) products. The article provides support to the LD50 values that were supported by the unpublished data on the Tween (Polysorbate) products cited in the unpublished dated referred to in AGRN 000-0007.

Regarding the BIBRA reports cited, we were unable to find copies of the reports in our files. We contacted BIBRA, and have ordered the report referenced in AGRN 000-0005, "BIBRA Working Group (1991). Polydimethylsiloxane, Toxicity Profile, BIBRA Toxicology International." We expect to receive a copy of this report this week (it was mailed to us on 12/23/11), and will send it to you as soon as we do. Regarding the two BIBRA reports cited in AGRN 000-007, we were also unable to locate these in our files, unfortunately. We contacted BIBRA to re-order the reports, but were informed that these particular reports are no longer maintained in BIBRA's files (many of the old reports such as these have apparently been destroyed or sent back to the study sponsors). In lieu of these BIBRA reports, please see the attached "Reports of the Scientific Committee for Food (Fifteenth Series)," which was previously provided to you. We note that both the 1981 short-term (13 week) study in rats with polyoxyethylene (20) sorbitan monostearate (Footnote 49) and the 1983 review of the status of polysorbates (Footnote 51) are cited as references here (see bottom of page 7). Please let us know if this will be sufficient for your needs.

I look forward to speaking with you. As always, if you have any additional questions or concerns, or if you would like to set up a conference call to discuss, please do not hesitate to let us know.

Best regards,


Devon Hill

Devon Wm. Hill

1/12/2012

Partner

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1001 G Street, N.W., Suite 500 West | Washington, D.C. 20001

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Writer's Direct Access
Devon Wm. Hill
(202) 434-4279
hill@khlaw.com

February 22, 2012

Via Electronic Mail and Federal Express

Dr. Andrea Krause, Ph.D.
Food and Drug Administration
Division of Animal Feeds (HFV-224)
Center for Veterinary Medicine
7519 Standish Place
Rockville, Maryland 20855

**Re: Amendment to AGRN 000-007; GRAS Notification for
Polyoxyethylene 20 Sorbitan Monostearate; Our File No. EM13458-01**

Dear Dr. Krause:

On behalf of our client, Emerald Carolina Chemicals, LLC (the Notifier), we hereby respectfully submit the enclosed Amendment to the Generally Recognized as Safe (GRAS) notification for polyoxyethylene 20 sorbitan monostearate, designated AGRN 000-007, filed on April 8, 2011. As discussed in detail in AGRN 000-007, the Notifier's defoamer product is added to the condensed distillers solubles (*i.e.*, thin stillage concentrate) to assist in separating out corn oil during processing of grain from ethanol distillation. Accordingly, the polyoxyethylene 20 sorbitan monostearate defoamer component may be present at minute levels as an impurity in distillers grains fed to the food-producing animals.

Pursuant to our telephone conferences on February 3, 2012, you asked us to provide (1) assurance that the Notifier's polyoxyethylene 20 sorbitan monostearate meets the specifications set forth in 21 C.F.R. § 172.826(b) ("Polysorbate 60"); (2) a description for how the polyoxyethylene 20 sorbitan monostearate functions as a defoamer; (3) a revised GRAS Status Claim which specifies the food-producing target animal species that are subject to the notification; and (4) a description of why turkeys, egg laying hens and goats should be included among the types of food-producing target animal species subject to this GRAS notification.

Accordingly, the enclosed Amendment to AGRN 000-007 includes the following:

- (1) Signed Letter, dated February 10, 2012, from (b) (4) (b) (4), stating that (b) (4) Poegasorb 60K and Poegasorb 60 polyoxyethylene 20 sorbitan monostearate products meet all the specifications listed in 21 C.F.R. § 172.826(b).

KELLER AND HECKMAN LLP

Food and Drug Administration

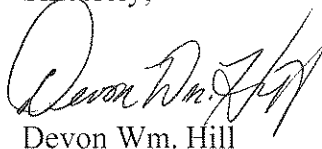
February 22, 2012

Page 2

- (2) A detailed description of polyoxyethylene 20 sorbitan monostearate's chemical and physical properties that enable it to function as a defoamer (*i.e.*, its defoaming mechanism).
- (3) A detailed description and dietary intake calculations demonstrating why turkeys, egg laying hens and goats should be included among the types of food-producing target animal species subject to this GRAS notification.
- (4) A revised GRAS Status Claim which states that the polyoxyethylene 20 sorbitan monostearate is GRAS when present as an impurity in animal feed for the following food-producing target animal species: beef cattle, dairy cattle, poultry (turkey, broiler chickens and egg laying hens), sheep, goat and swine.

The enclosed Amendment to AGRN 000-007 is provided in triplicate. We trust that this Amendment satisfies the Agency's needs, and will be deemed accepted and complete. Should any questions arise, please contact us, preferably by telephone or e-mail, so that we can promptly respond.

Sincerely,



Devon Wm. Hill

Cc: Geoffrey Wong, Ph.D.

Enclosure

**Amendment to AGRN 000-007
Generally Recognized as Safe (GRAS) Notification for
Polyoxyethylene 20 Sorbitan Monostearate
(CAS Reg. No. 9005-67-8)**

Prepared for:

U.S. Food and Drug Administration
Center for Veterinary Medicine
Division of Animal Feeds (HFV-224)
7519 Standish Place
Rockville, MD 20855

Notifier:

Emerald Carolina Chemical, LLC
8309 Wilkinson Boulevard
Charlotte, NC 28214-9052

February 22, 2012

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I. Assurance Letter from Hi-Mar Specialty Chemicals, LLC

Please see attached (**Attachment 1**) the signed letter, dated February 10, 2012, from (b) (4), stating that (b) (4) Poegasorb 60K and Poegasorb 60 polyoxyethylene 20 sorbitan monostearate products meet all the specifications listed in 21 C.F.R. § 172.826(b), which are as follows:

Saponification number: 45-55
Acid number: 0-2
Hydroxyl number: 81-96
Oxyethylene content: 65-69.5%

II. Polyoxyethylene 20 Sorbitan Monostearate Mechanism

Polyoxyethylene (20) sorbitan monostearate is used as a component of a defoamer that is added to condensed distillers solubles (CDS) prior to processing in a mechanical centrifuge that separates corn oil from the aqueous CDS. A defoamer is a chemical additive that functions to reduce and inhibit the formation of foam in industrial process liquids. This action eliminates problems that occur with the presence of surface foam or entrapped air that can lead to reduced efficiency in industrial processes such as pumping, separation, and centrifugation.

Foam is frequently produced in hydrophilic-hydrophobic mixtures, and is expected to be formed during the separation of hydrophobic corn oil from aqueous concentrated stillage or CDS in the production of distillers grains at ethanol production plants. Generally a defoamer is insoluble in the foaming medium and has surface active properties such that it has an affinity to the air-liquid surface where it destabilizes foam lamellas (foam film) causing the rupture of air bubbles and breakdown of surface foam.

The properties of a defoamer which facilitate the rupture of the foam film include (1) insolubility in the foam medium, (2) facile dispersibility in the foam medium, (3) chemical inertness, and (4) a lower surface tension than the foam medium. Insolubility is important because if a defoamer was soluble in a foam film, its surfactant properties would lead to reinforced foam formation. Easy dispersibility allows the defoamer to be dispersed in the medium quickly with agitation. Chemical inertness is important to ensure that a defoamer will not react with any components in the medium.

Polyoxyethylene (20) sorbitan monostearate, with hydrophobic and hydrophilic moieties in its structure, is easily dispersed in the CDS medium from which it is transferred to the air-liquid surface. Once it reaches the air-liquid surface, it enters the foam interface forming micelles with its hydrophobic moieties that disrupt the foam film structure, thereby inhibiting foaming.

III. Inclusion of Turkeys, Egg Laying Hens and Goats to List of Target Animal Species

AGRN 000-007 provides that, although the animal species tested were predominantly rats, the toxicology data are equally applicable to the following food-producing target animal species: beef cattle, dairy cattle, poultry (broiler chickens), sheep and swine. For the reasons set forth herein, **turkeys, egg laying hens and goats** should be included in the list of food-producing target animals subject to this notification. The calculations below demonstrate that the maximum dietary intake of polyoxyethylene (20) sorbitan monostearate for each of the new target animal species is below the conservative Acceptable Daily Intake (ADI) of 10 mg/kg-bw/day.

First, we calculate the amount of distillers grains consumed on a dry basis for each animal. Next, using the maximum residual level of 13.9 mg/kg of polyoxyethylene (20) sorbitan monostearate in the distillers grains on a dry basis, we calculate the maximum amount of polyoxyethylene (20) sorbitan monostearate consumed (*i.e.*, the maximum dietary intake) for each target animal species. This value is then compared to the very conservative ADI for polyoxyethylene (20) sorbitan monostearate for the target animal species.

a. Amount of Distillers Grains Consumed by Target Animal Species

An egg laying hen has an average body weight of 4.2 lb (1.9 kg) and consumes 52 g of dry feed per day for a food consumption of $52 \text{ g} / 1.9 \text{ kg} = 27 \text{ g/kg bw/day}$ ¹. Assuming that egg laying hens consume no more than 15% by weight dry distillers grains in feed², the maximum daily consumption of distillers grains for egg laying hens is $27 \text{ g/kg bw/day} \times 15\% = 4.1 \text{ g/kg bw/day}$.

A female turkey is reported to have an average body weight of 8.1 kg and consumes 2.23 kg of dry feed per week or 320 g/day ($2.23 \text{ kg/wk} \times 1000 \text{ g/kg} \div 7 \text{ days/wk} = 320 \text{ g/day}$) for a daily feed intake of $320 \text{ g/day} \div 8.1 \text{ kg bw} = 39.5 \text{ g/kg bw/day}$ ³. Additionally, a male turkey is reported to have an average body weight of 12.8 kg and consumes 3.6 kg of dry feed per week or 514 g of feed per day ($3.6 \text{ kg} \times 1,000 \text{ g/kg} \div 7 \text{ days/wk} = 514 \text{ g}$) for a daily intake of $514 \text{ g} / 12.8 \text{ kg bw} = 40 \text{ g/kg bw/day}$. Assuming that female turkeys consume no more than 15%

¹ "Revisions of Feedstuffs in Table 1 of OPPTS Test Guideline 860.100 and Guidance on Constructing Maximum Reasonably Balanced Diets (MRBD)," United States Environmental Protection Agency, Jan. 30, 2008. page 5, available at: <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0155-0003>.

² Using Distillers Grains in the U.S. and International Livestock and Poultry Industries, *see* http://www.card.iastate.edu/books/distillers_grains/pdfs/distillers_grains_book.pdf.

³ J.D. Furman. Nutrient Requirements of Chickens and Turkeys. 8th ed. 1984. National Academy Press. Washington, D.C., *see* <http://www.afn.org/~poultry/flkman9.htm>.

by weight of dry distillers grains⁴, and male turkeys consume no more than 20% by weight dry distillers grains⁵, the maximum daily amount of distillers grains consumed is 6 g/kg bw/day⁶ for female turkeys and 8 g/kg bw/day for male turkeys.⁷

The maximum daily dry feed intake for goats is 4% of their body weight or 40 g/kg bw/day (0.04 kg/kg bw/day x 1000 g/kg = 40 g/kg bw/day).⁸ Assuming a goat consumes no more than 30% by weight dry distillers grains in their feed², the maximum daily consumption of distillers grains is 40 g/kg bw/day x 30% = 12 g/kg bw/day.

b. Maximum Dietary Intake of Polyoxyethylene (20) Sorbitan Monostearate for each Target Animal Species

As the concentration of polyoxyethylene (20) sorbitan monostearate in distillers grains is 13.9 mg-polyoxyethylene (20) sorbitan monostearate /kg-distillers grains, the maximum dietary intake of the substance in turkey, egg laying hens, and goats are presented in the following revised tables:

⁴ B.A. Babcock, D.J. Hayes, J.D. Lawrence. Using Distillers Grains in the U.S. and International Livestock and Poultry Industries. The Midwest Agribusiness Trade Research and Information Center. 2008. p.128, available at http://www.card.iastate.edu/books/distillers_grains/pdfs/distillers_grains_book.pd.

⁵ See “Value-added Feed Source for Beef, Dairy Beef, Dairy, Poultry, Swine, Sheep,” National Corn Growers Association (NCGA), September 9, 2008, available at http://www.ethanol.org/pdf/contentmgmt/NCGA_Distillers_Grain_908-1.pdf.

⁶ 39.5 g/kg bw/day x 15% = 6 g/kg bw/day.

⁷ 40 g/kg bw/day x 20% = 8 g/kg bw/day.

⁸ M. Rashid, “Goats and their Nutrition,” Manitoba Goat Association, *see* <http://www.gov.mb.ca/agriculture/livestock/goat/pdf/bta01s08.pdf>.

² T. Hutchens and R. Harmon. Adding Value to Kentucky Products by Feeding Distillers Dried Grains (Report Summer 2005). Goat Producer’s Newsletter. University of Kentucky. November 2005. Available at http://www.distillersgrains.org/files/feedsource/Hutchens_11_05.pdf

TABLE 1. Feeding Data for Food-Producing Target Animals

Target Animal Species	Weight (kg)	Food Consumed (g/day)	Distillers Grains (dry weight basis) Consumed per Day		
			(%)	(g/day)	g/kg bw/day
Beef Cattle	500	10,000	30%	3,000	6
Dairy Cattle	500	10,000	30%	3,000	6
Poultry ¹⁰ (broiler)	2.5	232.5	15%	34.9	14
Egg laying hen	1.9	52	15%	7.8	4.1
Female turkey	8.1	320	15%	48	6
Male turkey	12.8	514	20%	103	8
Sheep	60	2,400	30%	720	12
Swine	60	2,400	30%	720	12
Goat	-	4% (maximum of body weight)	30%		12 ¹¹

With a maximum residual level of 13.9 mg/kg of polyoxyethylene (20) sorbitan monosteareate in distiller's grains on a dry weight basis, a maximum dietary intake for laying hens is calculated as follows:

$$4.1 \text{ g-distillers grain/kg bw} \times (13.9 \text{ mg- POESM/kg-distillers grains}) \times (\text{kg}/1000 \text{ g})$$

$$= 0.06 \text{ mg POESM/kg bw/day}$$

The dietary intake of polyoxyethylene (20) sorbitan monosteareate by the other food-producing target animals is similarly calculated and presented in the table below:

TABLE 2. EDIs for Target Animals

Target Animal Species	EDI (mg/kg-bw/day) for Polyoxyethylene (20) sorbitan monosteareate
Beef Cattle	0.08
Dairy Cattle	0.08

¹⁰ The feed consumption for broiler chickens is reported to be 93 mg/kg bw/day – Predicting Feed Intake of Food-Producing Animals, Subcommittee on Feed Intake, Committee on Animal Nutrition, Board on Agriculture, National Research Council, National Academy Press, Washington, D.C., 1987.

¹¹ 40 g/kg bw/day x 30% = 12 g/kg bw/day.

Target Animal Species	EDI (mg/kg-bw/day) for Polyoxyethylene (20) sorbitan monostearate
Poultry (Broiler)	0.2
(Egg Laying Hen)	0.06 ¹²
(Turkey- Female)	0.08 ¹³
(Turkey- Male)	0.1 ¹⁴
Sheep	0.17
Swine	0.17
Goat	0.17 ¹⁵

IV. Revised GRAS Status Claim

Polyoxyethylene (20) sorbitan monostearate is GRAS based on scientific procedures, when present at levels up to 20 ppm in the defoamer, as an impurity in animal feed for food-producing target animal species (e.g., beef cattle, dairy cattle, poultry (turkey, broiler chickens and egg laying hens), sheep, goat and swine) as a result of its use as an emulsifier in the production of wet and dried distillers grain with added solubles (WDGS and DDGS, respectively). Polyoxyethylene (20) sorbitan monostearate serves no technical purpose in the animal feed itself. Accordingly, the GRAS substance that is the subject of this notification is only present as a potential impurity in the WDGS and DDGS due to its use in the processing of the CDS.

The use of polyoxyethylene (20) sorbitan monostearate in this manner as a component of the Notifier's defoamer product has been determined to be exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 301 *et. seq.*).

¹² 4.1 g-distillers grain/kg bw x (13.9 mg-POESM/kg-distillers grains) x (kg/1000 g) = 0.06 mg POESM/kg bw/day.

¹³ 6 g-distillers grain/kg bw x (13.9 mg-POESM/kg-distillers grains) x (kg/1000 g) = 0.08 mg POESM/kg bw/day.

¹⁴ 8 g-distillers grain/kg bw x (13.9 mg-POESM/kg-distillers grains) x (kg/1000 g) = 0.1 mg POESM/kg bw/day.

¹⁵ 12 g-distillers grain/kg bw x (13.9 mg-POESM/kg-distillers grains) x (kg/1000 g) = 0.17 mg POESM/kg bw/day.

Attachment 1

(b) (4)



February 10, 2012

Virginia Littleton
Emerald Performance Materials
8309 Wilkerson Blvd.
Charlotte, NC 28214

Dear Ms. Littleton,

Per your request of February 6, 2012 regarding the POE 20 Sorbitan Monostearate (*i.e.*, Poegasorb 60K, Polysorbate 60), FDA needs a statement from your supplier (FCI) that the substance meets the specifications set forth in 21 CFR 172.836(b). Those specifications are as follows:

Saponification number: 45-55
Acid number: 0-2
Hydroxyl number: 81-96
Oxyethylene content: 65-69.5%

I can confirm that Poegasorb 60K and Poegasorb 60 does complies with all the specifications set forth in 21CFR 172.836, which includes all paragraphs and paragraph (b) being just one of the sections.

Thank you for your interest in (b) (4) and if you have any additional needs, please let your representative, (b) (4) or me, at (b) (4), know and we will be happy to see address those needs.

Sincerely,

(b) (4)



(b) (4)

