#761

February 20, 2018

Office of Food Additive Safety HFS-200
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD, 20740

Dear Sir or Madam:

Accompanying this letter is a notice pursuant to regulations of the Food and Drug Administration found at 21 CFR Part 170 setting forth the basis for the conclusion reached by the submitter, Choco Finesse, LLC, that esterified propoxylated glycerol (EPG) is generally recognized as safe under the intended conditions of use described in the notice. The notice is contained in a binder. In addition, we include a CD that contains a complete copy of the notice. I hereby certify that the electronic files contained on the flash drive were scanned for viruses prior to submission, and thus certified as being virus-free using Symantec Endpoint Protection.

Sincerely,

(b) (6)

20 Feb 2018

David Rowe
President
Phone: 317-694-3601
Email: drowe@chocofinesse.com

GRAS NOTICE FOR ESTERIFIED PROPOXYLATED GLYCEROL (EPG) FOR USE IN SELECT COMMERCIAL FRYING APPLICATIONS

Prepared for:
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740

Prepared by:
Choco Finesse, LLC
5019 N. Meridian Street
Indianapolis, Indiana
46208

February 20, 2018

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GRAS Notice for Esterified Propoxylated Glycerol (EPG) for Use in Select Commercial Frying Applications

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GRAS Notice for Esterified Propoxylated Glycerol (EPG) for Use in Select Commercial Frying Applications

Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §§170.203 through 170.285 (U.S. FDA, 2017), Choco Finesse LLC (Choco Finesse) hereby informs the U.S. Food and Drug Administration (FDA) that esterified propoxylated glycerol (EPG), manufactured by Choco Finesse, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Choco Finesse's view that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Section 1.3 below. In addition, as a responsible official of Choco Finesse, David Rowe hereby certifies that all data and information presented in this notice represents a complete, representative, and balanced submission, and which considered all unfavorable as well as favorable information known to Choco Finesse and pertinent to the evaluation of the safety and GRAS status of EPG as an ingredient in select commercial frying applications.

Signed,

(b) (6)

David Rowe
President

February 20, 2018
Date

1.1 Name and Address of Notifier

Choco Finesse, LLC
5019 N. Meridian Street
Indianapolis, IN 46208

Contact Name: David Rowe
Phone: 317-694-3601
E-mail: drowe@chocofinesse.com

1.2 Common Name of Notified Substance

Esterified Propoxylated Glycerol (EPG)

1.3 Conditions of Use

EPG has been the subject of 2 previous GRAS Notifications, both of which have received letters of 'no objection' from FDA. GRN 583 detailed the use of "confectionary" EPG as a fat replacer at up to 34.5% in confectionary coatings used in baked goods and baking mixes (biscuit items, bakery items and ice-cream inclusions), frozen dairy desserts and mixes (ice cream novelties), grain products and pasta (snack and meal replacement bars), hard candy and cough drops (panned confectionary items, sugar-shelled confectionary pieces), snack foods (snack items, trail mix) and soft candy (molded and hollow molded confectionery pieces) (U.S. FDA, 2015). In GRN 640, the use of "spreadable" EPG as a fat replacer at levels up to 38 percent by weight in baked goods and baking mixes, frozen dairy desserts and mixes, grain products and pasta, gravies and sauces, nuts and nut products, and soft candy was described (U.S. FDA, 2016).

Choco Finesse now intends to market a version of spreadable EPG for a new use in commercial frying of french fries (80:20 blend; EPG: vegetable oil) and doughnuts (60:40 blend; EPG:vegetable oil) in the United States (U.S.). In order to determine exposure to EPG from these applications, ChocoFinesse analyzed EPG absorption in french fries and doughnuts. Control frying was done in high-oleic sunflower oil (HOS) while test frying was performed using 80:20 and 60:40 blends of spreadable EPG with HOS. EPG absorption into food products was determined by analyzing the fatty acid composition of oils extracted from these foods.

The total content of EPG in a medium serving of french fries is 9.46 g (8.1% by weight). This value is very similar to a theoretical 9.9 g EPG in medium serving of McDonald's fries. Theoretical exposure to EPG in french fries ranges from 2.5 g in the kids serving to 12.3 g in the large serving size. In experimental frying of frozen doughnuts, the content of EPG is 3.6 g in a 49.1 g doughnut.

The individual proposed food-uses and use-levels for EPG employed in the current intake analysis are summarized in Table 1.3-1. Food codes representative of each proposed food-use were chosen from the 2011-2012 National Health and Nutrition Examination Survey (NHANES) (CDC, 2015; USDA, 2014). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (U.S. FDA, 2017).

Table 1.3-1 Summary of the Individual Proposed Food Uses and Use Levels for EPG in the U.S.

Food Category (21 CFR 170.3)	Foods-Uses*	EPG Level (g/serving)†	Serving size (g)‡		Residual EPG Levels in the Food (% w/w)**
Processed vegetables	French fries	12.3	Large	154	8.0
		9.46	Medium	117	**8.1**
		5.7	Small	71	8.0
		2.5	Kid	31	8.1
Baked good	Doughnuts	7.7	Large	71	**10.8**
		5.8	Medium	54	10.7
		1.5	Small	14	10.7

CFR = Code of Federal Regulations; EPG = esterified propoxylated glycerol; U.S. = United States.

* EPG is intended to be used in frying applications. The proposed food-uses represent foods that are intended to be fried in EPG.

† EPG level is the amount of EPG absorbed in the food following the intended frying application, as measured by Choco Finesse.

‡ Serving sizes were provided by Choco Finesse.

** The highest, **bolded**, values were used for each food category in the current intakes assessment to examine worst-case EPG exposure.

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a) and (b) of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2017), EPG manufactured by Choco Finesse, has been concluded to have GRAS status for use as an ingredient for addition to specified conventional food products, as described in Table 1.3-1 on the basis of scientific procedures.

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be made available to the FDA for review and copying upon request during business hours at the offices of:

Choco Finesse, LLC
5019 N. Meridian Street
Indianapolis, Indiana
46208

In addition, should the FDA have any questions or additional information requests regarding this notification during or after the Agency`s review of the notice, Choco Finesse will supply these data and information.

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Choco Finesse's view that all data and information presented in Parts 2 through 7 of this notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. 552.

Part 2. §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Description

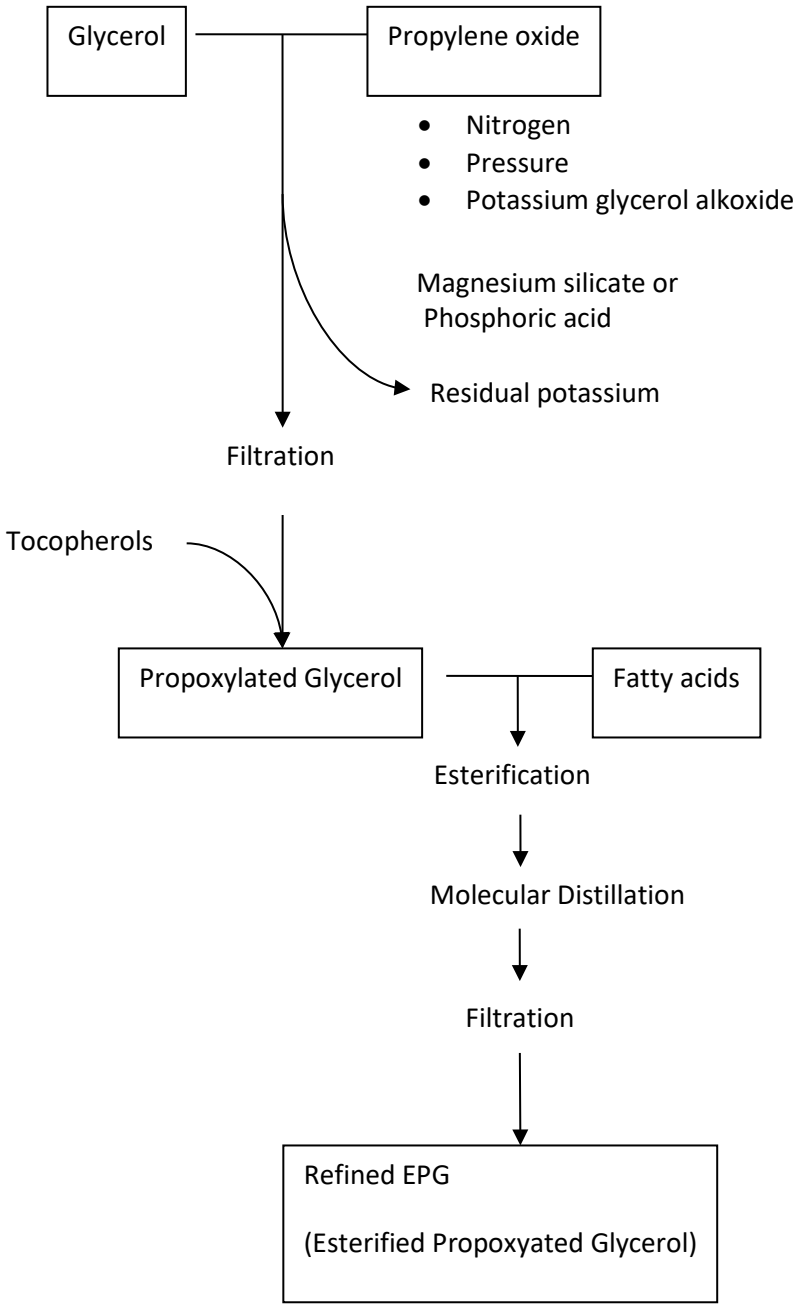
EPGs are a family of fat- and oil-like substances that resemble triglycerides in structure and appearance, but they have been modified to prevent or limit their digestion when consumed in food. Due to the nature of the manufacturing process, a large number of versions of EPG are made available through modification of the fatty acid moieties of the triglyceride and the extent of the propoxylation of the glycerol. The subject of this notification is a version of spreadable EPG (*i.e.*, H-EPG-05) that was the subject of GRN 640 (U.S. FDA, 2016) for which FDA had "no questions".

2.2 Manufacturing

2.2.1 Overview

EPG is manufactured in compliance with current Good Manufacturing Practice (cGMP) regulations. Briefly, the production of EPG consists of 2 basic processes: (1) propoxylation of glycerol; and (2) esterification of propoxylated glycerol with fatty acids. Propoxylation of glycerol involves reacting food grade glycerol with propylene oxide under base catalysis to form the tri-functional polyether polyol (propoxylated glycerol). The esterification is carried out without catalyst using an excess of fatty acids. The unsaturated fatty acids are derived from splitting natural edible fats and oils, while saturated fatty acids are produced by splitting fully hydrogenated edible oils. The unreacted fatty acids are removed from crude EPG by molecular distillation. Note that the more efficient molecular distillation step replaces the steam deodorizing step in the previous GRAS-Notified manufacturing process. The improved manufacturing process for EPG is depicted in Figure 2.2-1.

Figure 2.2-1 Schematic Overview of the Manufacturing Process for EPG



EPG = esterified propoxylated glycerol

2.2.2 Raw Material Specifications

The raw materials used in the manufacture of EPG include Kosher vegetable fatty acids (*i.e*., oleic acid, stearic acid, palmitic acid, behenic acid, and arachidic acid). These fatty acids are all derived from vegetable sources (rape, palm kernel, *etc*.). The composition and purity of all fatty acids used to make EPG is supported by the supplier's Certificate of Analysis which includes the Manufacturer name, lot or batch number, and the ratio of fatty acids and other components in each batch or lot.

Vegetable oils are mixtures of triacylglycerols of fatty acids, the relative ratios of the fatty acids can vary markedly in composition and availability between growing seasons. In order to assemble the correct ratio of fatty acid inputs necessary to achieve consistent physical and functional EPG, ChocoFinesse works closely with fatty acid suppliers to assemble the consistent EPG batch composition, based upon fatty acid availability and cost. The fatty acids ChocoFinesse purchases are >70% pure and typically have other related fatty acids to make up the balance. For instance, the oleic acid, 18:1, is typically 80 to 90% oleic with some 18:2 and 18:3 fatty acids. One exception is that the arachidic acid is a blend of arachidic and behenic acids. All are sourced from USA-based companies, but may originate in Canada or southeast Asia.

Kosher glycerin and propylene oxide are also used. A limit of 1 ppm has been established for residual propylene oxide in the final EPG product (see Table 2.3.1-1). A certificate of analysis for propylene oxide is provided in Appendix 1. Mixed tocopherols extracted from vegetable oils are used at levels compliant with cGMP requirements. The safety of this tocopherol mix is addressed in Appendix 2 herein. Citric acid, meeting United States Pharmacopeia specifications and food-grade phosphoric acid are also used.

2.2.3 Detailed Description of the Manufacturing Process

Crude EPG obtained in the esterification step undergoes bleaching to remove any "impurities" prior to stripping of excess fatty acid and deodorization. While undesirable volatile components present in crude EPG are readily distilled off during stripping of free fatty acid, polar compounds are not removed by distillation and negatively affect the stability of finished EPG. Polar compounds, such as polymers and oxidation compounds, are created during hydrogenation, splitting of fatty acids, and during esterification. Also, hydrogenation is typically done on fatty acids derived from degummed-only oils (not finished) which still contain residual phospholipids, residual pigments and low levels of other compounds present in degummed oils. These compounds undergo degradation at high processing temperatures. Therefore, removal of these impurities from crude EPG is essential to producing a high quality, stable finished product.

Pretreatment with concentrated (85%) food grade phosphoric acid is very effective in reacting with polar compounds which can then be removed with standard adsorbents used for bleaching of edible fats and oils. Adding mixed tocopherols at the pretreatment stage adds additional protection for EPG when it is exposed to air while adding bleaching clay, filter aid and activated carbon. With good mixing, reaction with H_3PO_4 for 20 minutes at 80°C is sufficient to render impurities readily absorbable. After H_3PO_4 pretreatment, bleaching clay, filter aid and activated carbon are added, temperature in the bleacher is raised to 105°C under vacuum and intense mixing continues for 30 minutes. Afterwards, the bleacher contents are cooled to 80°C and filtered.

The bleached and nitrogen protected EPG is treated with 0.10% citric acid aqueous solution (20% concentration) in preparation for stripping excess fatty acids and deodorization. Citric acid chelates traces of metals that might be present in bleached EPG thus improving oxidative stability of the finished product.

In a change from the manufacturing process described in GRN 640, steam-refining deodorization has been replaced with molecular distillation (U.S. FDA, 2016). In the past, steam distillation (steam stripping) was employed to reduce the levels of unreacted free fatty acid in esterified propoxylated glycerol (EPG) products. While this industry standard technique is effective, it has now been found that steam distillation may lead to partial hydrolysis of the EPGs, thereby producing some amount of mono-

and di-esterified propoxylated glycerols in admixture with fully esterified propoxylated glycerol. Additionally, steam distillation may result in color development in the EPGs. Another disadvantage of steam distillation involves the mixing of recovered fatty acids with a significant quantity of water (steam condensate), which must then be removed in order for the recovered fatty acid to be suitable for reuse, leading to increased cost of EPG on a commercial scale.

As a result, a short path distillation technique for removing and recovering unreacted fatty acid from EPG products was employed on a commercial scale. During the short path distillation, EPGs are subjected to very high vacuum (*e.g*., less than 1 mm Hg absolute pressure) at temperatures 250 to 270°C for very short time (fewer than 3 seconds). Centrifugal stills are particularly effective in removing free fatty acids EPGs. Centrifugal stills are commonly employed for distillation of heat-sensitive components such as flavoring compounds, vitamins and highly unsaturated fatty acids. In a centrifugal still, crude EPGs are introduced into the proximity of the center of a heated rotating disk housed in a vacuum chamber. Prior to being deposited on the heated rotating disk, the EPGs may be subjected to a degassing step, to remove dissolved oxygen. The feed material is spread by centrifugal force in a thin film (typically, about 0.002 to about 0.03 mm). Free fatty acids evaporate under the low-pressure conditions present in the vacuum chamber and condense on a condenser, such as an internal water-cooled condenser. The heavier, unevaporated residue, (*e.g*., EPG having a reduced content of unreacted fatty acid) slides off the rotating disk into a collecting gutter. Both fractions may separately flow by gravity to discharge pumps and are transferred out of the still (to a distillate collector, in the case of the distilled fatty acid, or to a residue collector, in the case of the purified EPG). Typically, the feed rate of crude EPG to the centrifugal still is controlled so that under the still conditions selected the residence time on the heated rotating disk is less than about 3 seconds and only 1 pass through the centrifugal still is needed in order to achieve the desired level of residual unreacted fatty acid in the purified EPG.

The quality of finished EPGs is improved by substituting short path distillation for standard steam-refining deodorization. The finished EPG exhibits lighter color, due to minimal exposure to high temperatures, and has a low content of free fatty acid, optimizing its suitability for use as a reduced calorie fat substitute in various food products. The unreacted fatty acids are removed in a pure form suitable for direct reuse in a further esterification.

2.3 Product Specifications

Choco Finesse has made minor changes to the product specifications from the EPG intended for spreadable and baked goods originally notified in GRN 640 (U.S. FDA, 2016). Table 2.3.1-1 compares specifications for Spreadable EPG published in GRN 640 and adjustments to the specifications made for commercial scale up (U.S. FDA, 2016). These changes include the following:

- Lovibond color was added because molecular distillation produced very light EPG (desirable attribute);
- Slight adjustments were made in several fatty acids ranges to account for variability in fatty acid concentrations from different batches/suppliers;
- Minor adjustments were made in Solid Fat Content reflecting the variability in fatty acid composition received from the suppliers; and

- Smoke Point was increased by 5°C because molecular distillation reduces free fatty acid to a lower level than steam stripping/deodorization.

In addition, for the current frying applications, the specifications were modified to allow for extra tocopherols (38% increase) to ensure protection of the frying medium from oxidation in multiple frying cycles.

2.3.1 Proposed Chemical Specifications

The chemical specifications for EPG are presented in Table 2.3.1-1. Changes from the specifications included in GRAS Notice 640 are highlighted in yellow.

Table 2.3.1-1 Chemical Specifications for EPG

General Attribute	Specifics	Old Specification (GRN 640) (U.S. FDA, 2016)	Current Specification	Method
Appearance	Solid, white < 30°C	Solid, off white	Solid, off white	Visual (internal procedure)
Color, Lovibond 5.25"	Red/Yellow	Not specified	2.0R/20Y	AOCS Cc 13b-45
Melting point	Mettler Dropping Point	38 to 43°C (100.4 to 109.4°F)	38 to 43°C (100.4 to 109.4°F)	AOCS Tr la-64
Taste	Sensory	Flavorless	Flavorless	Taste (internal procedure)
Texture	Sensory	Waxy	Waxy	Taste (internal procedure)
Free Fatty Acid, %	% FFA as oleic	<0.5	< 0.5	AOCS Ca 5a-40
Peroxide Value	Meq. peroxide/ 1,000g	0 to 1	0 to 1	AOCS Cd 8b-90
Anisidine Value	p-Anisidine	1to 10	1to 10	AOCS Cd18-90
Phosphorus, ppm		< 3	< 3	AOCS Ca 12a-02
Trace Metals, ppm	Calcium	<0.5	< 0.5	AOCS Ca17-1
	Copper	<0.01	< 0.01	AOCS Ca17-1
	Iron	<0.1	<0.1	AOCS Ca17-1
	Magnesium	<0.5	<0.5	AOCS Ca17-1
	Arsenic	<0.05	<0.05	ICP-MS/AOAC 993.14
	Lead	<0.05	<0.05	ICP-MS/AOAC 993.14
Hydroxyl Value	mg KOH/g	<5	<5	ASTM D4274
Iodine Value	mg Iodine/g	15to 30	15 to 30	AOCS Cd 1-25
Tocopherols, ppm	*alpha*	120 to 230	120 to 320 [4]	AOCS Ce 8-89
	beta	5 to 30	5 to 40	
	gamma	500 to 800	500 to 1,100	
	delta	150 to 250	150 to 350	
	Total	900 to 1,300	775 to 1,800	
Fatty Acid Composition	Palmitic, C16:0	0 to 1.0	0 to 1.0	AOCS Ce 1-62
(% as oleic)	Stearic, C18:0	4 to 8	4 to 15	
	Arachidic + Behenic	50 to 70	40 to 70	
Total saturates		70 to 80	70 to 80	
	Oleic, C18:1	20 to 30	18 to 30	

Table 2.3.1-1 Chemical Specifications for EPG

General Attribute	Specifics	Old Specification (GRN 640) (U.S. FDA, 2016)	Current Specification	Method
	Linoleic, C18:2	1.0 to 3.0	1.0 to 3.0	
	Linolenic, C18:3	0 to 0.5	0 to 0.5	
Total unsaturated		21 to 33	21 to 33	
Trans fat, %	Total "trans"	<1.0[3]	<1.0[3]	AOCS Ce 1h-05
Solid Fat Content	@ 10°C	65 to 75	71 to 78	Cd16b-IUPAC
	@ 20°C	58 to 68	59 to 70	
	@ 25°C	43 to 55	44 to 58	
	@ 30°C	30 to 40	30 to 43	
	@ 35°C	18 to 25	18 to 28	
	@ 40°C	0 to 0.5	0 to 0.5	
Smoke Point	Open Cup, °C	>220	>225	AOCS Cc 9a-48
Flash Point	Open Cup, °C	>265	>265	AOCS Cc 9a-48
Caloric content	Kcal/g	0.7	0.7	Official rodent protocol
EPG purity, %	Ester content	>99.5	>99.5	Calculated (esters – FFA)

EPG = esterified propoxylated glycerol; FFA = free fatty acid; R = red; Y = yellow.
Range [1] = originally specified in Table 2.2 of the Dossier for Spreadable H-EPG-05 for baked goods
Range [2] = adjusted range for Spreadable H-EPG-05 used for frying of french fries and doughnuts
[3] Trans-fat contributed by Pamolyn oleic acid used for esterification of EPG. Less than 8% of the total amount present is bioavailable which amounts to less than 0.05% trans fatty acid that may be absorbed from EPG. Conjugated linoleic acid (CLA) not included in total trans values.
[4] For multiple frying cycles, Spreadable H-EPG-05 fortified with additional tocopherols to ensure maintaining an acceptable tocopherol levels in the frying medium. The amount of total tocopherols is consistent with amount reported to occur naturally in soybean oil (Przybylski *et al.*, 2013).

2.3.2 Microbiological Specifications

The microbiological specifications for EPG remain unchanged from those in GRN 640 and are presented in Table 2.3.2-1 (U.S. FDA, 2016).

Table 2.3.2-1 Microbiological Specifications for EPG

Attribute	Specifics	Limit	Method
Aerobic Plate Count	Petrifilm	<10/g	AOAC 990.12
Coliform	Petrifilm	<10/g	AOAC 991.14
Escherichia coli	Petrifilm	<10/g	
Salmonella	ELFA	Negative/25g	AOAC 2004.03
Yeast		<10/g	FDA-BAM, 7th ed.
Mold		<10/g	

ELFA = enzyme linked fluorescent assay; EPG = esterified propoxylated glycerol.

2.4 Product Analysis

2.4.1 Chemical Analysis

Analysis of 3 non-consecutive lots of EPG demonstrates that the manufacturing process as described in Section 2.3 produces a consistent product that meets specifications. A summary of the chemical analysis for the 3 lots of EPG is presented in Table 2.4.1-1. As no batch analysis data are available for commercial batches of EPG intended for use in frying applications, data presented are for the version of EPG intended for use in spreadable and baked goods presented in GRN 640 (U.S. FDA, 2016). The adjustments to the spreadable/baked goods EPG specifications for frying applications are minor. Most reflect typical scale-up changes from the bench-top to a commercial process, and allow for improved consistency, color, taste, and product stability *via* adoption of molecular distillation or other short path distillation. In addition, an increased amount of mixed tocopherols are added to ensure an acceptable antioxidant tocopherol level in the frying medium. None of these changes are anticipated to make a difference in terms of consumer safety. For these reasons, Choco Finesse considers these data representative of the version of EPG intended for frying applications.

Table 2.4.1-1 Batch Analysis Data for H-EPG-05 for Use in Spreadable and Baked Goods

Attribute	Specification	Manufacturing Lot		
		Batch B160 7/15/15	Batch B168 8/12/15	Batch 169 8/12/15
Appearance	Solid, white < 30°C	White, solid	White, solid	White, solid
Mettler Dropping Point, °C	38 to 43	38.9	38.5	38.6
Taste	Flavorless	Flavorless	Flavorless	Flavorless
Texture	Waxy	Waxy	Waxy	Waxy
Free Fatty Acid, % (as oleic)	0.50 max.	0.32	0.50	0.49
Peroxide Value	0 to 1	0.6	0.1	0.2
Anisidine Value	1 to 10	1.6	1.4	1.0
Hydroxyl Value	5.0 max.	5.0	2.7	2.7
Iodine Value	15 to 30	19.5	21.4	20.4

Table 2.4.1-1 Batch Analysis Data for H-EPG-05 for Use in Spreadable and Baked Goods

Attribute	Specification	Manufacturing Lot		
		Batch B160 7/15/15	Batch B168 8/12/15	Batch 169 8/12/15
Purity, % EPG (100-%FFA)	>99.5	99.68	99.50	99.51
Trace Metals				
Calcium, ppm	<0.5	<0.5	<0.5	<0.5
Copper, ppm	<0.01	<0.01	<0.01	<0.01
Iron, ppm	<0.1	<0.1	<0.1	<0.1
Magnesium, ppm	<0.5	<0.5	<0.5	<0.5
Arsenic, ppm	< 0.05	< 0.006	< 0.005	< 0.007
Lead, ppm	< 0.05	< 0.006	< 0.004	< 0.007
Tocopherols				
alpha, ppm	120 to 230	168	166	158
beta, ppm	5 to 40	19	19	17
gamma, ppm	500 to 800	691	713	598
delta, ppm	150 to 250	234	246	203
Total, ppm	900 to 1,300	1,112	1,114	976
Fatty Acid Composition,%				
Palmitic, C16:0	0 to 1.0	0.4	0.4	0.2
Stearic, C18:0	4 to 10	5.6	5.4	5.5
Oleic, C18:1	20 to 30	23.5	24.9	24.7
Linoleic, C18:2	1 to 3.0	1.3	1.4	1.3
Linolenic, C18:3	0 to 0.5	0.3	0.2	0.2
Arachidic, C20:0	38 to 48	44.0	43.1	43.3
Behenic, C22:0	20 to 30	23.4	22.9	23.0
Arachidic + Behenic	58 to 78	67.4	66.0	66.3
Total saturates	67 to 79	73.4	71.8	72.0
Total unsaturates	21 to 33	26.6	28.2	28.0
Trans fat, %	<1.0	0.8	0.9	0.8
Solid Fat Content				
@10°C	65 to 75	70.4	68.4	68.8
@20°C	58 to 68	61.7	59.5	60.0
@25°C	43 to 55	49.2	45.3	46.1
@30°C	30 to 40	33.4	30.4	30.7
@35°C	17 to 25	20.2	17.6	18.2
@40°C	0 to 0.5	0.13	0.04	0.16
Smoke Point, °C	>200	220	220	226
Flash Point, °C	>265	310	310	320
Heavy Metals				
Arsenic	<0.05	<0.006	<0.005	<0.007
Lead	<0.05	<0.006	<0.004	<0.007

EPG = esterified propoxylated glycerol; FFA = free fatty acid; max = maximum.

2.4.2 Microbiological Analysis

A summary of the microbiological analysis for the 3 lots of EPG is presented in Table 2.4.2-2.

Table 2.4.2-1 Summary of the Microbiological Product Analysis for 3 Lots of EPG

Attribute	Specification	Manufacturing Lot		
		Batch B160 7/15/15	Batch B168 8/12/15	Batch 169 8/12/15
Aerobic Plate Count	<10/g	<10/g	<10/g	<10/g
Coliform	<10/g	<10/g	<10/g	<10/g
Escherichia coli	<10/g	<10/g	<10/g	<10/g
Salmonella	Negative/25 g	Negative/25 g	Negative/25 g	Negative/25 g
Yeast	<10/g	<10/g	<10/g	<10/g
Mold	<10/g	<10/g	<10/g	<10/g

EPG = esterified propoxylated glycerol.

2.4.3 Additional Analytical Information

2.4.3.1 Acid and Lipase Resistance and Caloric Availability

As detailed in the GRAS Notification for EPG for confectionary applications (GRN No. 583), EPG-05 versions are resistant to hydrochloric acid and pancreatic lipase, and yield 0.7 kcal/g (U.S. FDA, 2015).

2.4.3.2 Thermal Stability

Lower solid fat content in spreadable EPGs ensures softer, less brittle texture, and the broader melting profile desired in spreads (*e.g.*, nut butters, margarines and cooking/baking shortenings). These properties are achieved by incorporating unsaturated fatty acids up to 30% of the total fatty acid content in EPG.

It is also desirable that EPG used in frying (as well as in spreads, cooking and baking) is as resistant to oxidation and thermal decomposition as current edible fats, oils and shortenings. A standard method for the assessment of the comparative stability of edible oils is the Oxidative Stability Index (OSI) (Medallion Laboratories, undated). This method measures relative resistance of fats and oils to oxidation, as well as decomposition of oxidation products such as, hydroperoxides at a temperature of 110°C (230°F). Table 2.4.3.2-1 shows OSI results for 3 spreadable EPG samples; as controls, and for direct comparison, 2 Crisco shortenings commonly used in cooking and baking were included in the same OSI test. Results demonstrated that EPG samples exhibit superior resistance to oxidation compared to shortenings commonly used in cooking and baking.

Table 2.4.3.2-1 OSI Results for EPG and Crisco Samples

Sample	OSI hours @ 110 °C (230 °F)
Spreadable EPG B156	99.7
Spreadable EPG B160	110.1
Spreadable EPG B161	105.3
Crisco Vegetable shortening	16.9
Crisco Butter shortening	15.4

EPG = esterified propoxylated glycerol; OSI = Oxidative Stability Index.

Another example of edible oils recently developed specifically for cooking and baking are high oleic varieties of canola and sunflower oils (Cargill, 2017). The published OSI data for Cargill's Clear Valley® brand are shown in Table 2.4.3.2-2. The resistance of these new Cargill oils to oxidation, and the thermal degradation of oxidation compounds, is similar to Crisco shortenings and inferior to the stability of spreadable EPGs.

Table 2.4.3.2-2 OSI Results for Cargill's Clear Valley® Brand

Sample	OSI hours @ 110°C (230°F)
Clear Valley® 65 High Oleic Canola Oil	11
Clear Valley® 80 High Oleic Canola Oil	20
Clear Valley® High Oleic Sunflower Oil	15

OSI = Oxidative Stability Index.

Two additional measures of the thermal stability of fats and oils are their Smoke and Flash points (ChartsBin, 2011; Institute of Shortening and Edible Oils, Inc., undated). Smoke Point is the temperature at which a sample heated in the presence of air shows the first signs of bluish smoke rising from the surface. Flash Point is the lowest temperature at which an oil generates sufficient vapors to form an ignitable mixture in air.

The values in the Table 2.4.3.2-3 represent typical temperatures for Smoke and Flash points as determined by the AOCS test method. As the values indicate, the thermal stability of spreadable EPGs is comparable to common vegetable oils used in cooking and baking. This is expected as EPG contains fatty acids derived from vegetable oils.

Table 2.4.3.2-3 Smoke and Flash Points for EPG and Common Vegetable Oils*

Fat/Oil Type	Smoke Point, °C (°F)	Flash Point, °C (°F)
Spreadable EPG B160	220 (428)	310 (590)
Spreadable EPG B168	220 (428)	310 (590)
Spreadable EPG B169	226 (439)	320 (608)
Canola oil	236 (457)	326 (619)
Coconut oil	177 (351)	295 (563)
Cottonseed oil	216 (421)	319 (606)
Corn oil	236 (457)	325 (617)
Peanut oil	231 (448)	334 (633)
Palm oil	230 (446)	324 (615)
Soybean oil	241 (466)	330 (626)

EPG = esterified propoxylated glycerol.

* Determined by AOCS Cc 9a-48 method, Cleveland Open Cup

In conclusion, spreadable EPGs are significantly more resistant to oxidation compared to edible fats and oils as measured by OSI, and comparable to edible fats and oils in thermal stability as measured by Smoke and Flash points.

2.4.3.3 Frying Studies

Deep-fat frying produces known changes to the quality of the oil by hydrolysis, oxidation, and polymerization. Hydrolysis increases the amount of free fatty acids, mono- and diacylglycerols, and glycerol in oils. Oxidation produces hydroperoxides and then low molecular volatile compounds such as aldehydes, ketones, carboxylic acids, and short chain alkanes and alkenes. Dimers and polymers are also formed in oil by radical and Diels-Alder reactions during deep-fat frying. The physical conditions of heating such as temperature, length of heating time, oil surface area, and more importantly, the chemical and physical characteristics of the oil all determine the type of degradation products and the amount of oxidation occurring. In addition, replenishment of fresh oil, frying conditions, quality of frying oil, food materials, fryer, antioxidants, and oxygen concentration affect the quality and flavor of oil during deep-fat frying.

Exploratory studies of the stability of earlier versions of EPG are available in the published scientific literature. It is notable that these early investigations examined an EPG with a higher degree of propoxylation (8) and therefore more liquid than the current commercial versions (5).

Artz *et al.* (1999) compared the free fatty acid value, the *p*-anisidine value, and degree of polymerization of 2 oil samples (EPG-00 soyate [transesterified soybean oil] and soy oil esterified propoxylated glycerol [EPG-08 soyate]) after heating at approximately 190°C. Heating continued for 12 hours/day until the polymer content of the oil reached 20%. While the EPG-00 soyate sample reached a polymer content of >20% after 48 hours, the EPG-08 soyate required only 36 hours. After 48 hours, the free fatty acid value (FFA) of the EPG-00 soyate increased from 0.19 to 0.79, the acid value (AV) increased from 0.10 to 1.59, and the *p*-anisidine value (p-AV) increased from 1.6 to 195.4. Similar values were seen with the EPG-08 soyate at 36 hours: FFA, AV, and p-AV increased from 0.19 to 0.71, from 0.26 to 1.36, and from 1.1 to 191.7, respectively. The triacylglycerol substrate degradation rate for EPG-00 soyate was $k = 0.0126 \pm 0.0003\ h^{-1}$, while the rate for EPG-08 soyate was $k = 0.0166 \pm 0.0017\ h^{-1}$. The color intensity, dielectric constant, and ultra violet (UV) absorbance, other markers of hydrolysis and thermal

oxidative generation, were also greater for the EPG-08 soyate sample than for the EPG-00 soyate sample. These results suggested that the EPG-00 soyate was slightly more stable than EPG-08 soyate under the heating conditions of this study.

Mahungu *et al.* (1999) evaluated the decomposition products of EPG-08 linoleate produced during heating at deep-fat frying temperatures using static headspace and capillary gas chromatography/infrared spectroscopy-mass spectrometry. Approximately 4 L of EPG-08 linoleate or was heated in a deep-fat fryer at 192 ± 8°C for 12 hours each day until the oil sample contained >20% polymeric material, which occurred after 24 hours of heating. The major volatile compounds both in heated EPG-08 linoleate included pentane, hexanal, 2-heptenal, 1-octen-3-ol, 2-pentylfuran, 2-octenal, and 2,4-decadienal. These compounds are those generally expected from the oxidative and thermal decomposition of the 9- and 13-hydroperoxides of linoleate, except acetoxyacetone (1-acetoxy-2-propanone), which was found at 2.1, 3, and 2.4 ppm in the unheated, 12-hours heated, and 24-hours heated samples, respectively. The absence of unusual volatiles from the oxidative decomposition of EPG-08 linoleate (except for relatively low concentrations of acetoxyacetone) suggested that the high rate of reactivity of linoleate may limit the participation of the EPG polyol backbone in volatile compound formation in EPG oil samples that contain even modest amounts of linoleic or more unsaturated fatty acids.

Part 3. §170.235 Dietary Exposure

3.1 History of Use in Food

EPG has been the subject of 2 previous GRAS Notifications, both of which have received letters of 'no objection' from FDA. GRN 583 detailed the use of EPG as a fat replacer at up to 34.5% in confectionary coatings used in baked goods and baking mixes (biscuit items, bakery items and ice-cream inclusions), frozen dairy desserts and mixes (ice cream novelties), grain products and pasta (snack and meal replacement bars), hard candy and cough drops (panned confectionary items, sugar-shelled confectionary pieces), snack foods (snack items, trail mix) and soft candy (molded and hollow molded confectionery pieces) (U.S. FDA, 2015). In GRN 640, the use of EPG as a fat replacer at levels up to 38 percent by weight in baked goods and baking mixes, frozen dairy desserts and mixes, grain products and pasta, gravies and sauces, nuts and nut products, and soft candy was described (U.S. FDA, 2016). Cumulatively, these uses resulted in mean and 90th percentile all-user exposures of 11.06 and 24.11 g/day, respectively.

3.2 Intended Use of EPG and Levels of Use in Foods

In order to determine exposure to EPG from the proposed commercial frying applications, ChocoFinesse analyzed EPG absorption in french fries and doughnuts. Control frying was done in high-oleic sunflower oil (HOS) while test frying was performed using either a 60:40 blend of spreadable EPG with HOS in the case of donuts or an 80:20 EPG-HOS blend in the case of french fries. EPG absorption was determined by analyzing the fatty acid composition of oils extracted from these foods.

Calculations of EPG absorption in french fries and doughnuts is shown in Tables 3.2-1 through 3.2-4. As data in Table 3.2-1, the total content of EPG in a medium serving of french fries is 9.46 g (8.1% by weight). This value is very similar to a theoretical 9.9 g EPG in medium serving of McDonald's fries. Theoretical exposure to EPG in french fries ranges from 2.5 g in the kids serving to 12.3 g in the large

serving size. In experimental frying of frozen doughnuts, the content of EPG is 3.6 g in a 49.1 g doughnut. This value is well within the theoretical range calculated for doughnuts of various sizes. EPG absorbed from the EPG-HOS frying blend equals 1.5 g in a mini-doughnut, 5.4 g in a medium doughnut, and 7.4 g in a large doughnut. Of interest is the exchange of moisture in raw substrates for oil absorbed during frying. In french fries, for each 3.2 to 3.4 g of water loss during frying, 1g of fat is absorbed. In doughnuts, for each 1g of water lost, 1.07 to 1.09 g fat is absorbed. This explains significant weight reduction in finished fries and slight weight gain in doughnuts.

Table 3.2-1 EPG Absorption in French Fries

Product	Component	Content in product	Calculations	Reference
Frozen, blanched french fries* Starting batch = 200.5 g	Moisture, %	70.1	(70.1 x 200.5)/100	1
	Moisture, g/batch	140.5		3,4,5
	Fat, %	4.1		1
	Fat in 200.5 g raw fries, g/batch	8.2	(4.1 x 200.5)/100	3,5
	Carbohydrates + protein, %	25.8	(100-4.1-70.1)	3
	Carbohydrates + protein, g/batch	51.7	(200.5 x 25.8)/100	3
Control Fries in 100% high-oleic sunflower oil (HOS) Fried, finished batch	Moisture %	47.8		4
	Moisture in finished batch, g	67.4	(47.8 x 141)/100	3,5
	Fat in raw, blanched fries, g	8.2		3,5
	Total fat after frying %	15.3		3,5
	Total fat in finished batch, g	21.6	(15.3 x 141)/100	3
	Fat absorbed during frying, g	13.4	(21.6-8.2)	3
	EPG content, %	**0**		
Test fries cooked in EPG-HOS, 80:20 blend Fried, finished batch = 141 g	Moisture, %	46.9		4
	Moisture, g	66.1	(46.9x141)/100	3,4,5
	Fat in raw, blanched fries, g	8.2		1,3,5
	Total fat after frying, %	15.9		1
	Total fat in finished batch, g	22.4	(15.9x141)/100	3,5
	Fat absorbed during frying, g	14.2	(22.4-8.2)	3,4,5
	EPG content, g (141 g batch)	11.4	(14.2 x 0.8)	3,4
	EPG content in medium serving of McDonald's fries, g Serving size = 117 g	**9.46**	(11.4/141) x 117	3 2

EPG = esterified propoxylated glycerol; HOS = high-oleic sunflower oil.

* Ore-Ida Golden Fries.

1. AOCS Ca 2d-25. Stratas analytical data
2. Nutrition Facts. Product label
3. Calculations
4. A study of the use of Epogee™-05-S as a frying medium for french fries and doughnuts. Tetramer, 5/1/2017.
5. A. Millhouse, e-mail 4/14/2017. Mass balance calculations.

Table 3.2-2 Theoretical EPG Exposure for French Fries Fried in the EPG-HOS (80:20) Blend)*

Specifics	Large	Medium	Small	Kids	References
Weight of raw, frozen fries with 70.1% moisture, g/serving	205.8	156.3	94.8	41.4	1
Weight of finished fries, g/serving	**154**	**117**	**71**	**31**	[a]
Total dry substance, g/serving	97.6	74.1	45	19.6	2
Total moisture of finished fries, g/serving	56.4	42.9	26.0	11.4	[a]
Moisture in finished fries, %	36.6	36.6	36.6	36.6	[a]
Total fat in finished fries, %	15.5	15.5	15.5	15.5	[a]
Total fat in finished fries, g/serving	23.9	18.2	11.0	4.8	[a]
Fat (4.1%) from raw, frozen fries, g/serving	8.4	5.8	3.9	1.7	3
Fat absorbed from frying medium, g/serving	15.4	12.4	7.1	3.1	4
EPG content, g/serving	**12.3**	**9.9**	**5.7**	**2.5**	5

EPG = esterified propoxylated glycerol; HOS = high-oleic sunflower oil.

* USDA National Nutrition Database for Standard Reference. Release 28 slightly revised May 2016. Basic Report 21238, McDonald's french fries. Report Date: May 10, 2017 (USDA, 2017)

1. Calculation: Raw, frozen french fries total moisture = (g moisture in finished fries x % moisture in raw, frozen fries) / % moisture in finished fries.
   Example: For Large serving fries, total moisture = (56.4 g x 70.1%)/36.6% = 108.2g
   Total raw fries weight = 108.2g (moisture) + 97.6g (dry substance) = 205.8g
2. By difference: dry substance, g = (total weight, g – total moisture, g)
3. Calculation: g fat from raw, frozen fries = (4.1% x weight of frozen fries)/100%
   Example for Large raw, frozen fires: fat, g = (4.1% x 205.8g) / 100% = 8.4g
4. By difference: Absorbed fat from frying = fat in finished fries – fat in raw fires
   Example for Large fires: (23.9g – 8.4g) = 15.5g
5. Calculation: EPG absorbed from frying EPG:HOS blend = total absorbed fat x 0.8

Table 3.2-3 EPG Absorption in Doughnuts

Product	Component	Content in product	Calculations	Reference
Frozen doughnut dough* 48 g/doughnut	Moisture, %	37.4		1,3
	Moisture in doughnut dough, g/serving	18.3	(37.4x49)/100	5
	Fat in doughnut dough, %	7.1		1
	Fat in doughnut dough, g/serving	3.4	(7.1x48g/100)	4,5
	Carbohydrates + protein in dough, %	55.5	(100-37.4-7.1)	
	Carbohydrates + protein in dough, g	26.6	(48x 55.5)/100	
Control doughnuts in 100% high-oleic sunflower oil (HOS) 48g/doughnut	Moisture in finished doughnut, %	28.6		4
	Moisture in finished doughnut, g/serving	14.2	(28.6x49.8)/100	5
	Total fat in finished doughnut, %	19.5		4
	Fat in finished doughnut, g/serving	9.7	(19.5x49.8)/100	3,4
	Fat absorbed during frying, g/serving	6.3	9.7g-3.4g=6.3g	5
	EPG content, g/serving	**0**		
Test doughnuts fried in EPG-HOS, 60:40 blend Fried, finished 49.1 g/doughnut	Moisture in finished doughnut, %	29.1		1,3
	Moisture in finished doughnut, g/serving	14.3	(29.1x49.1)/100	5
	Total fat in finished doughnut, %	19.2		4
	Fat in finished doughnut, g/serving	9.4	(19.2x49.1)/100	5
	Fat absorbed during frying, g/serving	6.0	(9.4 g-3.4g) = 6.0g	5
	EPG content, g/serving	**3.6**	(6.0 x 0.06) = 3.6 g	5

EPG = esterified propoxylated glycerol; HOS = high-oleic sunflower oil.

* Rich's Rich Classic Raised Ring Yeast Doughnut Dough, 49g, 1.75 Ounce (Mfg. #3877)

1. AOCS Ca 2d-25
2. Nutrition Facts
3. A study on the use of EpogeeTM-050S as a frying medium for french fries and doughnuts, Tetramer, 5/1/2017
4. A. Millhouse, email 4/14/2017 Mass balance calculations
5. Calculations

Table 3.2-4 Theoretical EPG Exposure for Doughnuts Fried in the EPG-HOS (60:40) Blend)*

Specifics	Large doughnut, 4" diameter	Medium doughnut, 3-1/4" diameter	Mini doughnut, 1-1/2" diameter	Reference
Weight of raw, frozen dough for 1 doughnut, g	69.51	52.94	13.76	3
Weight of finished doughnut, g/serving	71	54	14	[a]
Moisture in frozen dough, %	37.4	37.4	37.4	1
Moisture in frozen dough, g/doughnut	26.55	20.19	5.23	2
Moisture in finished doughnut, %	20.82	20.82	20.82	[a]
Total moisture in finished doughnut, g/serving	14.78	11.24	2.91	[a]
Total fat in finished doughnut, %	24.93	24.93	24.93	[a]
Total fat in finished doughnut, g/serving	17.70	13.46	3.49	[a]
Fat from doughnut dough, g/serving	4.9	3.8	1.0	4
Fat absorbed during frying, g/serving	12.8	9.66	2.49	5
EPG content, g/serving	**7.7**	**5.8**	**1.5**	6

EPG = esterified propoxylated glycerol; HOS = high-oleic sunflower oil.

* USDA National Nutrition Database for Standard Reference. Release 28 slightly revised May 206. Basic Report 18248, Doughnuts, cake-type, plain (includes unsugared, old-fashioned). Report Date: May 10, 2017 (USDA, 2017).

1. AOCS Ca 2d-25
2. Calculation: Moisture in frozen doughnut dough, g = (moisture in finished doughnut, g x moisture in frozen dough, %)/moisture in finished doughnut, %
   Example: Large doughnut dough moisture, g = (14.78 x 37.4)/20.82 = 26.55 g
3. Contents of carbohydrates and proteins are not affected by frying and represent constant percentage of raw or fried doughnuts. Therefore, the weight of raw dough needed for finished doughnut can be calculated by adding moisture and fat in the raw dough plus carbohydrates and proteins in the finished doughnut.
4. Calculation: Fat in doughnut dough, g = (fat in dough, % x weight of frozen dough, g)/100
   Example: Fat in a large doughnut dough, g = (7.1x69.51)/100 = 4.9g
5. Calculation: Fat absorbed during frying, g = (fat in finished doughnuts, g – fat in doughnut dough, g). For a large doughnut, absorbed fat = (17.70-4.9) = 12.8g.
6. Calculation: EPG content, g/serving = fat absorbed during frying x. 0.6. For large doughnut, EPG content, g/serving = 12.8 x 0.6 = 7.15 g

ChocoFinesse also examined the possible leaching of fat from the french fries or doughnuts into the fryer oil by comparing the fatty acid profile of frying oils after 10 frying cycles frying. This analysis, presented in Table 3.2-5, demonstrates no difference in fatty acid composition in the frying medium before frying and after the 10th frying cycle. These results suggest that any leaching of original oil from the substrates is negligible and does not change fry medium composition during multiple frying cycles.

Table 3.2-5 Fatty acid Composition of Frying Medium for Doughnuts and French Fries at 0 and 10th Fry

Fatty Acid	Doughnuts	Doughnuts	Doughnuts	Doughnuts	French Fries	French Fries	French Fries	French Fries
	Control 0 Fry	Control 10th Fry	EPG-blend 0 Fry	EPG-blend 10th Fry	Control 0 Fry	Control 10th Fry	EPG-blend 0 Fry	EPG-blend 10th Fry
Palmitic, C16:0	3.9	4.0	2.8	2.1	3.6	3.9	1.2	1.6
Stearic, C18:0	2.9	2.8	4.6	5.7	3.4	3.4	7.2	7.1
Oleic, C18:1	87.3	87.4	50.2	50.2	86.5	85.9	35.2	35.3
Linoleic, C18:2	5.0	5.0	3.8	2.9	4.7	5.5	2.1	2.9
Arachidic, C20:0	0.0	0.0	26.6	26.8	0.4	0.3	37.3	36.4
Behenic,C22:0	1.1	0.9	12.4	12.5	1.1	1.0	17.1	16.7

EPG = esterified propoxylated glycerol.

The fatty acid data shown above indicate remarkable agreement between the fresh frying medium (0 fry) and after the 10th fry cycle for both the doughnuts and french fries. The minor differences are most likely due to analysis at 2 different labs.

3.3 Estimated Consumption of EPG from All Intended Conditions of Use in Food

3.3.1 Dietary Intake Survey

Choco Finesse, LLC (Choco Finesse) intends to market EPG as an ingredient for use in commercial frying applications for french fries and doughnuts in the U.S. In order to estimate potential exposure to this component, an assessment was conducted based on the highest residual levels, as measured by Choco Finesse in both french fries and doughnuts, combined with food consumption data for these items by the U.S. population.

Food consumption data included in the U.S. National Center for Health Statistics' NHANES 2013-2014 (CDC, 2015, 2016; USDA, 2016). Calculations for the mean and 90th percentile *per capita* and consumer-only intakes were performed for all proposed food-uses of EPG and the percentage of consumers were determined. Similar calculations were used to estimate the intake of EPG resulting from each individual proposed food-use, including the calculations of percent consumers. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

Infants and Young children, up to and including 2 years;
Children, ages 3 to 11;
Female teenagers, ages 12 to 19;
Male teenagers, ages 12 to 19;
Female adults, ages 20 and up;
Male adults, ages 20 and up; and
Total population (all age and gender groups combined).

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of EPG by the U.S. population[1]. Estimates for the daily intake of EPG represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2013-2014; these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of EPG averaged over all individuals surveyed, regardless of whether they consumed food products that are intended to be fried in EPG, and therefore includes individuals with "zero" intakes (*i.e.*, those who reported no intake of food products that are intended to be fried in EPG during the 2 survey days). "Consumer-only" intake refers to the estimated intake of EPG by those individuals who reported consuming food products that are intended to be fried in EPG. Individuals were considered "consumers" if they reported consumption of 1 or more food products that are intended to be fried in EPG on either Day 1 or Day 2 of the survey.

Mean and 90th percentile intake estimates based on sample sizes of less than 30 and 80, respectively, may not be considered statistically reliable due to the limited sampling size (CDC, 2013). As such, the reliability of estimates for the intake of EPG based on consumption estimates derived from individual population groups of a limited sample size should be interpreted with caution. These values are marked with an asterisk in the relevant data tables. The full intake report is available in Appendix 3.

3.3.2 Estimated Consumption of EPG from Proposed Food-Uses

Table 3.3.2-1 summarizes the estimated total intake of residual EPG (g/person/day) in different U.S. population groups based on the intended frying applications. Table 3.3.2-2 presents this data on a per kilogram body weight basis (mg/kg body weight/day). The percentage of users of foods intended to be fried in EPG was low among all age groups evaluated in the current intakes assessment, wherein 18.1 to 32.6% of the population groups consisted of consumers of food products that are intended to be fried in EPG (Table 3.3.2-1). The consumer-only intakes are more applicable to the assessment of safety as they are more likely to represent exposure in the target populations. Consequently, only the consumer-only intake results will be discussed in detail below.

Among the total population (all ages), the mean and 90th percentile consumer-only intakes of EPG were determined to be 4.0 and 7.6 g/person/day, respectively. Of the individual population groups, female teenagers, male teenagers and male adults were determined to have the greatest mean consumer-only intakes of EPG on an absolute basis of 4.7 g/person/day, and female teenagers were determined to have the greatest 90th percentile intakes of 11.9 g/person/day. Infants and young children had the lowest mean and 90th percentile consumer-only intakes of 2.1 and 4.6 g/person/day, respectively (Table 3.3.2-1).

[1] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2016). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

Table 3.3.2-1 Summary of the Estimated Daily Intake of Residual EPG from Intended Frying Applications in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 3	0.4	1.3	18.1	121	2.1	4.6
Children	4 to 11	0.8	3.4	25.6	351	3.2	5.3
Female Teenagers	12 to 19	1.2	3.5	25.5	165	4.7	11.9
Male Teenagers	12 to 19	1.5	5.3	32.6	168	4.7	8.7
Female Adults	20 and up	0.8	3.4	22.4	531	3.6	6.7
Male Adults	20 and up	1.2	4.6	24.3	512	4.7	9.8
Total Population	All Ages	1.0	3.5	23.9	1,848	4.0	7.6

EPG = esterified propoxylated glycerol; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

On a body weight basis, the total population (all ages) mean and 90th percentile consumer-only intakes of EPG were determined to be 64 and 130 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 154 and 348 mg/kg body weight/day, respectively (Table 4.1-2). Female adults had the lowest mean and 90th percentile consumer-only intakes of 46 and 83 mg/kg body weight/day, respectively.

Table 3.3.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Residual EPG from Intended Frying Applications in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 3	28	105	18.2	121	154	348
Children	4 to 11	27	111	25.3	349	108	205
Female Teenagers	12 to 19	21	64	25.6	163	83	161
Male Teenagers	12 to 19	22	79	32.8	167	67	143
Female Adults	20 and up	10	43	22.5	530	46	83
Male Adults	20 and up	14	52	24.5	512	56	106
Total Population	All Ages	15	55	24.0	1,842	64	130

bw = body weight; EPG = esterified propoxylated glycerol; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

In summary, on consumer-only basis, the resulting mean and 90th percentile intakes of EPG by the total U.S. population from all intended frying applications were estimated to be 4.0 g/person/day (64 mg/kg body weight/day) and 7.6 g/person/day (130 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean consumer-only intakes of EPG were determined to be 4.7 g/person/day in female teenagers, male teenagers and male adults (83, 67 and 56 mg/kg body weight/day, respectively), and the highest 90th percentile intakes were determined to be 11.9 g/person/day (161 mg/kg body weight/day) in female teenagers. Infants and young children had

the lowest mean and 90th percentile consumer-only intakes of 2.1 g/person/day (154 mg/kg body weight/day) and 4.6 g/person/day (348 mg/kg body weight/day), respectively.

When intakes were expressed on a body weight basis, infants and young children had the highest mean and 90th percentile consumer-only intakes of 154 and 348 mg/kg body weight/day. Female adults had the lowest mean and 90th percentile consumer-only intakes of 46 and 83 mg/kg body weight/day, respectively.

3.3.3 Cumulative Intakes

Among the total population (all ages), the addition of EPG in frying applications resulted in mean and 90th percentile consumer-only intakes of EPG of 11.9 and 25.0 g/person/day, respectively; increased from 11.1 and 24.1 g/person/day, respectively, as calculated in the 2016 assessment. Of the individual population groups, male teenagers were determined to have the greatest mean and 90th percentile consumer-only cumulative intakes of EPG on an absolute basis, at 13.3 and 30.3 g/person/day, respectively; this age group was noted to have the highest increase in mean and P90 intakes from the current proposed use in frying applications of 1.6 and 5.9 g/day, respectively. Infants and young children were determined to have the lowest mean and 90th percentile consumer-only cumulative intakes of 8.8 and 18.9 g/person/day, respectively. Overall, the inclusion of EPG in frying applications resulted in an increase of between 0.4 and 1.6 g/day at the mean and between 0.5 and 5.9 mg/day at the 90th percentile of intake.

On a body weight basis, the addition of EPG in frying applications increased the total population (all ages) mean and 90th percentile consumer-only intakes of EPG to 216 and 486 mg/kg body weight/day, respectively; these intakes were previously 203 and 469 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90th percentile consumer-only cumulative intakes of any population group, of 632 and 1,355 mg/kg body weight/day, respectively. Female adults were identified as having the lowest mean consumer-only cumulative intakes of 153 mg/kg body weight/day, whereas male adults were identified as having the lowest 90th percentile consumer-only cumulative intakes of 320 mg/kg body weight/day. Overall, the inclusion of EPG in commercial applications resulted in increases of between 7 and 34 mg/kg body weight/day (mean) and between 11 and 71 mg/kg body weight/day (P90).

Part 4. §170.240 Self-Limiting Levels of Use

Use of EPG in french fries and donuts will be limited by the amount absorbed from the frying oils.

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable

Part 6. §170.250 Narrative and Safety Information

6.1 Introduction

Preclinical studies were conducted with H-EPG-05 HR/SO 9:1 (Mettler dropping point 106.9°F) unless otherwise stated. EPG-05 HR/ST 45:55 (Mettler dropping point 104.3°F) is a somewhat softer version at average normal body temperature and was selectively investigated in safety studies. It is worthwhile to note that, unlike olestra, EPG is not strongly hydrophobic and exhibits far less interaction with fat-soluble substances including fat soluble vitamins. As such, vitamin fortification of animal diets was not required in any of the EPG preclinical safety studies including lifetime studies in rats and mice as well as up to 3 generations in reproductive and development studies. This differed from studies conducted with olestra, which required vitamin fortification. It is also important to note that residues of EPG were not found in any tissues from any animals placed on study, indicating efficient clearance and absence of accumulation even following lifetime administration.

A battery of preclinical feeding studies was initiated to assess the safety of the core compound, H-EPG-05 HR/SO 9:1, including carcinogenic activity and the potential to cause developmental anomalies in several animal species. In addition, a series of mutagenicity studies were conducted with H-EPG-05 HR/SO 9:1, as well as other EPG versions (*e.g*., H-EPG-05 soyate and H-EPG-14 soyate). It is worthwhile to note that, unlike olestra, EPG is not strongly hydrophobic and exhibits far less interaction with fat-soluble substances including fat soluble vitamins. As such, vitamin fortification of animal diets was not required in any of the EPG preclinical safety studies including lifetime studies in rats and mice as well as up to 3 generations in reproductive and development studies. This differed from studies conducted with olestra, which required vitamin fortification. It is also important to note that residues of EPG were not found in any tissues from any animals placed on study, indicating efficient clearance and absence of accumulation even following lifetime administration. In addition, the stability and homogeneity of prepared diets for the safety studies was established.

The preclinical studies showed no adverse treatment related changes to the general health and appearance of the animals, or on the conventional parameters measured including but not limited to: growth, feed consumption, body weight, clinical chemistry, hematology, reproductive performance and fetal development. Feeding EPG to rats, mice, rabbits, dogs and micro-pigs produced no observed adverse findings in the gastrointestinal (GI) tract structure or function. Minor fluctuations in fat soluble vitamin status were evident in preclinical studies, however, the concentrations of fat soluble vitamins in the liver (*e.g*., vitamins A and E) and serum (*e.g*., vitamin D) remained within the historical limits of species traditionally used in animal studies involving lifetime dietary exposures. Refer to Section 7.2 for additional information concerning the effect of EPG on fat-soluble vitamin status.

Articles for the following preclinical studies have been published in Regulatory Toxicology and Pharmacology: (i) mutagenicity assays; (ii) 90-Day Dietary Safety Study with Esterified Propoxylated Glycerol (EPG) in Rats; (iii) 90-Day Safety Study of Esterified Propoxylated Glycerol (EPG) Administered in the Feed to Yucatan Micro-Pigs® (SUS SCROFA); (iv) One Generation Reproduction Study of Esterified Propoxylated Glycerol (EPG) Administered in the Feed to CD® (Sprague-Dawley) Rats; and (v) Developmental Toxicity Evaluation of Esterified Propoxylated Glycerol (H-EPG-05 HR/SO [9:1]) Administered in the Diet to New Zealand White Rabbits. Additional unpublished studies (*i.e*., subchronic toxicity studies in mice and beagle dogs, 2-year combined chronic dietary safety study and carcinogenicity studies in rats and mice, a 1-year chronic safety studies in beagle dogs and micropigs, a

3-generation reproduction study [with a teratology phase] in rats, and irritation and sensitization) provide corroborative evidence of safety.

6.2 Literature Search

An updated search of the literature was performed through November of 2017. No additional studies relevant to the toxicity of EPG were identified.

6.3 Absorption, Distribution, Metabolism, and Excretion

The pharmacokinetics of 2 radiolabeled EPG versions (H-EPG-08 oleate [a semi-solid] and H-EPG-14 oleate [a liquid]) were evaluated in male and female Crl:CD®BR rats to determine the absorption, distribution, metabolism and excretion (ADME) profile of these materials. Two separate studies were conducted with H-EPG-08 oleate; one in which the material was ^{14}C-radiolabeled on the C_1-carbon of the propylene glycol units (Section 5.1.2.1), and a second in which it was ^{14}C-radiolabeled on the carboxyl carbon of the fatty acid portion (Section 5.1.2.2). In the latter study, thin-layer chromatography (TLC) was used to confirm the presence ^{14}C-oleic acid in liver tissue extracts, as incorporation of the radiolabeled fatty acid into tissues was expected. Finally, 1 study was conducted with H-EPG-14 oleate radiolabeled on the C_1-carbon of the propylene glycol units (Section 5.1.2.3).

In each study, 5 rats/sex/group were administered a single oral dose of 1.0 g/kg bw or 3.0 g/kg body weight by gavage. A third dose group was given 1.0 g/kg body weight of the radiolabeled version after 2 weeks of daily EPG administration of the non-labeled version at the same dose. In addition, to simulate the worst-case scenario of complete absorption of the EPGs, ADME studies were conducted on rats receiving a dose of 35 mg/kg of each version intravenously in a liposome suspension. Expired air, feces, urine, organs, tissue samples and the carcass were monitored for radioactivity for up to 1 week after EPG administration when, essentially, there was complete recovery of the dose administered.

The results of these studies indicate that the 2 EPG versions evaluated were poorly absorbed from the GI tract and could not be found intact in any tissues after oral dosing. EPG-08 oleate was degraded approximately 20%, while EPG-14 oleate was degraded by approximately 10%. There was some evidence that possible bacterial degradation in the GI tract was taking place, particularly in the colon. The pattern of distribution of the radiolabel observed in the body of the rats was consistent with GI absorption of fatty acids and the propylene glycol units modified glycerol, both of which were partially oxidized to carbon dioxide. A significant portion of the fatty acids absorbed were incorporated into triglycerides and stored in adipose tissue.

In the 2 studies where the propoxylated glycerol units were radiolabeled, small amounts of radiolabel were detected in the liver and other metabolically active tissues, indicating that a small portion of this material was assimilated into normal body constituents during the oxidation process. This absorption, disposition, metabolism and excretion pattern for EPG was considered predictable and similar to that which would be expected from normal triglycerides. When given intravenously in fine liposome emulsion, the 2 versions of EPGs tested were rapidly oxidized to fatty acids and glycerol containing propoxylated glycerol units. The disposition pattern was similar to that *via* the oral route, except that larger portions of the metabolites of the EPGs were deposited in the liver and lungs. The route by which the metabolites of the various versions of EPGs were excreted appeared to be governed by their molecular weights. The greater the molecular weight, the more of the metabolites of the EPGs excreted into the feces, and the less into the urine.

More detailed summaries of the ADME studies are provided in Section 6.3.1.

6.3.1 ADME Studies

6.3.1.1 Metabolism and Disposition of EPG-08 Oleate in Rats (COVANCE 6226-104; E-012)

The metabolism and disposition of oxypropylene -^{14}C-EPG-08 oleate were studied in Crl:CD®BR rats. Forty-four animals (22/sex) were divided into 5 groups and exposed to oxypropylene-^{14}C-EPG-08 oleate by gavage or intravenously. A preliminary group was exposed to a single dose of 1,000 mg/kg, and used to assess the potential for excretion of radioactivity in expired air. Since approximately 2 to 8% of oxypropylene -^{14}C-EPG-08 oleate was recovered in the expired air of rats in the preliminary group, expired air was also collected from the rats in the remaining groups. The remaining 4 groups were administered a single intravenous dose (30 mg/kg), a single oral dose (1,000 or 3,000 mg/kg), or 1,000 mg/kg of non-radiolabeled EPG-08 oleate for 14 days followed by a single radiolabeled, oral administration on the 15th day. Mean recovery of radioactivity in this study is summarized in Table 6.3.1.1-1.

Excretion of oxypropylene -^{14}C-EPG-08 oleate was measured in the feces, urine, and expired air (presumably as CO_2). Oxypropylene -^{14}C-EPG-08 oleate *via* intravenous (i.v.), was excreted predominantly in the urine with 49.9 and 59.9% of the test material excreted by males and females, respectively. Recovery of radioactivity in expired air averaged 29.9 and 19% for males and females, respectively. Recovery of test material in the feces was 9.09 and 9.73% for males and females, respectively. In contrast, the fecal route was the predominant route of excretion following oral administration of oxypropylene -^{14}C-EPG-08 oleate, demonstrated by 66.1 to 84.2% of recovered test material in feces. Recovery of radiolabeled test material in urine ranged from 7.96 to 14.3%, while recovery in expired air ranged from 2.87 to 8.11%.

Rats were sacrificed 7 days after the administration of the radiolabeled dose and various tissues[2] were collected and analyzed for total radioactivity. Rats exposed intravenously had the highest concentrations of radioactivity in the spleen, liver, lungs, kidneys, and bone. Approximately 6% of the administered substance was recovered from the tissues and carcasses of these animals. Rats exposed orally had the highest concentrations of radioactivity in the liver, kidneys, spleen, lungs, and stomach. However, tissues and carcasses of these rats accounted for less than 0.5% of the administered test material.

In conclusion, oxypropylene-^{14}C-EPG-08 oleate was poorly absorbed *via* the oral route in rats. Administration of oxypropylene -^{14}C-EPG-08 oleate *via* i.v. results in de-esterification of the material to form diester, monoester, and base polyol. The polyol may be further metabolized to products that are excreted in urine, incorporated into endogenous components, or eliminated as CO_2.

[2] Tissues included bone (femur), brain, fat, heart, kidneys, large intestine, liver, lungs, muscle (thigh), ovaries, small intestine, spleen, stomach, tail, testes and uterus.

Table 6.3.1.1-1 Mean Recovery (%) of Radioactivity from ^{14}C-EPG-08 Oleate in Rats (5/group) After 7 Days

Route	Dosage (mg/kg bw)	Males								Females							
		Cage wash*	CO_2†	Feces	Urine	Volatiles	Carcass	Tissues	Total	Cage wash*	CO_2†	Feces	Urine	Volatiles	Carcass	Tissues	Total
Intravenous (single dose)	30	0.29	29.9	9.09	49.9	<0.01	1.85	4.38	95.4	0.79	19.0	9.73	59.9	<0.01	1.28	4.62	95.3
Oral (single dose)	1,000	0.63	7.11	74.5	11.6	<0.01	0.52	0.12	94.5	1.17	3.85	78.9	11.4	<0.01	0.27	0.05	95.7
Oral (multiple dose; radiolabeled EPG on Day 15)	1,000	3.21	8.11	66.1	14.3	<0.01	0.66	0.18	92.6	0.31	3.32	78.5	10.4	<0.01	0.22	0.04	92.8
Oral (single dose)	3,000	1.16	7.19	74.7	11.0	<0.01	0.61	0.15	94.8	0.22	2.87	84.2	7.96	<0.01	0.17	0.04	95.4

bw = body weight; EPG = esterified propoxylated glycerol.

* Includes cage wash/wipe with 1% trisodium phosphate and hexane

† Ethoxyethanol:ethanolamine trap; includes backup.

6.3.1.2 Metabolism and Disposition of EPG-08 [14C] – Oleate in Rats (HWI 6226-108; E-013)

The purpose of this study was to assess the bioavailability and extent of absorption, distribution, elimination, and biotransformation of EPG-08 [^{14}C] - oleate administered orally to Crl:CD®BR rats (131 to 182 g). Forty treated animals were divided into 4 groups of 10 animals (5/sex) as follows: single i.v. low dose (30 mg/kg), single oral low dose (1,000 mg/kg), multiple oral low dose (1,000 mg/kg; 14 daily nonradiolabeled doses followed by a single radiolabeled dose on the 15th day), and single oral high dose group (3,000 mg/kg). All groups had urine, feces, expired carbon dioxide, and organic volatiles collected. The animals were sacrificed 7 days after the administration of the radiolabeled dose and various tissues[3] were collected and analyzed for total radioactivity. Mean recovery of radioactivity in this study is summarized in Table 6.3.1.2-1.

Most of the radioactivity was found in the feces in all oral dose groups with values ranging from 77.1 to 83.3% of the dose. The next most important route of elimination was expiration of CO_2, representing 5.38 to 10.4% of the dose. The least significant route of elimination was excretion in urine with values ranging from 0.24 to 0.52% of the dose. In contrast, following i.v. administration expiration as CO_2 was the predominant route of excretion representing 47.4 and 56.9% of the dose for males and females, respectively. Excretion in urine was minor with 1.30 and 1.64% of the dose excreted by males and females, respectively. Similarly, feces contained 1.42 and 1.54% of the dose for males and females, respectively. The remainder of the radioactivity was not excreted by the 7-day sacrifice time. The highest percentage of the unexcreted dose was recovered in the residual carcass for both males (26.4%) and females (15.9%). Of the discrete tissues collected, the liver (7.62% of the dose in males and 9.12% of the dose in females), tail (2.23% of the dose in males and 1.57% of the dose in females), and fat (2.27% of the dose in males and 1.25% of the dose in females) contained the most radioactivity. Material balance was high for all dose groups, ranging from 89.6 to 96.5%.

With respect to concentrations of radioactivity in the carcass and tissues following oral administration, the highest percent of dose was recovered in the carcass (1.42 to 5.31%). Tissues accounted for 0.17 to 0.60% of the radioactivity recovered, with the fat, large intestine, small intestine, stomach, lungs, ovaries, and uterus containing the highest concentrations of all the tissues examined. The radioactivity in the liver and lung tissue of the oral dose groups was believed to most likely be the result of absorption of oleic acid or oleic acid fragments resulting from chemical hydrolysis or enzymatic attack of the oxypropylene linkage in the digestive tract. In order to substantiate this hypothesis, the lipid components of tissues were extracted and the remaining residues were subjected to protease digestion. The resulting extracts were analyzed by liquid scintillation counting and by TLC to characterize the ^{14}C-components. The presence of ^{14}C-oleic acid was confirmed by TLC profiling of liver tissue extracts from all dose groups. The distribution of radioactivity in tissue components appeared to be due to the incorporation of oleic acid. With respect to the i.v. dose, the tissues that contained the highest concentrations of radioactivity were fat, spleen, liver, lungs, and carcass.

[3] Tissues included bone (femur), brain, fat, heart, kidneys, large intestine, liver, lungs, muscle (thigh), ovaries, small intestine, spleen, stomach, tail, testes and uterus.

Table 6.3.1.2-1 Mean Recovery (%) of Radioactivity from EPG-08 14C-Oleate in Rats (5/group) After 7 Days

Route	Dosage (mg/kg bw)	Males								Females							
		Cage wash*	CO_2†	Feces	Urine	Volatiles	Carcass	Tissues	Total	Cage wash*	Feces	Urine	Urine	Volatiles	Carcass	Tissues	Total
Intravenous (single dose)	30	<0.01	47.4	1.42	1.30	0.03	26.4	14.5	91.1	0.03	56.9	1.54	1.64	0.05	15.9	14.9	90.9
Oral (single dose)	1,000	0.50	7.59	83.3	0.30	0.01	3.75	0.42	95.9	1.65	10.4	79.9	0.35	<0.01	3.55	0.59	96.5
Oral (multiple dose; radiolabeled EPG on Day 15)	1,000	1.68	5.38	78.3	0.52	<0.01	3.34	0.36	89.6	0.89	6.86	80.7	0.24	<0.01	1.42	0.17	90.3
Oral (single dose)	3,000	0.58	5.90	80.1	0.25	<0.01	5.31	0.60	92.7	1.26	9.92	77.1	0.35	0.01	2.84	0.52	92.0

bw = body weight; EPG = esterified propoxylated glycerol.

* Includes cage wash/wipe with 1% trisodium phosphate and hexane.

† Ethoxyethanol:ethanolamine trap; includes backup.

6.3.1.3 Metabolism and Disposition of EPG-14 Oleate in Rats (HWI 6226-109; E-014)

The purpose of this study was to assess the bioavailability and extent of absorption, distribution, elimination, and biotransformation of oxypropylene-^{14}C-EPG-14 oleate in Crl:CD®BR rats (110 to 189 g). Forty-four treated animals were divided into 5 groups. A preliminary group of 4 animals (2/sex) was dosed to determine whether expired air and organic volatiles needed to be collected from subsequent groups. The remaining 40 animals were divided into 4 groups of 10 animals (5/ sex): single i.v. low dose group at 30 mg/kg, single oral low dose group at 1,000 mg/kg, multiple oral low dose at 1,000 mg/kg (14 daily non-radiolabeled doses followed by a single radiolabeled dose on the 15th day), and single oral high dose group at 3,000 mg/kg. All groups had urine, feces, expired carbon dioxide (CO_2), and organic volatiles collected. The animals were sacrificed 7 days after the administration of the radiolabeled dose and various tissues[4] and blood were collected and analyzed for total radioactivity. Mean recovery of radioactivity in this study is summarized in Table 6.3.1.3-1.

The majority of the radioactivity was found in the feces in all oral dose groups, with values ranging from 79.7 to 89.4% of the dose. A relatively minor amount of the dose was excreted in the urine with values ranging from 1.73 to 6.80% of the dose. The other significant route of elimination was through CO_2, which represented from 3.11 to 7.15% of the dose. The excretion pattern after i.v. administration was different than that observed for the oral groups. Urine and CO_2 were the predominant means of excretion, with 34.4 and 37.3% of the dose excreted in urine, and 41.8 and 36.7% of the dose excreted as CO_2 by males and females, respectively. Feces represented 11.2 and 13.5% of the dose for males and females, respectively.

Tissue residue levels were in general very low after oral administration. The tissues that contained the highest concentrations of radioactivity in all dose groups were liver, kidney, spleen, and lungs. The majority of the dose after oral administration passed unabsorbed through the gastrointestinal tract and into the feces where it was recovered. Parent EPG-14 oleate was the major component of the feces radioactivity, although the mono-and diester were also found indicating that degradation of EPG's are occurring in the gastrointestinal tract. The highest mean percentage of radioactive dose recovered in tissues after i.v. administration was in the residual carcass for males 3.20% and in the liver for females 4.09%. For males, the liver contained the next highest mean percentage of dose with 2.71%. For females, the residual carcass contained the next highest mean percentage of dose with 2.36%.

[4] Tissues included bone (femur), brain, fat, heart, kidneys, large intestine, liver, lungs, muscle (thigh), ovaries, small intestine, spleen, stomach, tail, testes and uterus.

Table 6.3.1.3-1 Mean Recovery (%) of Radioactivity from ^{14}C-PGU-EPG-14 Oleate in Rats (5/group) After 7 Days

Route	Dosage (mg/kg bw)	Males								Females							
		Cage wash*	CO_2†	Feces	Urine	Volatiles	Carcass	Tissues	Total	Cage wash*	CO_2†	Feces	Urine	Volatiles	Carcass	Tissues	Total
Intravenous (single dose)	30	0.31	41.8	11.2	34.4	0.03	3.20	5.11	96.1	0.93	36.7	13.5	37.3	0.04	2.36	5.59	96.5
Oral (single dose)	1,000	0.28	3.11	89.4	1.73	<0.01	0.26	0.06	94.9	1.07	4.88	79.7	5.86	0.01	0.29	0.07	91.9
Oral (multiple dose; radiolabeled EPG on Day 15)	1,000	0.99	6.30	85.4	3.81	<0.01	0.60	0.18	97.3	1.23	7.15	84.0	4.89	0.01	0.46	0.11	97.8
Oral (single dose)	3,000	0.95	5.84	86.5	5.59	<0.01	0.69	0.12	99.7	1.79	5.42	85.8	6.80	<0.01	0.55	0.11	100

bw = body weight; EPG = esterified propoxylated glycerol.

* Includes cage wash/wipe with 1% trisodium phosphate and hexane.

† Ethoxyethanol:ethanolamine trap; includes backup.

6.4 Pivotal Toxicology Studies

The safety of EPG is based on published subchronic studies in rats and micropigs, a 1-generation reproductive toxicity study in rats, a developmental toxicity evaluation in rabbits, and genotoxicity studies of EPG.

6.4.1 Repeat-Dose Toxicity

6.4.1.1 90-Day Dietary Safety Study with Esterified Propoxylated Glycerol (EPG) in Rats (Christian and Bechtel, 2014)

The purpose of this study was to assess the subchronic toxicity of a representative version of EPG when given to Crl:CD®BRVAF/Plus® rats (Sprague-Dawley derived; approximately 36 days old; males weighed between 147 and 193 g, and females weighed between 116 and 162 g) by dietary admixture for at least 90 days. Rats (n=700) were randomly assigned to 5 groups (70 animals/sex/group, subdivided into subsets A through F for each sex) and administered concentration levels of 0, 0.5, 1.0, and 2.0 g EPG/kg of body weight/day (g/kg/day) through adjusted diets, or a fixed intake of 5.0% (w/w) in the diet. The latter is expected to result in a decrease in EPG intake over time; the result of feed consumption in g/day remaining relatively constant and the mean body weights increasing markedly over time so that mean feed intake in g/kg/day decreases markedly over time. All diets were prepared weekly and provided *ad libitum*.

Animals were housed individually in stainless steel, screen bottom cages, and were observed twice daily (a.m. and p.m.) for mortality, moribundity, and signs of toxicity. Body weights and food consumption were recorded weekly. Ophthalmic examinations were performed on all animals before study initiation and during Week 13 for animals given 0 g/kg and 5.0% (w/w) EPG. Hematology and clinical chemistry evaluations were done on 10 animals/sex/group before sacrifice at Weeks 5 (Subset A) and 14 (Subset C). Liver vitamin A (trans-retinol) and E (ex-tocopherol) and serum vitamin D level (25-0H vitamin D, total) determinations were done on 10 animals/sex/group during Weeks 5 (Subset A, animals necropsied) and 14 (Subset D, animals discarded without necropsy). Samples of liver, kidney, spleen, and adipose tissue were collected for EPG level determinations from 10 animals/sex killed and discarded without necropsy during Weeks 5 (Subset B) and 14 (Subset F); assays were done on the tissues from controls and animals given 5.0% (w/w) EPG. Ten animals/sex/group (Subset F) were housed in metabolism cages for the collection of fecal matter for analysis of EPG, EPG metabolites, cholesterol, fatty acids, and total bile acids. In addition, ten animals/sex/group were sacrificed and subjected to pathological evaluation at Weeks 5 (Subset A) and 14 (Subset C), and 20 additional animals/sex/group (Subset E) were sacrificed at Week 14 for pathological evaluation. At scheduled necropsies, animals were anesthetized, weighed, and exsanguinated. Macroscopic observations were recorded; selected organs were weighed; and selected tissues were preserved. Microscopic examinations were performed on all Subset A, C, and E animals in the control and 5.0% (w/w) EPG groups; on the gastrointestinal tract, lungs, liver, kidneys, and all macroscopic lesions from animals in the remaining groups; and on all animals (regardless of subset designation) that died or were sacrificed in a moribund condition.

Results of the study showed that dietary administration of EPG at levels of 0.5, 1.0, and 2.0 g/kg, or 5% (w/w) to rats for at least 13 weeks was not associated with any adverse effects. The levels of liver vitamins A and E and serum vitamin D were generally decreased in EPG-treated animals at all concentration levels. However, there was no evidence of vitamin deficiency as assessed by growth, clinical observations, clinical pathology or anatomical pathology endpoints. Prothrombin time (PT),

measured as an indicator of vitamin K status, was not significantly affected. Based on the results of this study, it was not possible to establish a no-observable-effect level (NOEL). The possible effect of EPG on vitamin levels in the absence of any clinical signs of deficiency was not considered "adverse" *per se*. As such, the adjusted concentration of 2 g/kg and the fixed intake of 5% EPG (equivalent to an average EPG intake of approximately 6 g/kg body weight/day in the beginning of the study and declining to approximately 2 g/kg body weight/day) were considered to represent no-observable-adverse-effect levels (NOAEL).

6.4.1.2 90-Day dietary toxicity study with esterified propoxylated glycerol (EPG) in Micropigs (Wedig and Bechtel, 2014)

The subchronic (90-day) toxicity of EPG was assessed in Yucatan micropigs (approximately 8 to 10 months old). Animals (5/sex/group) received feed (Certified Agway® Prolab® Minipig Diet Meal) containing 5, 10, and 17% EPG, mixed accordingly throughout the study to deliver 1.5, 3, and 5 g/kg/day of EPG, respectively. Corn oil served as the vehicle control (0 g/kg body weight/day). The study design is also summarized in Table 6.4.1.2-1.

Table 6.4.1.2-1 EPG Concentration and Group Composition

Group	Treatment		Number of animals/sex
	EPG*	Dietary Concentration†	
Control (AVI1)‡	0 g/kg/day	0% (w/w)	5
Low EPG (AVI2)	1.5 g/kg/day	5% (w/w)	5
Mid EPG (AVI3)	3 g/kg/day	10% (w/w)	5
High EPG (AVI4)	5 g/kg/day	17% (w/w)	5

EPG = esterified propoxylated glycerol.

* Approximate levels; animals received feed containing 5, 10 and 17% EPG, mixed accordingly throughout the study to deliver 1.5, 3.0 and 5.0 g/kg bw/day, respectively.

† % (w/w) = weight of EPG per weight of basal diet including corn oil

‡ The basic diet was supplemented with 4% (w/w) corn oil; test diets contained EPG and 4% (w/w) corn oil as vehicle.

Micropigs were observed twice daily for toxicological, pharmacological, and behavioral effects. Feed consumption and dietary levels of EPG were determined on a weekly basis. Physical and ophthalmic examinations, body weights, urinalysis, hematology, clinical chemistry, water intake, bowel transit times, organ weight, organ tissue analysis for EPG, fecal assays, vitamin assays, gross necropsy, and histopathology were used to evaluate the effects of EPG.

EPG was palatable up to 5 g/kg/day in the diet. No treatment-related morbidity/mortality occurred. No consistent or distinct EPG treatment-related adverse pharmacological/toxicological or behavioral effects were noted. No treatment-related effects were observed during the physical and ophthalmic examinations. Analysis of body weight gain, feed efficiency, water consumption, bowel transit times, hematology and serum chemistry parameters, urinalysis data, feces, and organ weights indicated no treatment-related effects. Chemical analysis of liver, kidney, spleen, and adipose tissue yielded negative data for EPG residue. Gross necropsy and histopathology examinations indicated no treatment-related effects.

PT and activated partial thromboplastin time (APTT), measured as indicators of vitamin K status, were not significantly affected. EPG significantly affected liver vitamin A and serum vitamin D. A significant decrease in the liver vitamin A content was observed in animals fed 5 g of EPG/kg/day. EPG

demonstrated a concentration-dependent effect on the levels of total vitamin D and the biologically active vitamin D metabolite, 25-OH-vitamin D. Specifically, total vitamin D serum levels were significantly reduced in all groups, while serum levels of 25-OH-vitamin D were significantly reduced in animals administered 3 or 5 g of EPG/kg/day. Although a NOEL for effects of dietary EPG on total vitamin D serum levels was not established, a NOEL for effects on 25-OH-vitamin D levels was determined to be 1.5 g of EPG/kg/day, or 5% dietary EPG concentration.

6.4.2 Reproductive and Developmental Toxicity Studies

6.4.2.1 1-Generation Reproduction Study of Esterified Propoxylated Glycerol (EPG) Administered in the Feed to CD® (Sprague-Dawley) Rats (Tyl and Bechtel, 2014a)

This study investigated the reproductive effects following continuous exposure of Crl:CD® (SD)Br rats (approximately 6 weeks old; mean male weight 183.9 ± 1.1 g, mean female weight 151.5 ± 1.0 g) to EPG in the diet (30 animals/sex/group) at 0.0% (group 0), target levels of 0.5% g/kg/day (group 1), 1.0 g/kg/day (group 2), and 2.0 g/kg/day (group 3), and fixed 5.0% EPG (w/w) (group 4), all in 6% corn oil (vehicle). Dietary concentrations of EPG for groups receiving 0.5, 1.0, and 2.0 g/kg/day of test material were adjusted weekly to maintain target EPG intake, throughout the prebreed period. Animals were exposed for a 13-week prebreed period, and through 2 breeding cycles for F0 parental animals, and up to Postnatal Day (PND) 91 for F1a and F1b offspring. Results from this study aided in the design of a subsequent 3-generation reproductive toxicity study (refer to Section 7.6.2).

Parameters examined included, body weights, weight gains, feed consumption, clinical signs, reproductive indices and offspring litter sizes, pup survival and body weights, and histopathology of parental reproductive organs. The study also examined possible effects on blood clotting, parental and offspring immunologic status, histopathology of organs related to immunological function, neurological effects in parents and offspring, developmental effects in offspring, liver and serum fat-soluble vitamin status, and the possible presence of the test material in selected organs of parental and offspring animals.

Results indicated that dietary administration of EPG at levels of 0.5, 1.0, and 2.0 g/kg, and 5% (w/w) to rats for at least 13 weeks was not associated with adverse effects, except for that on liver vitamin status. Vitamin E levels exhibited concentration-related statistically significant reductions in all evaluated groups, with the exception of F1b(A) male weanlings and satellite group F1b(B) males and females. There was no evidence of vitamin D deficiency except in F0 parental, F1a(A) and F1b(A) weanling females, and no evidence of vitamin A deficiency except in F0 parental, F1a(C) and F1b(C) PND 91 females. There were no effects on reproduction of the F0 parental animals for either F1a or F1b mating; evidence for dystocia was present in all groups, including the vehicle control group, with no concentration-dependent response pattern. No treatment-related effects on postnatal growth or development (physical or behavioral), immunological status, blood clotting, and parental general status were observed. EPG was not detected in any of the 360 liver samples from the high concentration and control groups. With respect to kidney and spleen samples, there were 2 and 7 positive samples, respectively, out of 360 total samples for each organ. According to the authors, the positive samples were not the result of contamination during necropsy or analyses, were evenly divided between high concentration and control animals, and were not associated with other measures indicative of *in vivo* systemic exposure.

Based on the results of this study, the NOAEL was 5.0% EPG. Also, in the absence of any effects on behavioral development, immunologic status, and blood clotting, and with group 4 animals tolerating a fixed dietary EPG percentage, it was recommended that the 3-generation study with 2 litters per generation utilize fixed dietary percentages with the highest concentration 5.0% EPG, and endpoints examined not include behavioral, immunologic, or coagulation assessments.

6.4.2.2 Developmental Toxicity Evaluation of Esterified Propoxylated Glycerol (EPG) Administered in the Diet to New Zealand White Rabbits (Tyl and Bechtel, 2014b)

Seventy-two female New Zealand White rabbits (18/group) were fed EPG (0.0, 2.5, 5.0, and 10.0% [w/w]) in Modified Purina Certified Rabbit Chow #5322, supplemented with 6% corn oil (w/w), for 26 days (Day -7 through Gestational Day 19) to assess effects of EPG on the developing conceptus.

All maternal animals were observed for mortality, signs of gross toxicity, clinical signs, body weights, food consumption, and gestational parameters. A significant concentration-related downward trend was observed for "maternal" weight change only for Day -7 to Day 0 (the first week of dietary exposure, prior to insemination) with no significant pairwise comparisons to the concurrent control group. For "maternal" feed consumption (in g/kg/day), significant concentration-related downward trends were observed for Day -14 to -13, Day -11 to -10 and Day -14 to -7, with no significant pairwise comparisons, and all intervals prior to the initiation of administration of EPG (which began on Day -7). At necropsy, all fetuses were dissected from the uterus and examined for skeletal malformations or variations, body weights, and crown-rump length. No evidence of maternal or developmental toxicity was found in this study. A NOAEL of 10% EPG (approximately 4.76 g/kg body weight/day), the highest dose tested for both maternal and developmental toxicity is proposed based on the results of this study.

6.4.3 Genotoxicity Testing of Esterified Propoxylated Glycerol (EPG) (Bechtel, 2014)

6.4.3.1 Ames Assays

6.4.3.1.1 Study to Determine the Ability of H-EPG-05 HR/SO (9:1) to Induce Mutation in 4 Histidine-Requiring Strains of *Salmonella Typhimurium* and 2 Tryptophan-Requiring Strains of *Escherichia Coli* (CLE Study Number ACU 1/S; E-026)

The ability of H-EPG-05 HR/SO (9:1) to induce mutations in 4 histidine-requiring strains (TA98, TA100, TA1535, and TA1537) of *Salmonella typhimurium* (*S. typhimurium*) and 2 tryptophan-requiring strains (WP2 pKM101 and WP2 uvrA Pkm101) of *Escherichia coli* (*E. coli*) was tested in the presence and absence of metabolic activation from the rat liver post-mitochondrial fraction (S9) in 2 separate Ames assays. Negative (acetone) and positive controls (2-nitrofluorene [2NF], sodium azide [NaN_3], 9-aminoacridine [AAC], 4-nitroquinoline 1-oxide [NQO], 2-aminoantracene [AAN], depending on the strain of the bacteria) were also used.

Based on results of a toxicity range-finder experiment conducted in TA100 (results not discussed herein), *Salmonella* and *E. coli* strains were exposed to 1.6, 8, 40, 200, or 1,000 µg/plate of H-EPG-05 HR/SO (9:1), with and without S9, in Experiment 1. Precipitation of EPG was observed at the highest concentration. In addition, a slight thinning of the bacterial lawn in strain TA98 when exposed to 1,000 µg/plate of H-EPG-05 HR/SO (9:1) in the absence of S9, was considered a toxic effect. In Experiment 2, *Salmonella* and *E. coli* strains were exposed to 62.5, 125, 250, 500, or 1,000 µg/plate of H-EPG-05 HR/SO (9:1) with and without S9; treatments in the presence of S9 included a pre-incubation

step prior to plating. Precipitation of H-EPG-05 HR/SO (9:1) was observed at the 1,000 µg/plate concentration level in all strains, as well as at the 500 µg/plate concentration level in *E. coli* strain WP2 uvrA pKM101 only. No evidence of toxicity was observed.

The number of revertant colonies in the negative control treatments fell within normal ranges and the number of revertant colonies in the positive control treatments increased dramatically. There were no reproducible increases in revertant colonies in the presence and absence of S9 in *Salmonella* and *E. coli* strains following treatment with H-EPG-05 HR/SO (9:1) at concentrations up to its limit of solubility. As such it was concluded under the conditions of this study H-EPG-05 HR/SO (9:1) is not mutagenic.

6.4.3.1.2 Study to Determine the Ability of Heated and Unheated H-EPG-05 HR/SO 9:1 and EPG-05 HR/ST 45:55 to Induce Mutation in 2 Histidine-Requiring Strains of *Salmonella Typhimurium*

A modified (preincubation) Ames assay was used to assess the mutagenic potential of heated and unheated H-EPG-05 HR/SO 9:1 and EPG-05 HR/ST 45:55. These oils were compared to heated and unheated palm/rapeseed oil (60:40) and heated and unheated cottonseed oil, respectively.

S. typhimurium histidine strains TA98 and TA100 were incubated with the test materials at concentrations of 100, 250, 500, 1,000, 2,500, and 5,000 µg/plate, in the presence and absence of S9 mix. Vehicle (acetone) and positive controls (AAN, 2NF, NaN3) were tested concurrently in the presence and absence of S9. The study consisted of 2 independent tests, the initial mutagenicity assay and a confirmatory assay (Experiments 1 and 2, respectively) and in each experiment, EPG (all concentrations), vehicle control, and positive controls were plated in triplicate. A response was considered positive if the test material produced a concentration-related increase of at least 2-fold in the mean number of revertants per plate in at least 1 of the tester strains over the appropriate vehicle control value.

Heated and unheated versions of H-EPG-05 HR/SO 9:1 and EPG-05 HR/ST 45:55 (*vs*. palm/rapeseed oil 60:40 and cottonseed oil, respectively) did not increase the number of histidine revertants per plate of *S. typhimurium* strains TA98 and TA100 exposed in the presence and absence of a metabolic fraction. Under the conditions of this assay, heated and unheated H-EPG-05 HR/SO 9:1 and EPG-05 HR/ST 45:55 are not mutagenic in this system.

6.4.3.1.3 Additional Studies

Separate Ames assays were conducted using the same protocol to evaluate the mutagenicity of H-EPG-05 soyate[5] and H-EPG-14 soyate[6]. Bacterial strains were exposed to 1.6, 8.0, 40, 200 and 1,000 µg/plate (Experiment 1) and 62.5, 125, 250, 500 and 1,000 µg/plate (Experiment 2) of each form of EPG in the presence and absence of metabolic activation. Precipitation of EPG was restricted to the highest concentration and no evidence of mutagenicity was observed in assays conducted with either H-EPG-05 soyate or H-EPG-14 soyate.

[5] Study to determine the ability of H-EPG-05 Soyate to Induce Mutation in Four Histidine-Requiring Strains of *Salmonella typhimurium* and Two Tryptophan-Requiring Strains of *Escherichia coli*. (CLE study number ACU 2/S)
[6] Study to determine the ability of H-EPG-14 Soyate to Induce Mutation in Four Histidine-Requiring Strains of *Salmonella typhimurium* and Two Tryptophan-Requiring Strains of *Escherichia coli*. (CLE study number ACU 3/S).

6.4.3.2 Mouse Lymphoma Assays

6.4.3.2.1 Study to Determine the Ability of H-EPG-05 HR/SO (9:1) to Induce Mutations at the Thymidine Kinase (tk) Locus in Mouse Lymphoma L5178Y Cells Using a Fluctuation Assay (CLE study number: ACU 1/TK; E-017)

The ability of H-EPG-05 HR/SO (9:1) to induce mutation at the thymidine kinase (tk) locus (5-trifluorothymidine resistance) in L5178Y tk +/- mouse lymphoma cells was evaluated using a fluctuation assay. The assay was performed in the absence and presence of rat liver post-mitochondrial fraction S9. Based on results of a toxicity range-finder experiment (results not discussed) cells were exposed to 0, 12.5, 25, 50, 100, or 200 µg/mL of H-EPG-05 HR/SO (9:1) in Experiment 1 and 2; the highest concentration was selected based on the solubility limit of H-EPG-05 HR/SO (9:1). Negative (acetone) and positive controls (benzo[a]pyrene [with S9] and 4-nitroquinoline 1-oxide [without S9]) were also included. No statistically significant increases in mutants were observed after exposure to H-EPG-05 HR/SO (9:1). Positive control chemicals exhibited statistically significant frequencies of mutants in culture, whereas negative control cultures demonstrated mutants within normal ranges. Thus, it was concluded, that under the conditions of this assay, H-EPG-05 HR/SO (9:1) does not induce mutations, with or without metabolic activation, at the tk locus in mouse lymphoma cells.

6.4.3.2.2 Additional Studies

Separate mouse lymphoma fluctuation assays were conducted using the same protocol to evaluate the mutagenicity of H-EPG-05 soyate[7] and H-EPG-14 soyate[8]. Cells were exposed to H-EPG-05 soyate or H-EPG-14 soyate at concentrations 0, 25, 50, 75 or 100 µg/mL in the presence and absence of S9. No statistically significant increases in mutants were observed after exposure to either form of EPG. Thus, it was concluded, H-EPG-05 soyate and H-EPG-14 soyate do not induce mutations, with or without metabolic activation, at the tk locus in mouse lymphoma cells.

6.4.3.3 Chromosomal Aberrations Assays

6.4.3.3.1 Study to Evaluate the Chromosome Damaging Potential of H-EPG-05 HR/SO (9:1) by its Effects on Cultured Human Lymphocytes Using an *in Vitro* Cytogenetics Assay (CLE Study Number: ACU 1/HLC; E-020)

An *in vitro* cytogenetics assay using human peripheral blood lymphocyte cultures from a male and a female donor was used to assess the ability of H-EPG-05 HR/SO (9:1) to induce structural aberrations in 2 separate experiments. Both experiments were performed in the absence and presence of rat liver post-mitochondrial fraction S9. Negative (acetone) and positive controls (4-nitroquinoline 1-oxide [without S9] and cyclophosphamide [with S9], following a 20+0-hour exposure and a 3+17-hour exposure, respectively) were also used.

A toxicity range-finder experiment (results not discussed) was conducted in order to establish the concentration range for Experiments 1 and 2; the highest concentration was selected based on the solubility limit of EPG-05 HR/SO (9:1). In Experiment 1, cells were exposed to 0.9887 to 50.00 µg/mL of

[7] Study to Determine the Ability of H-EPG-05 Soyate to Induce Mutations at the Thymidine Kinase (*tk*) Locus in Mouse Lymphoma L5178Y Cells Using a Fluctuation Assay (CLE study number: ACU 2/TK)
[8] Study to Determine the Ability of H-EPG-14 Soyate to Induce Mutations at the Thymidine Kinase (*tk*) Locus in Mouse Lymphoma L5178Y Cells Using a Fluctuation Assay (CLE study number: ACU 3/TK)

EPG-05 HR/SO (9:1) for 20 hours in the absence of metabolic activation, and 3 hours in the presence of metabolic activation, followed by 17 hours of recovery. Based on the effects of H-EPG-05 HR/SO (9:1) on the mitotic index, cells exposed to 24.5, 35, and 50 µg/mL of EPG-05 HR/SO (9:1) were analyzed to assess the frequency of chromosomal aberrations. Results demonstrated no clear indications of mitotic inhibition.

In Experiment 2, cells were exposed to 2.112 to 50.00 µg/mL of EPG-05 HR/SO (9:1) for 20 hours in the absence of metabolic activation and 3 hours in the presence of metabolic activation, followed by 17 hours of recovery. Due to the indefinite results in Experiment 1, Experiment 2 also included delayed sampling times (44+0 and 3+41) and a pulse treatment (3+17) at the highest concentration. Based on the effects of EPG-05 HR/SO (9:1) on the mitotic index, cells exposed to 28.13, 37.5, and 50 µg/mL of EPG-05 HR/SO (9:1) were analyzed to assess the frequency of chromosomal aberrations. Afterwards, cells were exposed to the highest concentration of 50 µg/mL of EPG-05 HR/SO (9:1) in a delayed harvest study conducted in the presence (3 hours of exposure and 41 hours of recovery) and absence (44 hours of exposure) of metabolic activation, and in a pulse treatment (3 hours of exposure and 17 hours of recovery) study without metabolic activation.

Positive controls demonstrated statistically significant increases in the proportion of cells with structural aberrations, and the negative control demonstrated a proportion of cells with structural aberrations within normal ranges, confirming the validity of the assay. No significant differences were observed either in the absence or presence of metabolic activation between the H-EPG-05 HR/SO (9:1)-treated and negative control cultures. Thus, it was concluded that H-EPG-05 HR/SO (9:1) does not induce chromosome aberrations in cultured human peripheral blood lymphocytes when tested to its limit of solubility.

6.4.3.3.2 Additional Studies

Separate chromosome aberration tests were conducted using the same protocol to evaluate the mutagenicity of H-EPG-05 soyate[9] and H-EPG-14 soyate[10]. Cultures were treated with H-EPG-05 soyate, at concentrations ranging from 0.9887 to 50.00 µg/mL (Experiment 1) and 6.674 to 50.00 µg/mL (Experiment 2). Cells exposed to 24.5, 35, and 50 µg/mL of H-EPG-05 soyate in Experiment 1 and 28.13, 37.5, and 50 µg/mL of H-EPG-05 soyate in Experiment 2 were analyzed to assess the frequency of chromosome aberrations. A small, but statistically significant increase in aberrant cells was observed in Experiment 1 following treatment with H-EPG-05 soyate, however, it was not considered biologically significant because it was not reproducible in Experiment 2. Due to the indefinite results in Experiment 1, Experiment 2 also included delayed sampling times (44+0 and 3+41) and a pulse treatment (3+17) at the highest concentration of 50.00 µg/mL. With respect to H-EPG-14 soyate, cultures were treated with the test material at concentrations ranging from 1.186 to 60.00 µg/mL (Experiment 1) and 8.009 to 60.00 µg/mL (Experiment 2), in the presence and absence of metabolic activation. Cells exposed to 29.4, 42, and 60 µg/mL of H-EPG-14 soyate in Experiment 1 and 29.4, 42, and 60 µg/mL of H-EPG-14 soyate in Experiment 2 were analyzed to assess the frequency of chromosome aberrations. Due to the indefinite results in Experiment 1, Experiment 2 also included delayed sampling times (44+0 and 3+41) and a pulse treatment (3+17) at the highest concentration of 60.00 µg/mL. A small, but statistically significant increase in aberrant cells was observed in

[9] Study to Evaluate the Chromosome Damaging Potential of H-EPG-05 Soyate by its Effects on Cultured Human Lymphocytes Using an *in Vitro* Cytogenetics Assay (CLE Study Number: ACU 2/HLC)
[10] Study to Evaluate the Chromosome Damaging Potential of H-EPG-14 Soyate by its Effects on Cultured Human Lymphocytes Using an *in Vitro* Cytogenetics Assay (CLE Study Number: ACU 3/HLC)

Experiment 2; however, it was not considered biologically significant since the number of aberrant cells present were within normal range of traditional values for the negative control. Thus, it was concluded that H-EPG-05 soyate and H-EPG-14 soyate did not induce chromosome aberrations in cultured human peripheral blood lymphocytes when tested to their limit of solubility.

6.4.3.4 Unscheduled DNA Synthesis Assays

6.4.3.4.1 Study to Evaluate the Potential of H-EPG-05 HR/SO (9:1) to Induce Unscheduled DNA Synthesis in Rat Liver Using an *in Vivo/in Vitro* Procedure (CLE study number ACU 1/ILU; E-023)

This study investigated the ability of H-EPG-05 HR/SO (9:1) to induce unscheduled DNA synthesis (UDS) in the livers of rats following oral administration. Groups of 6 male Wistar rats (46 to 59 days old, weighing 214 to 329 g) were treated with the negative control, positive control, or 632.5 and 2,000 mg/kg of H-EPG-05 HR/SO (9:1), *via* oral gavage, in 2 experiments. Corn oil, the vehicle for H-EPG-05 HR/SO (9:1), was used as the negative control chemical; positive controls were 2-acetamidofluorene (75 mg/kg, 12 to 14-hour exposure) and dimethylnitrosamine (10 mg/kg, 2 to 4-hour exposure) for Experiments 1 and 2, respectively. A toxicity range-finder experiment (results not discussed) was performed to obtain concentration levels for Experiments 1 and 2. In both Experiment 1 and 2, animals were sacrificed and their livers were used to establish a primary hepatocyte culture. Cultures were treated with [^{3}H] thymidine, slides were fixed with hepatocytes, and dipped with photographic emulsion to prepare autoradiograms. Slides were examined microscopically to calculate the net grains/nucleus (NG = nuclear grain count – mean cytoplasmic grain count) and percentage of cells in repair (net grain ≥5) for each slide, animal, and concentration group.

Negative control animals demonstrated a mean NG value less than 0 with only 0 to 0.6% cells in repair. The positive control animals demonstrated mean NG values greater than 5 with more than 50% cells in repair. Treatment with 632.5 or 2,000 mg/kg of H-EPG-05 HR/SO (9:1) produced mean NG values no more than -0.9 with no more than 1.4% cells in repair. On this basis, it was concluded that H-EPG-05 HR/SO (9:1) was not genotoxic under the conditions of this assay.

6.4.3.4.2 Additional Studies

Separate *in vivo*/*in vitro* unscheduled DNA synthesis tests were conducted using the same protocol to evaluate the mutagenicity of H-EPG-05 soyate[11] and H-EPG-14 soyate[12]. Negative control animals demonstrated a mean NG value less than 0 with only 0 to 1.8% cells in repair in both studies. Treatment with 632.5 or 2,000 mg/kg of H-EPG-05 soyate or H-EPG-14 soyate produced mean NG values no more than -0.5 with no more than 0.4% cells in repair, and mean NG values no more than -0.8 with no more than 1.4% cells in repair, respectively. Therefore, it was concluded that H-EPG-05 soyate and H-EPG-14 soyate were not genotoxic under the conditions of these assays.

[11] Study to Evaluate the Potential of H-EPG-05 Soyate to Induce Unscheduled DNA Synthesis in Rat Liver Using an *in Vivo/in Vitro* Procedure (CLE study number ACU 2/ILU)

[12] Study to Evaluate the Potential of H-EPG-14 Soyate to Induce Unscheduled DNA Synthesis in Rat Liver Using an *in Vivo/in Vitro* Procedure (CLE study number ACU 3/ILU)

6.5 Corroborative Evidence of Safety

6.5.1 Repeat-Dose Toxicity

6.5.1.1 90-Day Dietary Safety Study with Esterified Propoxylated Glycerol (EPG) in Mice (unpublished, HWI 6226-123; E-008)

The purpose of this study was to assess the subchronic toxicity of a representative version of EPG when given to Crl:CD-1®(ICR)BR mice (approximately 7 weeks old; males weighed between 24 and 33 g and females weighed between 18 and 26 g) by dietary admixture for at least 90 days. Mice (n=800) were randomly assigned to 5 groups (80 animals/sex/group, subdivided into subsets A through H for each sex) and administered concentration levels of 0, 1.0, 2.0, and 5.0 g EPG/kg of body weight/day through adjusted diets, or a fixed intake of 5.0% (w/w) in the diet. All diets were prepared weekly and provided *ad libitum*.

Animals were housed individually in stainless steel, screen-bottom cages, and were observed twice daily (a.m. and p.m.) for mortality, moribundity, and signs of toxicity. At least once weekly, each animal was removed from its cage and examined. Body weights and food consumption were recorded weekly. Ophthalmic examinations were performed on all animals before study initiation and during Week 13 for animals given 0 g/kg/day and 5.0% (w/w) EPG. Hematology (10 animals/sex/group) and clinical chemistry (10 animals/sex/group) evaluations were performed before the interim sacrifice at Week 6 (Subsets A and B) and the terminal sacrifice at Week 14 (Subsets D and E). Liver vitamin A and E and serum vitamin D (25-0H vitamin D, total) level determinations were performed on samples collected from 10 animals/sex/group during Weeks 6 (Subset C) and 14 (Subset G). EPG level determinations were done on liver, spleen, and kidney tissue from 10 animals/sex in Groups 1 and 5 collected during Weeks 6 (Subset A) and 14 (Subset H). Ten animals/sex/group were sacrificed and subjected to pathological evaluation at Weeks 6 (Subset B) and 14 (Subset D), and 20 additional animals/sex/group (Subsets E and F) were also sacrificed at Week 14 for pathological evaluation. At scheduled necropsies, animals were anesthetized, weighed, and exsanguinated. Macroscopic observations were recorded, selected organs were weighed, and selected tissues were preserved. Microscopic examinations of all preserved tissue were performed on all animals in the control group and 5.0% (w/w) EPG group (Subsets B, D, E, and F). Microscopic examination of lesions, gastrointestinal tract, lungs, liver, and kidneys was also performed on all animals in the remaining groups.

Results of the study showed that dietary administration of EPG at levels of 1.0, 2.0, and 5.0 g/kg, and at 5.0% (w/w) for at least 90 days was not associated with adverse effects. Body weight gains were slightly increased over the treatment period in animals given 5.0% (w/w) EPG. The mean liver vitamin A levels at Week 14 were statistically lower for males given 5.0 g/kg and 5% EPG. For females at Week 14, liver vitamin A levels were lower for all EPG-treated groups, however, not in a dose-related manner and statistical significance was noted for only for females given 1.0 g/kg. At Week 14, concentration-related decreases in liver vitamin E were noted for both males and females exposed to the test material. At Week 14, serum vitamin D (25-0H vitamin D, total) levels in males given 5.0 g/kg and 5.0% EPG and females given 2.0 and 5.0 g/kg and 5.0% EPG were reduced; these test material-related differences were statistically significant. Males given EPG at levels of 1.0 and 2.0 g/kg and females given 1.0 g/kg had serum vitamin D levels similar to those of controls. There was no evidence of vitamin deficiency in any of the animals as assessed by growth, clinical observations, clinical pathology, or anatomical pathology endpoints. Based on these results, the NOAEL was 5.0% (w/w) EPG in the diet, equivalent to 6.2 g/kg (males) and 8.1 g/kg (females) at study termination.

6.5.1.2 Thirteen Week Safety Study of EPG Administered in the Feed to Beagle Dogs (unpublished, T.P.S. Study No. 460D-502-634-92; E-009)

This study was designed to evaluate the toxicity of EPG when administered as a dietary admix to beagle dogs, daily, for at least 13 weeks. Male and female (5/sex/group) purebred beagle dogs (approximately 7 to 8 months old) were fed modified Teklad Basal Dog Diet Certified Meal supplemented with 6% corn oil which contained EPG; the experimental design of this study was as follows:

Table 6.5.1.2-1 Design of 13-Week Feeding Study in Beagle Dogs

Group No.	EPG Theoretical Concentration Level*		EPG Mean Actual Dose (g/kg bw/day)*		Number of Dogs per Group	
	(g/kg bw/day)	% Diet (wt/wt)	Male	Female	Male	Female
AVK1 (Control)	0.0	0	0	0	5	5
AVK2	1.5	5	1.6	1.6	5	5
AVK3	3.0	10	3.2	3.2	5	5
AVK4	5.0	17	5.2	5.3	5	5

bw = body weight; EPG = Esterified Propoxylated Glycerol; wt = weight.

* Approximate levels; animals received feed containing 5, 10 and 17% EPG, mixed accordingly throughout the study to deliver 1.5, 3.0 and 5.0 g/kg bw/day, respectively.

Dogs ate the isocaloric diets offered to them over the 2-hour daily feeding period and food consumption was measured daily. They were housed individually in adjacent runs with chain linkwire sides, epoxy coated floors, and elevated resting boards, and were observed twice daily for pharmacological, toxicological, and behavioral effects; feces were inspected for phase separation (*i.e.*, appearance of oily layers or deposits in the stools). Detailed physical examinations including ophthalmoscopy were conducted pretest, during Week 7, and just prior to necropsy. A general physical examination was done each week on each animal. Body weights were recorded pretest and weekly thereafter. Blood samples from fasted animals were obtained at pretest and during Weeks 6 and 13 for evaluation of hematologic and clinical chemistry parameters. Serum samples obtained just prior to necropsy were analyzed for total Vitamin D and 25-OH-Vitamin D concentrations. Urinalysis (including urine chemistries) and water consumption evaluations were conducted pretest and during Weeks 6 and 13/14. Fecal samples obtained during Weeks 6 and 13/14 were analyzed for total fat, total cholesterol, total fatty acids and profile, total fecal bile acid, and calcium. A separate fecal sample was obtained during Weeks 6 and 13/14 for possible gut microflora assay. At necropsy samples of liver (all lobes), kidney, spleen and adipose tissue were taken for EPG analysis; an additional liver sample (all lobes) was analyzed for vitamin A and E content. All dogs were subjected to a complete postmortem examination.

No treatment-related mortality/morbidity occurred. No consistent or distinct EPG treatment related adverse pharmacological/toxicological or behavioral effects were noted during this evaluation. Physical and ophthalmic examinations at termination indicated no treatment-related effect. The dogs ate the isocaloric diets offered to them (over each 2-hour daily feeding period) indicating EPG was palatable up to 5.0 g/kg/day in the diet. Visual inspection of the feces daily indicated no phase separation *(i.e.*, appearance of oily layers or deposits in the stools). Analysis of body weight gain, feed efficiency, water consumption, bowel transit times, hematology and serum chemistry parameters, urinalysis data (including urine chemistries), feces (for total fat, total fatty acid and calcium), organ weights (absolute, relative to body or brain weight) and vitamin A concentration of the liver did not indicate a treatment-related effect.

Results of chemical analyses of liver, kidney, spleen and adipose tissue showed that there was no EPG found in any of the tissues examined, within the limits of detection. Fecal chemical assay results for EPG and the mono and diester metabolite concentrations showed that all fecal samples from dogs receiving 17% EPG triester diet contained EPG triester, diester, and monoester after 6 weeks of dosing at levels of 11.7 to 29.2 g, 1.21 to 3.07 g, and 0.183 to 0.517 g, respectively, for males; 15.2 to 35.3 g, 1.44 to 3.61 g and 0.348 to 0.784 g of triester, diester and monoester were present in the feces from female dogs in a 24-hour period during Week 6 of dosing. The amounts of EPG triester, diester, and monoester found in the feces of male dogs in a 24-hour period during Week 13 of dosing were 5.08 to 37.8 g, 0.494 to 3.25 g and 0.121 to 0.553 g, respectively; 13.4 to 27.3 g, 1.50 to 3.03 g, and 0.321 to 0.596 g of triester, diester, and monoester were present in the feces of female dogs from a 24-hour period during Week 14 of dosing.

Chemical analysis of feces collected during Week 6 and 13/14 indicated a treatment-related significant increase in cholesterol and decrease in total bile acid at 5 g EPG/kg/day. A significant decrease in the liver vitamin E content of the Group AVK4 animals was considered to be treatment-related. A concentration-dependent relationship was apparent with respect to the total vitamin D serum levels which were significantly reduced in all groups. The serum level of 25-OH-Vitamin D was significantly reduced at 3 and 5 g EPG/kg/day in a concentration-dependent manner. Effects of EPG on these serum vitamin levels were considered to be treatment-related. PT and APTT, measured as indicators of vitamin K status, were not significantly affected.

Gross necropsy and histological examination of all tissues indicated no distinct or consistent treatment-related effects. In summary, an increase in fecal cholesterol, a decrease in total fecal bile acid, and a decrease in vitamin E content of the liver were considered treated-related at a level of 17% EPG in the diet, or 5 g EPG/kg/day. Associated with dietary administration of EPG, the NOEL for serum vitamin D was <1.5 g EPG/kg/day, as serum vitamin D levels were significantly reduced in all groups even though there were no indications of vitamin deficiency expressed clinically. The NOEL for the biologically active 25-OH-Vitamin D metabolite was 1.5 g EPG/kg/day.

6.5.1.3 90-Day Dietary Toxicity Study of EPG-05 HR/ST (45:55) in Rats (unpublished, MPI Study Identification No. 728-006; E-045)

In a 90-day toxicity study, Crl:CD®BR (VAF/Plus) rats (20/sex/group; approximately 4 weeks of age) were administered EPG-05 HR/ST (45:55) at concentration levels of 0, 0.5, 1.0, or 2.0 g/kg body weight/day through adjusted diets, or a fixed intake of 5.0% (w/w) in the diet. Rats were individually housed in suspended stainless steel cages with wire-mesh floors and were observed twice daily for mortality, morbidity, and signs of toxicity. Detailed observations, body weight, food consumption, and EPG-05 HR/ST (45:55) consumption were monitored weekly. Individual body weight and an ophthalmoscopic examination were conducted before dosing. Ophthalmoscopic examination, clinical pathology assessment, and serum sample evaluation were conducted in the last week of the study. Necropsies were performed at study termination. Results showed that serum total vitamin D (25-OH D_2 plus 25-OH D_3) levels decreased in a dose-related manner for all groups of males and females administered EPG-05 HR/ST (45:55), and were statistically significant in the 2.0 g/kg/day and 5.0% (w/w) groups. The mean liver vitamin E concentration was statistically significantly decreased in all groups of males and females receiving EPG-05 HR/ST (45:55) in the diet when compared to controls. In females, the decreases were dose-related. The mean concentration of liver vitamin A in males was statistically significantly decreased in the 1.0 and 2.0 g/kg/day and the 5% (w/w) groups, although the decreases were not dose-related. No effect on liver vitamin A levels were noted in males receiving 0.5 EPG-05 HR/ST (45:55)

g/kg/day. In females, the mean liver vitamin A concentrations were decreased in all groups administered EPG-05 HR/ST (45:55) in the diet. The decreases were not dose-related, but were statistically significant in the 0.5 and 2.0 g/kg/day and the 5.0% (w/w) females. No adverse clinical signs, gross, or microscopic pathologic evidence of vitamin A, E, or D deficiency were observed. PT and APTT, measured as indicators of vitamin K status, were not significantly affected.

6.5.1.4 Combined Chronic Dietary Safety Study and Carcinogenicity Study with Esterified Propoxylated Glycerol (EPG) in Rats (unpublished, HWI 6226-120; E-001)

The purpose of this study was to assess the potential chronic toxicity and carcinogenicity of the test material, EPG, when fed to Crl:CD®BR VAF/Plus® rats (Sprague-Dawley derived) for at least 104 weeks. Rats (n=1,400) were randomly assigned to 5 groups (140 animals/sex/group) and administered EPG at concentration levels of 0, 0.5, 1.0, or 2.0 g/kg body weight/day through adjusted diets, or a fixed intake of 5.0% (w/w) in the diet. All diets were prepared weekly and available *ad libitum*. Fifty rats/sex/group were designated for interim sacrifice and were terminated after 52 weeks of treatment.

Animals were housed individually in stainless steel, screen-bottom cages and were observed twice daily (a.m. and p.m.) for mortality, moribundity, and signs of toxicity. Body weights were recorded on the first day of treatment, weekly for 16 weeks and every 4 weeks thereafter: food consumption was recorded weekly for 16 weeks and every 4 weeks thereafter. Ophthalmic examinations were performed on all animals before study initiation and during Weeks 13, 26, 39, 54, 65, 78, and 91 for animals in the control and 5.0% EPG groups. Ophthalmic examinations were also done for all animals scheduled for interim (Week 52) and terminal (Week 104) sacrifice. Hematology evaluations were done on 20/sex/group at Weeks 26 and 78, and hematology and clinical chemistry evaluations were done for 20/sex/group before the interim sacrifice (Week53/54) and terminal sacrifices (Week 105/106). Liver vitamin A, vitamin E, and serum vitamin D (25-OH vitamin D, total) level determinations were done for 10/sex/group necropsied at the interim and terminal sacrifice. Ten animals/sex/group were housed in metabolism cages for the collection of fecal matter for analysis of EPG, EPG metabolites, cholesterol, fatty acids, and total bile acids. Forty rats/sex/group were sacrificed and subjected to pathological evaluation at the Week 53/54 interim sacrifice; and all surviving animals were sacrificed at the Week 105/106 terminal sacrifice. Microscopic examinations were performed on the animals in the control and 5.0% EPG groups; on all animals that died or were sacrificed; and on the gastrointestinal tract, lungs, liver, kidney, and all macroscopic lesions from animals in the remaining groups.

Results of the study showed that dietary administration of EPG to rats at concentration levels of 0.5, 1, and 2 g/kg, or 5.0% (w/w) in the diet for at least 104 weeks was not associated with adverse effects nor were there neoplasms whose incidence suggested an association with EPG administration. Body weights were slightly increased in males given 1 g/kg, 2 g/kg, and 5.0% EPG, and in females given 5.0% EPG. The levels of liver vitamins A and E and serum vitamin D (25-OH vitamin D, total) were generally decreased in EPG-treated animals. However, there was no evidence of vitamin deficiency as assessed by growth, clinical observations, clinical pathology or anatomical pathology endpoints. PT, measured as an indicator of vitamin K status, was not significantly affected. The increased incidence of palpable masses in females given 1 g/kg, 2 g/kg and 5.0% EPG which correlated with higher incidences of mammary fibroadenomas and increased incidence of thyroid C-cell adenomas, were not considered important because these are commonly occurring spontaneous neoplasms in female rats and furthermore, the incidence at which they were observed was comparable to that observed in control animals of previous studies. Based on the results of this study, the NOAEL was 5.0% (w/w) in the diet, equivalent to 2 g/kg/day at study termination.

6.5.1.5 Combined Chronic Dietary Safety Study and Carcinogenicity Study with Esterified Propoxylated Glycerol (EPG) in Mice (unpublished, HWI 6226-121; E-002)

The purpose of this study was to assess the chronic toxicity and potential carcinogenicity of the test material, EPG, when fed to Crl:CD-1® (ICR) BR VAF/Plus® mice (51 (males) or 52 (females) days old; males weighed between 20.4 and 33.7 g, and females weighed between 18.9 and 27.7 g) for at least 104 weeks. Mice (n=1,500) were randomly assigned to 5 groups (150 animals/sex/group) and administered EPG at concentration levels of 0, 0.5, 1.0, or 2.0 g/kg body weight/day through adjusted diets, or a fixed intake of 5.0% (w/w) in the diet. All diets were prepared weekly and available *ad libitum*. Fifty mice/sex/group were designated for interim sacrifice and were terminated after 52 weeks of treatment.

Animals were housed individually in stainless steel, screen-bottom cages, and were observed twice daily (a.m. and p.m.) for mortality, moribundity, and signs of toxicity. Body weights and food consumption were recorded weekly for 16 weeks and at least every 4 weeks thereafter. Body weights were also recorded at the time of death for all animals that died or were sacrificed at an unscheduled interval. Ophthalmic examinations were performed on all animals before study initiation, on all animals scheduled for interim and terminal sacrifice (Weeks 52 and 104) and on all animals in Groups 1 and 5 (control and 5.0% EPG) during Weeks 13, 26, 39, 55, 77, and 91. Hematology evaluations were done on 10 animals/sex/group at Weeks 27 and 78 and after 104 weeks; hematology and clinical chemistry evaluations were done on 10 animals/sex/group before the interim sacrifice (Week 53). Liver vitamin A, vitamin E, and serum vitamin D (25-OH vitamin D, total) level determinations were done for 10 mice/sex/group necropsied at the interim and terminal sacrifices. Ten animals/sex/group were housed in metabolism cages for the collection of feces for analysis of EPG, EPG metabolites, cholesterol, total fatty acids, and bile acids; collections were initiated during Weeks 1, 4, 13, 26, 52, 78, and 103. During these collections, body weight, food consumption, and fecal output measurements were performed daily. At scheduled necropsies, animals were fasted overnight, anesthetized, weighed, exsanguinated, and necropsied. Macroscopic observations were recorded; selected organs were weighed and selected tissues were preserved. Microscopic examinations were performed on the animals in the control and 5.0% EPG groups; on all animals that died or were sacrificed in a moribund condition; and on the gastrointestinal tract, lungs, liver, kidneys, and all macroscopic lesions from animals in the remaining groups.

Based on the results of the study, dietary administration of EPG at concentration levels of 1.0, 2.0, and 5.0 g/kg, or 5.0% (w/w), for at least 104 weeks was associated with no adverse effects. In addition, since there were no statistically significant differences in the incidence of neoplasms between the control and treated groups, EPG and was not carcinogenic. Body weights were increased slightly over the treatment period which may be related to slight increases in food consumption. At Week 53, mean liver vitamin A levels were lower in males and females given 2.0 and 5.0 g/kg, and 5% EPG compared with those of the control group; these differences were not statistically significant but were dose-related for males. Mean liver vitamin A levels at Week 106 were lower for animals given all dose levels of EPG when compared to control animals. For animals given 5.0% EPG, liver vitamin A levels were reduced by approximately 16% for males and 24% for females; only the value for females was statistically significant. Group mean liver vitamin E levels were similar for control and EPG-treated males at all dose levels at Week 53. For EPG-treated females, liver vitamin E levels were reduced in a dose-dependent manner; these differences were statistically significant for animals given 5.0 g/kg and 5.0% EPG. At Week 106, mean liver vitamin E levels were reduced in a dose-dependent manner for males and females. The differences were statistically significant for males given 5.0 g/kg and for females given 2.0 or 5.0 g/kg or 5.0% EPG. There

was no effect of EPG administration on serum levels of vitamin D in males or females. Despite reductions in liver vitamin A and E levels compared to control values, there was no evidence of vitamin deficiency as assessed by growth, clinical observations, clinical pathology, or anatomical pathology endpoints. The increased incidence and severity of skin lesions in males given 5.0% (w/w) EPG were associated with the clinical observation of an increased incidence of oily hair coat; this finding is likely secondary to dermal contact with the diet (oily consistency) and is not considered an adverse effect of the test material. Based on these results, the NOAEL was 5.0% (w/w) in the diet, equivalent to 2 g/kg/day at study termination.

6.5.1.6 A 1-Year Chronic Safety Study of H-EPG-05 HR/SO (9:1) Esterified Propoxylated Glycerol (EPG) Administered in the Feed to the Beagle Dogs (unpublished, MPI Study Identification No. 728-001)

Forty purebred beagle dogs (approximately 5 to 6 months of age) were fed EPG at levels of 0, 1, 2, and 3 g/kg body weight/day in Modified Teklad Basal Mix for Dog Diet #7058 supplemented with 6% (w/w) corn oil (5/sex/group), for 1 year in order to evaluate potential effects attributed to long-term EPG consumption. Animals were individually housed in standard nonspecialized stainless steel dog cages and were observed for mortality, signs of gross toxicity, clinical signs, body weights, food consumption/food efficiency, and water consumption, as well as electrocardiogram, opthalmoscopic, hematologic and urinalysis findings. EPG concentrations were measured in adipose tissue, liver, kidney, and spleen samples. Additional tests were conducted to measure EPG its metabolites, as well as fatty acids in the feces, and recovery of EPG and its breakdown products in feces. Other parameters evaluated included: bowel transit times, vitamin concentrations in plasma, serum, and liver tissue, and mineral concentrations in liver tissue and bone. Necropsies were performed on all animals.

Effects attributed to EPG consumption included statistically significant increases in fecal fat and fatty acids in the 3 g/kg body weight/day group, statistically significant decreases in fecal calcium in the 3 g/kg body weight/day group, and statistically significant decreases in serum phosphorus in the 3 g/kg body weight/day females at 3 and 6 months, and in the 1 g/kg body weight/day females at 6 months. All treated groups, males and females, had decreases in serum vitamin E concentrations that were statistically significant at 3 g/kg body weight/day in males, and 2 and 3 g/kg body weight/day in females. The average concentrations of iron and vitamin E in liver tissue were found to be statistically significantly lower for males in all groups compared to controls. Additionally, bone zinc was statistically significantly decreased at 2 and 3 g/kg body weight/day in males, but was increased compared to controls at 1 g/kg body weight/day. PT and APTT, measured as indicators of vitamin K status, were not significantly affected. Intact EPG was not found at the level of detection in any tissues analyzed and no clinical signs of vitamin deficiency were observed in this study.

6.5.1.7 A 1-Year Chronic Safety Study of Esterified Propoxylated Glycerol H-EPG-05 HR/SO (9:1) (EPG) Administered in the Feed to Yucatan Micropigs® (Sus scrofa) (unpublished, MPI Study Identification 728-002; EPG-041)

The objective of this study was to evaluate the effects of H-EPG-05 HR/SO (9:1) administration in feed to Yucatan Micropigs® (Sus scrofa) for 1 year. Yucatan Micropigs® (approximately 9 months of age) were fed Certified Agway® Prolab® Minipig Diet Meal supplemented with 4% corn oil containing H-EPG-05 HR/SO 9:1 daily. Animals received 0, 1, 2, or 3 g/kg/day of H-EPG-05 HR/SO 9:1 through the diet (5/sex/group). All groups were fed H-EPG-05 HR/SO 9:1-containing micropig feed in the morning and at night. In addition, animals were fed 100 g/day of feed (containing no EPG) at noon to provide additional

calories; this amount was increased to 250 g/day starting Week 47. Animals were individually housed in pens consisting of solid floors and wire sides or chain link fencing as dividers, with laboratory grade ASPEN wood shavings. Feed intake, EPG consumption, water intake, physical and ophthalmic examinations, clinical and behavioral observations, body weight gain, hematology, serum chemistry, urinalysis, bowel transit times, organ weights, necropsy, histopathology, electrocardiograms, fecal assays, tissue assays for H-EPG-05 HR/SO 9:1, vitamin and mineral assays, and bone density, bone mineral content, bone mineral density, and tissue mass measurements were used to evaluate the effects of H-EPG-05 HR/SO 9:1.

Effects related to H-EPG-05 HR/SO 9:1 included decreased mean body weights (referable to caloric restriction, not toxicity) in all groups of treated males, increased fecal fatty acids in pigs administered 3 g/kg/day (referable to H-EPG-05 HR/SO 9:1 and its breakdown products), and decreased mean total serum 25-hydroxy vitamin D concentrations in all treated groups. Although a decrease in mean total serum 25-hydroxy vitamin D concentrations was noted in all treated groups, there were no clinical signs of a vitamin deficiency as evidenced by the lack of changes in bone density, bone mineral content, and bone mineral density upon measurement with a X-ray densitometer. No other parameters including PT and APTT, which were measured as indicators of vitamin K status, were affected by H-EPG-05 HR/SO 9:1 consumption. High performance liquid chromatography (HPLC) analysis confirmed that intact H-EPG-05 HR/SO 9:1 was not present in liver, kidney, spleen, or body fat.

6.5.2 Reproductive and Developmental Toxicity

6.5.2.1 3 Generation Reproduction Study (with a Teratology Phase) of Esterified Propoxylated Glycerol (H-EPG-05 HR/SO (9:1); EPG) Administered in the Feed to CD®(Sprague-Dawley) Rats (RTI Study Identification No. 65C-5304-02; E-003)

To evaluate the potential effects of EPG on reproduction and development, in 3 generations of 240 Crl:CD® (SD)Br rats (approximately 6 weeks old; mean male weights 182.0 to 184.6 g and mean female weights 151.8 to 154.6 g) were fed EPG in a modified NIH-07 diet *ad libitum*, 7 days/week, at concentration levels of 0, 1, 2, and 5%, in 6% corn oil vehicle (30 animals/sex/group). The parental generation (designated F0, F1a and F2a) was exposed over a 10-week pre-breed, 3-week mating, 3-week gestation, and 3-week lactation period. The study design is summarized in Table 6.4.2.2-1.

Table 6.4.2.2-1 Summary of Study Design for 3-Generation Reproduction Study With H-EPG-05 HR/SO (9:1)

Groups	No. of Animals	Animal Fate	Endpoints Assessed
F0, F1a parental animals*	30/sex/group	Males sacrificed at end of second mating Non-pregnant females sacrificed at least 3 weeks after last day of second cohabitation period Parent females sacrificed at weaning of each F1b litter or F2b litter	Mortality, clinical observations, reproductive and lactational indices, body weights, feed consumption, gross lesions, histopathology exams on animals in the high concentration and control groups only
F2a parental animals	30/sex/group	Males sacrificed at end of second mating Non- pregnant females sacrificed at least 3 weeks after last day of second cohabitation period Parent females were sacrificed on Gestational Day 20	Mortality, clinical observations, reproductive and lactational indices, body weights, feed consumption, gross lesions, histopathology exams on animals in the high concentration and control groups only; Reproductive organs from males and females that failed to mate; uterus plus 1 attached ovary in dams to examine ovarian corpora lutea and uterine contents
F1a, b offspring F2a, b offspring F3a, b offspring	F1a,b and F2a,b culled to 10 pups per litter on PND 4 F1a and F2a PND 21 weanlings assigned as parents for next generation F3a and F3b 10/sex/group	F1b, F2b, F3a sacrificed at weaning F3b fetuses part of teratology phase; c-sectioned from F2a dams on Gestational Day 20	Body weights, clinical observations, survival on PND 4, 7, 14 & 21, body weights recorded on PND 0 All live pups were counted, sexed, and examined grossly on PND 0, 4, 7, 14 & 21 F3b animals were measured for crown rump length, morphological abnormalities including cleft palate; external, skeletal, visceral exams performed
F1a(A) satellite	10/sex/group	Animals sacrificed at weaning	Body weights, clinical observations and liver weights
F1b(A) satellite	10/sex/group	Animals were sacrificed at weaning	Body weights, clinical observations and liver weights
F2a(A) satellite	10/sex/group	Animals were sacrificed at weaning	Body weights, clinical observations and liver weights
F2b(A) satellite	10/sex/group	Animals were sacrificed at weaning	Body weights, clinical observations and liver weights
F3a(A) satellite	10/sex/group	Animals were sacrificed at weaning	Body weights, clinical observations, total and differential leukocyte counts, total protein, albumin, and serum, albumin/globulin ratio, and liver weights
F3a(B) satellite	10/sex/group	Animals were sacrificed at weaning	Body weights, clinical observations and liver weights

EPG = esterified propoxylated glycerol; PND = Postnatal Day.

* The parental generation designated as F0, F1a, and F2a were given 0, 1.0, 2.0, and 5.0% EPG in 6% corn oil vehicle in the diet during a 10-week pre-breed period.

Animals were individually housed upon the initiation of the treatment period in solid bottom polycarbonate cages with stainless steel wire lids. Study animals were housed 2 per cage (1 male:1 female from the same dose level) during the mating periods. Females were caged separately and individually once they were successfully mated (or at the end of the mating periods). Females were housed individually with their litters during the lactation periods.

Endpoints assessed for the parental generation included mortality and clinical exams, body weights, feed consumption, complete necropsy with special attention to reproductive organs, and histopathological exams on animals in the high concentration and control groups.

F1a,b, F2a,b, and F3a,b were examined as soon as possible after birth to determine the number of viable and stillborn members of each litter. Where appropriate, litters were evaluated for survival on PND 4, 7, and 14, and at weaning (PND 21). Litter weights were measured at the time of parturition, and PND 4, 7, 14, and 21. On PND 4, the size of each litter was adjusted by eliminating extra pups by random selection to yield 10 pups per litter.

F3b fetuses were c-sectioned from F2a dams on Gestational Day 20 and measured for crown rump length, morphological abnormalities including cleft palate; external, skeletal, and visceral malformations and variations.

Liver weights, body weights, feed consumption, and clinical observations were collected on satellite group animals F1a,b (A), F2a,b (A), and F3a,b (A). In addition, F3a (A) animals had total and differential leukocyte counts, total protein, albumin, and serum albumin/globulin ratios evaluated.

There were no treatment-related deaths for the parental animals. Clinical observations indicated no treatment-related findings throughout the study. All reproductive and lactational indices were equivalent for all matings throughout the study, and at necropsies, there were no treatment-related gross lesions.

In conclusion, continuous dietary exposure to EPG at all concentration levels through 3 generations resulted in no indications of systemic, reproductive, developmental or postnatal toxicity.

6.5.3 Irritation and Sensitization Studies

6.5.3.1 Primary Skin Irritation Study in Rabbits, Primary Eye Irritation Study in Rabbits (unpublished, Hill Top Biolabs Report No. 87-1064-21 (A); E-060)

This study was conducted to evaluate the potential of EPG-08-oleate (lot # 606617 THPG08), to produce irritation to the skin and eye of New Zealand White rabbits. The study was conducted in compliance with the conditions specified in the Regulation for the Enforcement of the Federal Hazardous Substances Act (16 CFR 1500) (CPSC, 2017).

For the primary skin irritation study in rabbits, the test material, a clear light-yellow liquid, was applied undiluted to a 1-inch by 1-inch square surgical gauze patch. The patch was then applied to an intact skin area and an abraded skin area on 6 young adult rabbits (3M and 3F). Each animal received 0.5 mL of test material at each application site. Rabbits were housed individually in wire mesh suspension cages and allowed Purina Laboratory Rabbit Chow and tap water *ad libitum*. They were maintained on a 12-hour light/12-hour dark cycle. At the end of the 24-hour exposure period, the patches were removed and the sites were scored for erythema and edema and checked for tissue damage according to the

method of Draize (1959). Two days later (72-hour reading), sites were again scored for erythema and edema and checked for tissue damage. Results from the Primary Irritation Index were found to be 1.0 based on erythema and edema. No evidence of tissue damage was found. In conclusion, the test material is not classified as a primary irritant or as corrosive following dermal application.

For the primary eye irritation study in rabbits, the test material, a clear light-yellow liquid, was applied undiluted to the right eye of each of 6 New Zealand White rabbits (3M and 3F). It should be noted that these are not the same rabbits as used in the aforementioned primary skin irritation study. Each animal received 0.1 mL of the test material. The eye was not rinsed following the application. Rabbits were housed individually in wire mesh suspension cages and allowed Purina Laboratory Rabbit Chow and tap water *ad libitum*. They were maintained on a 12-hour light/12-hour dark cycle. The eyes of each rabbit were examined approximately 24 hours prior to treatment to assure that they had no pre-existing lesions which could compromise the study. The eyes were graded for corneal changes, conjunctival changes, and changes in the iris approximately 24 hours following test material administration (24-hour reading) and 1 and 2 days later (48-hour and 72-hour reading). Scoring of irritative effects was performed according to the method of Draize (1959), in which corneal, iris, and conjunctival effects are scored separately. An irritation score was calculated for each rabbit on a basis of 0 to 110. Results indicate that the eyes of none of the 6 rabbits were found to show evidence of positive corneal, iris, or conjunctival changes. Irritation scores in individual rabbits were all zeros. In conclusion, the material is not classified as an irritant following ocular application.

6.5.3.2 Guinea Pig Maximization Test (Magnusson and Kligman Method) (unpublished, Hill Top Biolabs Report No. 87-1064-21 (B); E-059)

This study was conducted to evaluate the potential of EPG-08-oleate (lot # 606617 THPG08), to cause delayed contact hypersensitivity in guinea pigs (Hartley albino) using the methodology of Magnusson and Kligman. The test material is a clear, light yellow liquid. Twenty test animals (weighing 319 to 871 g), ten positive control animals, and ten vehicle control animals were injected intradermally on Day 0 with preparations of test material, formaldehyde, and acetone, respectively. Each animal also received 2 intradermal injections of 50% v/v Freund's Complete Adjuvant (FCA) in distilled water and 2 injections of the respective test or control materials in the FCA/distilled water emulsion. On Day 6, these same groups of animals were exposed topically to a preparation of their respective test or control material. Patches were secured under ELASTOPLAST bandage wrappings for approximately 48 hours. On Day 19, all animals were topically challenged at a naive skin site using a preparation of their respective test or control material under ELASTOPLAST bandage wrappings for approximately 24 hours. Naive control animals were patched identically to and concurrently with the test and positive control animals. On the day after removal, the sites were depilated. Later that day and again the next day, the sites were graded for erythema responses (24- and 48-hour responses). On Day 27, all of the original test animals were topically rechallenged at a naive skin site using a preparation of the test material under ELASTOPLAST bandage wrapping for approximately 24 hours. Naive control animals were patched identically to and concurrently with the test animals. On the day after removal, the sites were depilated. Later that day and again the next day, the sites were graded for erythema responses (24- and 48-hour responses).

Following primary challenge, the incidence of grade 1 responses in the test group (0 of 20) was compared to that of the naive test control group (0 of 10). The incidence of these responses was comparable to that produced by the naive control group and resulted in a classification of weak sensitization. However, due to an increased incidence of ± reactions (slight patchy erythema) in the test

group, (15 of 20), as compared to the naive test control group, (3 of 10), a rechallenge was performed to more clearly define the mechanism. Following primary challenge, the incidence of grade 1 response or greater in the positive control group (10 of 10) was compared to that of the naive positive control group (0 of 10). The incidence of these responses was more pronounced than that produced by the naive positive control group and resulted in a classification of extreme sensitization. Following primary challenge, the incidence of grade 1 responses in the vehicle control group (0 of 10) resulted in a classification of weak sensitization. Eight days following the primary challenge application the 20 test animals were single patch rechallenged. Ten naive control animals were patched identically to and concurrently with the test animals. On the day after removal, the sites were depilated. Later that day and again the next day, the sites were graded for erythema responses (24- and 48-hour responses).

Following the single patch rechallenge, the incidence of grade 1 responses in the test group (0 of 20) was compared to that of the naive control group (0 of 10). The incidence of these responses was comparable to that produced by the naive control group. The incidence of ± reactions in the test group, (16 of 20), as compared to the naive test control group, (3 of 10), remained essentially unchanged from that of primary challenge confirming a classification of weak sensitization. As indicated above, classification in accordance with the protocol categorizes the test and vehicle materials as those exhibiting a weak rate of sensitization. It is important to note that this category includes materials which have induced a 0% sensitization rate. This 0% sensitization rate is consistent with the activity of EPG-08-oleate (lot # 606617 THPG08), and the acetone vehicle under the conditions of this protocol. In conclusion, under the conditions of this study, there is no evidence to suggest that EPG-08-oleate (lot # 606617 THPG08) is a dermal sensitizer.

6.6 Clinical Safety

Studies of human tolerance to EPG (H-EPG-05 HR/SO 9:1 and EPG-05 HR/ST 45:55), including single dietary exposure studies and incremental increasing multiple dietary exposure studies, demonstrated that food products prepared with EPGs were highly palatable compared to similar foods prepared with conventional fats. Furthermore, no untoward effects in human volunteers resulted from the consumption of up to 150 g of EPG per day.

It is worthwhile to note that a decline in serum HDL was observed in some subjects placed on very high EPG diets, which greatly reduced the available fat in their diet. This effect was consistent with reports in the published scientific literature which describe a transient drop in serum HDL in subjects placed on low fat diets (Schaefer *et al*., 1981; Knuiman *et al*., 1987; Brinton *et al*., 1990).

Table 6.6-1 provides of summary of human studies conducted with EPG; individual study summaries are provided in Sections 6.6.1 and 6.6.2. An article based on the study entitled, “Assessment of the Effect of Esterified Propoxylated Glycerol (EPG) on the Status of Fat-Soluble Vitamins and Select Water-soluble Nutrients following Dietary Administration to Humans for 8 Weeks” (Section 6.6.2.3), has been published in *Regulatory Toxicology and Pharmacology*.

Table 6.6-1 Summary of Human Studies with EPG in Foods

Study ID	Type of exposure	EPG consumption	Number of subjects completed the study	Observations
Bechtel, 2015	Rising EPG intake (with 2-day wash-out in between) H-EPG-05 HR/SO 9:1	Day 4: 0 g Day 7: 30 g Day 10: 60 g Day 13: 90 g Day 16: 120 g Day 19: 150 g	16	No effects on vital signs, hematology, or urinalysis. EPG was associated with ↓HDL cholesterol in all subjects; transient ↑liver transaminase (12 subjects). Except for flatus, GI-related adverse events decreased with decreasing fat and increasing EPG concentrations.
ICR 004251	Constant EPG intake H-EPG-05 HR/SO 9:1	Days 1 to 18: 120 g/day	15	No effects on hematology or urinalysis. EPG was associated with ↓HDL cholesterol in all subjects; transient ↑liver transaminase (10 subjects). Greater frequency of bowel movements after Day 4 (all subjects), abdominal pain (11 subjects), and oil leakage from rectum (3 subjects). 120 g/day considered well tolerated.
ICR 010197	Constant EPG intake EPG-05 HR/ST 45:55	Days 1 to 3: 0 g/day Days 4 to 21: 60 g/day	12	No effects on vital signs, hematology, urinalysis, body weight, serum 25-OH vitamin D, or ECG. EPG was associated with ↓HDL cholesterol in 7 subjects in Group 1 and 6 subjects of Group 2; ↑liver transaminase (AST, ALT). Three subjects withdrawn (1 receiving 60 g/day; 2 receiving ≤120 g/day) due to GI adverse events (abdominal pain, nausea, *etc.*) possibly/probably related to EPG. The most common GI-related adverse event was bowel movement with oil and colored matter. Greater frequency of bowel movements and incidence of abnormal bowel movements as study progressed. 60 g/day considered well tolerated.
	Declining EPG intake EPG-05 HR/ST 45:55	Days 1 to 3: 0 g/day Days 4 to 7: 120 g/day Days 8 to 16: 100 g/day Days 17 to 21: 76 g/day	14	

Table 6.6-1 Summary of Human Studies with EPG in Foods

Study ID	Type of exposure	EPG consumption	Number of subjects completed the study	Observations
ICR 011426	Constant EPG intake compared to margarine (ordinary triglycerides) EPG-05 HR/ST 45:55	Days 1 to 5: 0 g/day Days 6 to 23: 0, 10, 20, 30, or 40 g/day	10 to 12 per group	No effects on vital signs, clinical chemistry, hematology, or urinalysis. ↑Mean body weights in subjects receiving either margarine or 10 g/day EPG; ↓mean body weight in remaining groups. Statistical analysis showed no significant difference in the frequency of gastrointestinal symptoms for any EPG group or relationship to concentration (↑incidence of difficulty swallowing, excessive flatus, and vomiting at 10 and 40 g/day; ↑vomiting at 30 g/day). EPG was considered well tolerated.
CCRC EC-10*	Constant EPG intake compared to margarine (ordinary triglycerides) EPG-05 HR/ST 45:55	Days 1 to 56: 0, 10, 25, or 40 g/day	34 to 36 per group	No effects on vital signs, body weight, hematology, serum chemistry, urinalysis, PT, PTT, or circulating retinol, α-tocopherol, folate, vitamin B_{12}, zinc, iron, calcium, phosphorus, osteocalcin, RBP, and PTH. EPG was associated with ↓β-carotene, ↓phylloquinone, and ↑PIVKA-II; ↑25-OH D_3 but to a lesser extent than margarine. Greater incidence of GI adverse events (gas with discharge, diarrhea, oily spotting/evacuation/stool, liquid/soft stool) at 25 and 40 g/day. 10 g/day considered well tolerated.

↑ = increased; ↓ = decreased; EPG = Esterified Propoxylated Glycerol; GI = Gastrointestinal; HDL = High Density Lipoprotein; PIVKA-II = Proteins Induced in Vitamin K Absence; PT = Prothrombin Time; PTH = Parathyroid Hormone; PTT = Partial Thromboplastin Time; RBP = Retinol Binding Protein.

* Davidson and Bechtel, 2014

6.6.1 Tolerance Studies with H-EPG-05 HR/SO (9:1)

6.6.1.1 A Rising Multiple Dose Tolerance Dietary Study of a Solid Esterfied Propoxylated Glycerol Version H-EPG-05 HR/SO 9:1 in Normal Volunteers (Bechtel, 2015)

This single-center, domiciled, single-blind, increasing-concentration study was designed to evaluate human tolerance of foods containing a solid form of the fat substitute EPG, H-EPG-05 HR/SO 9:1. Sixteen healthy male volunteers received 0, 30, 60, 90, 120, and 150 g of EPG in baked foods and a butter-like spread, as a rising concentration given over a period of 19 days, each separated by a 2-day wash-out period. Vital signs, blood chemistry, hematology, urinalysis, bowel habit, and adverse events were monitored for 23 days. Corresponding fat levels in the diet for these days were approximately 302, 270, 239, 208, 183, and 149 g, respectively. EPG replaced 0, 10, 20, 30, 40, and 50% fat in the diet, respectively.

All subjects demonstrated decreased HDL cholesterol concentrations. Twelve out of 16 subjects exhibited increased transaminase level at some point during the study; the levels returned to normal by the end of the study or by the post-study follow-up visit. The authors indicated that adverse events related to gastrointestinal dysfunction were associated with large quantities of food and fat consumed. Adverse events, with the exception of flatus, decreased as the fat content decreased and the amount of EPG increased. No serious adverse events were reported. Overall, EPG was well tolerated.

6.6.1.2 A Repeated Dose, Tolerance Study of Dietary Esterified Propoxylated Glycerol (H-EPG-05 HR/SO 9:1) in Normal Volunteers (unpublished, ICR Project No. 004251; E-030)

Fifteen healthy male volunteers received 120 g/day of a solid form of EPG, H-EPG-05 HR/SO 9:1, in a single-center, domiciled, single-blind, repeated, constant concentration, tolerance study. EPG was incorporated into 3 meals and 3 snacks, in foods such as cinnamon buns, chocolate bars, scones, and a butter-like spread administered for 18 days. Hematology, clinical chemistry, and urinalysis were monitored on study Days 7, 14, and 22.

All subjects experienced an increase in the frequency of bowel movements after Day 4, 11 subjects reported abdominal pain at some point, and 3 subjects reported oil leakage per rectum. All subjects exhibited a decrease in HDL concentration over time. An increase of liver transaminases was observed in 10 subjects, the elevation was transient attributed to a high carbohydrate intake. Reported adverse events of a gastrointestinal nature demonstrated no trend. As such, it was concluded that ingestion of 120 g of EPG for 18 days was not associated with any serious adverse effects.

6.6.2 Tolerance Studies with EPG-05 HR/ST 45:55 (Softer Version on EPG)

6.6.2.1 Repeated, Parallel Group, Constant Dose Tolerance Study of 2 Consumption Levels of Dietary Esterified Propoxylated Glycerol (EPG-05 HR/ST 45:55) AOD2-09 (unpublished, ICR Project No. 010197; E-035)

This single-center, domiciled, constant-concentration study assessed the safety and tolerance of a solid form of the fat substitute EPG, EPG-05 HR/ST 45:55, as a dietary component of breakfast, lunch, and dinner snacks, such as cinnamon buns, chocolate bars, scones, and spread. In addition, the study was designed to collect data to support a NOEL for oil leakage per rectum, and for oil separation in bowel movements. Healthy male volunteers were randomized into 2 parallel groups for 22 days. Group 1

included 12 subjects that received 60 g/day of EPG-05 HR/ST 45:55 on Days 4 to 21 of the study. Group 2 included 16 subjects that received a declining EPG-05 HR/ST 45:55 concentration of 120, 100, and 76 g on Days 4 to 7, 8 to 16, and 17 to 21, respectively. Vital signs, blood chemistry, hematology, urinalysis, weight, bowel habit, serum 25-monohydroxy vitamin D, electrocardiogram (ECG) results, and adverse events were monitored.

No serious adverse events were observed. No effects were seen in vital signs, ECG, hematology, and urinalysis. Decreased HDL cholesterol concentrations were observed in 7 subjects from Group 1, and 6 subjects from Group 2. Increases in aspartate aminotransferase (AST) and alanine transaminase (ALT) were observed and could possibly be related to EPG intake. The frequency of bowel movements and incidences of abnormal bowel movements increased as the study progressed. A total of 895 out of 947 adverse events reported were related to the gastrointestinal tract; of these, the most common event was a bowel movement with oil and colored matter. One subject receiving 60 g/day was withdrawn due to abdominal pain. Two subjects from Group 2 (receiving 120 g/day initially and then reduced to 100 and 76 g/day) were withdrawn due to rectal fissure bleeding, and abdominal pain/nausea. These effects were considered possibly or probably related to EPG. No NOEL for oil leakage per rectum and for oil separation in bowel movements was established. The authors concluded that ingestion of 60 g/day of EPG-05 HR/ST 45:55 for 18 days was not associated with any serious adverse effects.

6.6.2.2 Protocol Number EC-09/011426. A Double-Blind, Parallel Group, Placebo Controlled Tolerance Study of 4 Doses of Dietary Esterified Propoxylated Glycerol (EPG-05 HR/ST 45:55) and Placebo in Normal Volunteers (unpublished, ICR Project No. 011426; E-038)

A randomized, domiciled, double-blind, parallel group, placebo-controlled study was conducted to assess the safety and tolerability of EPG-05 HR/ST 45:55 and possibly determine an approximate NOEL. The experimental design included a 5-day run-in period, during which subjects received ordinary triglycerides, followed by an 18-day double-blind period in which 55 subjects (10 to 12/group) were randomized to receive 0, 10, 20, 30, or 40 g of EPG/day in food products such as spread, chocolate, and muffins. Vital signs, adverse events, gastrointestinal symptoms, bowel movements and stool characteristics, hematology, blood chemistry, and body weight were monitored.

According to the principal investigator, no clinically-significant changes were observed in vital signs, ECGs, urinalysis, or hematology. No increase in abnormal bowel movements or loose soft stools was observed in subjects receiving EPG-05 HR/ST 45:55 when compared to ordinary triglycerides. However, there was an increase in the frequency of normal bowel movements in the group receiving 30 g of EPG-05 HR/ST 45:55/day. There was an increase in mean body weight of subjects receiving triglycerides and 10 g of EPG-05 HR/ST 45:55/day, and a decrease in groups receiving 20, 30, and 40 g of EPG-05 HR/ST 45:55/day. Decreased cholesterol and low-density lipoproteins (LDL) was observed in groups receiving 30 and 40 g of EPG-05 HR/ST 45:55/day; these decreases were small and not considered clinically significant.

No serious adverse events were reported. Ninety-five minor adverse events were observed, of these, headache was most common. Subjects receiving EPG-05 HR/ST 45:55 also reported difficulty swallowing, excess flatus, and vomiting. One subject receiving 30 g of EPG-05 HR/ST 45:55/day demonstrated increased levels of AST and ALT concentrations on Day 10, possibly related to the test EPG-05 HR/ST 45:55. No significant concentration-related trend in the occurrence of adverse events was observed. A slight increase in the number of adverse events in the group receiving 40 g of EPG-05

HR/ST 45:55/day was observed; however, this was considered unrelated to the administration of the test material. In conclusion, concentrations of 10, 20, 30, or 40 g of EPG-05 HR/ST 45:55/day were expected to cover the NOEL range. However, a NOEL was not established. Overall, EPG-05 HR/ST 45:55 was considered to be safe and well tolerated.

6.6.2.3 Assessment of the Effect of Esterified Propoxylated Glycerol (EPG) on the Status of Fat-Soluble Vitamins and Select Water-soluble Nutrients following Dietary Administration to Humans for 8 Weeks (Davidson and Bechtel, 2014)

A double-blind, randomized, controlled study was performed to assess the effect of EPG-05 HR/ST 45:55 (*i.e.*, a softer version of EPG) on fat-soluble vitamins and select nutrients in human subjects. For 8 weeks, 139 healthy volunteers (34 to 36/group) consumed a core diet providing adequate caloric and nutrient intakes. The diet included items (spread, muffins, cookies, and biscuits) providing EPG (10, 25, and 40 g/day) *vs*. margarine alone (control).

The variables measured at baseline and regular intervals were: physical exam, including vital signs; body weight; hematology; clinical chemistry; urinalysis; circulating levels of β-carotene, retinol (vitamin A), α-tocopherol (vitamin E), 25-OH D_2 (vitamin D, ergocalciferol), 25-OH D_3 (vitamin D, cholecalciferol), phylloquinone (vitamin K_1), PIVKA-II (proteins induced in vitamin K absence), serum folate, red blood cell folate, vitamin B_{12}, zinc, iron, calcium, phosphorus, osteocalcin, retinol-binding protein, intact parathyroid hormone, cholesterol, HDL-C (high-density lipoproteins), LDL-C, and triglycerides; PT and PTT (partial thromboplastin time); urine zinc, sodium, potassium, creatinine, calcium, and phosphorus; and tolerability. Tolerability was assessed by the incidence of 14 specific gastrointestinal adverse events: passing gas; gas with discharge; abdominal bloat/cramp; heartburn; diarrhea; constipation; urgency of bowel movement; fecal incontinence; oily spotting; oily evacuation; oily stool; liquid stool; soft stool; and hard stool.

Significant declines in β-carotene were seen over time, especially in the EPG groups, but with no apparent relationship to EPG concentration (more severe at 10 g/day and 40 g/day than at 25 g/day). It is possible that the apparent effect of EPG on circulating β-carotene was related to a lower dietary fat intake among subjects receiving EPG, since subjects had difficulty consuming all of the additional fat necessary to fully compensate for what EPG is displaced in the diet. In this case, as a lipid-like material, EPG might have affected the absorption of these nutrients strictly through physicochemical processes, acting as a lipid "sink" during transit in the gastrointestinal tract.

As shown in Figure 6.6.2.3-1, based on the Wilcoxon Rank Sum Test, there were no statistically significant changes from baseline at the primary endpoint (Day 56) in mean retinol levels in evaluable subjects receiving EPG 10, 25, and 40 g/day compared with subjects receiving placebo. Similarly, no other statistically significant differences in the mean change from baseline were noted between the EPG groups and placebo group at Days 14, 28, 42, 56 and the end point analysis with the exception of the EPG 25 g/day group at Day 14 (P=0.0141).

Likewise, the Wilcoxon Rank Sum Test revealed significant decreases in mean α-tocopherol levels from baseline in the EPG 25 g/day group at Days 14 (p=0.498), 28 (p=0.0014) and 42 (p=0.0001) and in the EPG 40 g/day group at Day 14 (p=0.0166), Day 42 (p=0.0030) compared to the placebo group. No other statistically significant differences in the change from baseline were noted between the EPG groups and the placebo group at Days 14, 28, 42, and 56. The end point analysis using the last observation carried forward was similar to the Day 56 results for each treatment group (Figure 6.6.2.3-2).

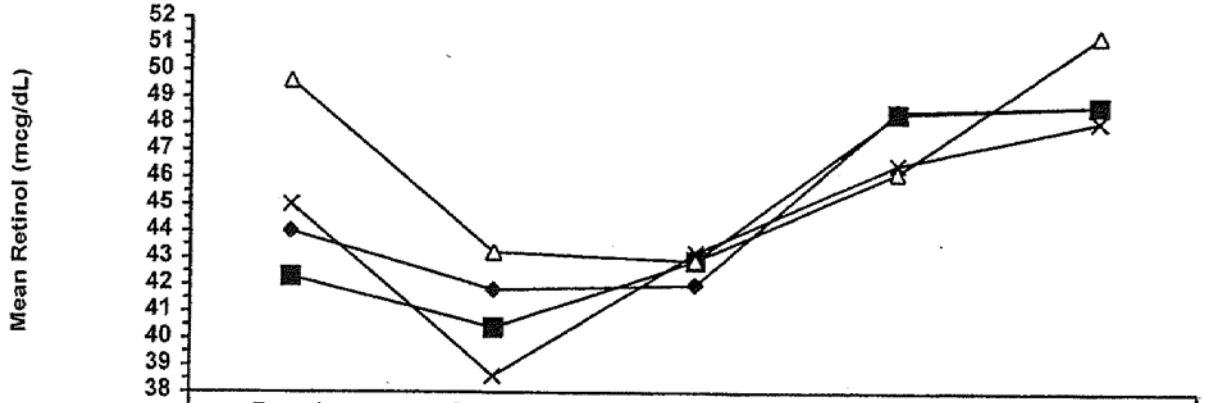
Circulating 25-OH D_3 levels increased over time in the EPG groups, but not to the same degree as the control group, which had an unexpected rise, despite attempts to control endogenous 25-OH-D_3 synthesis by conducting the study during the winter in Chicago, Illinois, USA (Figures 6.6.2.3-3 and 6.6.3.2-4).

EPG intake was associated with a slight decline in phylloquinone levels across all groups. However, the declines did not exceed 0.1 ng/mL and were not statistically significant within any of the individual groups; statistical significance was observed only when the differences within each EPG group were compared to the differences (none or positive) in the control.

By the end of the study, the levels of circulating proteins induced in vitamin K absence (PIVKA-II) had increased significantly in the EPG 25 and EPG 40 groups, compared to the control; in the EPG 10 group, the difference from baseline was comparable to the difference from baseline in the control. Combined with the phylloquinone results, these data suggest that EPG might have affected the synthesis of vitamin K-dependent clotting factors to some extent, but the changes were small, and there was no indication of any clinical manifestation. The changes in clotting parameters (PT and PTT) from baseline to the end of the study were comparable between the control and EPG groups.

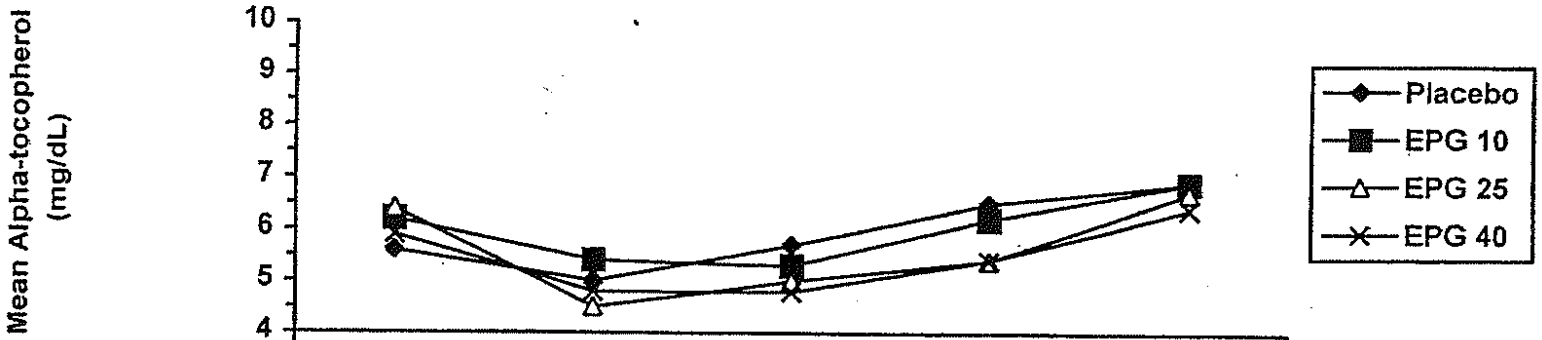
With the exception of gastrointestinal discomfort, all adverse events reported by subjects in this study were considered unrelated to EPG. Seven of the 14 pre-defined gastrointestinal adverse events (gas with discharge; diarrhea; oily spotting; oily evacuation; oily stool; liquid stool; soft stool) were reported more frequently by subjects receiving 25 or 40 g/day of EPG, especially females. In general, the incidence and duration of these symptoms correlated with EPG dietary concentration. The results suggest 10 g/day of EPG was reasonably well tolerated.

Figure 6.6.2.3-1 Mean Retinol Levels Over Time



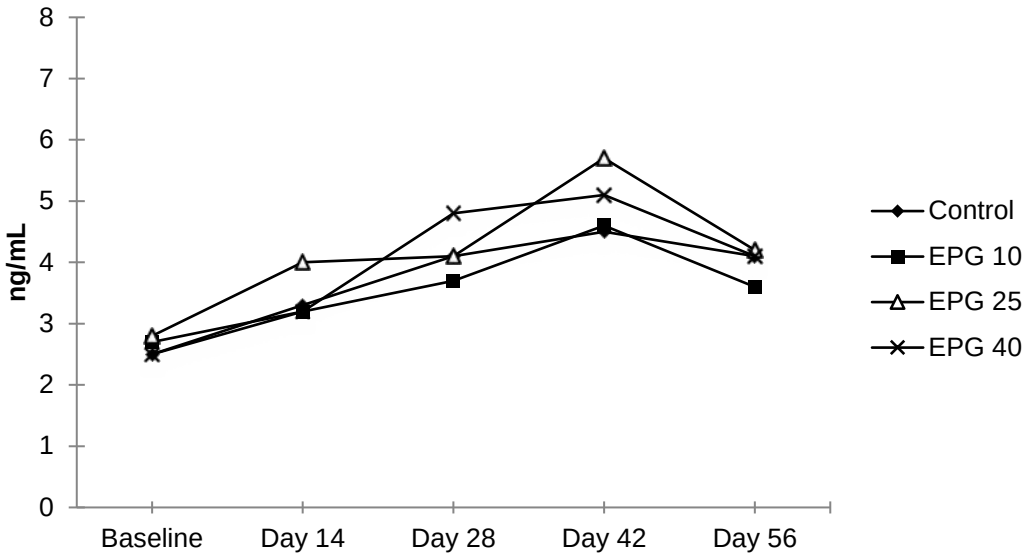
	Day -1	Day 14	Day 28	Day 42	Day 56
Placebo	44	41.8	42	48.5	48.7
EPG 10	42.3	40.4	42.9	48.4	48.7
EPG 25	49.6	43.2	42.9	46.2	51.3
EPG 40	45	38.6	43.2	46.5	48.1

Figure 6.6.2.3-2 Mean α-Tocopherol Levels Over Time



	Day -1	Day 14	Day 28	Day 42	Day 56
Placebo	5.6	5	5.7	6.5	6.9
EPG 10	6.2	5.4	5.3	6.2	6.9
EPG 25	6.4	4.5	5	5.4	6.7
EPG 40	5.9	4.8	4.8	5.4	6.4

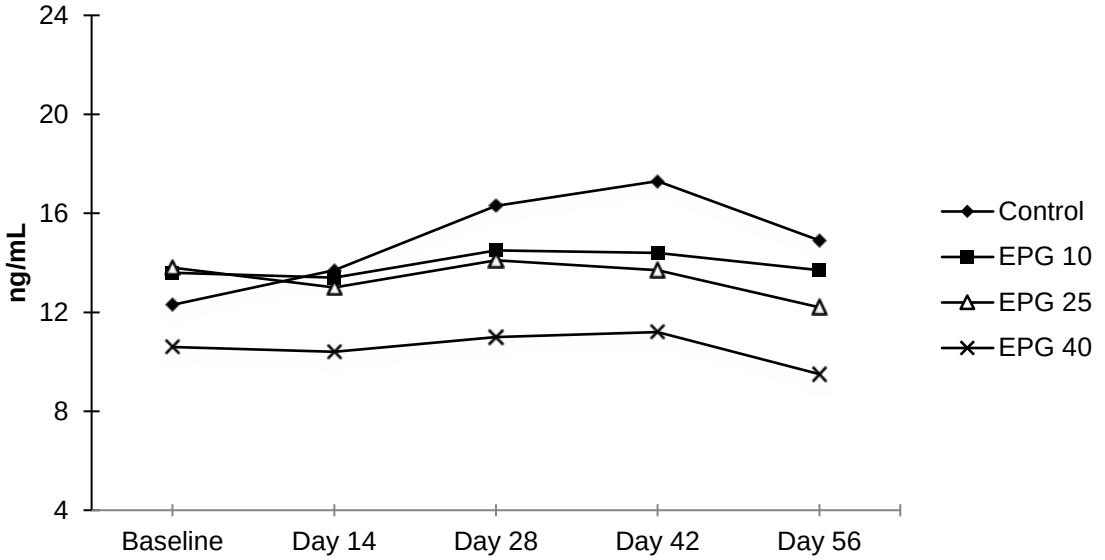
Figure 6.6.2.3-3 Mean Circulating 25-OH D2 (Ergocalciferol) Levels Over Time



	Control	EPG 10	EPG 25	EPG 40
Baseline				
N	27	27	24	25
Mean (SD)	2.5 (1.6)	2.7 (1.6)	2.8 (1.2)	2.5 (1.2)
Median	2.2	2.2	2.8	2.2
Range	1 to 8	1 to 7	1 to 6	1 to 5
Day 14				
N	27	27	23	25
Mean (SD)	3.3 (2.3)	3.2 (2.1)	4.0 (2.3)	3.2 (1.5)
Median	2.8	2.7	3.4	3.3
Range	1 to 10	1 to 11	1 to 12	1 to 7
Day 28				
N	27	27	23	25
Mean (SD)	4.1 (2.2)	3.7 (2.4)	4.1 (3.6)	4.8 (4.2)
Median	3.8	3.4	3.4	4.3
Range	1 to 11	1 to 14	1 to 19	1 to 24
Day 42				
N	25	27	24	25
Mean (SD)	4.5 (2.1)	4.6 (3.8)	5.7 (6.5)	5.1 (3.6)
Median	4.1	3.5	4.2	4.6
Range	1 to 10	2 to 22	3 to 35	2 to 21
Day 56				
N	27	27	23	24
Mean (SD)	4.1 (1.9)	3.6 (2.0)	4.2 (2.3)	4.1 (2.8)
Median	4.3	3.6	3.6	3.9
Range	1 to 8	1 to 9	1 to 11	1 to 16

EPG = esterified propoxylated glycerol; SD = standard deviation.

Figure 6.6.2.3-4 Mean Circulating 25-OH D3 (Cholecalciferol) Levels Over Time



	Control	EPG 10	EPG 25	EPG 40
Baseline				
N	27	27	24	25
Mean (SD)	12.3 (8.3)	13.6 (6.7)	13.8 (9.0)	10.6 (5.3)
Median	9.1	13	10.4	9.7
Range	4 to 35	4 to 26	5 to 41	5 to 28
Day 14				
N	27	27	23	25
Mean (SD)	13.7 (6.2)	13.4 (5.7)	13.0 (7.8)	10.4 (4.6)
Median	12.8	13.2	10	9.4
Range	6 to 31	5 to 23	6 to 35	5 to 24
Day 28				
N	27	27	23	25
Mean (SD)	16.3 (5.7)	14.5 (5.1)	14.1 (7.2)	11.0 (4.2)
Median	14.7	12.7	11.1	9.7
Range	10 to 33	8 to 24	7 to 33	7 to 24
Day 42				
N	25	27	24	25
Mean (SD)	17.3 (5.4)	14.4 (5.1)	13.7 (6.0)	11.2 (4.1)
Median	16.7	13	11.8	10.4
Range	9 to 31	6 to 25	8 to 31	6 to 25
Day 56				
N	27	27	23	24
Mean (SD)	14.9 (5.0)	13.7 (5.8)	12.2 (5.7)	9.5 (3.4)
Median	14.4	11.6	10	9.3
Range	7 to 27	5 to 29	5 to 29	5 to 19

EPG = esterified propoxylated glycerol; SD = standard deviation.

6.7 Information Potentially Inconsistent with GRAS

6.7.1 Gastrointestinal Discomfort

Olestra intake has reportedly been associated with gastrointestinal symptoms, including loose stools. This effect is considered similar to the effect of mineral oil, which interferes with the development of firm, well-formed stools (61 FR, 3118; January 30, 1996) (U.S. FDA, 1996).

The potential of EPG to induce similar effects was assessed through multiple studies. Administration of solid forms of EPG to experimental animals and humans has not resulted in any adverse effects on gastrointestinal physiology. All versions of EPG currently being considered for use in spreadable and baked goods are solids with melting points at or above 102°F. As such, the proposed forms are expected to be well tolerated and devoid of adverse gastrointestinal effects.

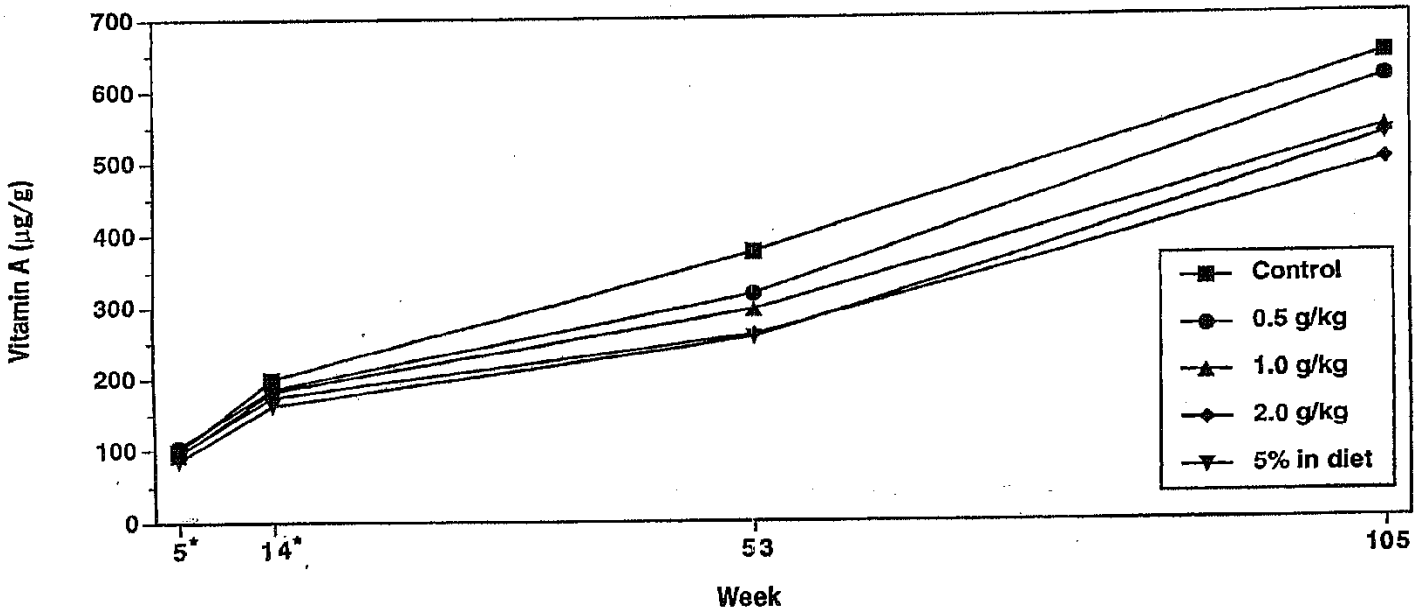
Occasional separation of the test material from stool bulk has been observed at the highest levels of EPG exposure (up to 150 g/day), but the incidence of loose stool and other gastrointestinal symptoms declines with decreasing dietary concentrations. For example, human volunteers receiving 25 or 40 g/day of a semi-solid form of EPG in food items (spread and baked goods) for 8 weeks, reported gastrointestinal adverse events (gas, soft stool, oily spotting, *etc.*) more frequently than subjects receiving margarine alone. However, at 10 g/day, the only statistically significant difference from the control (margarine) group was oily spotting (refer to Sections 6.6.1 and 6.6.2).

6.7.2 Effect on Nutrient (Fat-Soluble Vitamin) Status

EPG is intended to replace fats in selected spreadable and baked food products. Some fat-mimetic substances have been shown to interfere with the absorption of lipid-soluble nutrients from the gastrointestinal tract, and conduct of safety studies required the diet to be nutrient fortified. For example, pigs fed olestra for 4 to 26 weeks had lower liver and serum concentrations of vitamins A and E, and lower serum 25-OH vitamin D, in a dose-dependent manner (reviewed by Tulley *et al*., 2005). Olestra has also been associated with potentially clinically significant lower absorption of fat-soluble vitamins in humans (Schlagheck *et al*., 1997). Due to the intensity of this nutrient interaction, vitamin fortification of olestra is required by the FDA.

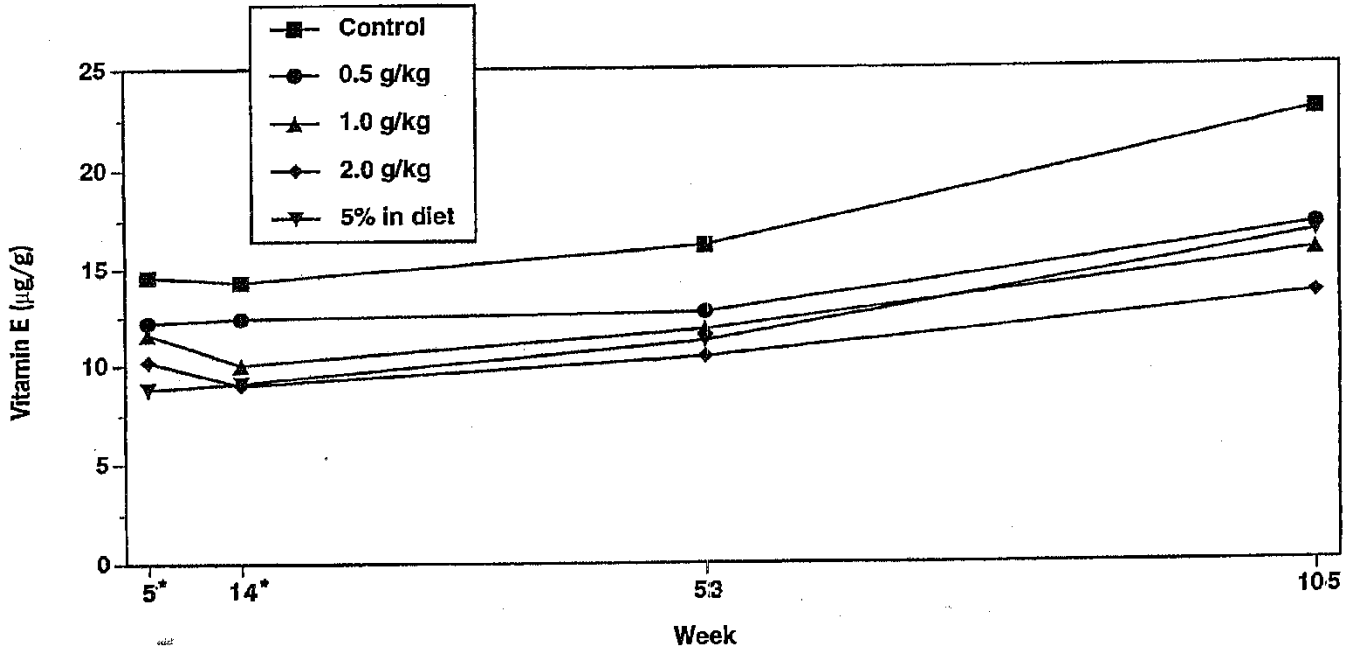
By contrast, EPG exhibits only a weak interaction and none of the safety or clinical studies required diets to be nutrient fortified. Experimental animal studies of EPG have shown some treatment-related effects on the levels of some fat-soluble vitamins (Figures 6.7.2-1 through 6.7.2-3). Specifically, dietary intake of EPG was associated with lower levels of liver vitamins A (retinol) and E (tocopherol), and serum vitamin D across multiple animal species, in both sexes, and generally in a concentration-dependent manner. Despite these observations, which were statistically significant in many cases, none of the animals in any of the studies exhibited clinical signs or microscopic evidence of vitamin deficiency. By the nature of the study designs, most effects would have been detected. In addition, there was no evidence that the vitamin levels detected in the EPG studies were significantly different from those reported in the published literature for normal control animals. In the long-term EPG studies, the levels of fat-soluble vitamins increased and stabilized over time to fall within normal ranges reported in the literature, despite being significantly lower statistically than those of the corresponding control group animals. As reported in Section 6.6.2.3, no significant effects were observed in fat soluble vitamin status among human subjects consuming up to 40 g/day of EPG for 8 weeks.

Figure 6.7.2-1 Liver Vitamin A in Male Rats



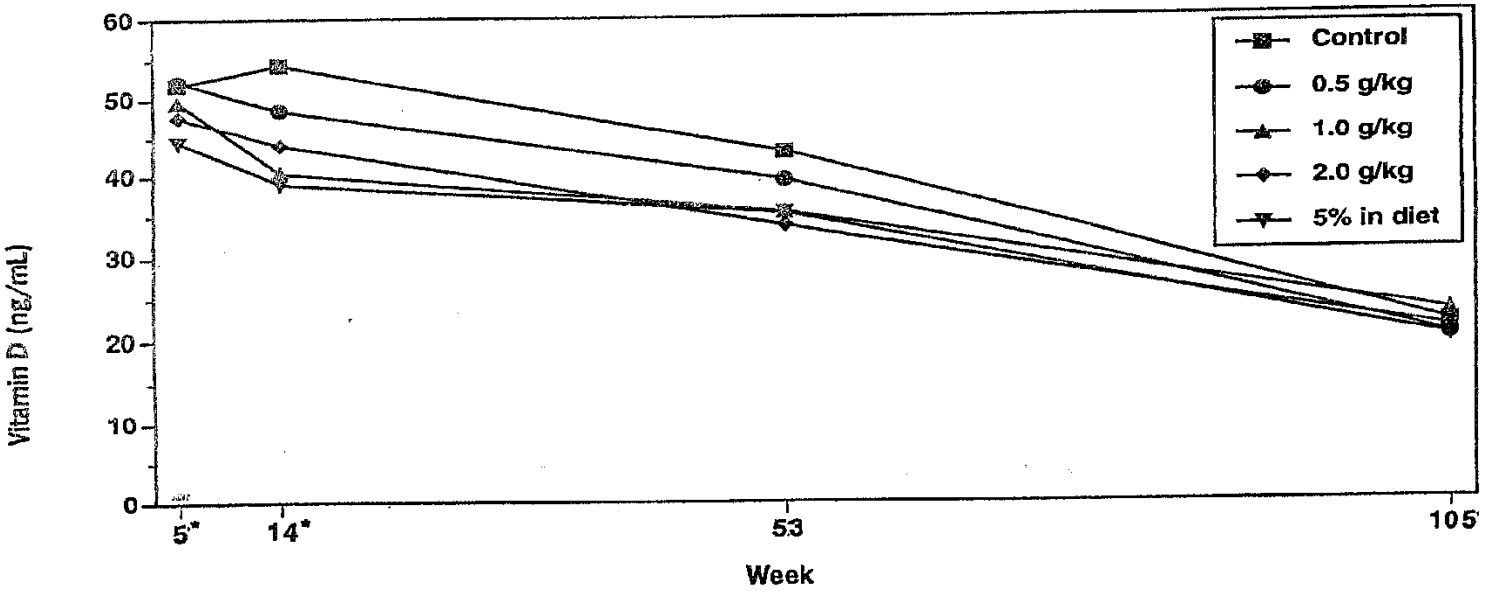
* Values from HWI 6226-122

Figure 6.7.2-2 Liver Vitamin E in Male Rats



* Values from HWI 6226-122

Figure 6.7.2-3 Serum Vitamin D in Male Rats



* Values from HWI 6226-122

6.7.3 Effect on β-Carotene Levels

As noted in Section 6.2.3, significant declines in β-carotene were reported in subjects receiving EPG; however, if the effect was indeed related to EPG intake, it is uncertain why there was no apparent relationship to EPG concentration (more severe at 10 g/day and 40 g/day than at 25 g/day).

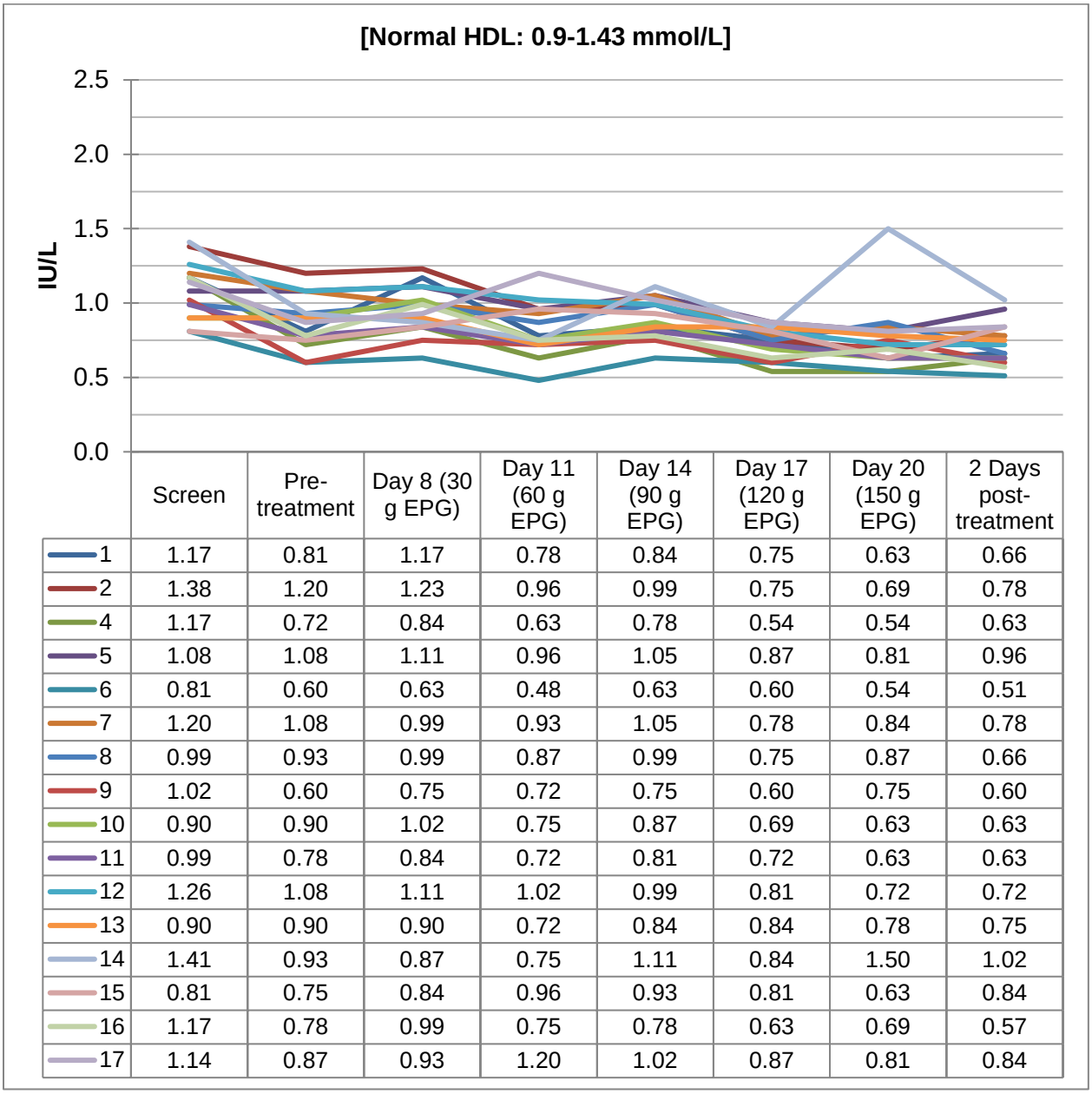
The bioavailability of carotenoids such as β-carotene can vary considerably based on the food matrix, concurrent fat intake, and serum/tissue concentrations. A study that explored, among others, the fate of β-carotene from carrot purée when administered to healthy human subjects, showed based on samples of blood, and stomach and duodenal contents, that the stomach initiates the transfer of carotenoids from the food matrix to the fat phase of a meal, but that the proportion of carotenoids recovered in the micellar phase of the duodenum is very low (<7%) (Tyssandier *et al*., 2003).

It is possible that the apparent effect of EPG on circulating β-carotene was related to the lower dietary fat intake among subjects receiving EPG. As previously mentioned, subjects might not have consumed all of the additional fat necessary to fully compensate for what EPG displaced in the diet. In this case, as a lipid-like material, EPG might have affected the absorption of these nutrients strictly through physicochemical processes, acting as a lipid "sink" during transit in the gastrointestinal tract. Substances known to reduce the bioavailability of carotenoids are lipid-lowering agents such as cholestryramine and probucol (Elinder *et al*., 1995), nonabsorbable fat substitutes such as sucrose polyester (olestra) (Peters *et al*., 1997; Schlagheck *et al*., 1997; Tulley *et al*., 2005; Neuhouser *et al*., 2006), plant sterol-enriched margarines (Gylling *et al.*, 1999; Law, 2000; Hendriks *et al*., 2003), and dietary fiber supplementation (Rock and Swendseid, 1992). No dietary reference intakes *per se* have been proposed by the Institute of Medicine for carotenoids, although existing recommendations for increased consumption of carotenoid rich fruits and vegetables are supported (IOM, 2000).

6.7.4 Effect on Serum HDL Levels

Serum HDL levels below the normal range were reported in some individuals receiving EPG in amounts between 60 and 150 g EPG/day for up to 18 days (Figure 6.7.4-1). The decrease in serum HDL levels was small and not reported in subsequent studies at lower doses for more extended periods. This effect is consistent with reports in the published scientific literature which describe a transient drop in serum HDL in subjects placed on low fat diets (Schaefer *et al*., 1981; Knuiman *et al*., 1987; Brinton *et al*., 1990). In addition, studies with EPG in micropigs, a species considered to be a good model for human digestion, for up to 1 year did not produce evidence of an effect on serum HDL (Figures 6.7.4-2a and 6.7.5-2b).

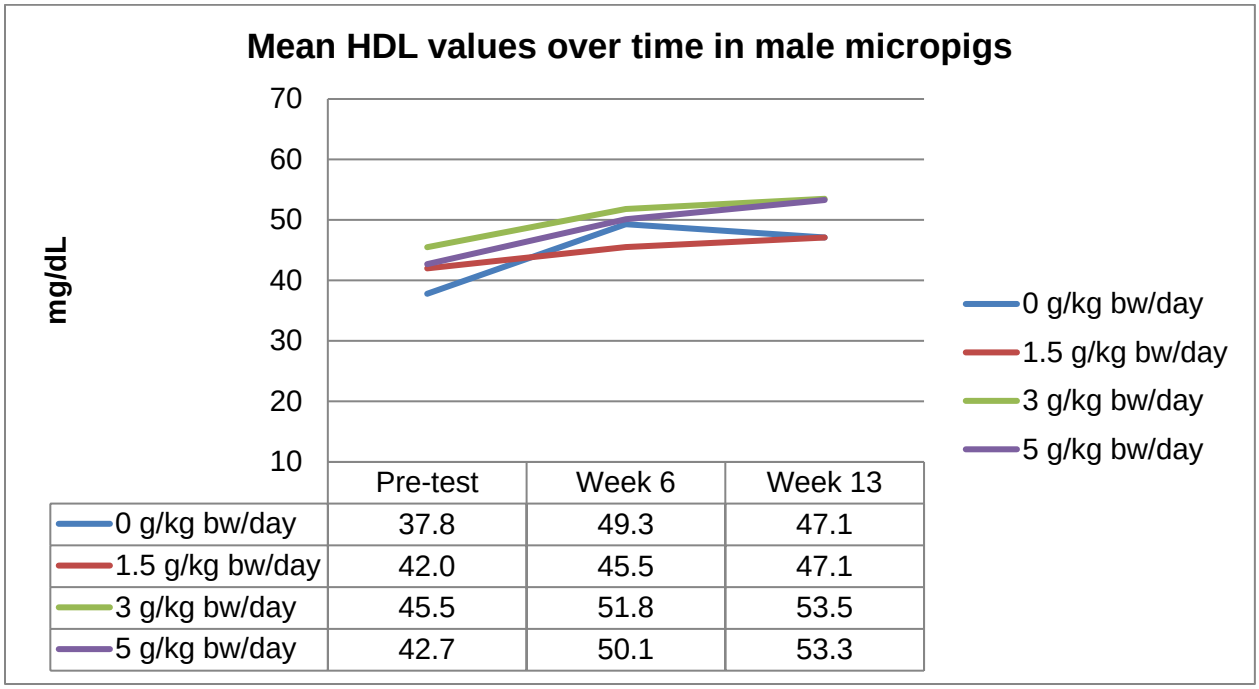
Figure 6.7.4-1 HDL Cholesterol Levels Over Time in Each of 16 Subjects Receiving Increasing EPG Concentrations



	Screen	Pre-treatment	Day 8 (30 g EPG)	Day 11 (60 g EPG)	Day 14 (90 g EPG)	Day 17 (120 g EPG)	Day 20 (150 g EPG)	2 Days post-treatment
1	1.17	0.81	1.17	0.78	0.84	0.75	0.63	0.66
2	1.38	1.20	1.23	0.96	0.99	0.75	0.69	0.78
4	1.17	0.72	0.84	0.63	0.78	0.54	0.54	0.63
5	1.08	1.08	1.11	0.96	1.05	0.87	0.81	0.96
6	0.81	0.60	0.63	0.48	0.63	0.60	0.54	0.51
7	1.20	1.08	0.99	0.93	1.05	0.78	0.84	0.78
8	0.99	0.93	0.99	0.87	0.99	0.75	0.87	0.66
9	1.02	0.60	0.75	0.72	0.75	0.60	0.75	0.60
10	0.90	0.90	1.02	0.75	0.87	0.69	0.63	0.63
11	0.99	0.78	0.84	0.72	0.81	0.72	0.63	0.63
12	1.26	1.08	1.11	1.02	0.99	0.81	0.72	0.72
13	0.90	0.90	0.90	0.72	0.84	0.84	0.78	0.75
14	1.41	0.93	0.87	0.75	1.11	0.84	1.50	1.02
15	0.81	0.75	0.84	0.96	0.93	0.81	0.63	0.84
16	1.17	0.78	0.99	0.75	0.78	0.63	0.69	0.57
17	1.14	0.87	0.93	1.20	1.02	0.87	0.81	0.84

EPG = esterified propoxylated glycerol; HDL = high-density lipoprotein.
* Values from ICR Project No. 0047047 (Section 6.1.1)

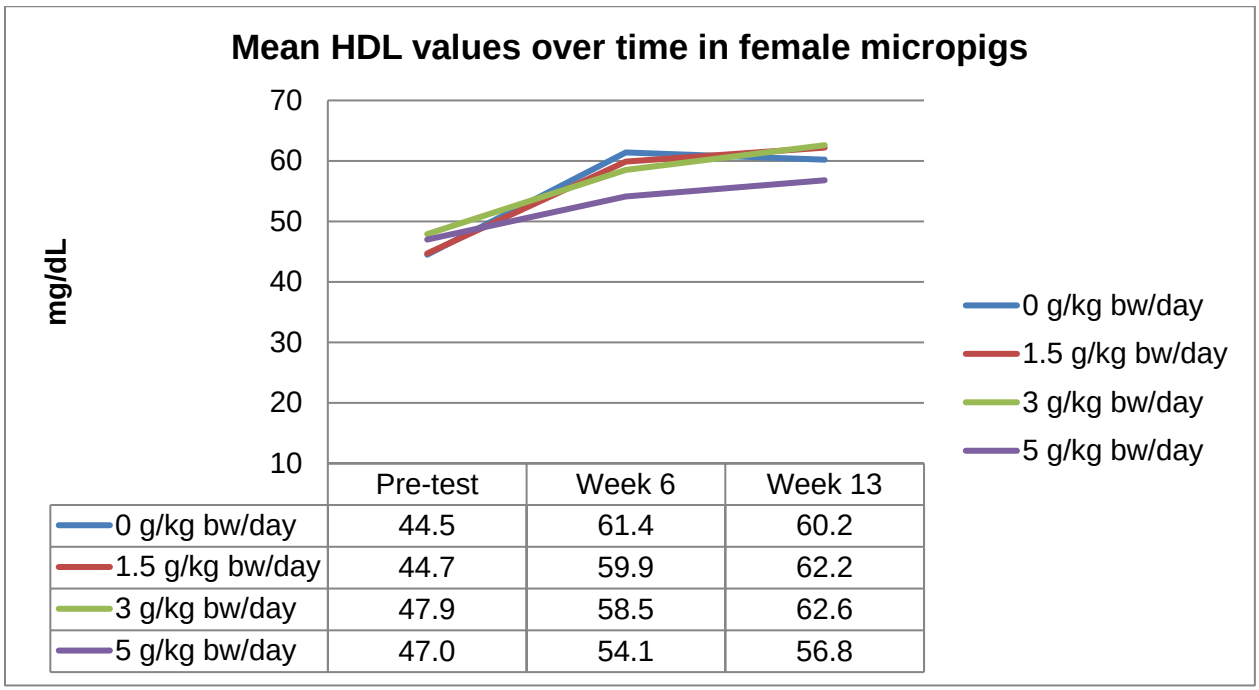
Figure 6.7.4-2a Mean HDL Values Over Time in Male Micropigs



	Pre-test	Week 6	Week 13
0 g/kg bw/day	37.8	49.3	47.1
1.5 g/kg bw/day	42.0	45.5	47.1
3 g/kg bw/day	45.5	51.8	53.5
5 g/kg bw/day	42.7	50.1	53.3

HDL = high-density lipoprotein.

Figure 6.7.4-2b Mean HDL Values Over Time in Female Micropigs



	Pre-test	Week 6	Week 13
0 g/kg bw/day	44.5	61.4	60.2
1.5 g/kg bw/day	44.7	59.9	62.2
3 g/kg bw/day	47.9	58.5	62.6
5 g/kg bw/day	47.0	54.1	56.8

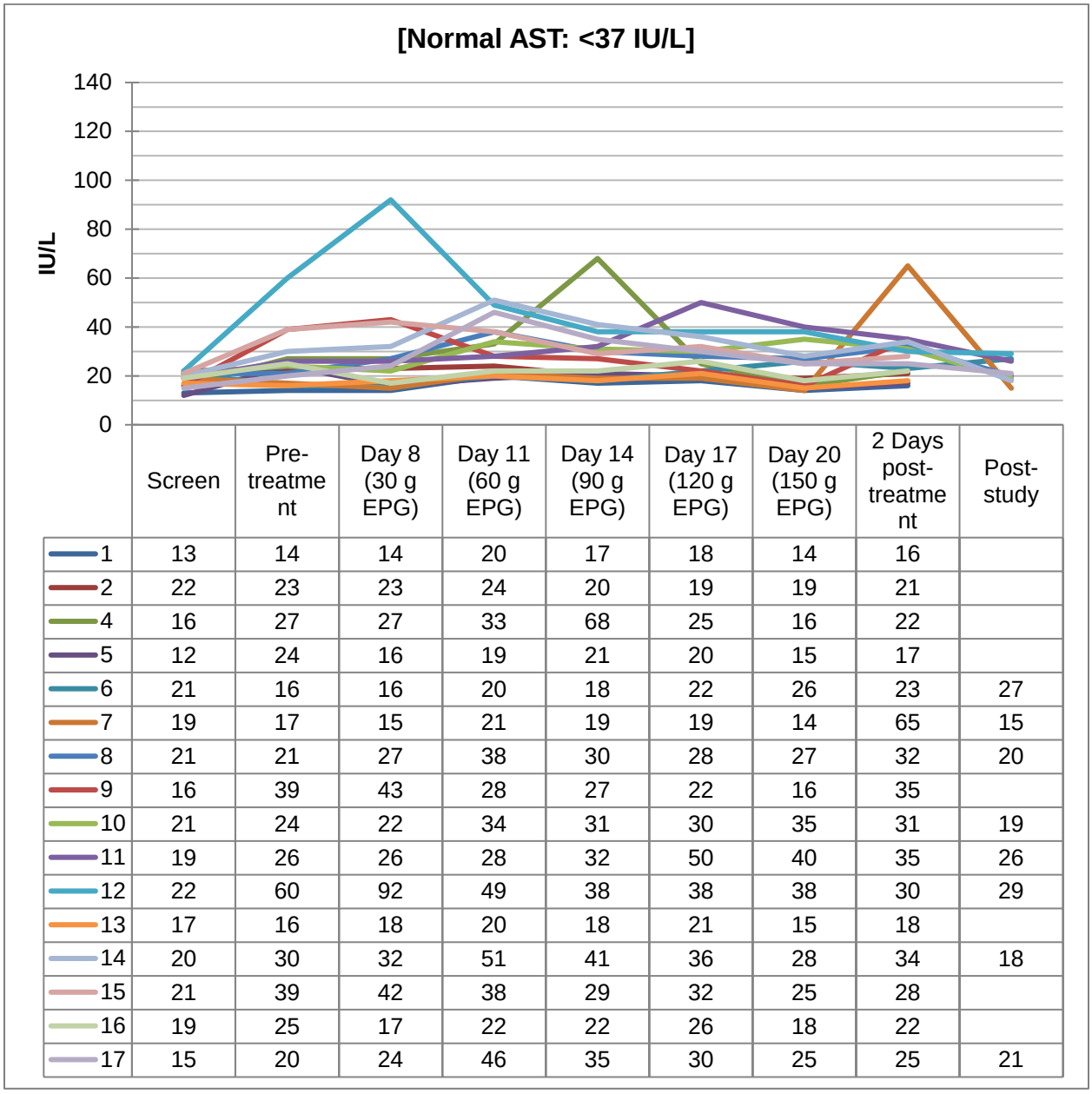
HDL = high-density lipoprotein.

* Values from 90-Day dietary toxicity study with esterified propoxylated glycerol (EPG) in Micropigs (Wedig and Bechtel, 2014)

6.7.5 Effect on Serum Transaminase Levels

Results of multiple human range-finding tolerance studies showed that serum transaminase (aspartate aminotransferase and/or alanine aminotransaminase) levels exceeded the normal range in some subjects receiving EPG in amounts between 60 and 150 g/day (Figures 6.7.5-1a through 6.7.5-1d). The occasional moderate increase in measured serum transaminase values often occurred in a transient manner, rising briefly and then returning to normal ranges. This response was not observed in more extended clinical studies designed to also measure vitamin and nutrient status. Likewise, no effect on serum transaminase activity was reported in any of the animal studies including lifetime studies in rats and mice, nor was there reported any evidence of liver damage or related pathology in these investigations. Since the excursion of transaminase activity observed in some preliminary clinical investigations at doses exceeding 60 g/day, was not observed at lower doses or in extended preclinical safety investigations, it is not considered relevant to the much lower intake of EPG expected from the current intended uses.

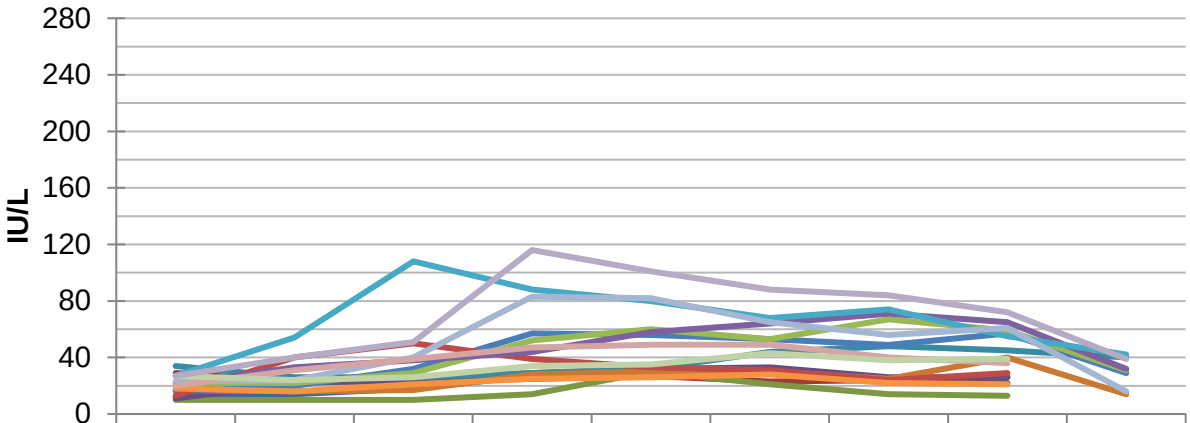
Figure 6.7.5-1a AST Levels Over Time in Each of 16 Subjects Receiving Increasing EPG Concentrations



	Screen	Pre-treatment	Day 8 (30 g EPG)	Day 11 (60 g EPG)	Day 14 (90 g EPG)	Day 17 (120 g EPG)	Day 20 (150 g EPG)	2 Days post-treatment	Post-study
1	13	14	14	20	17	18	14	16	
2	22	23	23	24	20	19	19	21	
4	16	27	27	33	68	25	16	22	
5	12	24	16	19	21	20	15	17	
6	21	16	16	20	18	22	26	23	27
7	19	17	15	21	19	19	14	65	15
8	21	21	27	38	30	28	27	32	20
9	16	39	43	28	27	22	16	35	
10	21	24	22	34	31	30	35	31	19
11	19	26	26	28	32	50	40	35	26
12	22	60	92	49	38	38	38	30	29
13	17	16	18	20	18	21	15	18	
14	20	30	32	51	41	36	28	34	18
15	21	39	42	38	29	32	25	28	
16	19	25	17	22	22	26	18	22	
17	15	20	24	46	35	30	25	25	21

AST = aspartate aminotransferase; EPG = esterified propoxylated glycerol.
* Values from ICR Project No. 0047047 (Section 6.6.1.1)

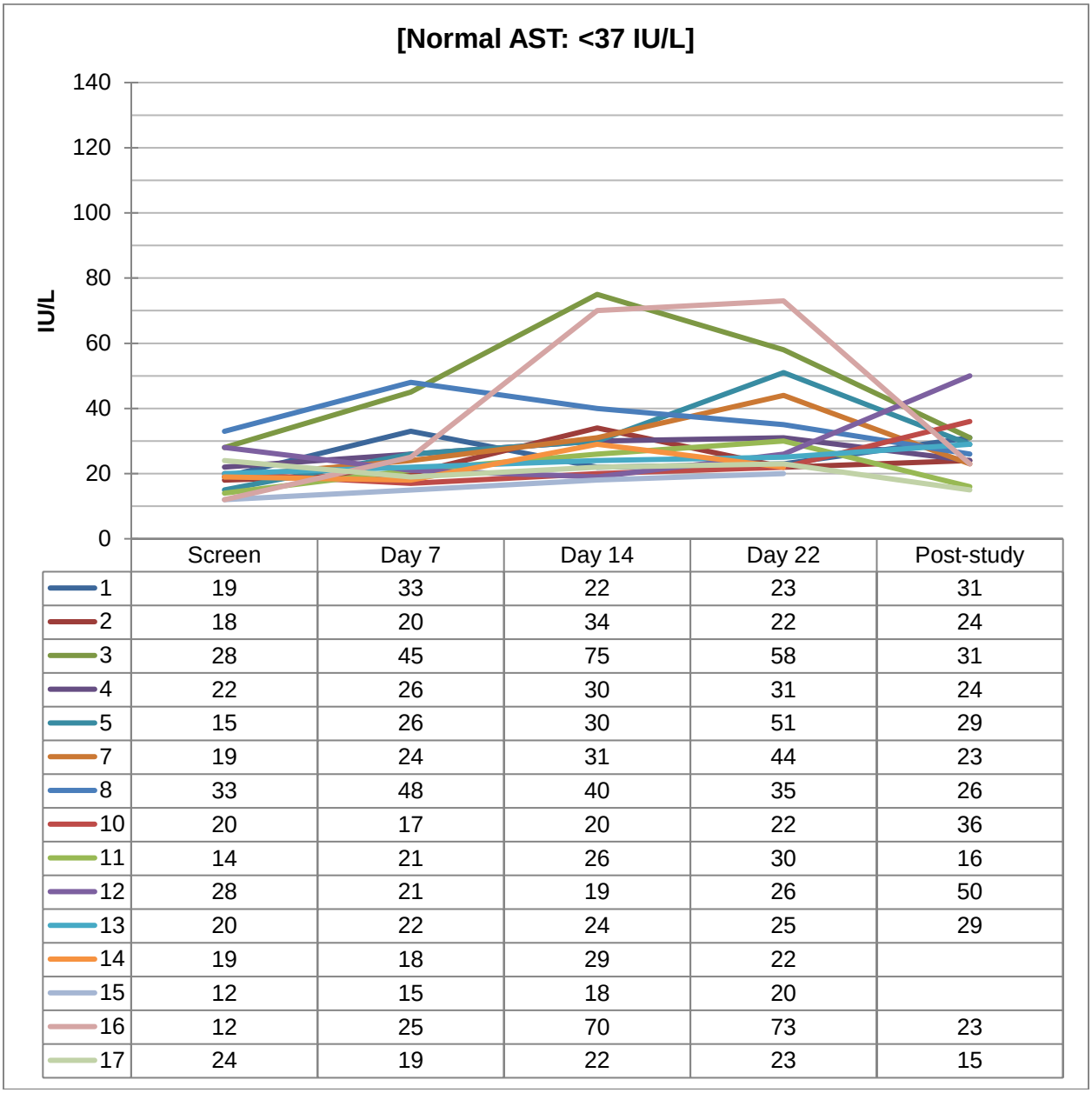
Figure 6.7.5-1b ALT Levels Over Time in Each of 16 Subjects Receiving Increasing EPG Concentrations



	Screen	Pre-treatment	Day 8 (30 g EPG)	Day 11 (60 g EPG)	Day 14 (90 g EPG)	Day 17 (120 g EPG)	Day 20 (150 g EPG)	2 Days post-treatment	Post-study
1	12	14	18	27	29	29	25	22	
2	29	23	23	25	27	23	24	26	
4	10	10	10	14	30	21	14	13	
5	11	22	24	29	32	33	26	26	
6	34	26	26	29	32	44	48	45	40
7	17	16	17	28	30	30	25	40	14
8	16	20	32	57	56	53	49	57	29
9	13	40	50	39	33	31	24	29	
10	22	22	29	52	60	53	67	59	31
11	21	33	38	44	58	64	71	65	32
12	27	54	108	88	80	68	74	55	42
13	18	16	21	25	26	28	22	21	
14	23	24	40	83	82	65	56	61	16
15	20	31	39	47	49	49	40	36	
16	27	24	26	34	35	43	38	39	
17	27	40	51	116	101	88	84	72	39

ALT = alanine transaminase; EPG = esterified propoxylated glycerol.
* Values from ICR Project No. 0047047 (Section 6.6.1.1)

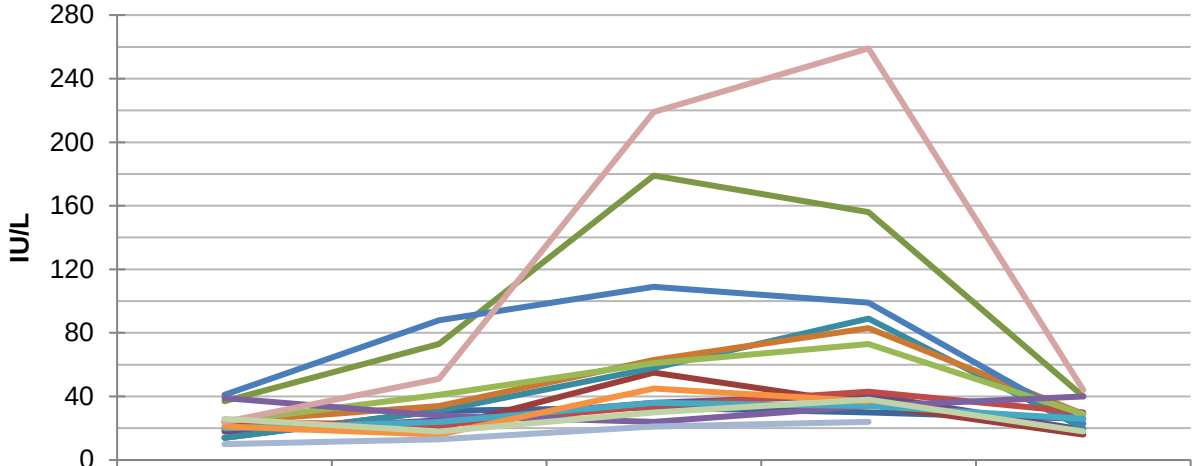
Figure 6.7.5-1c AST Levels Over Time in Each of 15 Subjects Receiving 120 g EPG/day



	Screen	Day 7	Day 14	Day 22	Post-study
1	19	33	22	23	31
2	18	20	34	22	24
3	28	45	75	58	31
4	22	26	30	31	24
5	15	26	30	51	29
7	19	24	31	44	23
8	33	48	40	35	26
10	20	17	20	22	36
11	14	21	26	30	16
12	28	21	19	26	50
13	20	22	24	25	29
14	19	18	29	22	
15	12	15	18	20	
16	12	25	70	73	23
17	24	19	22	23	15

AST = Aspartate Aminotransferase; EPG = Esterified Propoxylated Glycerol.
* Values from ICR Project No. 004251 (Section 6.6.1.2)

Figure 6.7.5-1d ALT Levels Over Time in Each of 15 Subjects Receiving 120 g EPG/day



	Screen	Day 7	Day 14	Day 22	Post-study
1	14	31	33	30	26
2	19	20	55	35	16
3	37	73	179	156	40
4	18	25	36	41	19
5	14	31	58	89	20
7	24	34	63	83	29
8	41	88	109	99	23
10	24	22	34	43	30
11	24	41	61	73	29
12	39	28	24	34	40
13	20	24	36	34	26
14	21	16	45	37	
15	10	13	21	24	
16	24	51	219	259	44
17	26	18	30	38	18

ALT = Alanine Transaminase; EPG = Esterified Propoxylated Glycerol.
* Values from ICR Project No. 004251 (Section 6.6.1.2)

6.8 Expert Panel Evaluation

ChocoFinesse has concluded that its EPG, manufactured consistent with cGMP and meeting food-grade specifications, is GRAS for use as in select commercial frying applications, as described in Part 1.3, on the basis of scientific procedures. Choco Finesse's conclusion on the GRAS status of EPG under the conditions of its intended use is based on data generally available in the public domain and includes a series of product-specific toxicology studies on EPG.

A Panel of Experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients unanimously concluded on the GRAS status of the EPG under conditions of its intended use. The Expert Panel consisted of the following qualified scientific experts: Dr. John Thomas (Adjunct Professor, Indiana University School of Medicine), Dr. Robert Nicolosi (Professor Emeritus,

University of Massachusetts Lowell) and Dr. Fergus Clydesdale (Distinguished Professor and Director of the Food Science Policy Alliance, Department of Food Science, University of Massachusetts)

The Expert Panel, convened by Choco Finesse, independently and critically evaluated all data and information presented herein and concluded that EPG, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice, is safe and suitable for use as an ingredient in select commercial frying applications, as described in Part 1.3, and is GRAS based on scientific procedures. A summary of data and information reviewed by the Expert Panel, and evaluation of such data as it pertains to the proposed GRAS uses of the EPG is presented in Appendix 4.

6.9 Conclusions

Based on data and information presented herein Choco Finesse has concluded that EPG can be determined to be Generally Recognized as Safe (GRAS) on the basis of scientific procedures.

The GRAS status of EPG is further supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training to evaluate the safety of food ingredients, who concluded that the intended use of EPG, as described herein, is GRAS.

Therefore, the intended use of EPG is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

Note on the use of unpublished data:

Consistent with FDA's new regulation, the safety of EPG was established on the basis of published results, including the clinical vitamin study. While many other unpublished studies were available and included herein these data were corroborative and thus not required for concluding that the proposed use of EPG is GRAS.

Part 7. §170.255 List of Supporting Data and Information

Artz WE, Soheili KC, Arjona IM (1999). Esterified propoxylated glycerol soyate, a fat substitute model compound, and soy oil after heating. J Agric Food Chem 47(9):3816-3821. DOI:10.1021/jf981059g.

Bechtel DH (2014). Genotoxicity testing of esterified propoxylated glycerol (EPG) Regul Toxicol Pharmacol 70(Suppl. 2):S131-S142. DOI:10.1016/j.yrtph.2014.11.013.

Bechtel, DH (2015) Tolerance of rising dietary concentrations of esterified propoxylated glycerol (EPG) among human volunteers. Regul Toxicol Pharmacol 73(1):413-8. DOI: 10.1016/j.yrtph.2015.07.030.

Brinton EA, Eisenberg S, Breslow JL (1990). A low-fat diet decreases high density lipoprotein (HDL) cholesterol levels by decreasing HDL apolipoprotein transport rates. J Clin Invest 85(1):144-151. DOI:10.1172/JCI114405.

Cargill (2017). *High Oleic Oils*. Minneapolis (MN): Cargill. Available at: http://www.cargillfoods.com/na/en/products/oils-shortenings/clear-valley/oils/index.jsp [©2017 Cargill, Incorporated].

CDC (2013). *National Health and Nutrition Examination Survey: Analytic Guidelines, 1999–2010*. (Vital and Health Statistics. Series 2, Number 161). Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://www.cdc.gov/nchs/data/series/sr_02/sr02_161.pdf [Last accessed October 30, 2016].

CDC (2015). *National Health and Nutrition Examination Survey (NHANES): 2013-2014*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/search/nhanes13_14.aspx [Page last updated: October 30, 2015].

CDC (2016). *National Health and Nutrition Examination Survey (NHANES): 2013-2014 – Dietary Data*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/Search/DataPage.aspx?Component=Dietary&CycleBeginYear=2013 [Date published: September 2016].

ChartsBin (2011). *Smoke Point of Different Cooking Oils*. ChartsBin Statistics collector team. Available at: http://chartsbin.com/view/1962 [Copyright 2011 ChartsBin.com. All rights reserved].

Christian BJ, Bechtel DH (2014). 90-Day dietary toxicity study with esterified propoxylated glycerol (EPG) in rats. Regul Toxicol Pharmacol 70(Suppl. 2):S95-S104. DOI:10.1016/j.yrtph.2014.11.017.

CPSC (2017). Part 1500—Hazardous substances and articles: administration and enforcement regulations. Section § 1500.41—Method of testing primary irritant substances. In: *U.S. Code of Federal Regulations (CFR). Title 16: Commercial Practices (Consumer Product Safety Commission)*. Washington (DC): U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

Davidson MH, Bechtel DH (2014). Assessment of the effect of esterified propoxylated glycerol (EPG) on the status of fat-soluble vitamins and select water-soluble nutrients following dietary administration to humans for 8weeks. Regul Toxicol Pharmacol 70(Suppl. 2):S143-S157.

Dazult Ltd. (2016). DaDiet - *The Dietary Intake Evaluation Tool [Software]*. (Version 16.05). Straffan, County Kildare, Ireland: Dazult Ltd. Available at: http://dadiet.daanalysis.com/.

Draize JH (1959). Dermal toxicity. In: *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics [FDA Grey Book] (3rd Printing, 1975)*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), and Austin (TX): Association of Food and Drug Officials of the United States, p. 46-59.

Elinder LS, Hadell K, Johansson J, Molgaard J, Holme I, Olsson AG, et al. (1995). Probucol treatment decreases serum concentrations of diet-derived antioxidants. Arterioscler Thromb Vasc Biol 15(8):1057-1063. DOI:10.1161/01.ATV.15.8.1057.

Gylling H, Puska P, Vartiainen E, Miettinen TA (1999). Retinol, vitamin D, carotenes and α-tocopherol in serum of a moderately hypercholesterolaemic population consuming sitostanol ester margarine. Atherosclerosis 145(2):279-285. DOI:10.1016/S0021-9150(99)00078-7.

Hendriks HF, Brink EJ, Meijer GW, Princen HM, Ntanios FY (2003). Safety of long-term consumption of plant sterol esters-enriched spread. Eur J Clin Nutr 57(5):681-692. DOI:10.1038/sj.ejcn.1601598.

Institute of Shortening and Edible Oils, Inc. [undated]. *Typical Smoke, Flash & Fire Points. Commercially Available Edible Fats and Oils*. Washington (DC): Institute of Shortening and Edible Oil, Inc. Available at: www.iseo.org/httpdocs/Smoke-Fire_Chart_0310.pdf.

IOM (2000). Vitamin E. In: *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. (National Academy of Sciences/NAS, Institute of Medicine/IOM, Food and Nutrition Board, Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of DRIs, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes). Washington (DC): National Academy Press (NAP), pp. 186-283. Available at: http://www.nap.edu/catalog/9810.html.

Knuiman JT, West CE, Katan MB, Hautvast JGAJ (1987). Total cholesterol and high density lipoprotein cholesterol levels in populations differing in fat and carbohydrate intake. Arteriosclerosis 7(6):612-619. DOI:10.1161/01.ATV.7.6.612.

Law M (2000). Plant sterol and stanol margarines and health. BMJ 320(7238):861-864. DOI:10.1136/bmj.320.7238.861.

Mahungu SM, Hansen SL, Artz WE (1999). Identification and quantitation of volatile compounds in two heated model compounds, trilinolein and linoleic acid esterified propoxylated glycerol. J Agric Food Chem 47(2):690-694. DOI:10.1021/jf9804138.

Medallion Laboratories [undated]. *Oil Stability Index: Technical Bulletin*. Golden Valley (MN): Medallion Laboratories. Available at: https://web.archive.org/web/20170329082645/http://www.medallionlabs.com/Downloads/Oil%20Stability%20Index.pdf.

Neuhouser ML, Rock CL, Kristal AR, Patterson RE, Neumark-Sztainer D, Cheskin LJ, et al. (2006). Olestra is associated with slight reductions in serum carotenoids but does not markedly influence serum fat-soluble vitamin concentrations. Am J Clin Nutr 83(3):624-631.

Peters JC, Lawson KD, Middleton SJ, Triebwasser KC (1997). Assessment of the nutritional effects of Olestra, a nonabsorbed fat replacement: Summary. J Nutr 127(8, Suppl.):1719S-1728S.

Przybylski R, Gruczynska, Aladedunye F (2013). Performance of regular and modified canola and soybean oils in rotational frying. J Am Oil Chem Soc 90:1271-1280. DOI:10.1007/s11746-013-2278-0.

Rock CL, Swendseid ME (1992). Plasma β-carotene response in humans after meals supplemented with dietary pectin. Am J Clin Nutr 55(1):96-99.

Schaefer EJ, Levy RI, Ernst ND, Van Sant FD, Brewer HB Jr (1981). The effects of low cholesterol, high polyunsaturdated fat, and low fat diets on plasma lipid and lipoprotein cholesterol levels in normal and hypercholesterolemic subjects. Am J Clin Nutr 34(9):1758-1763.

Schlagheck TG, Riccardi KA, Zorich NL, Torri SA, Dugan LD, Peters JC (1997). Olestra dose response on fat-soluble and water-soluble nutrients in humans. J Nutr 127(8, Suppl.):1646S-1665S.

Tulley RT, Vaidyanathan JA, Wilson JB, Rood JC, Lovejoy JC, Most MM, et al. (2005). Daily intake of multivitamins during long-term intake of olestra in men prevents declines in serum vitamins A and E but not carotenoids. J Nutr 135(6):1456-1461.

Tyl RW, Bechtel DH (2014a). One-generation reproduction study of esterified propoxylated glycerol (EPG) administered in the feed to CD® (Sprague-Dawley) rats. Regul Toxicol Pharmacol 70(Suppl. 2):S114-S122. DOI:10.1016/j.yrtph.2014.11.018.

Tyl RW, Bechtel DH (2014b). Developmental toxicity evaluation of esterified propoxylated glycerol (EPG) administered in the diet to New Zealand white rabbits. Regul Toxicol Pharmacol 70(Suppl. 2):S123-S130. DOI:10.1016/j.yrtph.2014.11.014.

Tyssandier V, Reboul E, Dumas JF, Bouteloup-Demange C, Armand M, Marcand J, et al. (2003). Processing of vegetable-borne carotenoids in the human stomach and duodenum. Am J Physiol Gastrointest Liver Physiol 284(6):G913-G923. DOI:10.1152/ajpgi.00410.2002.

U.S. FDA (1996). Food additives permitted for direct addition to food for human consumption; Olestra; Final rule [21 CFR Part 172, Docket No. 87F-0179]. Fed Regist (US) 61(20):3118-3173.

U.S. FDA (2015). *Agency Response Letter GRAS Notice No. GRN 000583 [Esterified propoxylated glycerol, Indianapolis (IN): Choco Finesse, LLC]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=583 [Nov. 12, 2015].

U.S. FDA (2016). *Agency Response Letter GRAS Notice No. GRN 000640 [Esterified propoxylated glycerol, Indianapolis (IN): Choco Finesse, LLC]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=640 [Sep. 21, 2016].

U.S. FDA (2017). *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (Food and Drug Administration)*. Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.ecfr.gov/cgi-bin/ECFR?SID=b3913f1005fd08fc916003094862a5e3&mc=true&page=browse [current to August 8, 2017].

Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	Section §	Section Title
170—Food additives	170.3	Definitions
	170.30	Eligibility for classification as generally recognized as safe (GRAS)
	170.203	Definitions
	170.285	Disposition of pending GRAS affirmation petitions

USDA (2014). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2011-2012*. Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: https://www.ars.usda.gov/northeast-area/beltsville-md/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/wweianhanes-overview/#release [Last Modified: October 13, 2016].

USDA (2016). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2013-2014*. Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release [Last Modified: October 13, 2016].

USDA (2017). Basic Report 18248, Doughnuts, cake-type, plain (includes unsugared, old-fashioned). In: *USDA National Nutrient Database for Standard Reference, Release 28, slightly revised*. (Software v.3.8.6.1, 2017-07-28). Beltsville (MD): U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Nutrient Data Laboratory. Available at: https://ndb.nal.usda.gov/ndb/search/list; https://ndb.nal.usda.gov/ndb/foods/show/5754?manu=&fgcd=&ds= [Last Modified: 2017-10-02].

Wedig J, Bechtel DH (2014). 90-Day dietary toxicity study with esterified propoxylated glycerol (EPG) in Micropigs. Regul Toxicol Pharmacol 70(Suppl. 2):S105-S113. DOI:10.1016/j.yrtph.2014.11.012.

APPENDIX 1
Certificate of Analysis – Propylene Oxide

Certificate 6428611 The Dow Chemical Company Page 1

Date: 01/07/2014 Certificate of Analysis

Quality Assurance
[redacted]
Fax:
WI 53546-2934 UNITED STATES

Cust P.O.: 058662 Dlvy Note: 72357178 10

Material: Propylene Oxide Spec: 00006141-S
Cust Mtl:
Vehicle: 8008
Ship from: THE DOW CHEMICAL COMPANY PLAQUEMINE LA UNITED STATES

DO NOT RETURN PRODUCT, for safety and security reasons, without prior consultation with a qualified DOW PO representative.

This material meets the requirements of the specification.

Feature	Units	Results 3A0701A502	Limits Minimum	Maximum	Method
Acidity As Acetic Acid	ppm	1	----	10	DOWM 101370
Appearance clear/matter free		Passes			DOWM 101967
Chlorides as Cl organic	ppm	5	----	10	DOWM 102001
Color, Pt-Co	-	3	----	10	ASTM D5386
NVM per 100 ml	mg	1	----	2	ASTM D1353
Water Content	ppm	15	----	100	ASTM E1064
Assay calculated	% wt	99.99	99.97	----	GC Method
Aldehydes Sum of Acetaldehyde and Propionaldehyde	ppm	5	----	30	GC Method
Total GC Impurities	ppm	31	----	200	GC Method

GC Method by DOWM 102001 or by DOWM 102563
Typical Properties: The values listed are considered physical properties and are not analyzed on a routine basis.
Sp. Grav. @20/20C 0.8290-0.8310
Sp. Grav. @25/25C 0.8250-0.8270

Plant Quality Coordinator

For inquiries please contact Customer Service at 1-800-232-2436 (USA).

APPENDIX 2
Tocopherol Safety Summary

1.0 TOCOPHEROL CONCENTRATE USED IN EPG

Choco Finesse LLC uses a tocopherol concentrate (Covi-ox® T-70, BASF Corporation) to stabilize EPG. The concentrate is produced from distillates of edible oils, predominantly from deodorization of soybean oil, and contains a minimum of 70% tocopherols, comprised of d-α-, d-β-, d-γ- and d-δ-tocopherols. The Certificate of Analysis (CoA) for Covi-ox® T-70 confirms the absence of heavy metals and microbiological purity (refer to Appendix A). In addition, the CoA indicates that Covi-ox® T-70 meets the monograph requirements of the current National Formulary (USP-NF, Tocopherols Excipient) and Food and Chemicals Codex (RRR-Tocopherols Concentrate, Mixed Low-alpha-Type), and current specification for food additives of European regulation (E306, Tocopherol-rich Extract).

For frying applications, Covi-ox® T-70 is added to EPG to provide total tocopherols at approximately 775 to 1,800 ppm (α-tocopherols: 120 to 320 ppm; β-tocopherols: 5 to 40 ppm; γ-tocopherols: 500 to 1,100 ppm; δ-tocopherols: 150 to 350 ppm).

2.0 BACKGROUND

Tocopherols are a group of chemically related substances which include α-, β-, γ- and δ-tocopherols, as well as the less saturated α-, β-, γ- and δ-tocotrienols (Tomassi and Silano, 1986). The substances are also collectively referred to as vitamin E. All of the tocopherols are comprised of a chromanol ring with a long aliphatic side chain, bound to the chromanol ring at the 2 position (EFSA, 2008). The Greek characters denote the number and position of the methyl groups at the 5, 7 and 8 positions, and natural α-tocopherol is designated (d) α-tocopherol, while synthetic α-tocopherol, usually synthesized from phytol or isophytol, is designated (d,l) *α*-tocopherol. Several biological activities have been associated with tocopherols; these include *in vivo* antioxidant properties, anti-hemolytic and anti-sterility.

Tocopherols occur naturally in a variety of foods including nuts, seeds, fruits and vegetables, at levels ranging from 0.01 to 200 mg/100 g food (Bauernfeind, 1980). The fats and oils in food products serve as major sources of tocopherols.

Several factors influence the relative proportions of α-, β-, γ- and δ-tocopherols in foods including, plant source, plant maturation stage, season, time and methods of harvesting, method of storage and genetics. Table 2-1 shows the variation in tocopherol content of a number of plant-derived oils.

Table 2-1 Tocopherol and Tocotrienol Content of Some Edible Plant Oils in mg/100 g Product (Ong, 1993; Sheppard *et al*., 1993)

Oil	Tocopherols (mg/100 g product)				Tocotrienols (mg/100 g product)			
	alpha	***beta***	***gamma***	***delta***	***alpha***	***beta***	***gamma***	***delta***
Canola	21.0	0.1	4.2	0.04	0.04	-	-	-
Castor	2.8	2.9	11.1	31.0	-	-	-	-
Coconut	0.5	-	-	0.6	0.5	0.1	1.9	-
Corn	11.2	5.0	60.2	1.8	-	-	-	-
Cottonseed	38.9	-	38.7	-	-	-	-	-
Olive	11.9	-	0.7	-	-	-	-	-
Palm	25.6	-	31.6	7.0	14.6	3.2	28.6	6.9
Palm Kernel	6.2	-	-	-	-	-	-	-
Peanut	13.0	-	21.4	2.1	-	-	-	-
Rice Bran	32.4	1.8	5.3	-	23.6	-	34.9	-
Safflower	34.2	-	7.1	-	-	-	-	-
Sesame	13.6	-	29.0	-	-	-	-	-
Soybean	7.5	1.5	79.7	26.6	0.2	0.1	-	0.03
Sunflower	48.7	-	5.1	0.8	-	-	-	-
Walnut	56.3	-	59.5	45.0	-	-	-	-
Wheat Germ	133.0	71.0	26.0	27.1	2.6	18.1	-	-

Tocopherol intake has been estimated for numerous populations based on analysis of diets consumed or calculations from food composition tables. For example, the average daily dietary intake of naturally occurring tocopherols in the United States reportedly ranged from 5 to 20 mg/person, of which, tocopherols added to foods as antioxidants accounted for a small percentage (Bieri and Evarts, 1973; Horwitt, 1974; Witting and Lee, 1975; U.S. FDA, 1978).

3.0 SAFETY

Tomassi and Silano (1986) conducted an assessment of the safety of α-, β-, γ- and δ-tocopherols as food additives. The authors reviewed a number of acute, subchronic, and chronic toxicity studies, as well as reproductive, teratogenicity and mutagenicity studies. Pharmacokinetic studies, clinical data and interactions with other vitamins were also considered. The authors' conclusions regarding safety, based on their review of available data are summarized in Table 3-1. Tomassi and Silano (1986) further indicated that: "*On the basis of these conclusions as well as of the chemical and biological similarity of the four tocopherols and the level of tocopherols used as food antioxidants, it is concluded that tocopherols are safe food additives.*"

Table 3-1 Conclusions Related to Safety Drawn from Experimental and Clinical Data on α-Tocopherols (Borrowed from Tomassi and Silano, 1986)

1	Tocopherols, particularly when administered at high levels, can interact with other vitamins; in particular, vitamin K activity is antagonized.
2	Toxicity of orally administered tocopherols in laboratory animals appears to be moderate; data are in effect available only for α-tocopherols.
3	There is no evidence of any carcinogenic or mutagenic potential of tocopherols.
4	Population groups exposed to excess vitamin E indicate marginal adverse effects, unless there is a deficiency of vitamin K.
5	Available data indicate that present intakes of tocopherols used as food additives are much lower than those capable of inducing any adverse effects in humans or laboratory animals.

More recently, the European Food Safety Authority's Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, evaluated the safety and bioavailability of mixed tocopherols (*i.e.*, a mixture containing all 4 tocopherols), tocotrienol tocopherol and tocotrienols as a source for vitamin E when added for nutritional purposes in food supplements (EFSA, 2008). According to the report, intake of mixed tocopherols from supplement use would be in accordance with the tolerable upper intake levels for vitamin E (as d-α-tocopherol) of 300 mg/day, set by the Scientific Committee on Food (SCF, 2003). The Panel also noted that "*mixed tocopherols underwent a safety evaluation by the SCF (SCF, 1989) and were approved as antioxidants for foods in general* ad quantum satis*, and as additives in nutrient preparations for use in infant formula, follow-on formulae and weaning foods within the European Community (European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners)*" (EC, 1995). Based on their review of the available biological and toxicological data, the Panel concluded the use of mixed tocopherols as a source of vitamin E in food supplements for the general population at the proposed levels of use is not of safety concern.

4.0 SUMMARY AND CONCLUSIONS

Choco Finesse LLC uses Covi-ox® T-70 to stabilize EPG. The concentrate is produced from distillates of edible oils, predominantly from deodorization of soybean oil, and contains a minimum of 70% tocopherols, comprised of d-α-, d-β-, d-γ- and d-δ-tocopherols.

Covi-ox® T-70 is added to EPG to provide total tocopherols at approximately 775 to 1,800 ppm (α-tocopherols: 120 to 320 ppm; β-tocopherols: 5 to 40 ppm; γ-tocopherols: 500 to 1,100 ppm; δ-tocopherols: 150 to 350 ppm).

In GRN 640 (U.S. FDA, 2016), it was estimated that an intake of 24.11 EPG/g from confectionary and spreadable EPG would result in a maximum α-tocopherol intake of 5.5 mg/day. Inclusion of the proposed frying applications of EPG contributes an additional 6.19 g EPG/day in male teenagers, the subpopulation group with the highest intake. Assuming a maximum α-tocopherol concentration of 320 ppm in the EPG used in these frying applications, these new uses would result in an intake of an additional α-tocopherol of 2.0 mg/day for a total of 7.5 mg/day. Therefore, based on the natural occurrence of tocopherols in foods and available safety data, their presence in EPG is not expected to be a safety concern.

5.0 REFERENCES

Bauernfeind J (1980). Tocopherols in food. In: Machlin LJ, editor. *Vitamin E: A Comprehensive Treatise*. New York (NY): Marcel Dekker, pp. 99-167. Cited In: Tomassi and Silano, 1986.

Bieri JG, Evarts RP (1973). Tocopherols and fatty acids in American diets. J Am Diet Assoc 62(2):147-151. Cited In: Tomassi and Silano, 1986.

EC (1995). European Parliament and Council Directive No. 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. Off J Eur Communities 38(L61):1-40. Available at: http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:31995L0002&qid=1472137038026 [Consolidated version: 12/11/2010].

EFSA (2008). Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission on mixed tocopherols, tocotrienol tocopherol and tocotrienols as sources for vitamin E (Question no EFSA-Q-2005-146, EFSA-Q-2005-172, EFSA-Q-2006-265, adopted: 22 February 2008 by European Food Safety Authority). EFSA J 640:1-34. DOI:10.2903/j.efsa.2008.640. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/640.

Horwitt MK (1974). Status of human requirements for vitamin E. Am J Clin Nutr 27(10):1182-1193. Cited In: Tomassi and Silano, 1986.

Ong A (1993). Natural sources of tocotrienols. In: Packer FJ, editor. Vitamin E in Health and Disease. New York (NY): Marcel Dekker Inc., pp. 3-8. Cited In: EFSA, 2008.

SCF (1989). Antioxidants (Opinion expressed on 11 December 1987 [Tocotrienols]. In: *Food Science and Techniques*. (Reports of the Scientific Committee for Food 22nd series). Brussels Belgium: Commission of the Europe Communities Scientific Committee for Food (SCF). Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_reports_22.pdf.

SCF (2003). *Opinion of the Scientific Committee on Food On the Tolerable Upper Intake Level of Vitamin E (Expressed on 4 April 2003)*. (SCF/CS/NUT/UPPLEV/31 Final). Brussels, Belgium: European Commission, Health & Consumer Protection Directorate-General, Scientific Committee on Food (SCF). Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out195_en.pdf.

Sheppard A, Pennington JAT, Weihrauch JL (1993). Analysis and distribution of vitamin E in vegetable oils and foods. In: Packer FJ, editor. Vitamin E in Health and Disease. New York (NY): Marcel Dekker Inc., pp. 9-31. Cited In: EFSA, 2008.

Tomassi G, Silano V (1986). An assessment of the safety of tocopherols as food additives. Food Chem Toxicol 24(10/11):1051-1061. DOI:10.1016/0278-6915(86)90288-7.

U.S. FDA (1978). Tocopherols and derivatives (21 CFR Parts 182 and 184) [Docket No. 78N-0213]; Proposed rule. Fed Regist (US)43(209):50193-50198. Cited In: Tomassi and Silano, 1986.

U.S. FDA (2016). *Agency Response Letter GRAS Notice No. GRN 000640 [Esterified propoxylated glycerol, Indianapolis (IN): Choco Finesse, LLC]* . College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=640 [Sep. 21, 2016].

Witting LA, Lee L (1975). Dietary levels of vitamin E and polyunsaturated fatty acids and plasma vitamin E[1, 2, 3]. Am J Clin Nutr 28(6):571-576. Cited In: Tomassi and Silano, 1986.

ANNEX A
Certificate of Analysis for Covi-ox® T-70

BASF CORPORATION

BASF
We create chemistry

Certificate of Analysis

Customer:		Product Number	:	50207050
POST WAREHOUSE CORP		Product Name	:	Covi-ox® T-70 10KG Plastics Buckets
3200 SHEFFIELD AVE				
HAMMOND IN 46327-1001		Vehicle	:	ME # 574761789
		Batch/Lot	:	0013618065
		Manuf.Date	:	May-01-2015
Attention:	BSICOANJ@BRENNTAG.COM	Shipped Date	:	Dec-29-2015
eMAIL:	bsicoanj@brenntag.com	Shipped Quantity	:	140.000 KG
Cust Prod:	896548	Delivery Date	:	Dec-29-2015
Cust Prod Name:	Covi-ox T-70 10KG IP22	Order Number	:	114322500 000010
Cust P.O.:	152232			
Cust P.O. Line:	1	Delivery Note	:	126374039 900001

Characteristic	Result	UOM	-----Specification----- Minimum	Maximum	Test Method
APPEARANCE-FCC III	CLEAR,AMBER				
ODOR	BLAND				
ACIDITY, ML	0.09		-	1.00	
ASSAY TOCOPHEROLS, %	71.5		70.0		
D-ALPHA TOC., MG/G	80.2				
D-BETA TOCOPHEROL, MG/G	11.3				
D-DELTA TOCOPHEROL, MG/G	178.0				
D-GAMMA TOCOPHEROL, MG/G	445.0				
TOTAL TOC., MG/G	715.0		700.0	-	
NON-ALPHA, MG/G	634.0		560.0	-	
NON-ALPHA OF TOTAL TOC, %	88.7		82.0	-	
IDENTIFICATION, NF (METHOD B)	Pass				
SPECIFIC ROTATION	CERTIFIED		20	35	
BAP, < 2 PPB	CERTIFIED				
HEAVY METALS (PB), PPM	CERTIFIED		-	10	
LEAD, PPM	CERTIFIED		-	2.0	
RESIDUAL SOLVENTS, USP (467)	CERTIFIED				
TOTAL PLATE COUNT, < 1000 CFU/g	CERTIFIED				USP 61
YEASTS AND MOLDS, < 100 CFU/g	CERTIFIED				USP 61
E. COLI, NEGATIVE/g	CERTIFIED				USP 62
STAPHYLOCCOCUS, NEGATIVE/g	CERTIFIED				USP 62
SALMONELLA, NEGATIVE/ 10 g	CERTIFIED				USP 62
COLIFORMS, < 10 CFU/g	CERTIFIED				FDA BAM
BEST BEFORE/RETEST DATE	-	04/30/2018			

Comments :
COUNTRY OF ORIGIN: USA
Manufacturing Location Kankakee, IL 60901

Natural source mixed tocopherol (Vitamin E) oil ,
d-alpha-tocopherol, d-beta-tocopherol, d-delta-tocopherol, and d-gamma-tocopherol

APPENDIX 3
Estimated Daily Intake of Esterified Propoxylated Glycerol (EPG) by the U.S. Population from intended Frying Applications (2013-2014 NHANES)

intertek
Total Quality. Assured.

ESTIMATED DAILY INTAKE OF ESTERIFIED PROPOXYLATED GLYCEROL (EPG) BY THE U.S. POPULATION FROM INTENDED FRYING APPLICATIONS (2013-2014 NHANES)

PREPARED FOR:
Choco Finesses, LLC
5019 N. Meridian Street
Indianapolis, Indiana
46208

PREPARED BY:
Intertek Scientific & Regulatory Consultancy
Health, Environmental & Regulatory Services (HERS)
2233 Argentia Road, Suite 201
Mississauga, Ontario, Canada
L5N 2X7

DATE:
15 September 2017; Updated 15 January 2018

Estimated Daily Intake of Esterified Propoxylated Glycerol (EPG) by the U.S. Population from Intended Frying Applications (2013-2014 NHANES)

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Estimated Daily Intake of Esterified Propoxylated Glycerol (EPG) by the U.S. Population from Intended Frying Applications (2013-2014 NHANES)

1.0 INTRODUCTION

Choco Finesse, LLC (Choco Finesse) intends to market Esterified Propoxylated Glycerol (EPG) as an ingredient for use in commercial frying applications for French fries and doughnuts in the United States (U.S.). In order to estimate potential exposure to this component, an assessment was conducted based on the highest residual levels, as measured by Choco Finesse in both French fries and doughnuts, combined with food consumption data for these items by the U.S. population.

Food consumption data included in the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) 2013-2014 (CDC, 2015, 2016; USDA, 2016). Calculations for the mean and 90th percentile *per capita* and consumer-only intakes were performed for all proposed food-uses of EPG and the percentage of consumers were determined. Similar calculations were used to estimate the intake of EPG resulting from each individual proposed food-use, including the calculations of percent consumers. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

Infants and Young children, up to and including 2 years;
Children, ages 3 to 11;
Female teenagers, ages 12 to 19;
Male teenagers, ages 12 to 19;
Female adults, ages 20 and up;
Male adults, ages 20 and up; and
Total population (all age and gender groups combined).

2.0 FOOD CONSUMPTION SURVEY DATA

2.1 Survey Description

NHANES for the years 2013-2014 are available for public use (CDC, 2015). NHANES are conducted as continuous, annual surveys, and are released in 2-year cycles. During each year of the ongoing NHANES program, individuals from the United States are sampled from up to 30 different study locations in a complex multi-stage probability design intended to ensure the data are a nationally representative sample of the U.S. population.

NHANES 2013-2014 dietary survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person, and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S., of which 30 PSUs are visited per year. Smaller contiguous counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or

more participants within a household were interviewed. For NHANES 2013-2014, 14,332 individuals were selected for the sample, 10,175 were interviewed (71.0%) and 9,813 were examined (68.5%).

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2013-2014 collected socio-economic, physiological and demographic information from individual participants in the survey, such as sex, age, body weight, and other variables (such as height and race-ethnicity) that may be useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. The primary sample design for NHANES 2013-2014 includes an oversample of Non-Hispanic Asian persons, Hispanic persons, non-Hispanic black persons, older adults, and "low income whites/others", however sample weights were incorporated to allow estimates from these subgroups to be combined to obtain national estimates that reflect the relative proportions of these groups in the population as a whole (CDC, 2015).

2.2 Statistical Methods

For the intake assessment, consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of EPG by the U.S. population[1]. Estimates for the daily intake of EPG represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2013-2014; these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of EPG averaged over all individuals surveyed, regardless of whether they consumed food products that are intended to be fried in EPG, and therefore includes individuals with "zero" intakes (*i.e.*, those who reported no intake of food products that are intended to be fried in EPG during the 2 survey days). "Consumer-only" intake refers to the estimated intake of EPG by those individuals who reported consuming food products that are intended to be fried in EPG. Individuals were considered "consumers" if they reported consumption of 1 or more food products that are intended to be fried in EPG on either Day 1 or Day 2 of the survey.

Mean and 90th percentile intake estimates based on sample sizes of less than 30 and 80, respectively, may not be considered statistically reliable due to the limited sampling size (CDC, 2013). As such, the reliability of estimates for the intake of EPG based on consumption estimates derived from individual population groups of a limited sample size should be interpreted with caution. These values are marked with an asterisk in the relevant data tables.

[1] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2016). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

3.0 FOOD USAGE DATA

To examine worst-case potential EPG exposure, the current intakes analysis considered the high residual levels of EPG measured by Choco Finesse in each food category (see Table 3-1 – **bolded** figures were utilized). Food codes representative of each food intended to be fried in EPG were chosen from the NHANES 2013-2014 (CDC, 2016). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (CFR, 2016). All food codes included in the current intake assessment are listed in Appendix C.

Table 3-1 Summary of Foods Intended to be Fried in EPG in the U.S. and Residual EPG Levels in the Food from Frying Applications

Food Category (21 CFR 170.3)	Foods-Uses[a]	EPG Level (g/serving)[b]	Serving size (g)[c]		Residual EPG Levels in the Food (%)[d]
Processed vegetables	French fries	12.3	Large	154	8.0
		9.46	Medium	117	**8.1**
		5.7	Small	71	8.0
		2.5	Kid	31	8.1
Baked good	Doughnuts	7.7	Large	71	**10.8**
		5.8	Medium	54	10.7
		1.5	Small	14	10.7

CFR = Code of Federal Regulations; EPG = Esterified Propoxylated Glycerol; U.S. = United States.
[a] EPG is intended to be used in frying applications. The proposed food-uses represent foods that are intended to be fried in EPG.
[b] EPG level is the amount of EPG absorbed in the food following the intended frying application, as measured by Choco Finesse.
[c] Serving sizes were provided by Choco Finesse.
[d] The highest, **bolded**, values were used for each food category in the current intakes assessment to examine worst-case EPG exposure.

4.0 FOOD SURVEY RESULTS

Estimates for the total daily intake of residual EPG from the intended frying applications in the U.S. are provided in Tables 4.1-1 and 4.1-2. Estimates for the daily intake of residual EPG from individual foods intended to be fried in EPG are summarized in Tables A-1 to A-7 and B-1 to B-7 of Appendices A and B, respectively. Tables A-1 to A-7 provide estimates for the daily intake of EPG on an absolute basis (g/person/day), whereas Tables B-1 to B-7 provide estimates for the daily intake of EPG on a per kilogram body weight basis (mg/kg body weight/day).

4.1 Estimated Daily Intake of Residual EPG from Intended Frying Applications in the U.S.

Table 4.1-1 summarizes the estimated total intake of residual EPG (g/person/day) in different U.S. population groups based on the intended frying applications. Table 4.1-2 presents this data on a per kilogram body weight basis (mg/kg body weight/day). The percentage of users of foods intended to be fried in EPG was low among all age groups evaluated in the current intakes assessment, wherein 18.1 to 32.6% of the population groups consisted of consumers of food products that are intended to be fried in EPG (Table 4.1-1). The consumer-only intakes are more applicable to the assessment of safety as they are more

likely to represent exposure in the target populations. Consequently, only the consumer-only intake results will be discussed in detail below.

Among the total population (all ages), the mean and 90th percentile consumer-only intakes of EPG were determined to be 4.0 and 7.6 g/person/day, respectively. Of the individual population groups, female teenagers, male teenagers and male adults were determined to have the greatest mean consumer-only intakes of EPG on an absolute basis of 4.7 g/person/day, and female teenagers were determined to have the greatest 90th percentile intakes of 11.9 g/person/day. Infants and young children had the lowest mean and 90th percentile consumer-only intakes of 2.1 and 4.6 g/person/day, respectively (Table 4.1-1).

Table 4.1-1 Summary of the Estimated Daily Intake of Residual EPG from Intended Frying Applications in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 3	0.4	1.3	18.1	121	2.1	4.6
Children	4 to 11	0.8	3.4	25.6	351	3.2	5.3
Female Teenagers	12 to 19	1.2	3.5	25.5	165	4.7	11.9
Male Teenagers	12 to 19	1.5	5.3	32.6	168	4.7	8.7
Female Adults	20 and up	0.8	3.4	22.4	531	3.6	6.7
Male Adults	20 and up	1.2	4.6	24.3	512	4.7	9.8
Total Population	All Ages	1.0	3.5	23.9	1,848	4.0	7.6

EPG = Esterified Propoxylated Glycerol; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

On a body weight basis, the total population (all ages) mean and 90th percentile consumer-only intakes of EPG were determined to be 64 and 130 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 154 and 348 mg/kg body weight/day, respectively (Table 4.1-2). Female adults had the lowest mean and 90th percentile consumer-only intakes of 46 and 83 mg/kg body weight/day, respectively.

Table 4.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Residual EPG from Intended Frying Applications in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 3	28	105	18.2	121	154	348
Children	4 to 11	27	111	25.3	349	108	205
Female Teenagers	12 to 19	21	64	25.6	163	83	161
Male Teenagers	12 to 19	22	79	32.8	167	67	143
Female Adults	20 and up	10	43	22.5	530	46	83
Male Adults	20 and up	14	52	24.5	512	56	106
Total Population	All Ages	15	55	24.0	1,842	64	130

bw = body weight; EPG = Esterified Propoxylated Glycerol; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

4.2 Estimated Daily Intake of EPG from Individual Foods Intended to be Fried in EPG in the U.S.

Estimates for the mean and 90th percentile daily intakes of EPG from each individual food category are summarized in Tables A-1 to A-7 and B-1 to B-7 on a g/day and mg/kg body weight/day basis, respectively. The total U.S. population was identified as being low to moderate consumers of French fries (15.4 to 24.3%) and doughnuts (3.7 to 10.1%).

In terms of contribution to total mean intake of EPG, French fries were the main source of intakes across all population groups (contributed 64.4 to 79.4% to total mean intakes), followed by doughnuts (contributed 20.6 to 35.6% to total mean intakes), see Appendices A and B.

5.0 SUMMARY AND CONCLUSIONS

Consumption data and information pertaining to the intended frying applications of EPG were used to estimate the *per capita* and consumer-only intakes of EPG for specific demographic groups and for the total U.S. population. There were a number of assumptions included in the assessment which render exposure estimates that may be considered suitably conservative. For example, while EPG is only intended to be used in commercial applications, it was not possible to identify only food items from retail outlets; as such, all food codes – which did not state 'homemade' in the description – were selected for inclusion – this is of particular relevance for French fries. Notably, only 1 food code for French fries was identified to be from a retail outlet (*i.e.*, 71401030 – White potato, French fries, from fast food / restaurant). Further, it has been assumed that all food products within a food category contain maximum EPG residual levels, as measured by Choco Finesse. In reality, residual EPG levels in specific foods will vary depending on the serving size (as indicated in Table 3-1), and only the maximum measured EPG levels were provided for each serving size in the different food categories for the purposes of this assessment. In addition, it is unlikely that EPG will have 100% market penetration for all intended commercial frying applications.

In summary, on consumer-only basis, the resulting mean and 90th percentile intakes of EPG by the total U.S. population from all intended frying applications were estimated to be 4.0 g/person/day (64 mg/kg body weight/day) and 7.6 g/person/day (130 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean consumer-only intakes of EPG were determined to be 4.7 g/person/day in female teenagers, male teenagers and male adults (83, 67 and 56 mg/kg body weight/day, respectively), and the highest 90th percentile intakes were determined to be 11.9 g/person/day (161 mg/kg body weight/day) in female teenagers. Infants and young children had the lowest mean and 90th percentile consumer-only intakes of 2.1 g/person/day (154 mg/kg body weight/day) and 4.6 g/person/day (348 mg/kg body weight/day), respectively.

When intakes were expressed on a body weight basis, infants and young children had the highest mean and 90th percentile consumer-only intakes of 154 and 348 mg/kg body weight/day. Female adults had the lowest mean and 90th percentile consumer-only intakes of 46 and 83 mg/kg body weight/day, respectively.

6.0 REFERENCES

CDC (2013). *National Health and Nutrition Examination Survey: Analytic Guidelines, 1999–2010*. (Vital and Health Statistics. Series 2, Number 161). National Center for Health Statistics. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://www.cdc.gov/nchs/data/series/sr_02/sr02_161.pdf [Last accessed October 30, 2016].

CDC (2015). *National Health and Nutrition Examination Survey (NHANES): 2013-2014*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/search/nhanes13_14.aspx [Page last updated: October 30, 2015].

CDC (2016). *National Health and Nutrition Examination Survey (NHANES): 2013-2014 – Dietary Data*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/Search/DataPage.aspx?Component=Dietary&CycleBeginYear=2013 [Date published: September 2016].

CFR (2016). Part 170—Food additives. Section §170.3—Definitions. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration)*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

Dazult Ltd. (2016). *DaDiet - The Dietary Intake Evaluation Tool [Software]*. (Version 16.11). Straffan, County Kildare, Ireland: Dazult Ltd. Available at: http://dadiet.daanalysis.com.

USDA (2016). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2013-2014.* Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release [Last Modified: October 13, 2016].

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Appendix A Estimated Daily Intake of EPG from Intended Frying Applications by Different Population Groups Within the U.S. (2013-2014 NHANES DATA)

Table A-1 Estimated Daily Intake of EPG from Intended Frying Applications by Infants and Young Children Aged Up to 3 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***0.4***	***1.3***	***18.1***	***121***	***2.1***	***4.6***
Processed Vegetables							
French Fries	73.6	0.3	0.9	15.4	108	1.8	3.8
Baked Goods							
Doughnuts	26.4	0.1*	na	3.7	22	2.8*	3.6*

EPG = esterified propoxylated glycerol; na = not available; NHANES = National Health and Nutrition Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-2 Estimated Daily Intake of EPG from Intended Frying Applications by Children Aged 4 to 11 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***0.8***	***3.4***	***25.6***	***351***	***3.2***	***5.3***
Processed Vegetables							
French Fries	64.4	0.5	2.5	19.5	271	2.7	5.3
Baked Goods							
Doughnuts	35.6	0.3	na	7.8	103	3.7	6.5

EPG = esterified propoxylated glycerol; na = not available; NHANES = National Health and Nutrition Survey; U.S. = United States.

Table A-3 Estimated Daily Intake of EPG from Intended Frying Applications by Female Teenagers Aged 12 to 19 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***1.2***	***3.5***	***25.5***	***165***	***4.7***	***11.9***
Processed Vegetables							
French Fries	79.4	1.0	3.4	21.7	138	4.4	8.4
Baked Goods							
Doughnuts	20.6	0.2	na	5.7	38	4.3	7.2*

EPG = esterified propoxylated glycerol; na = not available; NHANES = National Health and Nutrition Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-4 Estimated Daily Intake of EPG from Intended Frying Applications by Male Teenagers Aged 12 to 19 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***1.5***	***5.3***	***32.6***	***168***	***4.7***	***8.7***
Processed Vegetables							
French Fries	72.1	1.1	5.3	24.3	135	4.5	7.6
Baked Goods							
Doughnuts	27.9	0.4	0.7*	10.1	46	4.2	7.7*

EPG = esterified propoxylated glycerol; NHANES = National Health and Nutrition Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-5 Estimated Daily Intake of EPG from Intended Frying Applications by Female Adults Aged 20 Years and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***0.8***	***3.4***	***22.4***	***531***	***3.6***	***6.7***
Processed Vegetables							
French Fries	70.0	0.6	2.6	17.8	422	3.1	5.3
Baked Goods							
Doughnuts	30.0	0.2	na	6.1	143	3.9	7.0

EPG = esterified propoxylated glycerol; na = not available; NHANES = National Health and Nutrition Survey; U.S. = United States.

Table A-6 Estimated Daily Intake of EPG from Intended Frying Applications by Male Adults Aged 20 Years and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***1.2***	***4.6***	***24.3***	***512***	***4.7***	***9.8***
Processed Vegetables							
French Fries	77.5	0.9	3.4	21.0	420	4.3	6.9
Baked Goods							
Doughnuts	22.6	0.3	na	5.2	126	5.0	11.5

EPG = esterified propoxylated glycerol; na = not available; NHANES = National Health and Nutrition Survey; U.S. = United States.

Table A-7 Estimated Daily Intake of EPG from Intended Frying Applications by the Total US Population (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***1.0***	***3.5***	***23.9***	***1,848***	***4.0***	***7.6***
Processed Vegetables							
French Fries	73.6	0.7	3.3	19.5	1,494	3.6	6.7
Baked Goods							
Doughnuts	26.5	0.3	na	6.0	478	4.2	7.7

EPG = esterified propoxylated glycerol; na = not available; NHANES = National Health and Nutrition Survey; U.S. = United States.

Appendix B Estimated Daily Per Kilogram Body Weight Intake of EPG from Intended Frying Applications by Different Population Groups Within the U.S. (2013-2014 NHANES Data)

Table B-1 Estimated Daily Per Kilogram Body Weight Intake of EPG from Intended Frying Applications by Infants and Young Children Aged Up to 3 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***28***	***105***	***18.2***	***121***	***154***	***348***
Processed Vegetables							
French Fries	73.2	21	62	15.5	108	132	302
Baked Goods							
Doughnuts	26.5	7*	na	3.7	22	200*	270*

bw = body weight; EPG = esterified propoxylated glycerol; na = not available; NHANES = National Health and Nutrition Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-2 Estimated Daily Per Kilogram Body Weight Intake of EPG from Intended Frying Applications by Children Aged 4 to 11 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***27***	***111***	***25.3***	***349***	***108***	***205***
Processed Vegetables							
French Fries	61.9	17	73	19.2	269	88	154
Baked Goods							
Doughnuts	38.1	10	na	7.9	103	132	257

bw = body weight; EPG = esterified propoxylated glycerol; na = not available; NHANES = National Health and Nutrition Survey; U.S. = United States.

Table B-3 Estimated Daily Per Kilogram Body Weight Intake of EPG from Intended Frying Applications by Female Teenagers Aged 12 to 19 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***21***	***64***	***25.6***	***163***	***83***	***161***
Processed Vegetables							
French Fries	76.4	16	59	21.7	136	75	156
Baked Goods							
Doughnuts	23.6	5	na	5.8	38	87	179*

bw = body weight; EPG = esterified propoxylated glycerol; na = not available; NHANES = National Health and Nutrition Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-4 Estimated Daily Per Kilogram Body Weight Intake of EPG from Intended Frying Applications by Male Teenagers Aged 12 to 19 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***22***	***79***	***32.8***	***167***	***67***	***143***
Processed Vegetables							
French Fries	67.9	15	61	24.5	135	60	106
Baked Goods							
Doughnuts	32.3	7	8*	10.2	45	69	143*

bw = body weight; EPG = esterified propoxylated glycerol; NHANES = National Health and Nutrition Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-5 Estimated Daily Per Kilogram Body Weight Intake EPG from Intended Frying Applications by Female Adults Aged 20 Years and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***10***	***43***	***22.5***	***530***	***46***	***83***
Processed Vegetables							
French Fries	69.1	7	32	17.9	421	40	68
Baked Goods							
Doughnuts	31.4	3	na	6.1	143	53	100

bw = body weight; EPG = esterified propoxylated glycerol; na = not available; NHANES = National Health and Nutrition Survey; U.S. = United States.

Table B-6 Estimated Daily Per Kilogram Body Weight Intake of EPG from Intended Frying Applications by Male Adults Aged 20 Years and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***14***	***52***	***24.5***	***512***	***56***	***106***
Processed Vegetables							
French Fries	77.2	11	44	21.1	420	50	89
Baked Goods							
Doughnuts	23.2	3	na	5.2	126	61	131

bw = body weight; EPG = esterified propoxylated glycerol; na = not available; NHANES = National Health and Nutrition Survey; U.S. = United States.

Table B-7 Estimated Daily Per Kilogram Body Weight Intake of EPG from Intended Frying Applications by the Total US Population (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***15***	***55***	***24.0***	***1,842***	***64***	***130***
Processed Vegetables							
French Fries	70.8	11	42	19.6	1,489	56	109
Baked Goods							
Doughnuts	29.0	4	na	6.0	477	74	155

bw = body weight; EPG = esterified propoxylated glycerol; na = not available; NHANES = National Health and Nutrition Survey; U.S. = United States.

Appendix C Representative Food Codes for Foods Intended to be Fried in EPG in the U.S. (2013-2014 NHANES Data)

Representative Food Codes for Foods Intended to be Fried EPG in the U.S. (U.S. NHANES 2013-2014)

Food Intended to be Fried in EPG

French Fries
[EPG] = 8.1%

71401000	White potato, french fries, NS as to from fresh or frozen
71401010	White potato, french fries, from fresh, deep fried
71401030	White potato, french fries, from fast food / restaurant
71401032	White potato, french fries, from frozen, deep fried, from home or store
71401035	White potato, french fries, from frozen, NS as to deep fried or oven baked

Mixed Foods containing French Fries
Adjusted for a French Fry content of 90% **[EPG] = 7.29%**

71402500	White potato, french fries, with cheese
71402505	White potato, french fries, with cheese and bacon
71402510	White potato, french fries, with chili and cheese
71402520	White potato, french fries, with chili con carne

Doughnuts
[EPG] = 10.8%

53520000	Doughnut, NS as to cake or yeast
53520110	Doughnut, cake type
53520120	Doughnut, chocolate, cake type
53520140	Doughnut, cake type, chocolate covered
53520150	Doughnut, cake type, chocolate covered, dipped in peanuts
53520160	Doughnut, chocolate, cake type, with chocolate icing
53520200	Churros
53520500	Doughnut, Asian
53520600	Cruller, NFS
53520700	French cruller
53521100	Doughnut, chocolate, raised or yeast, with chocolate icing
53521110	Doughnut, raised or yeast
53521120	Doughnut, chocolate, raised or yeast
53521130	Doughnut, raised or yeast, chocolate covered
53521140	Doughnut, jelly
53521210	Doughnut, custard-filled
53521220	Doughnut, chocolate cream-filled
53521230	Doughnut, custard-filled, with icing

APPENDIX 4
Expert Panel Consensus Statement

Expert Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of Esterified Propoxylated Glycerol (EPG) for Use in Foods

December 19, 2017

INTRODUCTION

At the request of ChocoFinesse, LLC, an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether, under the conditions of intended use in select frying applications, esterified propoxylated glycerol (EPG) would be "Generally Recognized as Safe" (GRAS), based on scientific procedures. The Panel consisted of the below-signed qualified scientific experts: Fergus Clydesdale (University of Massachusetts), Robert J. Nicolosi (RJ Nicolosi, LLC), and Dr. John A. Thomas (Tom-Tox, LLC). For the purposes of the GRAS Expert Panel's evaluation, "safe" or "safety" means that there is a reasonable certainty of no harm under the intended conditions of use of the ingredients in foods, as stated in 21 CFR 170.30 (U.S. FDA, 2017).

The Panel, independently and collectively, critically examined a comprehensive package of publicly available scientific information and data compiled from the literature and other published sources based on searches of the published scientific literature conducted through November 2017. In addition, the Panel evaluated other information deemed appropriate or necessary, including data and information provided by Choco Finesse, LLC. The data evaluated by the Panel included information pertaining to the method of manufacture and product specifications, analytical data, intended use levels in specified food products, consumption estimates for all intended uses, and comprehensive literature on the safety of EPG and its individual components.

Choco Finesse previously submitted 2 notifications of GRAS status to the United States (U.S.) Food and Drug Administration (FDA) for the use of H-EPG-05 (i) as a fat replacer at levels up to 34.5% (w/w) in confectionary applications; and (ii) as a fat replacer at levels up to 38% (w/w expressed on a fat basis), in spreadable and baked goods. The FDA reviewed these GRAS notifications (GRN No. 583 and GRN No. 640, respectively), with "no resulting questions" (U.S. FDA, 2015, 2016).

Following independent, critical evaluation of such data and information, the Panel unanimously concluded that under the expanded conditions of intended use (*i.e.,* commercial frying of french fries and doughnuts as described herein), EPG, meeting appropriate food-grade specifications, and manufactured and used in accordance with current good manufacturing practice, is GRAS based on scientific procedures.

COMPOSITION, MANUFACTURING AND SPECIFICATIONS

EPG is manufactured in compliance with current Good Manufacturing Practice (cGMP) regulations. The production of EPG consists of 2 basic processes: (1) propoxylation of glycerol; and (2) esterification of propoxylated glycerol with fatty acids. Propoxylation of glycerol involves reacting food grade glycerol with propylene oxide under base catalysis to form the tri-functional polyether polyol (propoxylated glycerol). The esterification is carried out without catalyst using an excess of fatty acids. The unsaturated fatty acids are

derived from splitting natural edible fats and oils, while saturated fatty acids are produced by splitting fully hydrogenated edible oils. The unreacted fatty acids are removed from crude EPG by molecular distillation. Batch analysis data demonstrate that this manufacturing process produces a consistent product that meets specifications. EPG has been shown to resist oxidation and thermal decomposition as well as or better than current edible fats, oils and shortenings as measured by the oxidative stability index and the smoke and flash point. Decomposition products are similar to those seen with other unsaturated fatty acids.

INTENDED USE AND ESTIMATED EXPOSURE

EPG is intended for use in commercial frying of french fries (80:20 blend; EPG: vegetable oil) and doughnuts (60:40 blend; EPG:vegetable oil) in the U.S. In order to determine exposure to EPG from these applications, ChocoFinesse analyzed EPG absorption in french fries and doughnuts. Control frying was done in high-oleic sunflower oil (HOS) while test frying was performed using 80:20 and 60:40 blends of spreadable EPG with HOS. EPG absorption into food products was determined by analyzing the fatty acid composition of oils extracted from these foods. The total content of EPG in a medium serving of french fries is 9.46 g (8.1% by weight). In experimental frying of frozen doughnuts, the content of EPG is 3.6 g in a 49.1 g doughnut.

On a consumer-only basis, the resulting mean and 90th percentile intakes of EPG by the total U.S. population from all intended frying applications were estimated to be 4.0 g/person/day (64 mg/kg body weight/day) and 7.6 g/person/day (130 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean consumer-only intakes of EPG were determined to be 4.7 g/person/day in female teenagers, male teenagers and male adults (83, 67 and 56 mg/kg body weight/day, respectively), and the highest 90th percentile intakes were determined to be 11.9 g/person/day (161 mg/kg body weight/day) in female teenagers. Infants and young children had the lowest mean and 90th percentile consumer-only intakes of 2.1 g/person/day (154 mg/kg body weight/day) and 4.6 g/person/day (348 mg/kg body weight/day), respectively.

When intakes were expressed on a body weight basis, infants and young children had the highest mean and 90th percentile consumer-only intakes of 154 and 348 mg/kg body weight/day. Female adults had the lowest mean and 90th percentile consumer-only intakes of 46 and 83 mg/kg body weight/day, respectively.

EPG has been previously notified to be GRAS for use in confectionary coatings (GRN No. 583) and as a fat replacer (GRN No. 640) (Choco Finesse, LLC, 2015, 2016). A cumulative intake assessment was conducted encompassing these uses, as well as the current commercial frying applications. Cumulative mean and 90th percentile consumer-only intakes of EPG were estimated to be 11.9 and 25.0 g/person/day, respectively. Overall, the inclusion of EPG in frying applications resulted in an increase of between 0.4 and 1.6 g/day at the mean and between 0.5 and 5.9 mg/day at the 90th percentile of intake.

DATA PERTAINING TO SAFETY

The safety of EPG is based on published subchronic studies in rats and micropigs, a one-generation reproductive toxicity study in rats, a developmental toxicity evaluation in rabbits, and genotoxicity studies of EPG. Unpublished studies (*i.e.*, absorption, distribution, metabolism, and excretion studies, subchronic toxicity studies in mice and Beagle dogs, two year combined chronic dietary safety study and carcinogenicity studies in rats and mice, a 1-year chronic safety studies in Beagle dogs and micropigs, a 3-generation reproduction study [with a teratology phase] in rats, and irritation and sensitization) were available and reviewed by the panel, and providing corroborative evidence of safety.

Absorption, Distribution, Metabolism, and Excretion (ADME)

The pharmacokinetics of 2 separately radiolabeled EPG versions (H-EPG-08 oleate [a semi-solid] and H-EPG-14 oleate [a liquid]) were evaluated in male and female Crl:CD®BR rats to determine the absorption, distribution, metabolism and excretion (ADME) profile of these materials. Two separate studies were conducted with H-EPG-08 oleate; one in which the material was ^{14}C-radiolabeled on the C_1-carbon of the propylene glycol units, and a second in which it was ^{14}C-radiolabeled on the carboxyl carbon of the fatty acid portion. In the latter study, thin-layer chromatography was used to confirm the presence ^{14}C-oleic acid in liver tissue extracts, as incorporation of the radiolabeled fatty acid into tissues was expected. Finally, 1 study was conducted with H-EPG-14 oleate radiolabeled on the C_1-carbon of the propylene glycol units.

In each study, 5 rats/sex/group were administered a single oral dose of 1.0 g/kg body weight or 3.0 g/kg body weight by gavage. A third dose group was given 1.0 g/kg body weight of the radiolabeled version after 2 weeks of daily EPG administration of the non-labeled version at the same dose. In addition, to simulate the worst-case scenario of complete absorption of the EPGs, ADME studies were conducted on rats receiving a dose of 35 mg/kg of each version intravenously in a liposome suspension. Expired air, feces, urine, organs, tissue samples and the carcass were monitored for radioactivity for up to 1 week after EPG administration when, essentially, there was complete recovery of the dose administered.

The results of these studies indicate that the 2 EPG versions evaluated were poorly absorbed from the GI tract and could not be found intact in any tissues after oral dosing. EPG-08 oleate was degraded approximately 20%, while EPG-14 oleate was degraded by approximately 10%. There was some evidence that possible bacterial degradation in the gastrointestinal (GI) tract was taking place, particularly in the colon. The pattern of distribution of the radiolabel observed in the body of the rats was consistent with GI absorption of fatty acids and the propylene glycol units modified glycerol, both of which were partially oxidized to carbon dioxide. A significant portion of the fatty acids absorbed were incorporated into triglycerides and stored in adipose tissue.

In the 2 studies where the propoxylated glycerol units were radiolabeled, small amounts of radiolabel were detected in the liver and other metabolically active tissues, indicating that a small portion of this material was assimilated into normal body constituents during the oxidation process. This absorption, disposition, metabolism and excretion pattern for EPG was considered predictable and similar to that which would be expected from normal triglycerides. When given intravenously in fine liposome emulsion, the 2 versions of EPGs tested were rapidly oxidized to fatty acids and glycerol containing propoxylated glycerol units. The disposition pattern was similar to that *via* the oral route, except that larger portions of the metabolites of the EPGs were deposited in the liver and lungs. The route by which the metabolites of the various versions of EPGs were excreted appeared to be governed by their molecular weights. Namely, the greater the molecular weight, the more of the metabolites of the EPGs excreted into the feces, and the less into the urine.

Toxicological Studies

Preclinical studies were conducted with H-EPG-05 HR/SO 9:1 (Mettler dropping point 106.9°F) unless otherwise stated. EPG-05 HR/ST 45:55 (Mettler dropping point 104.3°F) is a softer version at average normal body temperature and was selectively investigated in safety studies. It is worthwhile to note that, unlike olestra, EPG is not strongly hydrophobic and exhibits far less interaction with fat-soluble substances including fat-soluble vitamins. As such, vitamin fortification of animal diets was not required in any of the EPG preclinical safety studies including lifetime studies in rats and mice as well as up to 3 generations in reproductive and development studies. This differed from studies conducted with olestra, which

required vitamin fortification. It is also important to note that residues of EPG were not found in any tissues from any animals placed on study, indicating efficient clearance and absence of accumulation even following lifetime administration. In addition, the stability and homogeneity of prepared diets for the safety studies was established.

Subchronic and Chronic Studies

The subchronic toxicity of a representative version of EPG when given to Sprague-Dawley rats by dietary admixture for at least 90 days was assessed by Christian and Bechtel (2014). Rats (n=700) were randomly assigned to 5 groups (70 animals/sex/group, subdivided into subsets A through F for each sex) and administered concentration levels of 0, 0.5, 1.0, and 2.0 g EPG/kg of body weight/day (g/kg/day) through adjusted diets, or a fixed intake of 5.0% (w/w) in the diet. The latter is expected to result in a decrease in EPG intake over time; the result of feed consumption in g/day remaining relatively constant and the mean body weights increasing markedly over time so that mean feed intake in g/kg/day decreases markedly over time. All diets were prepared weekly and provided *ad libitum*.

Results of this study showed that dietary administration of EPG at levels of 0.5, 1.0, and 2.0 g/kg, or 5% (w/w) to rats for at least 13 weeks was not associated with any adverse effects. The levels of liver vitamins A and E and serum vitamin D were generally decreased in EPG-treated animals at all concentration levels. However, there was no evidence of vitamin deficiency as assessed by growth, clinical observations, clinical pathology or anatomical pathology endpoints. Prothrombin time (PT), measured as an indicator of vitamin K status, was not significantly affected. Based on the results of this study, it was not possible to establish a no-observable-effect level (NOEL). The possible effect of EPG on vitamin levels in the absence of any clinical signs of deficiency was not considered "adverse" *per se*. As such, the adjusted concentration of 2 g/kg and the fixed intake of 5% EPG (equivalent to an average EPG intake of approximately 6 g/kg body weight/day in the beginning of the study and declining to approximately 2 g/kg body weight/day) were considered to represent no-observable-adverse-effect levels (NOAEL).

The subchronic (90-day) toxicity of EPG was also assessed in Yucatan micropigs (approximately 8 to 10 months old) by Wedig and Bechtel (2014). Animals (5/sex/group) received feed (Certified Agway® Prolab® Minipig Diet Meal) containing 5, 10, and 17% EPG, mixed accordingly throughout the study to deliver 1.5, 3, and 5 g/kg/day of EPG, respectively. Corn oil served as the vehicle control (0 g/kg body weight/day).

Micropigs were observed twice daily for toxicological, pharmacological, and behavioral effects. Feed consumption and dietary levels of EPG were determined on a weekly basis. Physical and ophthalmic examinations, body weights, urinalysis, hematology, clinical chemistry, water intake, bowel transit times, organ weight, organ tissue analysis for EPG, fecal assays, vitamin assays, gross necropsy, and histopathology were used to evaluate the effects of EPG.

EPG was palatable up to 5 g/kg/day in the diet. No treatment-related morbidity/mortality occurred. No consistent or distinct EPG treatment-related adverse pharmacological/toxicological or behavioral effects were noted. No treatment-related effects were observed during the physical and ophthalmic examinations. Analysis of body weight gain, feed efficiency, water consumption, bowel transit times, hematology and serum chemistry parameters, urinalysis data, feces, and organ weights indicated no treatment-related effects. Chemical analysis of liver, kidney, spleen, and adipose tissue yielded negative data for EPG residue. Gross necropsy and histopathology examinations indicated no treatment-related effects.

PT and activated partial thromboplastin time, measured as indicators of vitamin K status, were not significantly affected. EPG significantly affected liver vitamin A and serum vitamin D. A significant decrease in the liver vitamin A content was observed in animals fed 5 g of EPG/kg/day. EPG demonstrated a concentration-dependent effect on the levels of total vitamin D and the biologically active vitamin D metabolite, 25-OH-vitamin D. Specifically, total vitamin D serum levels were significantly reduced in all groups, while serum levels of 25-OH-vitamin D were significantly reduced in animals administered 3 or 5 g of EPG/kg/day. Although a NOEL for effects of dietary EPG on total vitamin D serum levels was not established, a NOEL for effects on 25-OH-vitamin D levels was determined to be 1.5 g of EPG/kg/day, or 5% dietary EPG concentration

Reproductive and Developmental Studies

Tyl and Bechtel (2014a) investigated the reproductive effects following continuous exposure of Crl:CD® (SD)Br rats (approximately 6 weeks old; mean male weight 183.9 ± 1.1 g, mean female weight 151.5 ± 1.0 g) to EPG in the diet (30 animals/sex/group) at 0.0% (group 0), target levels of 0.5% g/kg/day (group 1), 1.0 g/kg/day (group 2), and 2.0 g/kg/day (group 3), and fixed 5.0% EPG (w/w) (group 4), all in 6% corn oil (vehicle). Dietary concentrations of EPG for groups receiving 0.5, 1.0, and 2.0 g/kg/day of test material were adjusted weekly to maintain target EPG intake, throughout the prebreed period. Animals were exposed for a 13-week prebreed period, and through 2 breeding cycles for F0 parental animals, and up to postnatal Day 91 for F1a and F1b offspring.

Parameters examined included, body weights, weight gains, feed consumption, clinical signs, reproductive indices and offspring litter sizes, pup survival and body weights, and histopathology of parental reproductive organs. The study also examined possible effects on blood clotting, parental and offspring immunologic status, histopathology of organs related to immunological function, neurological effects in parents and offspring, developmental effects in offspring, liver and serum fat-soluble vitamin status, and the possible presence of the test material in selected organs of parental and offspring animals.

Results indicated that dietary administration of EPG at levels of 0.5, 1.0, and 2.0 g/kg, and 5% (w/w) to rats for at least 13 weeks was not associated with adverse effects, except for that on liver vitamin status. Vitamin E levels exhibited concentration-related statistically significant reductions in all evaluated groups, with the exception of F1b(A) male weanlings and satellite group F1b(B) males and females. There was no evidence of vitamin D deficiency except in F0 parental, F1a(A) and F1b(A) weanling females, and no evidence of vitamin A deficiency except in F0 parental, F1a(C) and F1b(C) postnatal Day 91 females. There were no effects on reproduction of the F0 parental animals for either F1a or F1b mating; evidence for dystocia was present in all groups, including the vehicle control group, with no concentration-dependent response pattern. No treatment-related effects on postnatal growth or development (physical or behavioral), immunological status, blood clotting, and parental general status were observed. EPG was not detected in any of the 360 liver samples from the high concentration and control groups. With respect to kidney and spleen samples, there were 2 and 7 positive samples, respectively, out of 360 total samples for each organ. According to the authors, the positive samples were not the result of contamination during necropsy or analyses, were evenly divided between high concentration and control animals, and were not associated with other measures indicative of *in vivo* systemic exposure.

Based on the results of this study in rats, the NOAEL was 5.0% EPG. Also, in the absence of any effects on behavioral development, immunologic status, and blood clotting, and with group 4 animals tolerating a fixed dietary EPG percentage, it was recommended that the 3-generation study with 2 litters per generation utilize fixed dietary percentages with the highest concentration 5.0% EPG, and endpoints examined not include behavioral, immunologic, or coagulation assessments.

In another study, 72 female New Zealand White rabbits (18/group) were fed EPG (0.0, 2.5, 5.0, and 10.0% [w/w]) in Modified Purina Certified Rabbit Chow #5322, supplemented with 6% corn oil (w/w), for 26 days (Day -7 through Gestational Day 19) to assess effects of EPG on the developing conceptus (Tyl and Bechtel, 2014b).

All maternal animals were observed for mortality, signs of gross toxicity, clinical signs, body weights, food consumption, and gestational parameters. A significant concentration-related downward trend was observed for "maternal" weight change only for Day -7 to Day 0 (the first week of dietary exposure, prior to insemination) with no significant pairwise comparisons to the concurrent control group. For "maternal" feed consumption (in g/kg/day), significant concentration-related downward trends were observed for Day -14 to -13, Day -11 to -10 and Day -14 to -7, with no significant pairwise comparisons, and all intervals prior to the initiation of administration of EPG (which began on Day -7). At necropsy, all fetuses were dissected from the uterus and examined for skeletal malformations or variations, body weights, and crown-rump length. No evidence of maternal or developmental toxicity was found in rabbits in this study. A NOAEL of 10% EPG (approximately 4.76 g/kg body weight/day), the highest dose tested for both maternal and developmental toxicity is proposed based on the results of this study.

Genotoxicity Studies

As reviewed in Bechtel (2014), various forms of EPG (i*.e.,* heated and Unheated H-EPG-05 HR/SO 9:1 and EPG-05 HR/ST 45:55) were not mutagenic in the bacterial reverse mutation test in Salmonella typhimurium or Escherichia coli. Similarly, EPG did not induce mutations, with or without metabolic activation, at the tk locus in mouse lymphoma cells. EPG did not induce chromosome aberrations in cultured human peripheral blood lymphocytes when tested to its limit of solubility in the i*n vitro* cytogenetics assay. EPG did not induce unscheduled DNA synthesis (UDS) in the livers of Wistar rats following oral administration.

Clinical Studies

Unpublished studies of human tolerance to EPG (H-EPG-05 HR/SO 9:1 and EPG-05 HR/ST 45:55), including single dietary exposure studies and incremental increasing multiple dietary exposure studies, demonstrated that food products prepared with EPGs were highly palatable compared to similar foods prepared with conventional fats. Furthermore, no untoward effects in human volunteers resulted from the consumption of up to 150 g of EPG per day.

A published double-blind, randomized, controlled study was performed to assess the effect of EPG-05 HR/ST 45:55 on fat-soluble vitamins and select nutrients in human subjects (Davidson and Bechtel, 2014). For 8 weeks, 139 healthy volunteers (34 to 36/group) consumed a core diet providing adequate caloric and nutrient intakes. The diet included items (spread, muffins, cookies, and biscuits) providing EPG (10, 25, and 40 g/day) *vs*. margarine alone (control). The variables measured at baseline and regular intervals were: physical exam, including vital signs; body weight; hematology; clinical chemistry; urinalysis; circulating levels of β-carotene, retinol (vitamin A), α-tocopherol (vitamin E), 25-OH D_2 (vitamin D, ergocalciferol), 25-OH D_3 (vitamin D, cholecalciferol), phylloquinone (vitamin K_1), PIVKA-II (proteins induced in vitamin K absence), serum folate, red blood cell folate, vitamin B_{12}, zinc, iron, calcium, phosphorus, osteocalcin, retinol-binding protein, intact parathyroid hormone, cholesterol, high-density lipoproteins, low-density lipoproteins and triglycerides; PT and PTT (partial thromboplastin time); urine zinc, sodium, potassium, creatinine, calcium, and phosphorus; and tolerability. Tolerability was assessed by the incidence of 14 specific gastrointestinal adverse events: passing gas; gas with discharge; abdominal bloat/cramp; heartburn; diarrhea; constipation; urgency of bowel movement; fecal incontinence; oily spotting; oily evacuation; oily stool; liquid stool; soft stool; and hard stool.

Significant declines in β-carotene were seen over time, especially in the EPG groups, but with no apparent relationship to EPG concentration (more severe at 10 g/day and 40 g/day than at 25 g/day). It is possible that the apparent effect of EPG on circulating β-carotene was related to a lower dietary fat intake among subjects receiving EPG, since subjects had difficulty consuming all of the additional fat necessary to fully compensate for what EPG is displaced in the diet. In this case, as a lipid-like material, EPG might have affected the absorption of these nutrients strictly through physicochemical processes, acting as a lipid "sink" during transit in the gastrointestinal tract.

Based on the Wilcoxon Rank Sum Test, there were no statistically significant changes from baseline at the primary endpoint (Day 56) in mean retinol levels in evaluable subjects receiving EPG 10, 25, and 40 g/day compared with subjects receiving placebo. Similarly, no other statistically significant differences in the mean change from baseline were noted between the EPG groups and placebo group at Days 14, 28, 42, 56 and the end point analysis with the exception of the EPG 25 g/day group at Day 14 (P=0.0141).

Likewise, the Wilcoxon Rank Sum Test revealed significant decreases in mean α-tocopherol levels from baseline in the EPG 25 g/day group at Days 14 (p=0.498), 28 (p=0.0014) and 42 (p=0.0001) and in the EPG 40 g/day group at Day 14 (p=0.0166), Day 42 (p=0.0030) compared to the placebo group. No other statistically significant differences in the change from baseline were noted between the EPG groups and the placebo group at Days 14, 28, 42, and 56. The end point analysis using the last observation carried forward was similar to the Day 56 results for each treatment group.

Circulating 25-OH D_3 levels increased over time in the EPG groups, but not to the same degree as the control group, which had an unexpected rise, despite attempts to control endogenous 25-OH-D_3 synthesis by conducting the study during the winter in Chicago, Illinois, USA.

EPG intake was associated with a slight decline in phylloquinone levels across all groups. However, the declines did not exceed 0.1 ng/mL and were not statistically significant within any of the individual groups; statistical significance was observed only when the differences within each EPG group were compared to the differences (none or positive) in the control.

By the end of the study, the levels of circulating proteins induced in vitamin K absence (PIVKA-II) had increased significantly in the EPG 25 and EPG 40 groups, compared to the control; in the EPG 10 group, the difference from baseline was comparable to the difference from baseline in the control. Combined with the phylloquinone results, these data suggest that EPG might have affected the synthesis of vitamin K-dependent clotting factors to some extent, but the changes were small, and there was no indication of any clinical manifestation. The changes in clotting parameters (PT and PTT) from baseline to the end of the study were comparable between the control and EPG groups.

With the exception of gastrointestinal discomfort, all adverse events reported by subjects in this study were considered unrelated to EPG. Seven of the 14 pre-defined gastrointestinal adverse events (gas with discharge; diarrhea; oily spotting; oily evacuation; oily stool; liquid stool; soft stool) were reported more frequently by subjects receiving 25 or 40 g/day of EPG, especially females. In general, the incidence and duration of these symptoms correlated with EPG dietary concentration. The results suggest 10 g/day of EPG was reasonably well tolerated.

SUMMARY

A battery of preclinical feeding studies was initiated to assess the safety of the core compound, H-EPG-05 HR/SO 9:1, including carcinogenic activity and the potential to cause developmental anomalies in several animal species. In addition, a series of mutagenicity studies were conducted with H-EPG-05 HR/SO 9:1, as well as other EPG versions (*e.g.*, H-EPG-05 soyate and H-EPG-14 soyate). The bacterial reverse mutation assay alone (with *Salmonella typhimurium* strains TA98 and TA100) was used to screen heated versions of EPG that might be used for frying and baking (*i.e.*, heated H-EPG-05 HR/SO 9:1 and EPG-05 HR/ST 45:55).

The preclinical studies showed no adverse treatment related changes to the general health and appearance of the animals, or on the conventional parameters measured including but not limited to: growth, feed consumption, body weight, clinical chemistry, hematology, reproductive performance and fetal development. Feeding EPG to rats, mice, rabbits, dogs and micro-pigs produced no observed adverse findings in the GI tract structure or function. Minor fluctuations in fat soluble vitamin status were evident in preclinical studies, however, the concentrations of fat soluble vitamins in the liver (*e.g.*, vitamins A and E) and serum (*e.g.*, vitamin D) remained within the historical limits of species traditionally used in animal studies involving lifetime dietary exposures.

Studies examining the influence of physical state, consumed mass and solubility properties of EPG indicated low potential for significant or biologically meaningful effects at intake amounts anticipated for consumers. Gastrointestinal tolerance and tidiness were found to be augmented through selection of versions that are solid at human body temperature. Similarly, the potential for these untoward effects was minimized through selection of initial food applications that result in moderate consumer intake. Finally, studies in both experimental animals and humans demonstrated that: (1) the potential for interaction with lipid-soluble nutrients and other substances present in the gastrointestinal lumen is minimized through version selection (*i.e.*, a solid form of EPG) and moderation of consumption; and (2) there is low potential for biologically-meaningful effects at the maximum anticipated consumer intake. This is consistent with the moderate organic nature and solubility properties of EPG (log Kow of approximately 3.2 to 3.4), and in strong contrast to the interaction of fat mimetics, such as olestra which have a log Kow in excess of 40.

CONCLUSION

We, the undersigned independent qualified members of the Expert Panel, have, independently and collectively, critically evaluated the data and information summarized above and conclude that esterified propoxylated glycerol (EPG), meeting appropriate food grade specifications and produced in according with current good manufacturing practice, is Generally Recognized as Safe (GRAS) based on scientific procedures under the conditions of intended use in commercial frying of french fries and doughnuts specified herein.

It is our opinion that other qualified experts would concur with these conclusions.

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REFERENCES

Bechtel DH (2014). Genotoxicity testing of esterified propoxylated glycerol (EPG) Regul Toxicol Pharmacol 70(Suppl. 2):S131-S142. DOI:10.1016/j.yrtph.2014.11.013.

Choco Finesse, LLC (2015). *Summary of Data Supporting a Determination That the Use of Esterified Propoxylated Glycerol (EPG) In Confectionary Foods is Generally Recognized as Safe (GRAS)*. (Submitted as U.S. FDA, 2015 - GRN 583). Prepared by Indianapolis (IN): Choco Finesse, LLC for submission to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm465255.pdf.

Choco Finesse, LLC (2016). *Summary of Data Supporting a Determination That the Use of Esterified Propoxylated Glycerol (EPG) in Spreadable and Baked Goods is Generally Recognized as Safe*. (Submitted as U.S. FDA, 2016 - GRN 640). Prepared by Indianapolis (IN): Choco Finesse, LLC for submission to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm508140.pdf.

Christian BJ, Bechtel DH (2014). 90-Day dietary toxicity study with esterified propoxylated glycerol (EPG) in rats. Regul Toxicol Pharmacol 70(Suppl. 2):S95-S104. DOI:10.1016/j.yrtph.2014.11.017.

Davidson MH, Bechtel DH (2014). Assessment of the effect of esterified propoxylated glycerol (EPG) on the status of fat-soluble vitamins and select water-soluble nutrients following dietary administration to humans for 8 weeks. Regul Toxicol Pharmacol 70(Suppl. 2):S143-S157.

Tyl RW, Bechtel DH (2014a). One-generation reproduction study of esterified propoxylated glycerol (EPG) administered in the feed to CD® (Sprague-Dawley) rats. Regul Toxicol Pharmacol 70(Suppl. 2):S114-S122. DOI:10.1016/j.yrtph.2014.11.018.

Tyl RW, Bechtel DH (2014b). Developmental toxicity evaluation of esterified propoxylated glycerol (EPG) administered in the diet to New Zealand white rabbits. Regul Toxicol Pharmacol 70(Suppl. 2):S123-S130. DOI:10.1016/j.yrtph.2014.11.014.

U.S. FDA (2015). *Agency Response Letter GRAS Notice No. GRN 000583 [Esterified propoxylated glycerol, Indianapolis (IN): Choco Finesse, LLC]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=583 [Nov. 12, 2015].

U.S. FDA (2016). *Agency Response Letter GRAS Notice No. GRN 000640 [Esterified propoxylated glycerol, Indianapolis (IN): Choco Finesse, LLC]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=640 [Sep. 21, 2016].

U.S. FDA (2017). Part 170—Secondary direct food additives permitted in food for human consumption. Section §170.30—Eligibility for classification as generally recognized as safe (GRAS). In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs*. (U.S. Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

Wedig J, Bechtel DH (2014). 90-Day dietary toxicity study with esterified propoxylated glycerol (EPG) in Micropigs. Regul Toxicol Pharmacol 70(Suppl. 2):S105-S113. DOI:10.1016/j.yrtph.2014.11.012.