ORIGINAL SUBMISSION

#1 583

Intertek Scientific & Regulatory Consultancy
1011 US Highway 22, Suite 200,
Bridgewater, New Jersey
08807-2950, U.S.A.

Phone: 908-429-9202
Fax: 908-429-9260
www.intertek.com

GRN 000583

May 26, 2015

Paulette Gaynor, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Dr. Gaynor:

In accordance with 21 CFR 170.36 (62 FR 18960; April 17, 1997), Intertek Scientific & Regulatory Consultancy is hereby submitting, on behalf of Choco Finesse, LLC, notice of a determination based on scientific procedures that the use of H-EPG-05, as defined in the enclosed documents, is generally recognized as safe (GRAS) under specific conditions of use as an ingredient in multiple food categories, and that it is therefore exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act.

Enclosed please find an electronic copy of the cover letter, GRAS Exemption Claim, and GRAS notice.

My contact information is provided below. Please feel free to contact me[1] by phone or e-mail if you have any questions regarding this GRAS notice.

Sincerely,

(b) (6)

David H. Bechtel, Ph.D., D.A.B.T.
Vice President New Jersey Office

cc: David Rowe, Choco Finesse, LLC

[1] Please note that Choco Finesse, LLC has authorized Dr. David Bechtel (David.Bechtel@Intertek.com) from Intertek Scientific & Regulatory Consultancy, located at 1011 U.S. Highway 22, Suite 200, Bridgewater, NJ 08827, to engage in discussions about any issues related to the enclosed GRAS notice. Dr. Bechtel may be reached by e-mail (shown above), by telephone at (908) 429-9202, or by FAX at (908) 429-9260.

chocofinesse
MAXIMUM FLAVOR. MINIMUM FAT.

12403 W.101 Terrace Lenexa, Kansas 66215 5019 N. Meridian Street Indianapolis, Indiana 46208
e: drowe@chocofinesse.com p: 317.694.3601

May 20, 2015

Paulette Gaynor, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Dr. Gaynor:

In accordance with 21 CFR 170.36 (62 FR 18960; April 17, 1997), Choco Finesse, LLC is hereby submitting notice of a determination based on scientific procedures that the use of H-EPG-05, as defined in the enclosed documents, is generally recognized as safe (GRAS) under specific conditions of use as an ingredient in multiple food categories, and that it is therefore exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act.

Enclosed please find an electronic copy of the GRAS notice, which includes a comprehensive summary of data supporting the safety of the ingredient and the signed statement of an expert panel regarding the value of these data in supporting a GRAS determination.

My contact information is provided below. Please feel free to contact me[1] by phone or e-mail if you have any questions regarding this GRAS notice.

Sincerely,

(b) (6)

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

[1] Please note that Choco Finesse, LLC has authorized Dr. David Bechtel (David.Bechtel@intertek.com) from Intertek Scientific & Regulatory Consultancy, located at 1011 U.S. Highway 22, Suite 200, Bridgewater, NJ 08827, to engage in discussions about any issues related to the enclosed GRAS notice. Dr. Bechtel may be reached by e-mail (shown above), by telephone at (908) 429-9202, or by FAX at (908) 429-9260.

chocofinesse
MAXIMUM FLAVOR. MINIMUM FAT.

12403 W.101 Terrace Lenexa, Kansas 66215 5019 N. Meridian Street Indianapolis, Indiana 46208
e drowe@chocofinesse.com p. 317.694.3601

GRAS Exemption Claim

Choco Finesse, LLC has determined that the use of H-EPG-05 under specific conditions of use as an ingredient in multiple food categories entails a reasonable certainty of no harm and is generally recognized as safe (GRAS) based on scientific procedures. Consequently, it is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act.

Signature (b) (6) Date May 20, 2015

David Rowe
President

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

GRAS Substance

The subject of this GRAS notice is H-EPG-05 marketed by Choco Finesse, LLC. The product is manufactured under current good manufacturing practices (cGMP) (21 CFR, part 110). Inspections and testing are performed at various points during the manufacturing process; every lot of H-EPG-05 is tested for compliance with the established specifications (*e.g.*, heavy metals, solid fat content, fatty acid composition and microbiological activity).

Intended Use and Projected Consumer Exposure

Choco Finesse, LLC intends to market H-EPG-05 for use as a fat replacer at levels up to 34.5% (w/w) in confectionery products and coatings as specified in Table 1.

Table 1 Summary of proposed food uses and maximum use levels

Coating Application	Food Category	Use level in coating (%)
Drizzle	Baked Goods and Baking Mixes	34.5
	Frozen dairy desserts and mixes	34.5
	Grain Products and Pasta	34.5
Enrobed	Baked Goods and Baking Mixes	34.5
		34.5
	Frozen dairy desserts and mixes	34.5
	Grain Products and Pasta	34.5
	Hard candy and cough drops	34.5
	Processed fruits and fruit juices	34.5
	Snack foods	34.5
Inclusion	Baked Goods and Baking Mixes	30
		30
	Grain Products and Pasta	30
	Snack foods	30
Solid	Hard candy and cough drops	30
	Soft candy	34.5
		34.5

Basis for GRAS Determination

To make the GRAS determination, Choco Finesse, LLC compiled information about the substance, specifications, manufacturing, proposed uses, and evidence of safety into a comprehensive dossier (GRAS Dossier); and sought the opinion of qualified experts (*i.e.*, expert panel) in determining whether there is consensus among their peers that the use of this substance as described entails a reasonable certainty of no harm and is generally recognized as safe based on the available scientific evidence.

All data and information that are the basis for this GRAS determination are available for FDA's review and copying at reasonable times at Intertek Scientific & Regulatory Consultancy, 1011 U.S. Highway 22, Suite 200, Bridgewater, NJ 08807, and will be sent to FDA upon request.

chocofinesse
MAXIMUM FLAVOR. MINIMUM FAT.

12403 W.101 Terrace Lenexa, Kansas 66215 5019 N. Meridian Street Indianapolis, Indiana 46208
e: drowe@chocofinesse.com p: 317.694.3601

SUMMARY OF DATA SUPPORTING A DETERMINATION THAT THE USE OF ESTERIFIED PROPOXYLATED GLYCEROL (EPG) IN CONFECTIONARY FOODS IS GENERALLY RECOGNIZED AS SAFE (GRAS)

Submitted to:

Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety

By:

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

May 20, 2015

GRAS EXPERT PANEL STATEMENT

SUMMARY OF DATA SUPPORTING A DETERMINATION THAT THE USE OF ESTERIFIED PROPOXYLATED GLYCEROL (EPG) IN CONFECTIONARY FOODS IS GENERALLY RECOGNIZED AS SAFE (GRAS)

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

Background

Choco Finesse, LLC (hereafter referred to as Choco Finesse) has commissioned an independent panel of recognized experts (hereafter referred to as the Expert Panel) to issue an opinion regarding the GRAS status of esterified propoxylated glycerols (EPG or EPGs hereafter). EPGs are a family of fat- and oil-like substances that resemble triglycerides in structure and appearance, but have been modified to prevent or limit their digestion when consumed in food. Specifically, the Expert Panel was asked to consider whether the use of H-EPG-05, the initial form of EPG selected for commercial development, at levels up to 34.5% (w/w) in specified confectionery products and coatings is GRAS based on scientific procedures. To facilitate the review, each Expert Panel member received a comprehensive package describing the intended use, manufacturing, specifications, analytical data, exposure estimates and data supporting the safety of EPGs.

In evaluating the GRAS status of H-EPG-05, the Expert Panel considered that:

- EPGs are produced by a three-step process: (1) fats and oils are split into glycerol and fatty acids; (2) glycerol is reacted with propylene oxide to produce glycerol with propylene glycol units (PGUs) inserted on its hydroxyl groups; and (3) the propylene glycol-substituted glycerol is reacted with fatty acids, resulting in their esterification with the hydroxyl moieties of the PGUs. The α-methyl group of the PGU sterically blocks access of lipase to the ester linkage, thus impairing normal lipid digestion of EPGs.

- A large variety of EPG versions can be manufactured using commercially available sources of fatty acids split from food-grade fats and oils. However, a core version, H-EPG-05, was selected as the representative form for initial commercial development, to assure a solid form.

- Analytical data for EPG-05 show that the final EPG product is consistently manufactured to food-grade specifications.

- H-EPG-05 is intended to replace triglyceride fats in foods such as 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 confectionery items, sugar-shelled confectionery pieces), snack foods (snack items, trail mix) and soft candy (molded and hollow molded confectionery pieces).

- Based on the intended use levels and representative food categories from the 2009-2010 National Health and Nutrition Examination Survey (NHANES), male adults would be expected to have the highest 90th percentile all-user intake, 19.96 g EPG/person/day, on an absolute basis.

- Unlike olestra, EPG is not strongly hydrophobic and exhibits far less interaction with fat-soluble substances including fat soluble vitamins. Consistent with this, the Experts noted that 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.

With respect to the safety of H-EPG-05, the Expert Panel noted that EPGs have been studied extensively, and, while the majority of this information is not publicly available, a series of articles addressing various aspects of safety was recently published.[1]

Ingested EPG is not absorbed or metabolized to any significant degree. The results of rat studies with radiolabeled EPG showed poor absorption from the gastrointestinal tract. About 70-80% of an oral dose was recovered in the feces, 10-20% in the urine, and the remaining fraction was recovered in expired air, presumably as carbon dioxide; no accumulation in tissues has been observed.

---

[1] Bechtel DH. 2014. Genotoxicity Testing of Esterified Propoxylated Glycerol (EPG). Regulatory Toxicology and Pharmacology, DOI 0.1016/j.yrtph.2014.11.013

Christian BJ and Bechtel DH. 2014. 90-Day Dietary Safety Study with Esterified Propoxylated Glycerol (EPG) in Rats. Regulatory Toxicology and Pharmacology, DOI 10.1016/j.yrtph.2014.11.017

Davidson MH and 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 Eight Weeks. Regulatory Toxicology and Pharmacology, DOI 10.1016/j.yrtph.2014.11.009

Tyl RW and Bechtel DH. 2014a. One-Generation Reproduction Study of Esterified Propoxylated Glycerol (EPG) Administered in the Feed to CD® (Sprague-Dawley) Rats. Regulatory Toxicology and Pharmacology, DOI 10.1016/j.yrtph.2014.11.018

Tyl RW and Bechtel DH. 2014b. Developmental Toxicity Evaluation of Esterified Propoxylated Glycerol (EPG) Administered in the Diet to New Zealand White Rabbits. Regulatory Toxicology and Pharmacology, DOI 10.1016/j.yrtph.2014.11.014

Wedig J and Bechtel DH. 2014. 90-Day dietary toxicity study with esterified propoxylated glycerol (EPG) in Micropigs. Regulatory Toxicology and Pharmacology, DOI 10.1016/j.yrtph.2014.11.012

As a substance that passes through the gastrointestinal tract largely unchanged, ingested EPG was not expected to result in systemic toxicity. Indeed, the Expert Panel acknowledged that experimental animal studies with EPG showed no acute (single-dose), subchronic (90-day), or chronic (1-year) oral toxicity; genotoxicity; carcinogenicity (2-year); skin or eye irritation; dermal sensitization; or adverse effects on reproduction and offspring development. Animals in these studies were exposed to diets containing a constant level of 5% EPG (w/w) or adjusted to deliver up to 5 g/kg of body weight/day. However, additional consideration was given to possible undesirable effects resulting from its presence in the intestine; specifically, possible interference with the absorption of nutrients (*e.g.*, lipid-soluble vitamins) and passive oil leakage in stool, two effects previously associated with olestra consumption. Among the factors the Expert Panel considered was the moderate organic nature and solubility of EPG. EPG is not severely hydrophobic, exhibiting an octanol/water partition coefficient (Kow) value in the range of 3.2-3.4, similar to the triglyceride fats it is intended to replace. This is in striking contrast to olestra, which has a Kow in excess of 40.

The Expert Panel recognized that administration of high concentrations of EPG in the diet was associated with some effects on fat-soluble nutrients in experimental animals and humans, an effect possibly related to the presence of unabsorbed material in the gastrointestinal tract, with EPG acting as a lipid "sink" or additional "compartment" for the distribution of lipid-soluble substances. In evaluating the significance of these findings, the Expert Panel considered it reassuring that: (1) none of the animals in any of the studies exhibited clinical signs or microscopic evidence of vitamin deficiency, and there was no evidence that the vitamin levels were significantly different from those reported in the published literature for normal control animals. In long-term EPG studies, the blood and tissue levels of fat-soluble vitamins increased and stabilized over time to fall within normal ranges reported in the literature; (2) the association between EPG consumption and effects on some fat-soluble nutrients seen in an 8-week dietary study in humans was uncertain. Beta-carotene was lower, for example, but showed no apparent relationship to EPG concentration (more evident at 10 and 40 g/day than at 25 g/day), and 25-OH D_3 (vitamin D, ergocalciferol) rose unexpectedly, but only in the control (margarine only) group. The Expert Panel concluded that the projected consumer exposure (90^{th} percentile of 19.96 g/day or less) from the anticipated initial food uses would not be expected to significantly affect normal absorption of lipid-soluble nutrients. Similarly, oily stools and other gastrointestinal symptoms occasionally seen at the highest EPG dietary concentrations (up to 150 g/day) were not considered by the Expert Panel to be likely to occur at the intended intake levels nor were they viewed as toxic effects.

During deliberation, the Expert Panel examined the clinical chemistry results of multiple human range-finding tolerance studies and noted 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. Likewise, high-density lipoprotein (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. Following a more detailed review, the Expert Panel found that the occasional moderate increase in measured serum transaminase values often occurred in a transient manner, rising briefly and then returning to normal ranges. The Panel was uncertain whether this was an adaptive response but found it reassuring that 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. For these reasons the Expert Panel concluded that 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, and was therefore, not relevant to the approximate 20 g or less intake of EPG expected from the uses under consideration.

With regard to a decline in serum HDL observed in some subjects in early studies at high doses, the Panel noted that the effect was small and not reported in subsequent studies at lower doses for more extended periods. Likewise, the Panel noted that studies 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. The Panel also noted reports that when humans are placed on a low fat diet they can exhibit a transient drop in serum HDL values and concluded that this effect may account for the observed serum HDL decline in some subjects placed on very high EPG diets which greatly reduced the available fat in their diet. Based on these considerations, the Panel concluded that the occasionally lower HDL values observed in early clinical studies was not evidence of a toxic effect and was not relevant to the intake of EPG expected from the uses under consideration.

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

Expert Panel Opinion

Having considered all the relevant information about EPG, the undersigned members of the Expert Panel conclude that there is reasonable certainty that no harm will result from the use of H-EPG-05 as a fat replacer at levels up to 34.5% (w/w) in specified confectionery products and coatings [*i.e.*, 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 confectionery items, sugar-shelled confectionery pieces), snack foods (snack items, trail mix) and soft candy (molded and hollow molded confectionery pieces)], and that such use is considered GRAS based on scientific procedures.

(b) (6)

2/9/15

Fergus M. Clydesdale, Ph.D. — Date
Distinguished Professor and Director of the Food Science Policy Alliance
Department of Food Science
University of Massachusetts Amherst, MA

(b) (6)

2/12/15

Robert J. Nicolosi, Ph.D., C.N.S — Date
Professor Emeritus of Clinical Laboratory and Nutritional Sciences
Adjunct Professor, Department of Medicine
University of Massachusetts Medical School, MA

(b) (6)

2/23/15

Robert M. Russell, M.D. — Date
Professor Emeritus
Friedman School of Nutrition Science and Policy
Tufts University, MA

(b) (6)

2/3/15

John A. Thomas, Ph.D. — Date
Adjunct Professor
Department of Pharmacology & Toxicology
Indiana University School of Medicine Indianapolis, IN

(b) (6)

3/3/15

Panel Scientific Facilitator — Date
David H. Bechtel, Ph.D., DABT
Intertek Scientific & Regulatory Consultancy
Vice President, New Jersey Office
Bridgewater, NJ

GRAS DOSSIER

SUMMARY OF DATA SUPPORTING A DETERMINATION THAT THE USE OF ESTERIFIED PROPOXYLATED GLYCEROL (EPG) IN CONFECTIONARY FOODS IS GENERALLY RECOGNIZED AS SAFE (GRAS)

SUMMARY OF DATA SUPPORTING A DETERMINATION THAT THE USE OF ESTERIFIED PROPOXYLATED GLYCEROL (EPG) IN FOODS IS GENERALLY RECOGNIZED AS SAFE (GRAS)

Table of Contents

List of Appendices

List of Figures and Tables

1.0 INTRODUCTION

1.1 Declaration of Intent

In the United States (U.S.), substances added to food are exempt from the definition of "food additive" and thus from the premarket approval requirements outlined in Section 201(s) of the Federal Food, Drug, and Cosmetic Act if their use is generally recognized as safe (GRAS). For substances not already on the list of GRAS substances[1] that are not considered harmful when added to food because of their intrinsic properties and/or because sufficient information about their safety exists, a proposed rule issued in 1997[2] provides a framework for self-determination of GRAS. A determination that a substance is GRAS requires both technical evidence of safety and a basis to conclude that this technical evidence of safety is generally known and accepted within the qualified scientific community. Self-determination of GRAS may be followed by notification to the Food and Drug Administration (FDA).

Esterified propoxylated glycerols (EPG or EPGs hereafter), 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. A core version, H-EPG-05 HR/SO 9:1, was selected as representative of the initial EPG-05 forms for commercial development. Selection was made based on the preference for: 1) a version that was solid at body temperature to minimize undesirable gastrointestinal effects; 2) high content of saturated fatty acids to reduce potential caloric availability; and 3) a mean degree of propoxylation (PO-5) that would assure all glycerol hydroxyls were blocked by at least one PGU unit to prevent susceptibility to lipase digestion. EPG-05 HR/ST 45:55 is a somewhat softer version at average normal body temperature and was selectively investigated in safety studies.

Choco Finesse, LLC (hereafter Choco Finesse) previously self-affirmed through scientific procedures that the use of H-EPG-05 as a fat replacer at levels up to 34.5% (w/w) in specified confectionery products and coatings, such as 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 confectionery items, sugar-shelled confectionery pieces), snack foods (snack items, trail mix) and soft candy (molded and hollow molded confectionery pieces), is GRAS (refer to

[1] 21 CFR 182 – Substances generally recognized as safe; 21 CFR 184 – Direct food substances generally recognized as safe; 21 CFR 186 – Indirect food substances affirmed as generally recognized as safe.
[2] 62 FR 18938; April 17, 1997.

Table 4-1 for a summary of the individual proposed food uses and use-levels). According to the intake assessment, when considering intakes by all-users, male adults were determined to have the highest 90th percentile all-user intake of EPG on an absolute basis at 19.96 g/person/day. The safety and tolerability of this level of intake has been clearly demonstrated in preclinical and human studies (refer to Sections 6.0 and 7.0).

The present document summarizes the available information supporting the safety of H-EPG-05, and provides the basis for the GRAS determination.

2.0 BACKGROUND INFORMATION

2.1 History of EPG Development

EPGs were invented in the 1980s by ARCO Chemical Company as a flexible technology for replacement of triglyceride fats in food products. ARCO entered into a joint agreement with Bestfoods (CPC, International) to develop EPG application in selected food products, and a comprehensive GLP- and GCP-compliant safety testing program was developed with FDA guidance. This research program was completed in the mid-1990s. However, for financial reasons, Bestfoods withdrew from the joint venture shortly thereafter, and ARCO was unable to continue the project. Activity on the project resumed in December 2003 when the technology was assigned to a non-profit organization affiliated with Kansas State University. A new partner, Choco Finesse, LLC, has now been granted development rights and is in the process of commercializing EPGs for selected food ingredient applications.

2.2 EPG is Not Olestra

EPG is significantly different from olestra in several important properties; a brief discussion of these differences follows.

1) The EPG-05 versions proposed for confectionery use have melting points of at least 102° F and are solid at average normal body temperature. As a result, they do not exhibit the untoward and untidy gastrointestinal effects associated with olestra consumption.

2) EPGs are modified triglycerides and similar in structure and solubility properties to the fats they are intended to replace. In contrast, olestra, a hybrid molecule of sucrose esterified with eight fatty acids, is not a triglyceride in conformation and exhibits extreme hydrophobic characteristics.

3) Olestra's extreme hydrophobic nature results in it exhibiting a strong, and in great part irreversible, sequestering effect when interacting with other organic molecules, particularly fat-soluble vitamins. As such, it was necessary to vitamin-fortify experimental diets used in

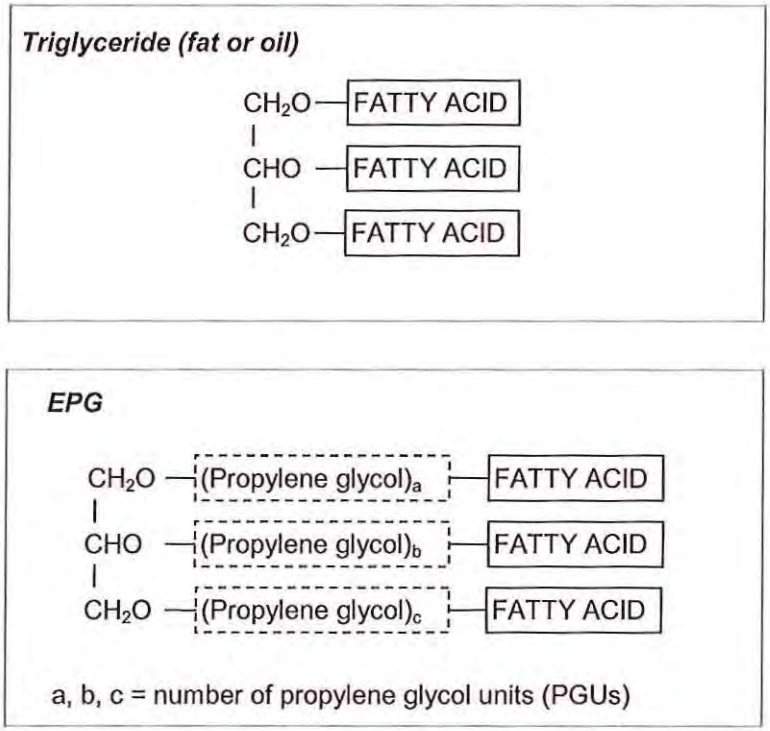
olestra animal safety studies to prevent rapid development of vitamin deficiency and possible death of the test animals. Likewise, commercial olestra used in foods for human consumption must also be vitamin-fortified. In comparison, EPG is not strongly hydrophobic and does not exhibit the strong vitamin-sequestering effect seen with olestra. Vitamin fortification of test diets was not needed during any of the EPG safety studies, including lifetime studies in rats and mice, a 1-year study in micropigs, a 3-generation study in rats and an 8-week clinical investigation. Accordingly, vitamin fortification of EPG for use in confectionery products is not expected to be required.

4) EPGs, while resistant to lipase, are susceptible to degradation by microfloral esterases in a manner similar to unmodified triglycerides. For that reason, EPG is much more readily degraded in wastewater and other environmental settings that have been reported for olestra.

2.3 Composition and Structure of EPG

EPGs are produced by a three-step process: first, fats and oils are split into glycerol and fatty acids. Then, glycerol is reacted with propylene oxide to produce glycerol with propylene glycol units (PGUs) inserted on its hydroxyl groups. Finally, the propylene glycol-substituted glycerol is reacted with fatty acids resulting in their esterification with the hydroxyl moieties of the PGUs to produce EPG. Importantly, the presence of the α-methyl group of PGU, situated adjacent to the ester linkage with the fatty acid, sterically blocks lipase activity. Figure 2-1 provides a schematic representation of the composition of EPGs compared to conventional triglycerides. It is important to note that fatty acid variations in EPG-05 would not materially affect safety considerations since the fatty acids are ordinary diet components and are in fact mostly non-bioavailable from EPG forms. Changes in fatty acids are chosen to affect physical characteristics of EPG to accommodate food technology parameters.

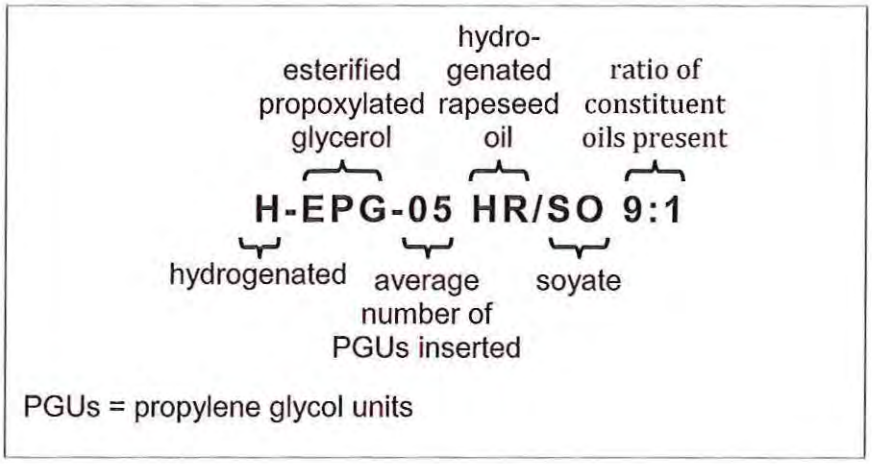
Figure 2-1 Basic structure of triglycerides *vs.* EPG



The EPG substance produced after esterification is then refined, filtered, hydrogenated (if necessary), and fortified with a commercial food-grade tocopherol mixture to improve the quality and stability of the product. This stabilizer is a mixture of α-, γ-, and δ-tocopherols added to prevent oxidation and to ensure a long shelf-life of this fat substitute without the development of undesirable off-odors and off-tastes with time.

A large variety of EPG versions can be manufactured using commercially available sources of fatty acids split from food-grade fats and oils. These versions can be made to resemble and function like vegetable oils, semi-solids like butter, or solids like lard, depending on the source of the fatty acids used and the number of PGUs inserted onto the glycerol backbone. Each form of EPG has a particular designation (Figure 2-2), where EPG is followed by a number that represent the average total PGUs per glycerol molecule, the source of the fatty acids, and for versions that use more than one source of fatty acids, the ratio. The letter "H" is used to indicate it is a hydrogenated EPG version.

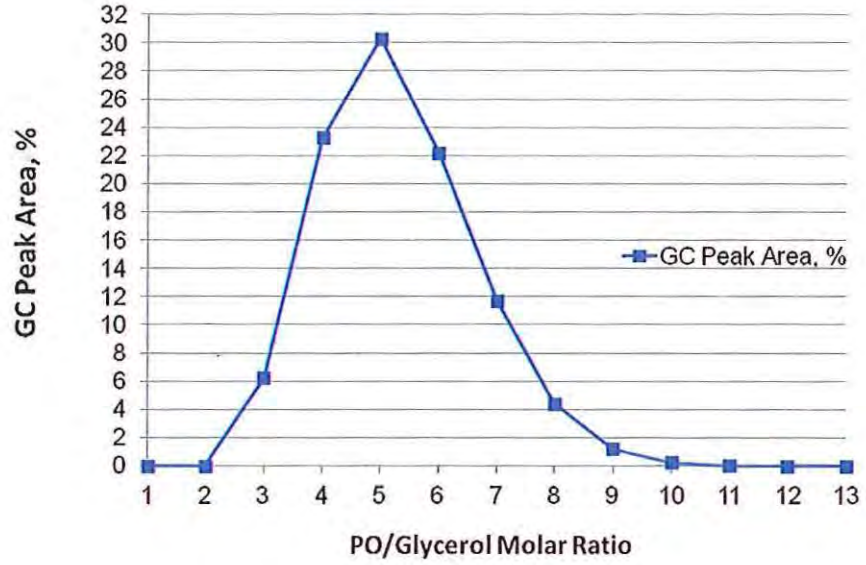
Figure 2-2 EPG Nomenclature



Because EPG formulations are actually mixtures consisting of multiple oligomers, the designations of EPG-05, EPG-08, EPG-14, *etc.* refer to the average number of PGUs in the predominant oligomer in the mixture; 5, 8, and 14, respectively, in the aforementioned formulations.

The graph in Figure 2-3 shows the oligomer distribution in EPG-05, a form of EPG that has been extensively studied. The oligomer distribution is reproducible and tightly controlled by thermodynamic factors (Cooper and Polley, 1995).

Figure 2-3 Oligomer distribution of glycerol PGU polyols in PG-05

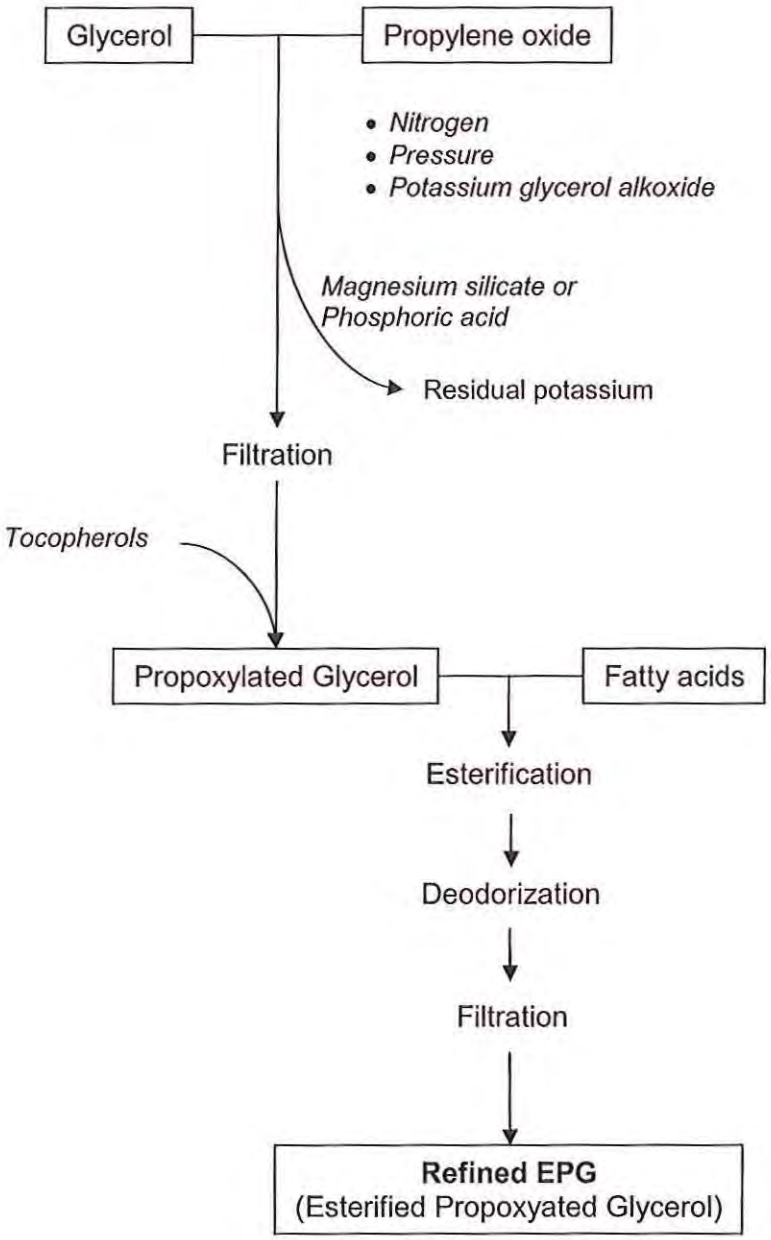


3.0 MANUFACTURING AND GRAS SUBSTANCE CHARACTERIZATION

3.1 Manufacturing Process

EPG is manufactured in compliance with current Good Manufacturing Practice (cGMP) regulations. Briefly, the production of EPG consists of two 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 standard steam stripping under vacuum (*e.g.*, during physical refining-deodorization). The manufacturing process for EPG is also depicted in Figure 3-1.

Figure 3-1 Manufacturing process of EPG



3.2 Food-Grade Specifications and Chemical Analysis

The master specifications for H-EPG-05 for confectionary use are summarized in Table 3-1.

Table 3-1 Master specifications for H-EPG-05 for confectionary use

Attribute	Specification	Range	Method
Appearance	Solid, white < 30°C	Sl. off white	Visual (internal procedure)
Melting point	Mettler Dropping Point	38-43° C	AOCS Tr Ia-64
Taste	Sensory	Flavorless	Taste (internal procedure)
Texture	Sensory	Waxy	Taste (internal procedure)
Free Fatty Acid, %	% FFA as oleic	<0.5	AOCS Ca 5a-40
Peroxide Value	Meq.peroxide/1000g	0-1	AOCS Cd 8b-90
Anisidine Value	p-anisidine	2-8	AOCS Cd18-90
Phosphorus, ppm		< 3	AOCS Ca 12a-02
Trace Metals, ppm	Calcium	<0.5	AOCS Ca17-1
	Copper	<0.01	
	Iron	<0.1	
	Magnesium	<0.5	
	Potassium	<2	ASTM D4668
	Sodium	<1	AOCS Ca17-1
Hydroxyl Value	mg KOH/g	<5	ASTM D4274
Iodine Value	mg Iodine/g	0-15	AOCS Cd 1-25
Tocopherols, ppm	Alpha	120-230	AOCS Ce 8-89
	Beta	5-30	
	Gamma	500-800	
	Delta	150-250	
	Total	900-1300	
Fatty Acid Composition (% as oleic)	Palmitic, C16:0	1.5-12	AOCS Ce 1-62
	Stearic, C18:0	47-70	
	Arachidic (C20:0) + Behenic (C22:0)	25-40	
	Oleic, C18:1	0-10	
	Linoleic, C18:2	0-6	
	Linolenic, C18:3	0-1	
	Erucic, C22:1*	0-2	
Total unsaturates		0-9	
Trans fat, %	Total "trans"	<0.25	AOCS Ce 1h-05
Solid Fat Content	@ 10°C	88-98	Cd16b-IUPAC
	@ 20°C	81-97	
	@ 25°C	70-96	
	@ 30°C	60-91	
	@ 35°C	35-62	
	@ 40°C	<1	
Smoke Point	Open Cup, °C	>225	AOCS Cc 9a-48
Flash Point	Open Cup, °C	>265	
Microbiological Screening	**Specification**	**Range**	**Method**
Aerobic Plate Count	Petrifilm	<10/g	AOAC 990.12
Coliform	Petrifilm	<10/g	AOAC 991.14
E. coli	Petrifilm	<10/g	
Salmonella	ELFA	Negative/25g	AOAC 2004.03
Yeast		<10/g	FDA-BAM, 7th ed.
Mold		<10/g	

* Percent of erucic in H-EPG-05 HR/SO 9:1 is consistent with the content of erucic acid in other vegetable oils such as canola.

Variations of fatty acid components, within the aforementioned specifications will yield EPGs for specific confectionary applications as discussed below.

The current base version of EPG-05 for confectionary applications utilizes fully hydrogenated fatty acid fractions from high erucic acid rapeseed oil (HEAR) providing concentrated arachidic and behenic acids. The second component is concentrated stearic acid which can be derived from fully hydrogenated soybean oil (SBO), canola oil (CAN), or separated from arachidic + behenic fraction during distillation of HEAR fatty acids.

The composition of the confectionary EPG-05 can be expressed as follows:

EPG-05 A+B/S 43:57 where A+B = Arachidic + Behenic, S = Stearic

Using the enriched fractions of specific fatty acids permits to consistently achieve EPG-05 with desirable properties, particularly its melting point and solid fat contents (SFCs). These are critical properties to control in order to achieve eutectic blends comparable in performance to cocoa butter and fractionated palm kernel oil in confectionary products.

The above base version of EPG-05 works well in compound chocolate when slightly "crumbly" texture is desirable. However, for applications which require a "smooth" texture, incorporation of low levels of unsaturated fatty in EPG-05 is necessary.

The composition of confectionary EPG-05 with unsaturated fatty acids (oleic + linoleic + erucic) can be expressed as follows:

EPG-05 A+B/S/U 46:45:9 where A+B = Arachidic + Behenic, S= Stearic, U = total Unsaturates (oleic + linoleic + erucic)

Examples of product compositions within the master specifications are provided in Table 3-2. The similarity among original EPG-05 core compound and current confectionary EPG-05 versions is evident when comparing their melting points and fatty acid compositions.

Table 3-2 Comparison of original core compound and current confectionary versions of EPG-05

Attribute	H-EPG-05 HR/SO 9:1[a]	EPG-05 A+B/S 43:57[b]	EPG-05 A+B/S/U 46:45:9[c]
Mettler Drop Point, $^0C/^0F$	41.6/106.9	40.2/104.3	39.2/102.6
FAC, %			
Palmitic, C16:0	4.8	2.0	2.0
Stearic, C18:0	39.8	68.2	49.4
Oleic, C18:1	4.2	0.7	6.5
Linoleic, C18:2	0.1	0.5	0.5
Erucic, C22:1	0	0	2.0
Total unsaturates Oleic+Linoleic+Erucic	4.3	1.2	9.0
Arachidic, C20:0	9.1	13.6	14.9
Behenic, C22:0	38.7	14.8	24.6
Arachidic + Behenic	47.8	28.4	39.5
Other	3.3	0.2	0.1

[a]**H-EPG-05 HR/SO 9:1,** where:

- **H** denotes hydrogenated EPG esterified with fatty acids derived from rapeseed and soybean oils
- **05** is the number of propylene glycol units per molecule of EPG
- **SO** indicates fatty acids derived from soybean oil
- **9:1** the proportion of rapeseed oil fatty acid to soybean oil fatty acid

[b]**EPG-05 A+B/S 47:53,** where:

- **05** is the number of propylene glycol units per molecule of EPG
- **A+B** is a sum of Arachidic + Behenic acids
- **S** is the content of Stearic acid
- **43:57** is the proportion of A+B:S

[c]**EPG-05 A+B/S/U 46:45:9**, where:

- **05** is the number of propylene glycol units per molecule of EPG
- **A+B** is a sum of Arachidic + Behenic acids
- **S** is the content of Stearic acid
- **U** is the total unsaturates (Oleic + Linoleic + Erucic acid)
- **46:45:9** is the proportion of A+B:S:U

Analyses of non-consecutive lots of H-EPG-05 for confectionary use against master specifications are presented in Table 3-3.

Table 3-3 Batch analysis data for esterified propoxylated glycerols (EPG-05 A+B/S 43:57) for confectionary use

Attribute	Specification	Lot Numbers				
		Batch 88b 6/11/14	Batch 89 6/11/14	Batch 91 6/19/14	Batch 95 8/05/14	Batch 96 8/05/14
Appearance	White, solid	White, solid	White, solid	White, solid	White, solid	White, solid
Mettler Dropping Point,°C	38-43	40.4	40.1	40.3	40.6	40.0
Taste	Flavorless	Flavorless	Flavorless	Flavorless	Flavorless	Flavorless
Texture	Waxy	Waxy	Waxy	Waxy	Waxy	Waxy
Free Fatty Acid, %	<0.50	0.25	0.10	0.43	0.25	0.28
Peroxide Value	0-1	0.2	0.4	0.5	0.2	0.1
Anisidine Value	2-8	4.3	4.5	6.0	4.1	1.2
Trace Metals						
Iron, ppm	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Copper, ppm	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Calcium, ppm	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Magnesium, ppm	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Hydroxyl Value	<5.0	3.3	2.1	0.8	1.8	1.7
Iodine Value	0 -15	2.3	3.0	3.2	2.7	2.9
Tocopherols						
Alpha, ppm	120-230	-	-	181	225	228
Beta, ppm	5-30	-	-	21	26	26
Gamma, ppm	500-800	-	-	540	761	749
Delta, ppm	150-250	-	-	179	245	244
Total, ppm	900-1300	-	-	921	1257	1247
Fatty Acid Composition,%						
Palmitic, C16:0	1.5-12	2.2	1.9	1.9	2.0	2.0
Stearic, C18:0	47-70	66.9	67.2	68.7	68.9	69.4
Arachidic, C20:0	6-20	13.2	13.8	13.8	13.8	13.6
Behenic, C22:0	14-30	14.6	15.7	14.9	14.5	14.3
Arachidic + Behenic	25-40	27.8	29.5	28.7	28.3	27.9
Oleic, C18:1	0-10	1.8	0.7	0.2	0.4	0.3
Linoleic, C18:2	0-6	1.0	0.5	0.3	0.3	0.2
Linolenic, C18:3	0-1	0.1	0	0	0	0
Erucic, C22:1	0	0	0	0	0	0
Total unsaturates	0.5-9	2.9	1.2	0.5	0.7	0.5
Trans fat, %	<0.25	0.2	0.1	0.1	0.1	0.1

Table 3-3 Batch analysis data for esterified propoxylated glycerols (EPG-05 A+B/S 43:57) for confectionary use (cont'd)

Attribute	Specification	Lot Numbers				
		Batch 88b 6/11/14	Batch 89 6/11/14	Batch 91 6/19/14	Batch 95 8/05/14	Batch 96 8/05/14
Solid Fat Content						
@10°C	88-98	97.2	97.2	97.1	97.0	96.9
@20°C	81-97	96.0	96.1	95.8	95.7	95.6
@25°C	70-96	94.8	94.8	94.5	94.4	94.3
@30°C	60-91	90.3	89.9	89.9	89.7	89.4
@35°C	35-62	59.4	58.6	59.0	58.4	56.1
@40°C	<1	0.3	0.2	0.1	0	0
Smoke Point, °C	>225	228	230	238	248	260
Flash Point, °C	>265	270	274	274	276	272
Microbiological Screening						
Aerobic Plate Count	<10/g	<10/g	<10/g	<10/g	<10/g	<10/g
Coliform	<10/g	<10/g	<10/g	<10/g	<10/g	<10/g
E. coli	<10/g	<10/g	<10/g	<10/g	<10/g	<10/g
Salmonella	Negative/ 25 g	Negative/ 25 g	Negative/ 25 g	Negative/ 25 g	Negative/ 25 g	Negative/ 25 g
Yeast	<10/g	<10/g	<10/g	<10/g	<10/g	<10/g
Mold	<10/g	<10/g	<10/g	<10/g	<10/g	<10/g

3.3 Analysis of EPG Versions for Acid Resistance and Lipase Resistance (Study E-037)

An *in vitro* hydrochloric acid and pancreatic lipase resistance study was performed on various EPG versions namely, EPG-05 oleate, EPG-05 stearate, EPG-08 oleate, EPG-08 stearate, EPG-14 oleate, and EPG-08 soyate, to determine their digestibility. The *in vitro* assays were set up to simulate the physiological hydrolytic conditions of the digestive process found in the stomach and small intestine in which hydrochloric acid and pancreatic lipase participate respectively. Either 0.1 N hydrochloric acid or porcine pancreatic lipase was used for each three hour incubation (at 37° C). An equal volume of corn oil served as a positive control for each incubation. The results of this study indicate that the EPG versions tested were resistant, under physiological conditions, to the action of pancreatic lipase, the primary enzyme responsible for fat digestion in the small intestine. EPG-05 oleate was somewhat less resistant to acid treatment then the other EPG versions. The remainder of the EPG versions tested were equally resistant to the acidified treatment.

3.4 Bioavailability Calorie Study with EPG-05 A+B/S 43:57 in Rats (Covance Study Number 8305133)

This study investigated caloric characteristics of EPG when administered daily in the diet to rats for at least 4 weeks following a modified method of that described by Fournier *et al.*, 1987.

Seventy male Crl:CD(SD) rats were assigned to seven groups (10 animals/group); at initiation of dosing, animals were 4 weeks old, and body weights ranged from 58 to 77 g. Each group received a dose preparation of control article (Crisco® corn oil) or EPG added to Certified Rodent Diet AIN-76A basal diet at 0, 0.4, 0.6, or 0.8 g/day as follows. The amount of diet (7.0 g) provided daily to each rat represented approximately 50% of the daily *ad libitum* consumption observed in young rats of this age/size. This assured that calories derived from the added oil or EPG would be reflected in increased body weight values.

Group/Material[a]	Diet Concentration (%)	Quantity of Diet Offered Per Animal per Day (g)[b]			No. of Animals (Males)
		Total	AIN-76A	Added Oil	
1 (Carrier)	0	7.0	7.0	0	10
2 (Control) Corn Oil	5.7	7.4	7.0	0.42	10
3 (Control) Corn Oil	8.6	7.6	7.0	0.65	10
4 (Control) Corn Oil	11.4	7.8	7.0	0.89	10
5 Test Article	5.7	7.4	7.0	0.42	10
6 Test Article	8.6	7.6	7.0	0.65	10
7 Test Article	11.4	7.8	7.0	0.89	10

a Group 1 received carrier (Certified Rodent Diet AIN-76A; basal diet) only.
b The quantity of basal diet offered per animal per day was an approximation of average consumption per animal and used for batch size calculations purposes only.

Animals were housed individually in stainless steel cages. Assessment of caloric determination was based on body weights, body weight gain, and food consumption as presented in Table 3.4.

All animals survived to the scheduled sacrifice.

Body weights were greater in a dose-dependent manner for all groups given corn oil compared with animals given basal diet alone. Mean body weights for animals fed EPG tended to be greater than those of controls but only transiently and only in animals given the highest dietary level.

The results indicated a mean caloric density value equal to approximately 9.5 kcal/g for corn oil. This is very close to but slightly higher than the standard accepted value of 9.0 kcal/g for corn oil and provides assurance that the assay results were consistent.

The mean calculated caloric density of EPG was approximately 0.7 kcal/g and ranged from 0.3 to 0.9 kcal/g among the three dose groups. While the value of 0.7 kcal/g will be used to characterize the caloric density of EPG-05 variants for confectionery use, the true caloric value in humans is almost assuredly less. The lowest calculated caloric value occurred among rats in the lowest EPG dose group. The EPG dose rate in this group was approximately 5 g EPG/kg body weight or approximately 10 fold greater than the highest expected consumer intake rate. It seems reasonable to view this lower caloric density value as probably more closely reflecting the value for human consumers. Even this value may be considered to be a maximum estimate.

Since fully propoxylated EPG is not susceptible to lipase or other usual lipid digestion processes, caloric value must derive from absorption and utilization of degradation products produced in the lower gut through the action of microfloral esterases. Rats possess a large microflora-rich cecum where such esterase degradation can occur; humans do not. Accordingly, it is again reasonable to conclude that the values derived in this rat bioassay represent the greatest values likely to occur in humans.

Table 3-4 Summary of mean caloric calculations

Group/Material	Food Consumption (g) Dosing Days 1-29	Body Weight Change (g) Dosing Days 1-29	Dietary Caloric Intake (kcal)	Caloric Density (kcal/g)	Relative Caloric Availability (%)
1 (Carrier)*	190.0 ± 3.2	39.1 ± 5.5	675.9 ± 31.9	NC	NC
2 (Control) Corn Oil	201.1 ± 1.4	57.3 ± 8.2	781.5 ± 47.8	9.6 ± 4.6	106.8 ± 50.9
3 (Control) Corn Oil	207.0 ± 2.8	65.1 ± 6.5	826.7 ± 37.5	9.1 ± 2.2	100.7 ± 25.0
4 (Control) Corn Oil	213.9 ± 1.9	76.9 ± 3.3	736.9 ± 19.3	9.7 ± 3.4	107.3 ± 38.2
5 Test Article	201.8 ± 3.2	40.3 ± 5.8	682.9 ± 33.4	0.3 ± 3.2	3.4 ± 35.5
6 Test Article	206.1 ± 2.7	41.6 ± 7.7	690.4 ± 44.5	0.9 ± 2.8	10.1 ± 31.1
7 Test Article	213.0 ± 1.7	42.9 ± 4.3	697.9 ± 25.1	0.8 ± 1.1	8.9 ± 11.8

* Carrier = Certified Rodent Diet AIN-76A; basal diet

4.0 PROPOSED USE AND ANTICIPATED CONSUMER INTAKE

4.1 Proposed Uses

For the purpose of this GRAS notification, Choco Finesse's EPG, manufactured in accordance with GMP as specified in 21 CFR 110, may be used at levels up to 34.5% (w/w) in confectionery coatings in the following categories of foods as defined in *21 CFR §170.3(n)*: 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 confectionery items, sugar-shelled confectionery pieces), snack foods (snack items, trail mix) and soft candy (molded and hollow molded confectionery pieces). The coating applications, classified as *Drizzle*, *Enrobed*, *Inclusion*, and *Solid*, and the individual proposed food uses and use levels, are summarized in Table 4-1. This information was used to estimate consumer intakes, which are discussed further below. The full intake assessment report is attached as Appendix 1 of the current report.

Table 4-1 Summary of the Individual Proposed Food Uses and Use-Levels for EPG Per Coating Application in the U.S. (2009-2010 NHANES Data)				
Coating Application	**Food Category**	**Proposed Food Use**	**Coating Inclusion (%)**	**Use level in coating (%)**
Drizzle	Baked Goods and Baking Mixes	Biscuit Items (cookies, sweet crackers)	5-30	34.5
	Frozen dairy desserts and mixes	Ice cream novelty (Ice cream bars, ice-cream sandwiches)	5-10	34.5
	Grain Products and Pasta	Snack & meal replacement bars (cereal and meal replacement bars)	3-10	34.5
Enrobed	Baked Goods and Baking Mixes	Biscuit Items (cookies, sweet crackers)	50-70	34.5
		Bakery items (buns, muffins, cakes, pastries, doughnuts, waffles)	5-30	34.5
	Frozen dairy desserts and mixes	Ice cream novelty (Ice cream bars, ice-cream sandwiches)	20-40	34.5
	Grain Products and Pasta	Snack & meal replacement bars (cereal and meal replacement bars)	20-30	34.5
	Hard candy and cough drops	Panned confectionery items (Coated popcorn, coated candy)	20-70	34.5
	Processed fruits and fruit juices	Panned fruit pieces (Dried fruit pieces)	40-60	34.5
	Snack foods	Snack items (Pretzels)	50-70	34.5
Inclusion	Baked Goods and Baking Mixes	Bakery items (buns, muffins, cakes, pastries, doughnuts, waffles)	15-35	30
		Ice cream inclusion (Ice cream inclusions and cones)	5-10	30
	Grain Products and Pasta	Snack & meal replacement bars (cereal and meal replacement bars)	10-25	30
	Snack foods	Trail mix (Nut/dried fruit mixes)	20-30	30
Solid	Hard candy and cough drops	Sugar shelled confectionery pieces (Sugar coated confectionery)	65-70	30
	Soft candy	Molded confectionery pieces	100	34.5
		Hollow molded confectionery pieces	100	34.5

To examine worst-case EPG exposure, the intake assessment considered the total daily intake of EPG from all applications, based on the maximum range of coating inclusion and the maximum EPG level per food category, as listed in Table 4-2. Food codes representative of each of these proposed foods were chosen from the NHANES 2009-2010 (CDC, 2011; USDA, 2012). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (CFR, 2014). Where required, product-specific adjustment factors were developed based on data provided in the standard recipe file for the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998 survey (USDA, 2000). All food codes included in the current intake assessment are listed in the full intake assessment report

(Appendix 1). A given food code may not be associated with both surveys; as with each new survey the food code list has been updated to reflect the availability of new foods and the discontinuation of certain obsolete codes.

Table 4-2 Summary of the Individual Proposed Food Uses and Use-Levels for EPG in the U.S. (2009-2010 NHANES Data)

Coating Application	Food Category	Proposed Food Use	Coating Inclusion (%)[a]	Use level in coating (%)[a]
All applications together	Baked Goods and Baking Mixes	Biscuit Items (cookies, sweet crackers)	5-70	34.5
		Bakery items (buns, muffins, cakes, pastries, doughnuts, waffles)	5-35	30-34.5
		Ice cream inclusion (Ice cream inclusions and cones)	5-10	30
	Frozen dairy desserts and mixes	Ice cream novelty (Ice cream bars, ice-cream sandwiches)	5-40	34.5
	Grain Products and Pasta	Snack & meal replacement bars (cereal and meal replacement bars)	3-30	30-34.5
	Hard candy and cough drops	Panned confectionery items (Coated popcorn, coated candy)	20-70	34.5
		Sugar shelled confectionery pieces (Sugar coated confectionery)	65-70	30
	Processed fruits and fruit juices	Panned fruit pieces (Dried fruit pieces)	40-60	34.5
	Snack foods	Snack items (Pretzels)	50-70	34.5
		Trail mix (Nut/dried fruit mixes)	20-30	30
	Soft candy	Molded confectionery pieces[b]	100	34.5
		Hollow molded confectionery pieces[b]	100	34.5

[a] The maximum proposed coating inclusion and EPG level per proposed food use was used in the intake assessments

[b] No intake for these products were reported in the NHANES 2009-2010, therefore these food uses were not included in the intake assessments.

4.2 Anticipated Consumer Intake

Estimates for the total daily intakes of EPG by various U.S. population groups from all proposed food applications together are provided in Tables 4-3 and 4-4. Table 4-3 summarizes the estimated total intake of EPG on a grams per person per day basis (g/person/day), and Table 4-4 presents these data on a per kilogram body weight per day basis (mg/kg bw/day). All-person intake refers to the estimated intake of EPG averaged over *all* individuals surveyed, regardless of whether they potentially consumed food products containing EPG, and therefore includes individuals with "zero" intakes (*i.e.*, those reporting no intake of foods like those for which EPG is intended during the 2 survey days). All-user intake refers to the estimated intake of EPG by individuals that reported consuming food products like those for which EPG is intended.

Individuals were considered *users* if they consumed 1 or more food products containing EPG on either Day 1 or Day 2 of the survey.

A total of 66.8% of participants were identified as users (*i.e.*, consumers) of foods intended to contain coatings with EPG (range of 56.9-79.9%). Estimates based on all-users revealed mean and 90th percentile EPG intakes of 8.38 g/person/day (158.3 mg/kg bw/day) and 17.57 g/person/day (352.7 mg/kg bw/day), respectively, for the total U.S. population. On an individual group basis, male adults had the greatest estimated mean and 90th percentile all-user intakes on an absolute basis (9.80 and 19.96 g/person/day, respectively). As might be expected, EPG intake estimates on a per kg body weight basis were greatest among younger (*i.e.*, smaller) individuals. Specifically, infants had the greatest projected intakes per body weight (464.7 and 960 mg/kg bw/day for the mean and 90th percentile, respectively). However, infants are not expected to be significant consumers of EPG-containing foods and would therefore be expected to have far smaller exposures to EPG (from only occasional consumption of EPG-containing foods) than the present (worst-case) intake assessment would indicate. None of the EPG-containing products would be targeted or marketed for consumption by infants; their exposure is expected to results from collateral ingestion of items being eaten by older persons.

Total population values are included for completeness. All-person mean and 90th percentile intakes of EPG from consumption of the proposed foods from all applications were estimated to be 5.60 g/person/day (105.8 mg/kg bw/day) and 14.85 g/person/day (276.6 mg/kg bw/day), respectively, for the total U.S. population. Within the individual population groups, children were determined to have the greatest estimated mean intake of EPG on an absolute basis, at 6.91 g/person/day, while male adults had the highest 90th percentile intake at 17.25 g/person/day.

Table 4-3 Summary of the Estimated Daily Intake of EPG from Proposed Food-Uses based on All Applications Together in the U.S. by Population Group (2009-2010 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants*	0 to 2	3.23	9.89	56.9	421	5.69	11.64
Children	3-11	6.91	16.26	79.9	1104	8.65	17.38
Female Teenagers	12 to 19	5.60	14.12	68.0	349	8.24	16.45
Male Teenagers	12 to 19	5.90	15.54	63.7	355	9.26	17.91
Female Adults	≥ 20	4.98	13.04	68.4	1733	7.29	15.54
Male Adults	≥ 20	6.10	17.25	62.2	1459	9.80	19.96
Total Population	All Ages	5.60	14.85	66.8	5421	8.38	17.57

*Infants are not expected to be significant consumers of EPG-containing foods, but were included in the intake assessment for completeness. No EPG-containing products will be targeted or marketed for consumption by infants.

Table 4-4 Summary of the Estimated Daily Per Kilogram Body Weight Intake of EPG from Proposed Food-Uses based on All Applications in the U.S. by Population Group (2009-2010 NHANES Data)							
Population Group	Age Group (Years)	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants*	0 to 2	264.3	766.1	56.9	421	464.7	960.0
Children	3-11	263.8	633.1	79.9	1104	330.2	691.3
Female Teenagers	12 to 19	99.2	259.0	68.0	349	145.8	303.2
Male Teenagers	12 to 19	95.8	248.8	63.7	355	150.4	310.9
Female Adults	≥ 20	71.1	191.4	68.4	1733	103.9	219.5
Male Adults	≥ 20	71.4	196.7	62.2	1459	114.7	242.3
Total Population	All Ages	105.8	276.6	66.8	5421	158.3	352.7

*Infants are not expected to be significant consumers of EPG-containing foods, but were included in the intake assessment for completeness. No EPG-containing products will be targeted or marketed for consumption by infants.

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 of Appendix 1. The total U.S. population was identified as being significant consumers of bakery items (20.9 to 36.4% users) and biscuit items (33.3 to 49.9% users).

In terms of contribution to total mean intake of EPG, bakery items (contributed 23.4 to 37.6% to total mean intakes) and biscuit items (contributed 40.1 to 49.1% to total mean intakes) were the 2 main sources of intake across all population groups on both an absolute and on a mg/kg body weight basis. Ice-cream inclusion and Trail mix each contributed <2% to total mean EPG intakes across all population groups (see Tables A-1 to A-7 and/or B-1 to B-7 of Appendix 1 for further details).

5.0 PHARMACOKINETICS

5.1 Overview of EPG Absorption, Distribution, Metabolism and Excretion

The pharmacokinetics of two 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 5.2.1), and a second in which it was ^{14}C-radiolabeled on the carboxyl carbon of the fatty acid portion (Section 5.2.2). 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, one study was conducted with H-EPG-14 oleate radiolabeled on the C_1-carbon of the propylene glycol units (Section 5.2.3).

In each study, five rats/sex/group were administered a single oral dose of 1.0 g/kg bw or 3.0 g/kg bw by gavage. A third dose group was given 1.0 g/kg bw of the radiolabeled version after two weeks of daily EPG administration of the non-labeled version at the same dose. In addition, to simulate the worse 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 one week after EPG administration when, essentially, there was complete recovery of the dose administered.

The results of these studies indicate that the two 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 two 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 two 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 5.2.

5.2 ADME Studies

5.2.1 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 five 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 four 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 5-1.

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* 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[3] 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.

---

[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 5-1 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[a]	CO_2[b]	Feces	Urine	Volatiles	Carcass	Tissues	Total	Cage wash[a]	CO_2[b]	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)	1000	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)	1000	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)	3000	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

[a]Includes cage wash/wipe with 1% trisodium phosphate and hexane.
[b]Ethoxyethanol:ethanolamine trap; includes backup.

5.2.2 Metabolism and Disposition of EPG-08 [^{14}C] – 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 intravenous (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[4] were collected and analyzed for total radioactivity. Mean recovery of radioactivity in this study is summarized in Table 5-2.

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

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

by liquid scintillation counting (LSC) 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.

Table 5-2 Mean recovery (%) of radioactivity from EPG-08 ^{14}C-oleate in rats (5/group) after 7 days

Route	Dosage (mg/kg bw)	Males								Females							
		Cage wash[a]	CO_2[b]	Feces	Urine	Volatiles	Carcass	Tissues	Total	Cage wash[a]	CO_2[b]	Feces	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)	1000	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)	1000	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)	3000	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

[a]Includes cage wash/wipe with 1% trisodium phosphate and hexane.
[b]Ethoxyethanol:ethanolamine trap; includes backup.

5.2.3 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 five 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 intravenous 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[5] and blood were collected and analyzed for total radioactivity. Mean recovery of radioactivity in this study is summarized in Table 5-3.

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%.

---

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

Table 5-3 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[a]	CO_2[b]	Feces	Urine	Volatiles	Carcass	Tissues	Total	Cage wash[a]	CO_2[b]	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)	1000	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)	1000	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)	3000	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

[a]Includes cage wash/wipe with 1% trisodium phosphate and hexane.
[b]Ethoxyethanol:ethanolamine trap; includes backup.

6.0 PRECLINICAL SAFETY

Preclinical studies were conducted with H-EPG-05 HR/SO 9:1 unless otherwise stated. EPG-05 HR/ST 45:55 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.

6.1 Overview of Preclinical Safety

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 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. Refer to Section 8.2 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 (Section 6.8); (ii) 90-Day Dietary Safety Study with Esterified Propoxylated Glycerol (EPG) in Rats (Section 6.2.1); (iii) 90-Day Safety Study of Esterified Propoxylated Glycerol (EPG) Administered in the Feed to Yucatan Micro-Pigs® (SUS SCROFA) (Section 6.2.4); (iv) One Generation Reproduction Study of Esterified Propoxylated Glycerol (EPG) Administered in the Feed to CD® (Sprague-Dawley) Rats (Section 6.6.1); 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 (Section 6.3).

6.1.1 Determination of the Homogeneity and Stability of Test Diets for Dietary Safety Studies with Esterified Propoxylated Glycerol (EPG) in Rodents E-057 (HWI 6226-124; E-057)

This study was conducted in order to establish homogeneity and stability of the prepared test diets for the following studies: (i)"Combined Chronic Dietary Safety Study and Carcinogenicity Study with Esterified Propoxylated Glycerol (EPG) in Rats" (HWI 6226-120); (ii) "Combined Chronic Dietary Safety Study and Carcinogenicity Study with Esterified Propoxylated Glycerol (EPG) in Mice" (HWI6226-121); (iii) "90-Day Dietary Safety Study with Esterified Propoxylated Glycerol (EPG) in Rats" (HWI 6226-122); and (iv) "90-Day Dietary Safety Study with Esterified Propoxylated Glycerol (EPG) in Mice" (HWI 6226-123). These studies will be referred to as the dietary EPG safety studies.

Stability of EPG and α-tocopherol was determined from five sets of samples of test diet (a set of samples consisted of two approximately 100 g samples). One set of samples was analyzed on the day after diet preparation; the second set was stored 5 days refrigerated (5° C ± 3°), then 9 days at room temperature and then an additional 7 days refrigerated (a total of 12 days refrigerated and 9 days at room temperature); the third set was stored for 3 weeks at room temperature; the fourth set was stored for 8 weeks refrigerated and then 11 days at room temperature; and the fifth set was stored for 7 weeks in a freezer set to maintain -20° C ± 10°. When the storage periods for each set of samples was completed, one sample from each set was analyzed for α-tocopherol content, and the other sample from each set was analyzed for EPG content. To evaluate stability of each test diet, analytical results of samples from the four types of storage conditions were compared with results of analyses done on the day of diet preparation.

Results of the analysis of stability samples for α-tocopherol and EPG content are shown in Table 6-1.

Table 6-1 Summary of results of stability analyses

Storage Conditions	α-Tocopherol (% of initial levels)	EPG Concentration (%, mean of replicate analyses)
Refrigerated for 12 days & 9 days at room temperature	94.5 – 102	98.8 – 99.6
Room temperature for 3 weeks	89.0 – 94.3	95.8 – 96.4
Frozen for 7 weeks	103 -106	97.2 – 98.7
Refrigerated for 8 weeks & 11 days at room temperature	81.6 – 90.1	93.6 – 95.9

The data from this study demonstrated that the mixing procedures used in the dietary EPG safety studies produced homogeneous diet preparations and that the diet preparations were stable under conditions used in the dietary EPG safety studies.

6.2 Subchronic Toxicity Studies with Esterified Propoxylated Glycerol (EPG)

6.2.1 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 five 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 bw/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 bw/day in the beginning of the study and declining to approximately 2 g/kg bw/day) were considered to represent no-observable-adverse-effect levels (NOAEL).

6.2.2 90-Day Dietary Safety Study with Esterified Propoxylated Glycerol (EPG) in Mice (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 five 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 bw/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.

6.2.3 Thirteen Week Safety Study of EPG Administered in the Feed to Beagle Dogs (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:

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

* 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 37.8g, 0.494 to 3.25g 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 activated partial thromboplastin time (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.

6.2.4 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 bw/day). The study design is also summarized in Table 6-2.

Table 6-2 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

* 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 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.

6.3 Subchronic Toxicity Study with EPG-05 HR/ST 45:55

Note that the EPG version tested in this study is somewhat softer than the "core" version tested in other studies, and softer than the version for confectionary use.

6.3.1 90-Day Dietary Toxicity Study of EPG-05 HR/ST (45:55) in Rats (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 bw/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.

6.4 Chronic Toxicity Studies with Esterified Propoxylated Glycerol (EPG)

6.4.1 Combined Chronic Dietary Safety Study and Carcinogenicity Study with Esterified Propoxylated Glycerol (EPG) in Rats (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 five groups (140 animals/sex/group) and administered EPG at concentration levels of 0, 0.5, 1.0, or 2.0 g/kg bw/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 four 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.

6.4.2 Combined Chronic Dietary Safety Study and Carcinogenicity Study with Esterified Propoxylated Glycerol (EPG) in Mice (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 five groups (150 animals/sex/group) and administered EPG at concentration levels of 0, 0.5, 1.0, or 2.0 g/kg bw/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 a 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 Chronic Toxicity Studies with H-EPG-05 HR/SO (9:1)

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

Forty purebred beagle dogs (approximately 5-6 months of age) were fed EPG at levels of 0, 1, 2, and 3 g/kg bw/day in Modified Teklad Basal Mix for Dog Diet #7058 supplemented with 6% (w/w) corn oil (5/sex/group), for one 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 bw/day group, statistically significant decreases in fecal calcium in the 3 g/kg bw/day group, and statistically significant decreases in serum phosphorus in the 3 g/kg bw/day females at 3 and 6 months, and in the 1 g/kg bw/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 bw/day in males, and 2 and 3 g/kg bw/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 bw/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.

6.5.2 A One-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) (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 one 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.

6.6 Reproductive and Developmental Toxicity

6.6.1 One-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 two breeding cycles for F0 parental animals, and up to postnatal day 91 for F1a and F1b offspring. Results from this study aided in the design of a subsequent three-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) 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 two and seven 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 three-generation study with two litters per generation utilize fixed dietary percentages with the highest concentration 5.0% EPG, and endpoints examined not include behavioral, immunologic, or coagulation assessments.

6.6.2 Three 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 three generations of 240 Crl:CD® (SD)Br rats (approximately 6 weeks old; mean male weights 182.0-184.6 g and mean female weights 151.8-154.6 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 6-3.

Table 6-3 Summary of study design for three-generation reproduction study with H-EPG-05 HR/SO (9:1)

Groups	No. of Animals	Animal Fate	Endpoints Assessed
F0, F1a parental animals[6]	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 one 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

[6] The parental generation designated as F0, F1a, 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 two per cage (one male:one 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 postnatal day (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 three generations resulted in no indications of systemic, reproductive, developmental or postnatal toxicity.

6.6.3 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 d 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.

6.7 Irritation and Sensitization Studies

6.7.1 Primary Skin Irritation Study in Rabbits, Primary Eye Irritation Study in Rabbits (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).

For the primary skin irritation study in rabbits, the test material, a clear light yellow liquid, was applied undiluted to a one-inch by one-inch square surgical gauze patch. The patch was then applied to an intact skin area and an abraded skin area on six 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 six 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 one and two 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 0-110. Results indicate that the eyes of none of the six 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.

6.7.2 Guinea Pig Maximization Test (Magnusson and Kligman Method) (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 two intradermal injections of 50% v/v Freund's Complete Adjuvant (FCA) in distilled water and two 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 twenty 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.

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

6.8.1 Ames Assays

6.8.1.1 Study to determine the ability of H-EPG-05 HR/SO (9:1) to Induce Mutation in Four Histidine-Requiring Strains of Salmonella Typhimurium and Two 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* 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 two 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 1000 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 1000 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 1000 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 1000 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.

6.8.1.2 Additional Studies

Separate Ames assays were conducted using the same protocol to evaluate the mutagenicity of H-EPG-05 soyate[7] and H-EPG-14 soyate[8]. 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.

---

[7] 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)
[8] 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).

6.8.2 Mouse Lymphoma Assays

6.8.2.1 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.

6.8.2.2 Additional Studies

Separate mouse lymphoma fluctuation assays were conducted using the same protocol to evaluate the mutagenicity of H-EPG-05 soyate[9] and H-EPG-14 soyate[10]. 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.

---

[9] 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)

[10] 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)

6.8.3 Chromosomal Aberrations Assays

6.8.3.1 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 two 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 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.

6.8.3.2 Additional Studies

Separate chromosome aberration tests were conducted using the same protocol to evaluate the mutagenicity of H-EPG-05 soyate[11] and H-EPG-14 soyate[12]. 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 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.

6.8.4 Unscheduled DNA Synthesis Assays

6.8.4.1 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 six 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 2000 mg/kg of H-EPG-05 HR/SO (9:1), *via* oral gavage, in two

---

[11] 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)
[12] 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)

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, 12-14 hour exposure) and dimethylnitrosamine (10 mg/kg, 2-4 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 2000 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.

6.8.4.2 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[13] and H-EPG-14 soyate[14]. 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 2000 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.

7.0 HUMAN 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.

---

[13] 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)
[14] 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)

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 (Knuiman *et al.*, 1987; Schaefer *et al.*, 1981; Brinton *et al.*, 1990).

Table 7-1 provides of summary of human studies conducted with EPG; individual study summaries are provided in Sections 7.1 and 7.2. 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 Eight Weeks" (Section 7.2.3), has been published in *Regulatory Toxicology and Pharmacology*.

r of human studies with EPG in foods

	EPG consumption	Number of subjects completed the study	Observations
2- ı)	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.
	Days 1-18: 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.
	Days 1-3: 0 g/day Days 4-21: 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.
	Days 1-3: 0 g/day Days 4-7: 120 g/day Days 8-16: 100 g/day Days 17-21: 76 g/day	14	

Table 7-1 Summary of human studies with EPG in foods (cont'd)

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 1-5: 0 g/day Days 6-23: 0, 10, 20, 30, or 40 g/day	10-12 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. Statistically 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 1-56: 0, 10, 25, or 40 g/day	34-36 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.

* Davidson and Bechtel, 2014

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

7.1.1 A Rising Multiple Dose Tolerance Dietary Study of a Solid Esterfied Propoxylated Glycerol Version H-EPG-05 HR/SO 9:1 in Normal Volunteers (ICR Project No. 0047047; E-029)

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 g, 270 g, 239 g, 208 g, 183 g, 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.

7.1.2 A Repeated Dose, Tolerance Study of Dietary Esterified Propoxylated Glycerol (H-EPG-05 HR/SO 9:1) in Normal Volunteers (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.

7.2 Tolerance Studies with EPG-05 HR/ST 45:55

7.2.1 A Repeated, Parallel Group, Constant Dose Tolerance Study of Two Consumption Levels of Dietary Esterified Propoxylated Glycerol (EPG-05 HR/ST 45:55) AOD2-09 (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 two parallel groups for 22 days. Group 1 included 12 subjects that received 60 g/day of EPG-05 HR/ST 45:55 on days 4-21 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 4-7, 8-16, and 17-21, 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.

7.2.2 Protocol Number EC-09/011426. A Double-Blind, Parallel Group, Placebo Controlled Tolerance Study of Four Doses of Dietary Esterified Propoxylated Glycerol (EPG-05 HR/ST 45:55) and Placebo in Normal Volunteers (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 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 material. Another subject in the same group experienced dyspepsia on Day 9, also possibly related to 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.

7.2.3 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 Eight Weeks (Davidson and Bechtel, 2014)

A 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. 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, RBC (red blood cell) folate, vitamin B_{12}, zinc, iron, calcium, phosphorus, osteocalcin, RBP (retinol-binding protein), intact PTH (parathyroid hormone), cholesterol, HDL-C (high-density lipoproteins), LDL-C (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.

As shown in Figure 7-1, 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 alpha-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 (LOCF) was similar to the Day 56 results for each treatment group (Figure 7-2).

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-

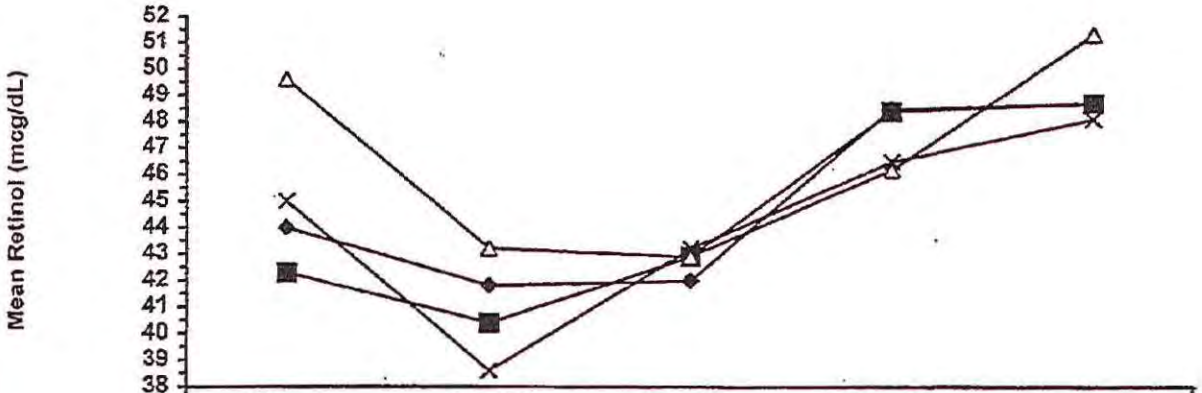
$OH\text{-}D_3$ synthesis by conducting the study during the winter in Chicago, Illinois, USA (Figures 7-3 and 7-4).

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.

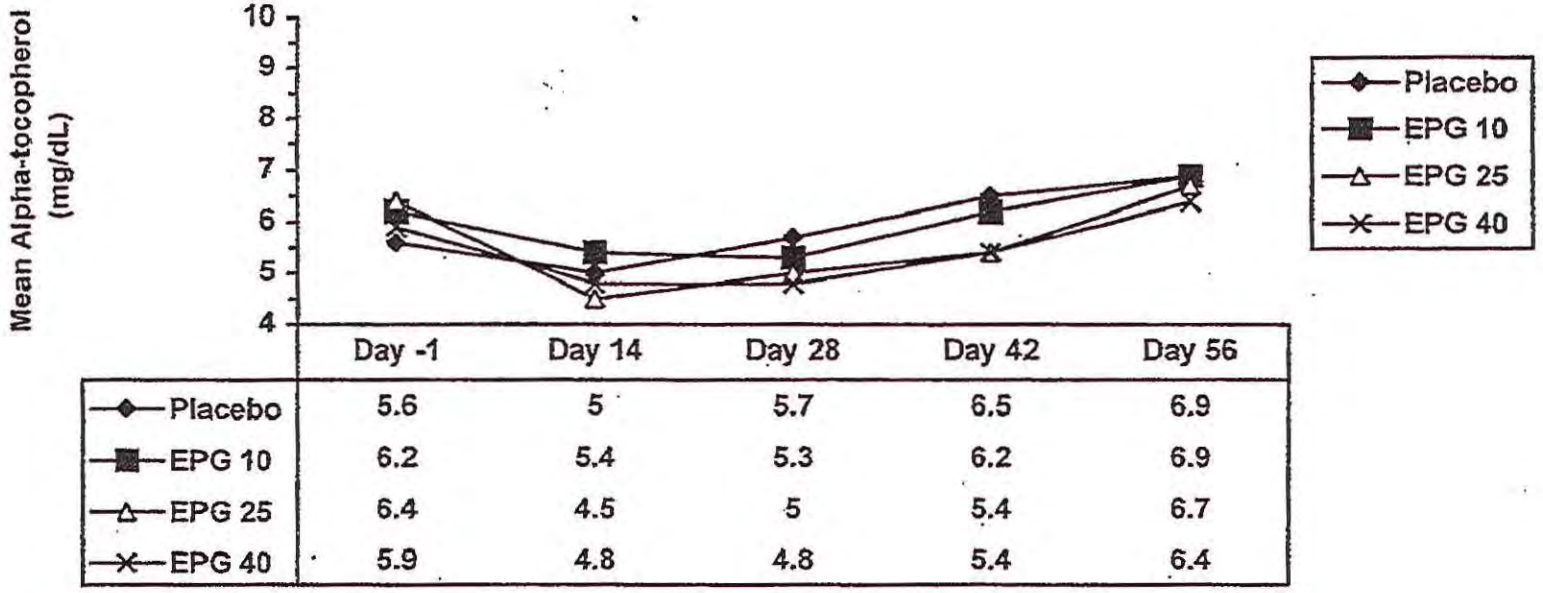
With the exception of gastrointestinal discomfort, all adverse events reported by subjects in this study were considered unrelated to EPG. Seven of the 14 pre-defined gastrointestinal adverse events (gas with discharge; diarrhea; oily spotting; oily evacuation; oily stool; liquid stool; soft stool) were 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 7-1 Mean retinol levels over time



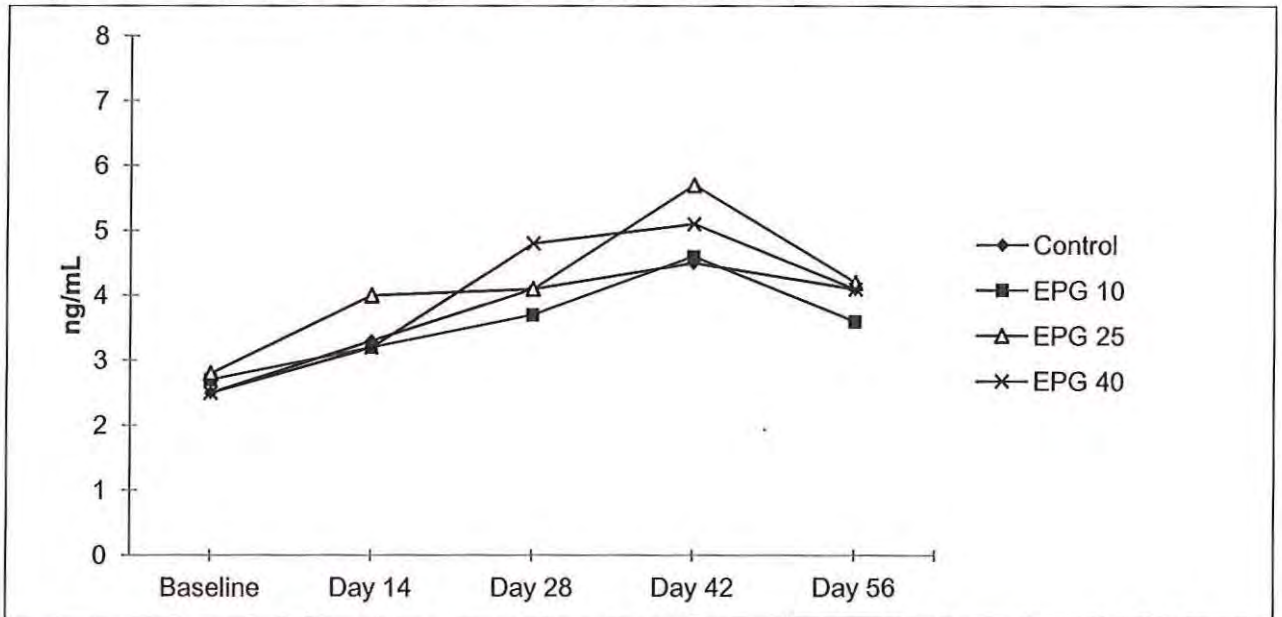
	Day -1	Day 14	Day 28	Day 42	Day 56
Placebo	44	41.8	42	48.5	48.7
EPG 10	42.3	40.4	42.9	48.4	48.7
EPG 25	49.6	43.2	42.9	46.2	51.3
EPG 40	45	38.6	43.2	46.5	48.1

Figure 7-2 Mean alpha-tocopherol levels over time



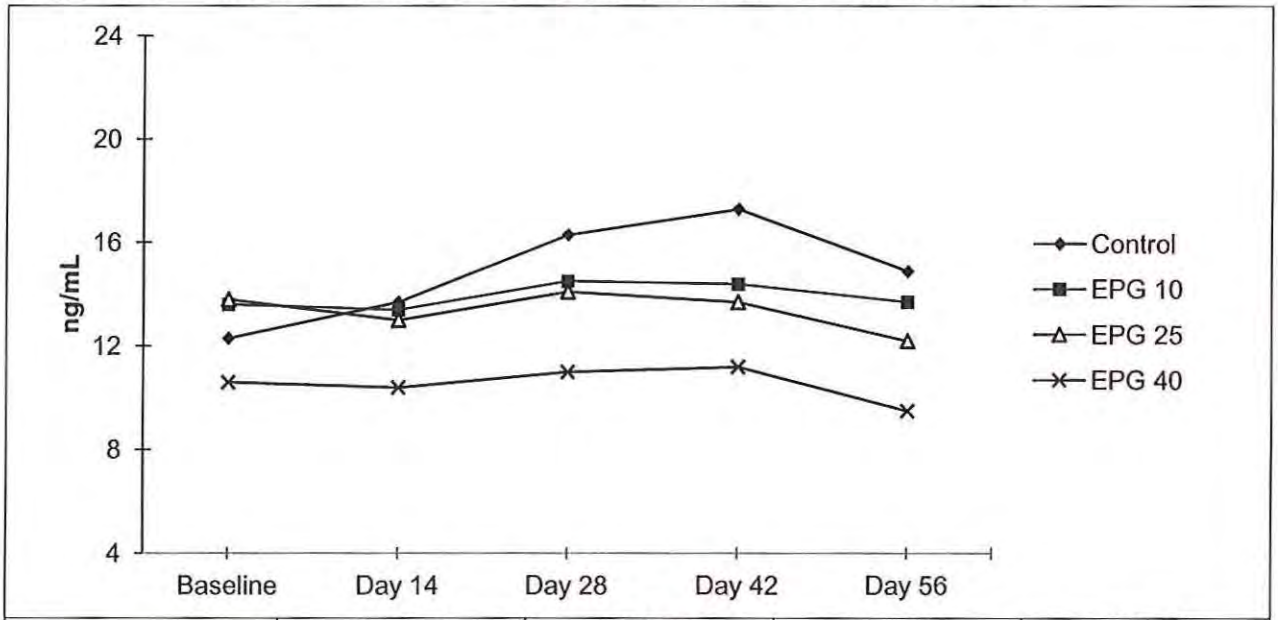
	Day -1	Day 14	Day 28	Day 42	Day 56
Placebo	5.6	5	5.7	6.5	6.9
EPG 10	6.2	5.4	5.3	6.2	6.9
EPG 25	6.4	4.5	5	5.4	6.7
EPG 40	5.9	4.8	4.8	5.4	6.4

Figure 7-3 Mean circulating 25-OH D_2 (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	1-8	1-7	1-6	1-5
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	1-10	1-11	1-12	1-7
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	1-11	1-14	1-19	1-24
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	1-10	2-22	3-35	2-21
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	1-8	1-9	1-11	1-16

Figure 7-4 Mean circulating 25-OH D_3 (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	4-35	4-26	5-41	5-28
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	6-31	5-23	6-35	5-24
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	10-33	8-24	7-33	7-24
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	9-31	6-25	8-31	6-25
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	7-27	5-29	5-29	5-19

8.0 INFORMATION POTENTIALLY INCONSISTENT WITH GRAS

8.1 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).

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 confectionary use 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 eight 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 7.1 and 7.2).

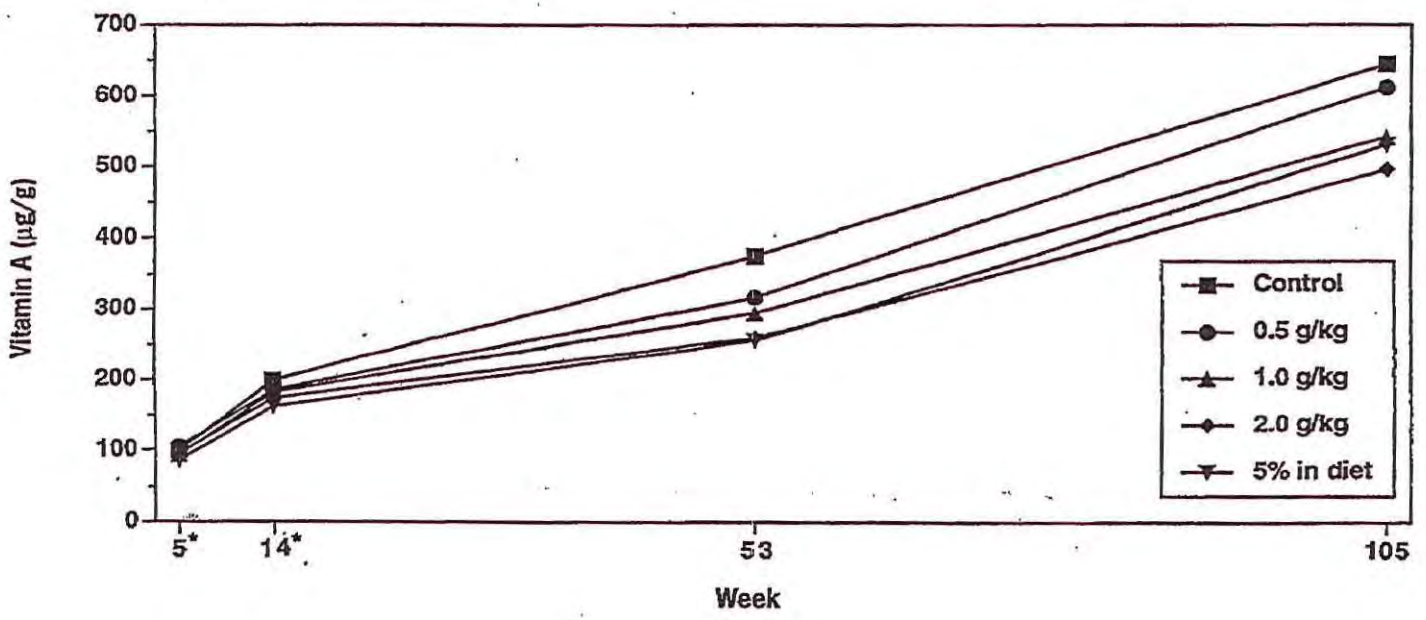
8.2 Effect on Nutrient (Fat-Soluble Vitamin) Status

EPG is intended to replace fats in selected confectionary 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 8-1 through 8-3). 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

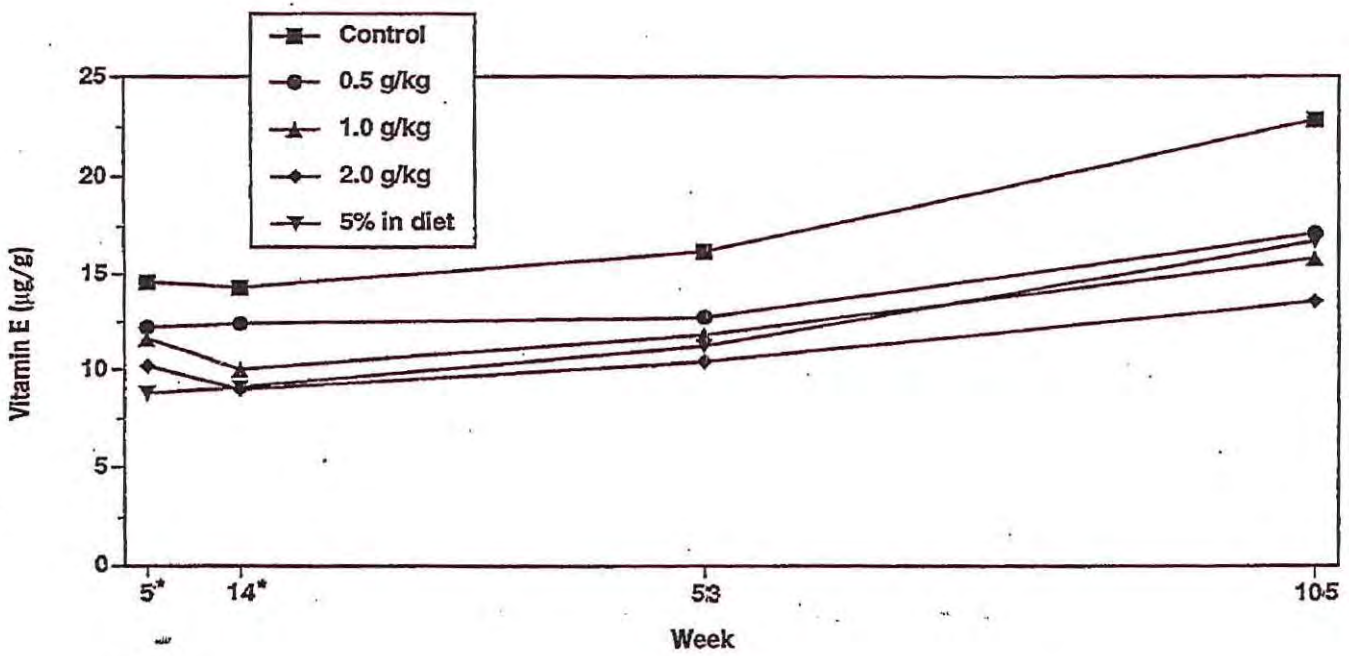
signs or microscopic evidence of vitamin deficiency. By the nature of the study designs, most effects would have been detected. In addition, there was no evidence that the vitamin levels detected in the EPG studies were significantly different from those reported in the published literature for normal 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 7.2.3, 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 8-1 Liver vitamin A in male rats



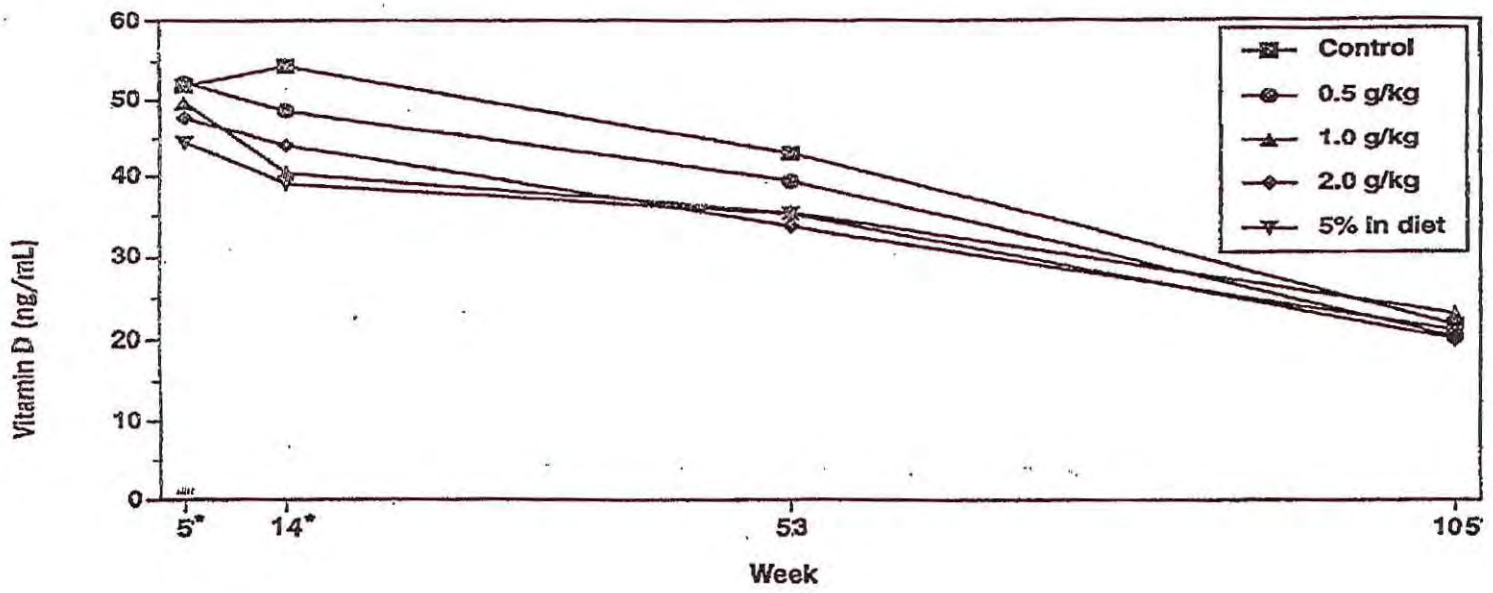
* Values from HWI 6226-122

Figure 8-2 Liver vitamin E in male rats



* Values from HWI 6226-122

Figure 8-3 Serum vitamin D in male rats



* Values from HWI 6226-122

8.3 Effect on β-Carotene Levels

As noted in Section 7.2.3, 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).

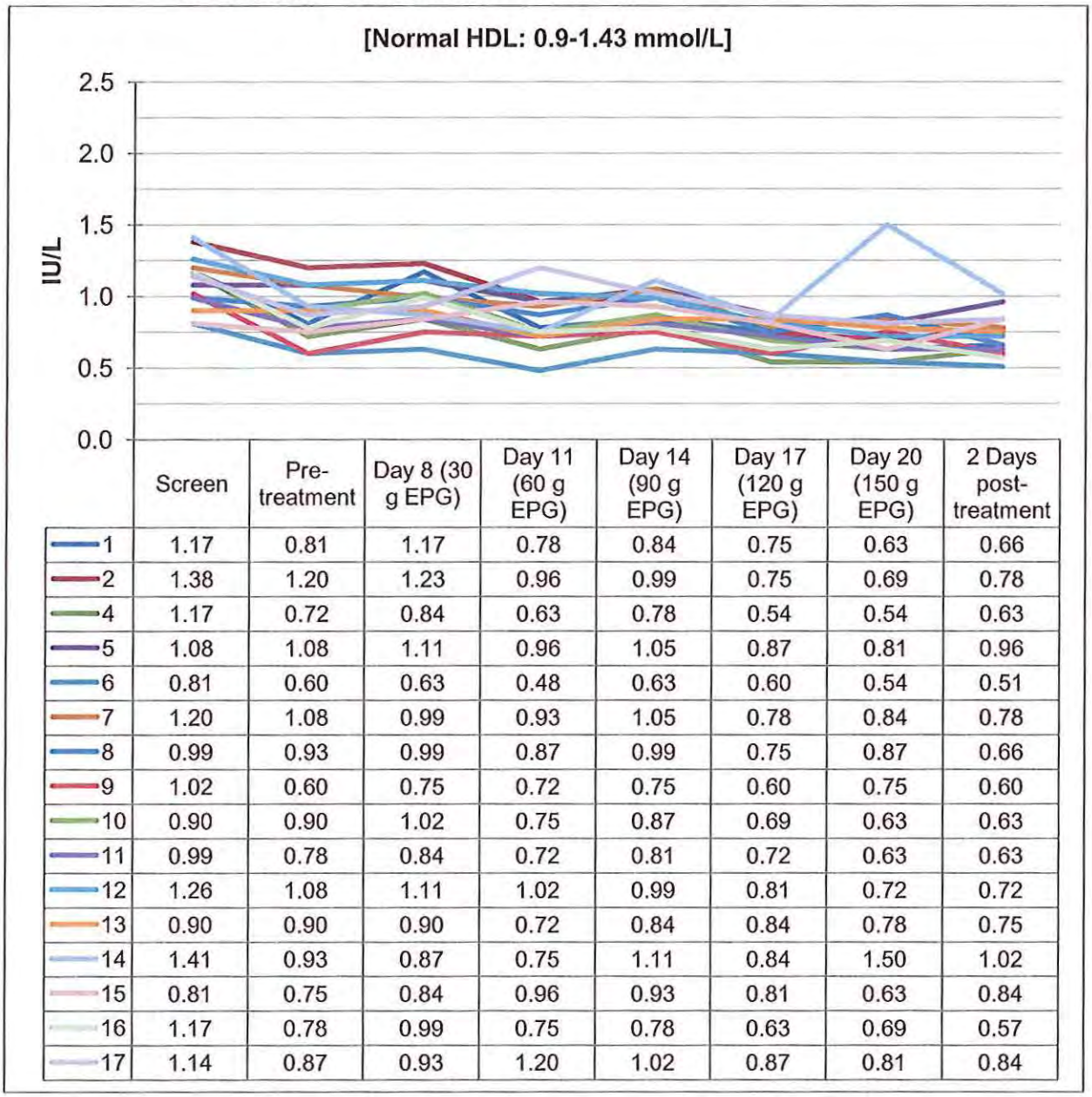
The bioavailability of carotenoids such as β-carotene can vary considerably based on the food matrix, concurrent fat intake, and serum/tissue concentrations. A study that explored, among others, the fate of β-carotene from carrot purée when administered to healthy human subjects, showed based on samples 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 (DRIs) *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).

8.4 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 8-4). 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 (Knuiman *et al.*, 1987; Schaefer *et al.*, 1981; 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 8-5a and 8-5b).

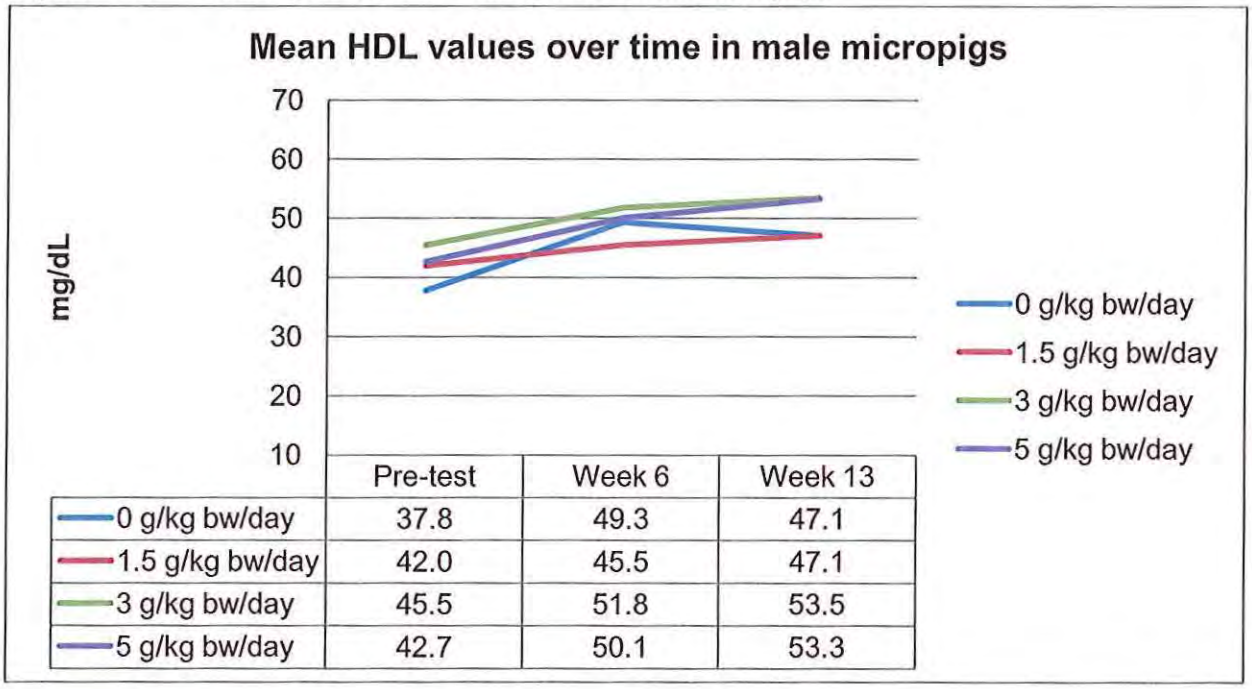
Figure 8-4 HDL cholesterol levels over time in each of 16 subjects receiving increasing EPG concentrations



	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

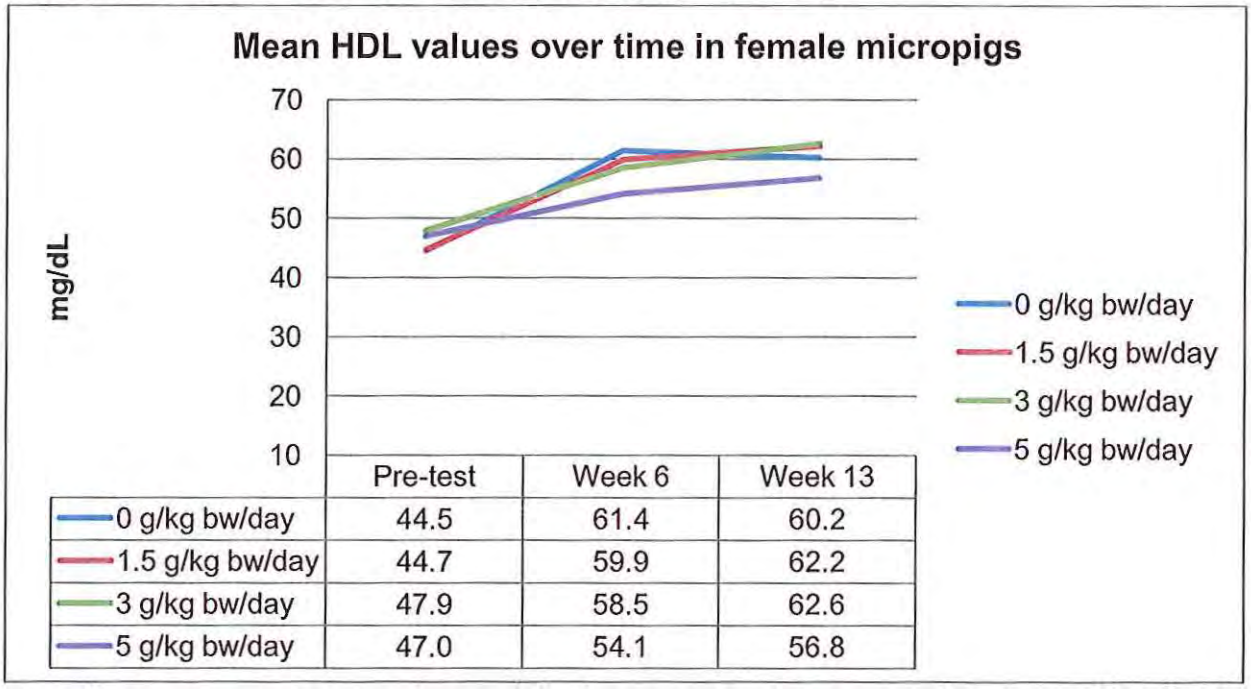
* Values from ICR Project No. 0047047 (Section 7.1.1)

Figure 8-5a 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

Figure 8-5b Mean HDL values over time in female micropigs



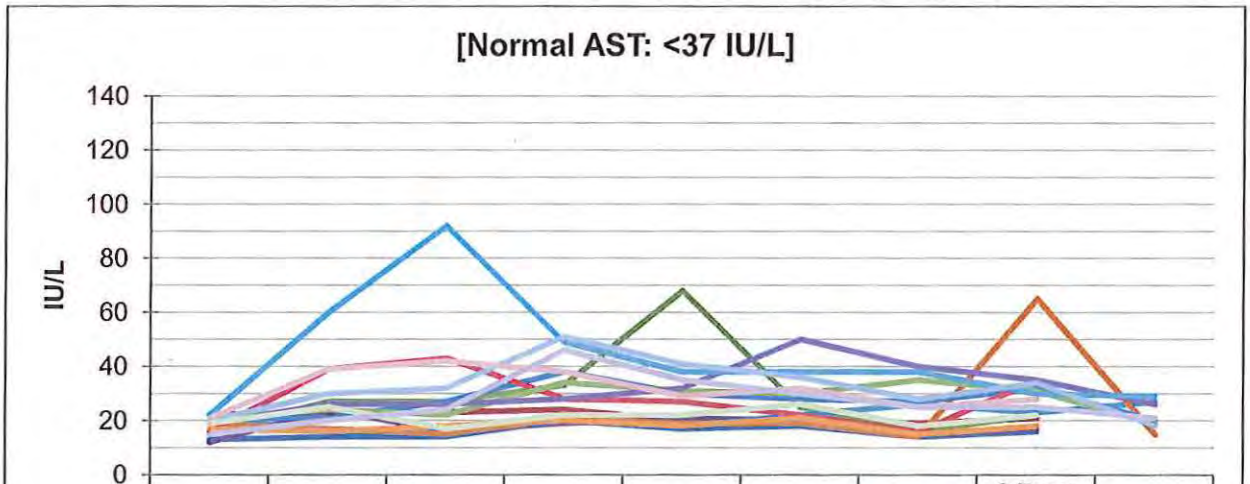
	Pre-test	Week 6	Week 13
0 g/kg bw/day	44.5	61.4	60.2
1.5 g/kg bw/day	44.7	59.9	62.2
3 g/kg bw/day	47.9	58.5	62.6
5 g/kg bw/day	47.0	54.1	56.8

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

8.5 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 8-6a through 8-6d). 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 approximate 20 g or less intake of EPG expected from the current intended uses.

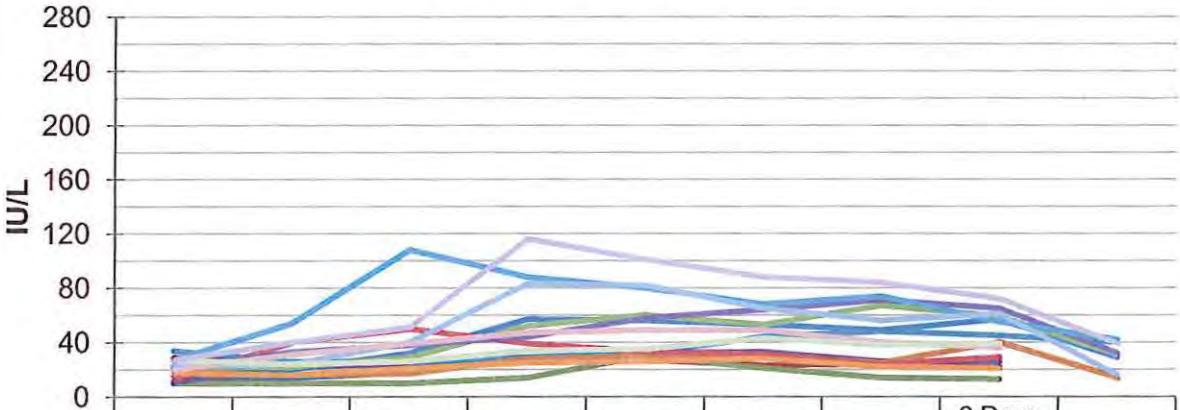
Figure 8-6a AST levels over time in each of 16 subjects receiving increasing EPG concentrations



	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

* Values from ICR Project No. 0047047 (Section 7.1.1)

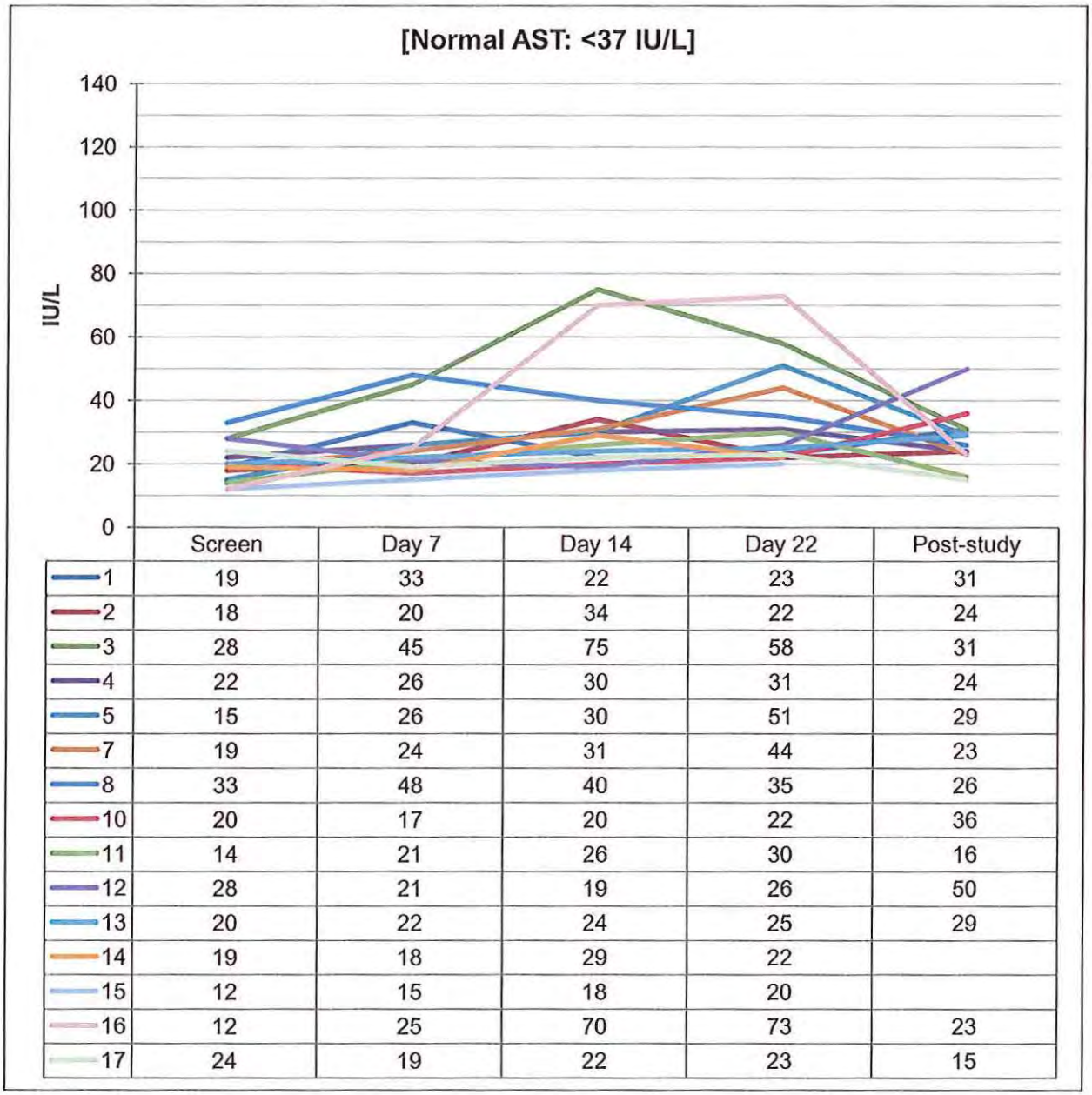
Figure 8-6b ALT levels over time in each of 16 subjects receiving increasing EPG concentrations



	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

* Values from ICR Project No. 0047047 (Section 7.1.1)

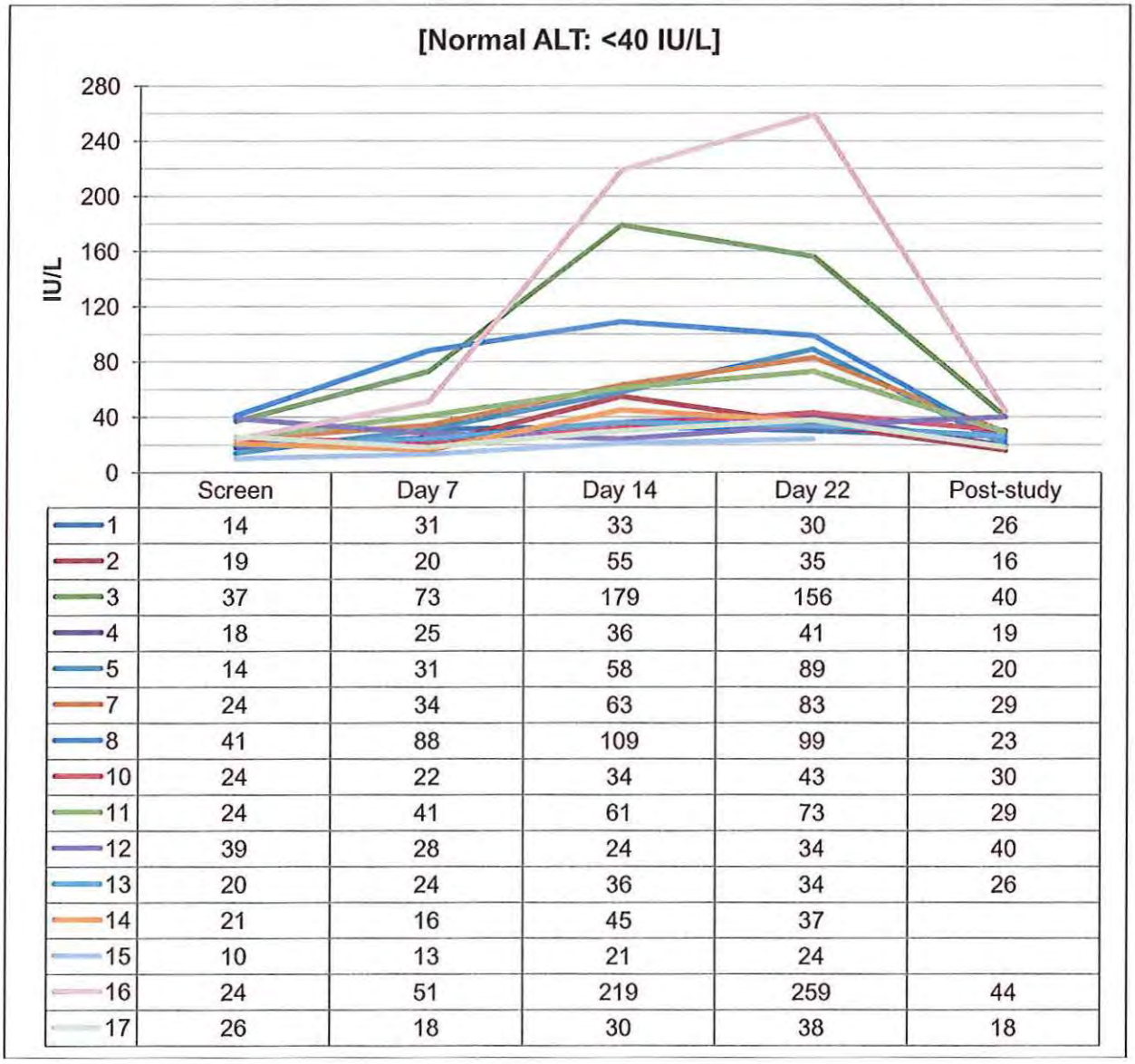
Figure 8-6c AST levels over time in each of 15 subjects receiving 120 g EPG/day



	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

* Values from ICR Project No. 004251 (Section 7.1.2)

Figure 8-6d 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

* Values from ICR Project No. 004251 (Section 7.1.2)

9.0 SUMMARY AND CONCLUSIONS

This document demonstrates that use of Choco Finesse's H-EPG-05 as a fat replacer at levels up to 34.5% (w/w) in specified confectionery products and coatings, such as 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 confectionery items, sugar-shelled confectionery pieces), snack foods (snack items, trail mix) and soft candy (molded and hollow molded confectionery pieces), is GRAS.

The following constitute the basis for the GRAS determination:

- The structure of EPGs, being comprised of components of edible fats and oils interrupted by simple propylene glycol units, provides a basis for a strong presumption of safety. This presumption has been confirmed by the results of an extensive array of preclinical investigations, including lifetime feeding studies and those examining reproduction and developmental endpoint at doses up to 5 g EPG/kg body weight/day. No indication of toxicity was observed in any study.

- Low digestibility and caloric release was confirmed, as was lack of absorption of EPG following oral administration; EPG was not found in any tissue after up to a lifetime of feeding in rats.

- Human and preclinical investigation of potential for nontoxic effects attributable to the 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 (Kow of approximately 3.2-3.4), and in strong contrast to the interaction of fat mimetics, such as olestra which has a Kow in excess of 40.

10.0 REFERENCES

Bechtel DH. 2014. Genotoxicity Testing of Esterified Propoxylated Glycerol (EPG). Regulatory Toxicology and Pharmacology 70:S131-S142.

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 85:144-151.

Christian BJ and Bechtel DH. 2014. 90-Day Dietary Safety Study with Esterified Propoxylated Glycerol (EPG) in Rats. Regulatory Toxicology and Pharmacology 70:S95-S104.

Cooper CF and Polley CW (1995). The structures of low molecular weight glycerin propoxylates: an experimental, semi-empirical, and Monte Carlo study. J Polymer Sci, Part A: Polymer Chemistry 33:31-47.

Davidson MH and 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 Eight Weeks. Regulatory Toxicology and Pharmacology 70:S143-S157.

Draize JH. 1959. *Dermal Toxicity*. In: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Association of Food and Drug Officials of the United States, Austin, Texas, p. 47.

Elinder LS, Hådell K, Johansson J, Mølgaard J, Holme I, Olsson AG, Walldius G (1995). Probucol treatment decreases serum concentrations of diet-derived antioxidants. Arteriosclerosis, Thrombosis, and Vascular Biology 15:1057-1063.

Fournier PE, Fournier-Desvaux M, Crouzette J, Palombo S, Vicaut E (1987). *In vivo* determination of the caloric value of gum arabic in the male rat-biological data. Food Additives and Contaminants 4(3):233-246

Gylling H, Puska P, Vartiainen E, Miettinen TA (1999). Retinol, vitamin D, carotenes and α-tocopherol in serum of a moderately hypercholesterolemic population consuming sitostanol ester margarine. Atherosclerosis 145:279-285.

Hendriks HFJ, Brink EJ, Meijer GW, Princen HMG, Ntanios FY (2003). Safety of long-term consumption of plant sterol esters-enriched spread. Eur J Clin Nutr 57:681-692.

IOM (2000). DRI Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Institute of Medicine, Food Nutrition Board, National Academy of Sciences, USA.

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 7:612-619.

Law M (2000). Plant sterol and stanol margarines and health. BMJ 320:861-864.

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

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

Rock CL and Swendseid (1992). Plasma beta-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 34:1758-1763.

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 127:1646S-1665S.

Tulley RT, Vaidyanathan J, Wilson JB, Rood JC, Lovejoy JC, Most MM, Volaufova J, Peters JC, Bray GA (2005). J Nutr 135:1456-1461.

Tyl RW and Bechtel DH. 2014a. One-Generation Reproduction Study of Esterified Propoxylated Glycerol (EPG) Administered in the Feed to CD® (Sprague-Dawley) Rats. Regulatory Toxicology and Pharmacology 70:S114-S122.

Tyl RW and Bechtel DH. 2014b. Developmental Toxicity Evaluation of Esterified Propoxylated Glycerol (EPG) Administered in the Diet to New Zealand White Rabbits. Regulatory Toxicology and Pharmacology 70:S123-S130.

Tyssandier V, Reboul E, Dumas JF, Bouteloup-Demange C, Armand M, Marcand J, Sallas M, Borel P (2003). Processing of vegetable-borne carotenoids in the human stomach and duodenum. Am J Physiol Gastrointest Liver Physiol 284:G913-G923.

Wedig J and Bechtel DH. 2014. 90-Day dietary toxicity study with esterified propoxylated glycerol (EPG) in Micropigs. Regulatory Toxicology and Pharmacology 70:S105-S113.

APPENDIX 1

Estimated Daily Intake of EPG by the U.S. Population from Proposed Food Uses

Estimated Daily Intake of Esterified Propoxylated Glycerols (EPG) in Confectionery Coatings by the U.S. Population from Proposed Food-Uses

- Final -

Prepared for:

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

Prepared by:

Intertek Scientific & Regulatory Consultancy
2233 Argentia Road, Suite 308
Mississauga, Ontario, Canada
L5N 2X7
www.intertek.com

October 2, 2014

Intertek

Estimated Daily Intake of Esterified Propoxylated Glycerols (EPG) in Confectionery Coatings by the U.S. Population from Proposed Food-Uses

Table of Contents

List of Tables

Intertek

Estimated Daily Intake of Esterified Propoxylated Glycerols (EPG) in Confectionery Coatings by the U.S. Population from Proposed Food-Uses

1.0 INTRODUCTION

Esterified propoxylated glycerols (EPG) are proposed for use in the United States (U.S.) in confectionery coatings in foods such as 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 confectionery items, sugar-shelled confectionery pieces), snack foods (snack items, trail mix) and soft candy (molded and hollow molded confectionery pieces).

Estimates for the intake of EPG were based on the proposed food-uses and use-levels for EPG in confectionery coatings in conjunction with food consumption data included in the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) 2009-2010 (CDC, 2011; USDA, 2012). Calculations for the mean and 90th percentile all-person and all-user intakes were performed for each of the individual proposed food-uses of EPG and the percentage of consumers were determined. Similar calculations were used to estimate the total intake of EPG resulting from all proposed food-uses of EPG combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

infants, ages 0 to 2;
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 2009-2010 are available for public use. NHANES are conducted as continuous, annual surveys, and are released in 2-year cycles. Each year about 7,000 people from 15 different locations across the U.S. are interviewed, and approximately 5,000 complete the health examination component of the survey. Any combination of consecutive years of data

collection is recognized and used as a nationally representative sample of the U.S. population. It is well-established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys, such as a 1-day dietary survey, may overestimate consumption compared to surveys conducted over longer time periods (Anderson, 1988). Because two 24-hour dietary recalls administered on 2 non-consecutive days are available from the NHANES 2009-2010 survey, these data were used to generate estimates for the current intake analysis.

NHANES 2009-2010 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 15 PSUs are visited per year. Small 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 2009-2010, 13,272 individuals were selected for the sample, 10,537 were interviewed (79.4%), and 10,253 were sampled (77.3%).

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2009-2010 collected socio-economic, physiological and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables 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. Sample weights were incorporated with NHANES 2009-2010 data to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2011; USDA, 2012).

2.2 Statistical Methods

Statistical analysis and data management were conducted in Creme software (www.cremeglobal.com) (Creme, 2014). For the deterministic 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 using Creme software. Estimates for the daily intake of EPG represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2009-2010 data; these average amounts comprised the distribution from which mean and percentile intake estimates were generated. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. All-person intake refers

to the estimated intake of EPG averaged over all individuals surveyed, regardless of whether they potentially consumed food products containing EPG, and therefore includes individuals with "zero" intakes (*i.e.* those who reported no intake of food products containing EPG during the 2 survey days). All-user intake refers to the estimated intake of EPG by those individuals who reported consuming food products containing EPG, hence the "all-user" designation. Individuals were considered 'users' if they consumed 1 or more food products containing 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 (LSRO, 1995). As such, the reliability of estimates for the intake of EPG based on the consumption of these foods may be questionable for certain individual population groups. These values were not considered when assessing the relative contribution of specific food uses to total EPG consumption and are marked with an asterisk in Appendices A and B.

3.0 FOOD USAGE DATA

Four types of coating applications were considered in the current intake assessment, including 'Drizzle', 'Enrobed', 'Inclusion', and 'Solid'. The individual proposed food-uses and use-levels for EPG in the coatings per application employed in the current intake analysis are summarized in Table 3-1.

Table 3-1 Summary of the Individual Proposed Food Uses and Use-Levels for EPG Per Coating Application in the U.S. (2009-2010 NHANES Data)

Coating Application	Food Category	Proposed Food Use	Coating Inclusion (%)	Use level in coating (%)
Drizzle	Baked Goods and Baking Mixes	Biscuit Items (cookies, sweet crackers)	5-30	34.5
	Frozen dairy desserts and mixes	Ice cream novelty (Ice cream bars, ice-cream sandwiches)	5-10	34.5
	Grain Products and Pasta	Snack & meal replacement bars (cereal and meal replacement bars)	3-10	34.5
Enrobed	Baked Goods and Baking Mixes	Biscuit Items (cookies, sweet crackers)	50-70	34.5
		Bakery items (buns, muffins, cakes, pastries, doughnuts, waffles)	5-30	34.5
	Frozen dairy desserts and mixes	Ice cream novelty (Ice cream bars, ice-cream sandwiches)	20-40	34.5
	Grain Products and Pasta	Snack & meal replacement bars (cereal and meal replacement bars)	20-30	34.5
	Hard candy and cough drops	Panned confectionery items (Coated popcorn, coated candy)	20-70	34.5

Table 3-1 Summary of the Individual Proposed Food Uses and Use-Levels for EPG Per Coating Application in the U.S. (2009-2010 NHANES Data)				
Coating Application	**Food Category**	**Proposed Food Use**	**Coating Inclusion (%)**	**Use level in coating (%)**
	Processed fruits and fruit juices	Panned fruit pieces (Dried fruit pieces)	40-60	34.5
	Snack foods	Snack items (Pretzels)	50-70	34.5
Inclusion	Baked Goods and Baking Mixes	Bakery items (buns, muffins, cakes, pastries, doughnuts, waffles)	15-35	30
		Ice cream inclusion (Ice cream inclusions and cones)	5-10	30
	Grain Products and Pasta	Snack & meal replacement bars (cereal and meal replacement bars)	10-25	30
	Snack foods	Trail mix (Nut/dried fruit mixes)	20-30	30
Solid	Hard candy and cough drops	Sugar shelled confectionery pieces (Sugar coated confectionery)	65-70	30
	Soft candy	Molded confectionery pieces	100	34.5
		Hollow molded confectionery pieces	100	34.5

EPG = Esterified propoxylated glycerols

In order to examine the intake of EPG from all uses and applications considered in one assessment, the maximum range of coating inclusion and the maximum EPG level per food category were used, as per Table 3-2. Food codes representative of each proposed food-use as per Table 3-2 were chosen from the NHANES 2009-2010 (CDC, 2011; USDA, 2012). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (CFR, 2014). Where required, product-specific adjustment factors were developed based on data provided in the standard recipe file for the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998 survey (USDA, 2000). All food codes included in the current intake assessment are listed in Appendix C. A given food code may not be associated with both surveys; as with each new survey the food code list has been updated to reflect the availability of new foods and the discontinuation of certain obsolete codes.

Table 3-2 Summary of the Individual Proposed Food Uses and Use-Levels for EPG in the U.S. (2009-2010 NHANES Data)				
Coating Application	**Food Category**	**Proposed Food Use**	**Coating Inclusion (%)[a]**	**Use level in coating (%)[a]**
All applications together	Baked Goods and Baking Mixes	Biscuit Items (cookies, sweet crackers)	5-70	34.5
		Bakery items (buns, muffins, cakes, pastries, doughnuts, waffles)	5-35	30-34.5
		Ice cream inclusion (Ice cream inclusions and cones)	5-10	30
	Frozen dairy desserts and mixes	Ice cream novelty (Ice cream bars, ice-cream sandwiches)	5-40	34.5
	Grain Products and Pasta	Snack & meal replacement bars (cereal and meal replacement bars)	3-30	30-34.5
	Hard candy and cough drops	Panned confectionery items (Coated popcorn, coated candy)	20-70	34.5
		Sugar shelled confectionery pieces (Sugar coated confectionery)	65-70	30
	Processed fruits and fruit juices	Panned fruit pieces (Dried fruit pieces)	40-60	34.5
	Snack foods	Snack items (Pretzels)	50-70	34.5
		Trail mix (Nut/dried fruit mixes)	20-30	30
	Soft candy	Molded confectionery pieces[b]	100	34.5
		Hollow molded confectionery pieces[b]	100	34.5

EPG = Esterified propoxylated glycerols
[a] The maximum proposed coating inclusion and EPG level per proposed food use was used in the intake assessments
[b] No intake for these products were reported in the NHANES 2009-2010, therefore these food uses were not included in the intake assessments.

4.0 FOOD SURVEY RESULTS

Estimates for the total daily intakes of EPG from proposed food-uses from all applications together are provided in Tables 4.1-1 and 4.1-2. Table 4.1-1 summarizes the estimated total intake of EPG (g/person/day) from all proposed food-uses in the U.S. population groups. Table 4.1-2 presents these data on a per kilogram body weight basis (mg/kg body weight/day).

4.1 Estimated Daily Intake of EPG from All Proposed Food-Uses and Applications Together in the U.S.

There was a total of 66.8% users of foods proposed to contain coatings with EPG from all applications together (range of 56.9 to 79.9% users) (Table 4.1-1). Taking all-person intakes into account, consumption of the proposed food-uses from all applications by the total U.S.

population resulted in an estimated mean and 90th percentile intake of EPG of 5.60 g/person/day (105.8 mg/kg body weight/day) and 14.85 g/person/day (276.6 mg/kg body weight/day), respectively. Within the individual population groups, children were determined to have the greatest estimated mean intake of EPG on an absolute basis, at 6.91 g/person/day, while male adults had the highest 90th percentile intake at 17.25 g/person/day. Infants had the lowest estimated mean and 90th percentile intakes of 3.23 and 9.89 g/person/day, respectively (Table 4.1-1).

Considering intakes by all-users , consumption of the proposed food-uses by the total U.S. population resulted in an estimated mean and 90th percentile intake of EPG of 8.38 g/person/day (158.3 mg/kg body weight/day) and 17.57 g/person/day (352.7 mg/kg body weight/day), respectively. Within the individual population groups, male adults were determined to have the greatest estimated mean and 90th percentile all-user intakes of EPG on an absolute basis, at 9.80 and 19.96 g/person/day, respectively, while infants had the lowest estimated mean and 90th percentile all-user intakes of 5.69 and 11.64 g/person/day, respectively (Table 4.1-1).

Table 4.1-1 Summary of the Estimated Daily Intake of EPG from Proposed Food-Uses based on All Applications Together in the U.S. by Population Group (2009-2010 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants	0 to 2	3.23	9.89	56.9	421	5.69	11.64
Children	3-11	6.91	16.26	79.9	1104	8.65	17.38
Female Teenagers	12 to 19	5.60	14.12	68.0	349	8.24	16.45
Male Teenagers	12 to 19	5.90	15.54	63.7	355	9.26	17.91
Female Adults	≥ 20	4.98	13.04	68.4	1733	7.29	15.54
Male Adults	≥ 20	6.10	17.25	62.2	1459	9.80	19.96
Total Population	All Ages	5.60	14.85	66.8	5421	8.38	17.57

EPG = Esterified propoxylated glycerols

On a body weight basis, taking all-person intakes into account, infants were the population group identified as having the highest mean and 90th percentile EPG intakes based on all applications together at 264.3 and 766.1 mg/kg body weight/day, respectively (Table 4.1-2). Female adults were identified as having the lowest mean and 90th percentile intakes of 71.1 and 191.4 mg/kg body weight/day, respectively.

The same patterns were noted on an all-user basis, infants were the population group identified as having the highest mean and 90th percentile EPG intakes at 464.7 and 960.0 mg/kg body weight/day, respectively (Table 4.1-2), whereas female adults were identified as having the

lowest mean and 90th percentile all-user intakes of 103.9 and 219.5 mg/kg body weight/day, respectively.

Table 4.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of EPG from Proposed Food-Uses based on All Applications in the U.S. by Population Group (2009-2010 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to 2	264.3	766.1	56.9	421	464.7	960.0
Children	3-11	263.8	633.1	79.9	1104	330.2	691.3
Female Teenagers	12 to 19	99.2	259.0	68.0	349	145.8	303.2
Male Teenagers	12 to 19	95.8	248.8	63.7	355	150.4	310.9
Female Adults	≥ 20	71.1	191.4	68.4	1733	103.9	219.5
Male Adults	≥ 20	71.4	196.7	62.2	1459	114.7	242.3
Total Population	All Ages	105.8	276.6	66.8	5421	158.3	352.7

EPG = Esterified propoxylated glycerols

4.2 Estimated Daily Intake of EPG from Individual Proposed Food-Uses 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 significant consumers of bakery items (20.9 to 36.4% users) and biscuit items (33.3 to 49.9% users).

In terms of contribution to total mean intake of EPG, bakery items (contributed 23.4 to 37.6% to total mean intakes) and biscuit items (contributed 40.1 to 49.1% to total mean intakes) were the 2 main sources of intake across all population groups on both an absolute and on a mg/kg body weight basis. Ice-cream inclusion and Trail mix each contributed <2% to total mean EPG intakes across all population groups (see Tables A-1 to A-7 and/or B-1 to B-7 for further details).

5.0 CONCLUSIONS

Consumption data and information pertaining to the individual proposed food-uses of EPG from confectionery coatings from four applications together were used to estimate the all-person and all-user intakes of EPG for specific demographic groups and for the total U.S. population. This type of intake methodology is generally considered to be 'worst case' as a result of several conservative assumptions made in the consumption estimates. For example, it was assumed that the maximum proposed coating inclusion rate and the maximum EPG level per proposed

food use was used in the intake assessments. In addition, it is well-established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently.

In summary, on an all-user basis, the highest mean and 90th percentile intakes of EPG by the U.S. population from all proposed food-uses in the U.S., as observed in male adults (aged 20 years plus) were estimated to be 9.80 g/person/day (114.7 mg/kg body weight/day) and 19.96 g/person/day (242.3 mg/kg body weight/day), respectively. On a body weight basis, infants were the population group identified as having the highest all-user mean and 90th percentile EPG intakes at 464.7 and 960.0 mg/kg body weight/day, respectively.

6.0 REFERENCES

Anderson SA, editor (1988). *Estimation of Exposure to Substances in the Food Supply* (Contract No. FDA 223-84-2059). Bethesda (MD): Federation of American Societies for Experimental Biology (FASEB), Life Science Research Office (LSRO).

CDC (2014). *National Health and Nutrition Examination Survey (NHANES): 2009-2010.* Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://wwwn.cdc.gov/nchs/nhanes/search/nhanes09_10.aspx [Page last updated: February 27, 2014].

CFR (2014). 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.

Creme (2014). *[Website].* Dublin, Ireland: Creme Software Ltd. Available at: http://www.cremeglobal.com/ [© 2005-2014 Creme Software Ltd].

LSRO (1995). *Third Report on Nutrition Monitoring in the United States.* Prepared by Bethesda (MD): Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB) for the Interagency Board for Nutrition Monitoring and Related Research. Washington (DC): U.S. Government Printing Office, vol 1, pp. 19-31 & III-1 to III-10 and vol 2, pp. VB-1 to VB-2.

USDA (2000). *1994-1996, 1998 Continuing Survey of Food Intakes by Individuals (CSFII) and Diet and Health Knowledge Survey (DHKS)* [On CD-ROM, PB2000-500027]. Riverdale (MD): U.S. Department of Agriculture (USDA).

USDA (2012). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2009-2010.* Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release [Last Modified: 10/2/2014].

Intertek

APPENDIX A
Estimated Daily Intake Esterified Propoxylated Glycerols (EPG) from Individual Proposed Food-Uses from All Applications Together by Different Population Groups Within the U.S.

Intertek

Table A-1 Estimated Daily Intake of EPG from Individual Proposed Food-Uses from all Applications Together by Infants Aged 0 to 2 Years Within the U.S. (2009-2010 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	*100*	3.23	9.89	56.9	421	5.69	11.64
Bakery items	23.4	0.76	2.94	20.9	130	3.63	7.79
Biscuit items	49.1	1.59	4.83	40.0	305	3.97	8.27
Ice cream inclusion	<0.1	<0.01*	na	0.7	9	0.11*	0.15*
Ice cream novelty	3.7	0.12*	na	3.7	26	3.18*	4.19*
Panned Confectionery items	<0.1	<0.01*	na	0.2	4	0.90*	1.29*
Panned fruit pieces	6.8	0.22	na	5.2	31	4.25	6.76*
Snack and meal replacement bars	4.5	0.14	na	8.0	46	1.81	2.56*
Snack items	10.6	0.34	na	7.8	49	4.36	9.56*
Sugar confectionery pieces	1.4	0.05*	na	4.3	22	1.09*	1.84*
Trail mix	0.4	0.01*	na	0.8	3	1.71*	2.18*

EPG = Esterified propoxylated glycerols; na = not available
* 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 Individual Proposed Food-Uses from all Applications Together by Children Aged 3 to 11 Years Within the U.S. (2009-2010 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	*100*	6.91	16.26	79.9	1104	8.65	17.38
Bakery items	33.3	2.30	7.33	36.4	496	6.32	11.71
Biscuit items	46.4	3.21	9.14	49.9	699	6.43	13.90
Ice cream inclusion	0.2	0.01	na	5.6	83	0.19	0.30
Ice cream novelty	3.9	0.27	na	6.4	99	4.23	6.06
Panned Confectionery items	0.5	0.03	na	1.7	30	2.00	4.66*
Panned fruit pieces	1.4	0.10	na	3.0	35	3.24	7.22*
Snack and meal replacement bars	3.8	0.26	1.24	12.3	141	2.14	3.92
Snack items	8.9	0.61	1.21	12.3	148	4.96	9.10
Sugar confectionery pieces	1.4	0.10	na	5.9	60	1.68	3.48*
Trail mix	0.2	0.01*	na	0.8	9	1.70*	2.10*

EPG = Esterified propoxylated glycerols; na = not available
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Intertek

Table A-3	Estimated Daily Intake of EPG from Individual Proposed Food-Uses from all Applications Together by Female Teenagers Aged 12 to 19 Years Within the U.S. (2009-2010 NHANES Data)						
Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	*100*	5.60	14.12	68.0	349	8.24	16.45
Bakery items	37.6	2.11	7.43	29.7	153	7.09	15.88
Biscuit items	40.1	2.25	7.25	36.0	201	6.23	11.53
Ice cream inclusion	0.3	0.01*	na	4.5	18	0.33*	0.97*
Ice cream novelty	5.1	0.29*	na	4.8	24	5.97*	8.29*
Panned Confectionery items	2.1	0.12*	na	2.6	16	4.43*	5.47*
Panned fruit pieces	2.1	0.12*	na	1.8	11	6.45*	14.23*
Snack and meal replacement bars	3.3	0.18	na	10.7	45	1.71	2.35*
Snack items	5.6	0.31	na	8.2	37	3.81	6.01*
Sugar confectionery pieces	3.1	0.17*	na	5.6	19	3.06*	6.97*
Trail mix	0.7	0.04*	na	1.5	3	2.78*	3.15*

EPG = Esterified propoxylated glycerols; na = not available
* 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 Individual Proposed Food-Uses from all Applications Together by Male Teenagers Aged 12 to 19 Years Within the U.S. (2009-2010 NHANES Data)						
Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	*100*	5.90	15.54	63.7	355	9.26	17.91
Bakery items	32.7	1.93	6.57	27.2	159	7.09	12.80
Biscuit items	44.6	2.63	8.22	37.1	211	7.09	13.19
Ice cream inclusion	0.2	0.01*	na	2.9	21	0.40*	1.34*
Ice cream novelty	4.2	0.25*	na	4.7	29	5.21*	7.73*
Panned Confectionery items	1.1	0.06*	na	1.4	8	4.44*	4.79*
Panned fruit pieces	0.1	<0.01*	na	0.5	4	1.10*	2.29*
Snack and meal replacement bars	2.6	0.15	na	6.9	43	2.23	3.73*
Snack items	12.2	0.72	na	9.3	42	7.72	23.65*
Sugar confectionery pieces	1.3	0.08*	na	2.7	14	2.94*	4.04*
Trail mix	1.1	0.06*	na	1.1	3	5.87*	9.65*

EPG = Esterified propoxylated glycerols; na = not available
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Intertek

Table A-5 Estimated Daily Intake of EPG from Individual Proposed Food-Uses from all Applications Together by Female Adults Aged 20 Years and Over Within the U.S. (2009-2010 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	*100*	4.98	13.04	68.4	1733	7.29	15.54
Bakery items	31.7	1.58	6.48	26.0	658	6.07	11.09
Biscuit items	40.5	2.02	7.00	37.0	981	5.46	10.87
Ice cream inclusion	0.1	0.01	na	2.5	65	0.26	0.63*
Ice cream novelty	4.3	0.22	na	4.3	119	5.00	8.26
Panned Confectionery items	2.3	0.12	na	3.9	99	3.00	4.80
Panned fruit pieces	5.8	0.29	na	8.0	171	3.58	7.50
Snack and meal replacement bars	4.0	0.20	na	9.2	184	2.18	3.73
Snack items	8.5	0.42	na	9.5	187	4.43	11.30
Sugar confectionery pieces	1.9	0.10	na	3.3	77	2.88	6.54*
Trail mix	0.9	0.04	na	1.8	36	2.35	4.30*

EPG = Esterified propoxylated glycerols; na = not available
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-6 Estimated Daily Intake of EPG from Individual Proposed Food-Uses from all Applications Together by Male Adults Aged 20 Years and Over Within the U.S. (2009-2010 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	*100*	6.10	17.25	62.2	1459	9.80	19.96
Bakery items	35.2	2.14	7.85	25.1	611	8.55	16.55
Biscuit items	40.1	2.45	8.45	33.3	822	7.35	15.70
Ice cream inclusion	0.1	0.01	na	2.5	51	0.26	0.67*
Ice cream novelty	5.4	0.33	na	5.0	110	6.62	11.64
Panned Confectionery items	2.1	0.13	na	2.9	78	4.51	9.22*
Panned fruit pieces	3.3	0.20	na	5.3	111	3.75	7.47
Snack and meal replacement bars	3.9	0.24	na	8.0	148	2.96	5.35
Snack items	7.0	0.43	na	7.3	136	5.86	14.25
Sugar confectionery pieces	2.4	0.15	na	3.3	60	4.39	8.73*
Trail mix	0.6	0.04*	na	1.0	21	3.53*	5.51*

EPG = Esterified propoxylated glycerols; na = not available
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Intertek

Table A-7	Estimated Daily Intake of EPG from Individual Proposed Food-Uses from all Applications Together by the Total US Population and Over Within the U.S. (2009-2010 NHANES Data)						
Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	*100*	5.60	14.85	66.8	5421	8.38	17.57
Bakery items	33.4	1.87	6.82	27.0	2207	6.94	13.66
Biscuit items	41.7	2.33	7.61	37.3	3219	6.26	13.28
Ice cream inclusion	0.1	0.01	na	2.9	247	0.25	0.82
Ice cream novelty	4.7	0.26	na	4.8	407	5.46	8.42
Panned Confectionery items	1.8	0.10	na	2.9	235	3.55	5.92
Panned fruit pieces	3.7	0.21	na	5.6	363	3.68	7.50
Snack and meal replacement bars	3.8	0.21	na	9.1	607	2.37	4.02
Snack items	8.1	0.45	na	8.9	599	5.06	12.33
Sugar confectionery pieces	2.0	0.11	na	3.8	252	3.04	6.91
Trail mix	0.7	0.04	na	1.3	75	2.78	5.51*

EPG = Esterified propoxylated glycerols; na = not available
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Intertek

APPENDIX B

Estimated Daily Per Kilogram Body Weight Intake of Esterified Propoxylated Glycerols (EPG) from All Applications Together from Individual Proposed Food-Uses by Different Population Groups Within the U.S.

Intertek

Table B-1 Estimated Daily Per Kilogram Body Weight Intake of EPG from Individual Proposed Food-Uses from all Applications Together by Infants Aged 0 to 2 Years Within the U.S. (2009-2010 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	*100*	264.3	766.1	56.9	421	464.7	960.0
Bakery items	22.7	60.0	203.4	20.9	130	287.5	545.2
Biscuit items	50.8	134.1	409.7	40.0	305	335.5	712.1
Ice cream inclusion	<0.1	0.1*	na	0.7	9	10.1*	15.2*
Ice cream novelty	3.6	9.5*	na	3.7	26	253.8*	415.0*
Panned Confectionery items	<0.1	0.1*	na	0.2	4	71.8*	109.5*
Panned fruit pieces	7.3	19.2	na	5.2	31	369.7	608.4*
Snack and meal replacement bars	4.4	11.6	na	8.0	46	146.0	212.8*
Snack items	9.4	25.0	na	7.8	49	318.9	622.2*
Sugar confectionery pieces	1.4	3.7*	na	4.3	22	86.8*	150.3*
Trail mix	0.4	1.0*	na	0.8	3	124.6*	156.6*

bw = body weight; EPG = Esterified propoxylated glycerols; na = not available
* 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 Individual Proposed Food-Uses from all Applications Together by Children Aged 3 to 11 Years Within the U.S. (2009-2010 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	*100*	263.8	633.1	79.9	1104	330.2	691.3
Bakery items	31.7	83.7	281.5	36.4	496	230.0	423.4
Biscuit items	47.1	124.3	367.6	49.9	699	249.2	491.5
Ice cream inclusion	0.1	0.3	na	5.6	83	5.5	8.0
Ice cream novelty	4.4	11.5	na	6.4	99	180.5	308.8
Panned Confectionery items	0.5	1.3	na	1.7	30	77.3	191.1*
Panned fruit pieces	1.9	5.1	na	3.0	35	168.1	445.8*
Snack and meal replacement bars	4.1	10.7	36.3	12.3	141	87.2	141.2
Snack items	8.1	21.4	39.6	12.3	148	173.1	369.0
Sugar confectionery pieces	1.8	4.6	na	5.9	60	78.5	187.4*
Trail mix	0.3	0.8*	na	0.8	9	96.2*	138.5*

bw = body weight; EPG = Esterified propoxylated glycerols; na = not available
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Intertek

Table B-3 Estimated Daily Per Kilogram Body Weight Intake of EPG from Individual Proposed Food-Uses from all Applications Together by Female Teenagers Aged 12 to 19 Years Within the U.S. (2009-2010 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	*100*	99.2	259.0	68.0	349	145.8	303.2
Bakery items	36.7	36.4	134.0	29.7	153	122.5	256.3
Biscuit items	40.9	40.5	128.3	36.0	201	112.4	216.0
Ice cream inclusion	0.2	0.2*	na	4.5	18	4.3*	11.1*
Ice cream novelty	5.2	5.1*	na	4.8	24	106.9*	155.0*
Panned Confectionery items	2.0	2.0*	na	2.6	16	77.8*	89.8*
Panned fruit pieces	1.8	1.8*	na	1.8	11	99.4*	256.8*
Snack and meal replacement bars	3.3	3.3	14.1*	10.7	45	30.5	44.9*
Snack items	5.8	5.7	na	8.2	37	69.5	108.7*
Sugar confectionery pieces	3.3	3.2*	na	5.6	19	58.1*	116.2*
Trail mix	0.8	0.8*	na	1.5	3	52.5*	73.0*

bw = body weight; EPG = Esterified propoxylated glycerols; na = not available
* 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 Individual Proposed Food-Uses from all Applications Together by Male Teenagers Aged 12 to 19 Years Within the U.S. (2009-2010 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	*100*	95.8	248.8	63.7	355	150.4	310.9
Bakery items	32.7	31.4	122.6	27.2	159	115.3	197.5
Biscuit items	43.0	41.2	130.7	37.1	211	111.0	196.5
Ice cream inclusion	0.2	0.2*	na	2.9	21	6.6*	19.4*
Ice cream novelty	4.2	4.0*	na	4.7	29	84.3*	137.1*
Panned Confectionery items	1.0	0.9*	na	1.4	8	64.6*	71.9*
Panned fruit pieces	0.1	0.1*	na	0.5	4	16.0*	30.0*
Snack and meal replacement bars	2.4	2.3	na	6.9	43	33.3	52.6*
Snack items	14.3	13.7	na	9.3	42	147.7	604.3*
Sugar confectionery pieces	1.3	1.3*	na	2.7	14	47.0*	75.2*
Trail mix	0.8	0.8*	na	1.1	3	70.8*	106.8*

bw = body weight; EPG = Esterified propoxylated glycerols; na = not available
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Intertek

Table B-5 Estimated Daily Per Kilogram Body Weight Intake of EPG from Individual Proposed Food-Uses from all Applications Together by Female Adults Aged 20 Years and Over Within the U.S. (2009-2010 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	*100*	71.1	191.4	68.4	1733	103.9	219.5
Bakery items	31.5	22.4	81.5	26.0	658	86.1	164.2
Biscuit items	41.0	29.1	96.6	37.0	981	78.8	166.3
Ice cream inclusion	0.1	0.1	na	2.5	65	3.7	9.1*
Ice cream novelty	4.2	3.0	na	4.3	119	69.1	104.6
Panned Confectionery items	2.2	1.5	na	3.9	99	39.8	70.4
Panned fruit pieces	6.1	4.3	na	8.0	171	53.9	137.3
Snack and meal replacement bars	4.0	2.8	na	9.2	184	30.9	51.8
Snack items	8.4	6.0	na	9.5	187	62.7	152.6
Sugar confectionery pieces	1.8	1.3	na	3.3	77	38.9	93.3*
Trail mix	0.8	0.6	na	1.8	36	30.9	56.4*

bw = body weight; EPG = Esterified propoxylated glycerols; na = not available
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-6 Estimated Daily Per Kilogram Body Weight Intake of EPG from Individual Proposed Food-Uses from all Applications Together by Male Adults Aged 20 Years and Over Within the U.S. (2009-2010 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	*100*	71.4	196.7	62.2	1459	114.7	242.3
Bakery items	35.1	25.1	97.4	25.1	611	99.9	202.4
Biscuit items	40.3	28.8	97.6	33.3	822	86.4	179.5
Ice cream inclusion	0.1	0.1	na	2.5	51	2.7	6.9*
Ice cream novelty	5.2	3.7	na	5.0	110	75.1	143.6
Panned Confectionery items	2.0	1.4	na	2.9	78	50.0	94.9*
Panned fruit pieces	3.5	2.5	na	5.3	111	46.8	89.9
Snack and meal replacement bars	3.9	2.8	na	8.0	148	34.7	62.6
Snack items	6.9	4.9	na	7.3	136	67.4	155.2
Sugar confectionery pieces	2.6	1.8	na	3.3	60	55.1	113.1*
Trail mix	0.5	0.4*	na	1.0	21	38.9*	62.8*

bw = body weight; EPG = Esterified propoxylated glycerols; na = not available
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Intertek

Table B-7 Estimated Daily Per Kilogram Body Weight Intake of EPG from Individual Proposed Food-Uses from all Applications Together by the Total US Population and Over Within the U.S. (2009-2010 NHANES Data)							
Food-Use Category	% Contribution to Total Mean Intake	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	*100*	105.8	276.6	66.8	5421	158.3	352.7
Bakery items	31.8	33.6	119.4	27.0	2207	124.7	257.3
Biscuit items	43.8	46.4	137.9	37.3	3219	124.3	273.2
Ice cream inclusion	0.1	0.1	na	2.9	247	4.1	10.6
Ice cream novelty	4.5	4.7	na	4.8	407	98.2	181.5
Panned Confectionery items	1.3	1.4	na	2.9	235	48.7	86.5
Panned fruit pieces	3.9	4.1	na	5.6	363	73.0	177.8
Snack and meal replacement bars	3.9	4.1	na	9.1	607	45.8	97.2
Snack items	8.2	8.7	na	8.9	599	97.3	207.8
Sugar confectionery pieces	2.0	2.1	na	3.8	252	55.7	125.1
Trail mix	0.5	0.6	na	1.3	75	43.4	76.5*

bw = body weight; EPG = Esterified propoxylated glycerols; na = not available
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Intertek

APPENDIX C
Representative NHANES Food Codes for Proposed Food-Uses of Esterified Propoxylated Glycerols (EPG) in Confectionery Coatings in the U.S.

Representative NHANES Food Codes for Proposed Beverage-Uses of EPG in Confectionery Coatings in the U.S.

Baked Goods and Baking Mixes

Biscuit Items
[Coating Inclusion Rate] = 70%
[EPG] = 34.5%

Food Code	Food Name
53201000	Cookie, NFS
53202000	Cookie, almond
53203000	Cookie, applesauce
53203500	Cookie, biscotti (Italian sugar cookie)
53204000	Cookie, brownie, NS as to icing
53204010	Cookie, brownie, without icing
53204100	Cookie, brownie, with icing
53204500	Cookie, brownie, with cream cheese filling, without icing
53204600	Cookie, brownie, with peanut butter fudge icing
53204800	Cookie, brownie, diet, NS as to icing
53204830	Cookie, brownie, lowfat, with icing
53204840	Cookie, brownie, lowfat, without icing
53204850	Cookie, brownie, fat free, cholesterol free, with icing
53204860	Cookie, brownie, fat free, without icing
53205250	Cookie, butterscotch, brownie
53205500	Cookie, butterscotch chip
53205600	Cookie, caramel coated, with nuts
53205750	Cookie, carob
53205760	Cookie, carob and honey brownie
53206000	Cookie, chocolate chip
53206010	Cookie, chocolate chip, with raisins
53206020	Cookie, chocolate chip, made from home recipe or purchased at a bakery
53206030	Cookie, chocolate chip, reduced fat
53206050	Cookie, rich, chocolate chip, with chocolate filling
53206100	Cookie, chocolate chip sandwich
53206500	Cookie, chocolate, made with rice cereal
53206550	Cookie, chocolate, made with oatmeal and coconut (no-bake)
53207000	Cookie, chocolate fudge, with/without nuts
53207050	Cookie, chocolate, with chocolate filling or coating, fat free
53208000	Cookie, chocolate-covered marshmallow
53208200	Cookie, marshmallow pie, chocolate covered
53209000	Cookie, chocolate, chocolate sandwich or chocolate-coated or striped

53209010	Cookie, chocolate-covered, sugar wafer, creme- or caramel-filled
53209020	Cookie, chocolate sandwich, reduced fat
53209050	Cookie, chocolate-covered, chocolate sandwich
53209100	Cookie, chocolate, sandwich, with extra filling
53209500	Cookie, chocolate and vanilla sandwich
53210000	Cookie, chocolate wafer
53210900	Cookie, graham cracker sandwich with chocolate and marshmallow filling
53210910	Cookie, graham cracker with marshmallow
53211000	Cookie bar, with chocolate, nuts, and graham crackers
53215500	Cookie, coconut
53216000	Cookie, coconut and nut
53220000	Cookie, fruit-filled bar
53220010	Cookie, fruit-filled bar, fat free
53220020	Cookie, date bar
53220030	Cookie, fig bar
53220040	Cookie, fig bar, fat free
53223000	Cookie, gingersnaps
53223100	Cookie, granola
53224000	Cookie, ladyfinger
53224250	Cookie, lemon bar
53225000	Cookie, macaroon, coconut-meringue type, no flour
53226000	Cookie, marshmallow, with coconut
53226500	Cookie, marshmallow, with rice cereal (no-bake)
53226550	Cookie, marshmallow, with rice cereal and chocolate chips
53226600	Cookie, marshmallow and peanut butter, with oat cereal (no-bake)
53227000	Cookie, marshmallow pies, non-chocolate coating
53228000	Cookie, meringue
53230000	Cookie, molasses
53231000	Cookie, Lebkuchen
53231400	Cookie, multigrain, high fiber
53233000	Cookie, oatmeal
53233010	Cookie, oatmeal, with raisins
53233020	Cookie, oatmeal, with fruit filling
53233030	Cookie, oatmeal, fat free, with raisins
53233040	Cookie, oatmeal, reduced fat, with raisins
53233050	Cookie, oatmeal sandwich, with creme filling
53233060	Cookie, oatmeal, with chocolate chips
53233080	Cookie, oatmeal sandwich, with peanut butter and jelly filling
53233100	Cookie, oatmeal, with chocolate and peanut butter (no-bake)
53233500	Cookie, oat bran
53234000	Cookie, peanut butter

53234010	Cookie, peanut butter, with oatmeal
53234100	Cookie, peanut butter, with chocolate
53234250	Cookie, peanut butter with rice cereal (no-bake)
53235000	Cookie, peanut
53235500	Cookie, with peanut butter filling, chocolate-coated
53235600	Cookie, Pfeffernusse
53236000	Cookie, pizzelle (Italian style wafer)
53236100	Cookie, pumpkin
53237000	Cookie, raisin
53237010	Cookie, raisin sandwich, cream-filled
53237500	Cookie, rum ball (no-bake)
53238000	Cookie, sandwich-type, not chocolate or vanilla
53239000	Cookie, shortbread
53239010	Cookie, shortbread, reduced fat
53239050	Cookie, shortbread, with chocolate filling
53241500	Cookie, butter or sugar cookie
53241600	Cookie, butter or sugar cookie, with fruit and/or nuts
53242000	Cookie, sugar wafer
53242500	Cookie, toffee bar
53243000	Cookie, vanilla sandwich
53243050	Cookie, vanilla sandwich, reduced fat
53243100	Cookie, rich, all chocolate, with chocolate filling or chocolate chips
53244010	Cookie, butter or sugar, with chocolate icing or filling
53244020	Cookie, butter or sugar, with icing or filling other than chocolate
53245000	Cookie, vanilla waffle creme
53246000	Cookie, tea, Japanese
53247000	Cookie, vanilla wafer
53247050	Cookie, vanilla wafer, reduced fat
53247500	Cookie, vanilla with caramel, coconut, and chocolate coating
53248000	Cookie, whole wheat, dried fruit, nut
53251100	Cookie, rugelach
53260030	Cookie, dietetic, chocolate chip
53260150	Cookie, lemon wafer, lowfat
53260200	Cookie, dietetic, oatmeal with raisins
53260300	Cookie, dietetic, sandwich type
53260400	Cookie, dietetic, sugar or plain
53270100	Cookies, Puerto Rican (Mantecaditos polvorones)
54101010	Cracker, animal
54102010	Crackers, graham
54102020	Crackers, graham, chocolate covered
54102050	Crackers, oatmeal

54102060	Crackers, Cuban
54102070	Crackers, Cuca
54102080	Crackers, graham, with raisins
54102100	Crackers, graham, lowfat
54102110	Crackers, graham, fat free
54102200	Crackers, graham, sandwich-type, with filling
91703200	TWIX Caramel Cookie Bars (formerly TWIX Cookie Bars)
91703250	TWIX Chocolate Fudge Cookie Bars
91703300	TWIX Peanut Butter Cookie Bars

Bakery Items
[Coating Inclusion Rate] = 35%
[EPG] = 34.5%

Food Code	Food Name
52301000	Muffin, NFS
52302010	Muffin, fruit and/or nuts
52302020	Muffin, fruit and/or nut, low fat
52302100	Muffin, fruit, fat free, cholesterol free
52302500	Muffin, chocolate chip
52302600	Muffin, chocolate
52302610	Muffin, chocolate, lowfat
52303010	Muffin, whole wheat
52303500	Muffin, wheat
52303550	Muffin, buckwheat
52304010	Muffin, wheat bran
52304040	Muffin, bran with fruit, lowfat
52304060	Muffin, bran with fruit, no fat, no cholesterol
52304100	Muffin, oatmeal
52304150	Muffin, oat bran
52304200	Muffin, oat bran with fruit and/or nuts
52306010	Muffin, plain
52306300	Muffin, cheese
52306500	Muffin, pumpkin
52306550	Muffin, zucchini
52306700	Muffin, carrot
52307020	Muffin, multigrain, with nuts
52307120	Muffin, multigrain, with fruit
52311010	Popover
53100100	Cake, NS as to type, with or without icing
53101000	Cake, angel food, NS as to icing

53101100	Cake, angel food, without icing
53101200	Cake, angel food, with icing
53101250	Cake, angel food, with fruit and icing or filling
53101300	Cake, angel food, chocolate, without icing
53102000	Cake, applesauce, NS as to icing
53102100	Cake, applesauce, without icing
53102200	Cake, applesauce, with icing
53102300	Cake, applesauce, diet, without icing
53102500	Cake, banana, NS as to icing
53102600	Cake, banana, without icing
53102700	Cake, banana, with icing
53102800	Cake, black forest (chocolate-cherry)
53103000	Cake, Boston cream pie
53103500	Cake, butter, NS as to icing
53103550	Cake, butter, without icing
53103600	Cake, butter, with icing
53104000	Cake, carrot, NS as to icing
53104100	Cake, carrot, without icing
53104260	Cake, carrot, with icing
53104300	Cake, carrot, diet
53104400	Cake, coconut, with icing
53105050	Cake, chocolate, devil's food, or fudge, made from home recipe or purchased ready-to-eat, NS as to icing
53105160	Cake, chocolate, devil's food, or fudge, without icing or filling, made from home recipe or purchased ready-to-eat
53105260	Cake, chocolate, devil's food, or fudge, with icing, coating, or filling, made from home recipe or purchased ready-to-eat
53105300	Cake, German chocolate, with icing and filling
53105500	Cake, chocolate, with icing, diet
53106100	Cake, Poor Man's (spice-type), without icing
53106500	Cake, cream, without icing or topping
53107000	Cake, cupcake, NS as to type or icing
53107100	Cake, cupcake, NS as to type, without icing
53107200	Cake, cupcake, NS as to type, with icing
53108000	Cake, cupcake, chocolate, NS as to icing
53108100	Cake, cupcake, chocolate, without icing or filling
53108200	Cake, cupcake, chocolate, with icing or filling
53109000	Cake, cupcake, not chocolate, NS as to icing
53109100	Cake, cupcake, not chocolate, without icing or filling
53109200	Cake, cupcake, not chocolate, with icing or filling
53109210	Cake, cupcake, not chocolate, with icing or filling, lowfat, cholesterol free
53109250	Cake, cupcake, not chocolate, with fruit and cream filling

53109270	Cake, cupcake, chocolate, with or without icing, fruit filling or cream filling, lowfat, cholesterol free
53109300	Cake, Dobos Torte (non-chocolate layer cake with chocolate filling and icing)
53110000	Cake, fruit cake, light or dark, holiday type cake
53110100	Cake, plum pudding
53111000	Cake, gingerbread, without icing
53111500	Cake, graham cracker, without icing
53112000	Cake, ice cream and cake roll, chocolate
53112100	Cake, ice cream and cake roll, not chocolate
53112150	Cake, frozen yogurt and cake layer, not chocolate, with icing
53112160	Cake, frozen yogurt and cake layer, chocolate, with icing
53113000	Cake, jelly roll
53113950	Cake, lemon, NS as to icing
53114000	Cake, lemon, without icing
53114100	Cake, lemon, with icing
53114150	Cake, lemon, lowfat, NS as to icing
53114200	Cake, lemon, lowfat, without icing
53114250	Cake, lemon, lowfat, with icing
53115000	Cake, marble, NS as to icing
53115100	Cake, marble, without icing
53115200	Cake, marble, with icing
53115300	Cake, nut, NS as to icing
53115310	Cake, nut, without icing
53115320	Cake, nut, with icing
53115400	Cake, oatmeal, without icing
53115410	Cake, oatmeal, with icing
53115450	Cake, peanut butter, with icing
53115500	Cake, pineapple, fat free, cholesterol free, without icing
53115600	Cake, poppyseed, without icing
53116000	Cake, pound, without icing
53116020	Cake, pound, with icing
53116270	Cake, pound, chocolate
53116280	Cake, pound, chocolate, fat free, cholesterol free
53116350	Cake, pound, Puerto Rican style (Ponque)
53116380	Cake, pound, fat free, cholesterol free
53116390	Cake, pound, reduced fat, cholesterol free
53116490	Cake, pumpkin, NS as to icing
53116500	Cake, pumpkin, without icing
53116510	Cake, pumpkin, with icing
53116550	Cake, raisin-nut, without icing
53116560	Cake, raisin-nut, with icing

53116570	Cake, Ravani (made with farina)
53116600	Cake, rice flour, without icing
53116650	Cake, Quezadilla, El Salvadorian style
53116750	Cake, soy flour, without icing
53117000	Cake, spice, NS as to icing
53117100	Cake, spice, without icing
53117200	Cake, spice, with icing
53118000	Cake, sponge, NS as to icing
53118100	Cake, sponge, without icing
53118200	Cake, sponge, with icing
53118300	Cake, sponge, chocolate, without icing
53118310	Cake, sponge, chocolate, with icing
53118350	Cake, sweetpotato, with icing
53118410	Cake, rum flavored, without icing (Sopa Borracha)
53118500	Cake, torte
53118550	Cake, tres leche
53118600	Cake, chiffon, NS as to icing
53118700	Cake, chiffon, without icing
53118800	Cake, chiffon, with icing
53118900	Cake, chiffon, chocolate, without icing
53118950	Cake, chiffon, chocolate, with icing
53119000	Cake, upside down (all fruits)
53120060	Cake, white, made from home recipe or purchased ready-to-eat, NS as to icing
53120160	Cake, white, without icing, made from home recipe or purchased ready-to-eat
53120260	Cake, white, with icing, made from home recipe or purchased ready-to-eat
53120400	Cake, white, eggless, lowfat
53120500	Cake, whole wheat, with fruit and nuts, without icing
53121060	Cake, yellow, made from home recipe or purchased ready-to- eat, NS as to icing
53121160	Cake, yellow, without icing, made from home recipe or purchased ready-to-eat
53121260	Cake, yellow, with icing, made from home recipe or purchased ready-to-eat
53122070	Cake, shortcake, biscuit type, with whipped cream and fruit
53122080	Cake, shortcake, biscuit type, with fruit
53123070	Cake, shortcake, sponge type, with whipped cream and fruit
53123080	Cake, shortcake, sponge type, with fruit
53123500	Cake, shortcake, with whipped topping and fruit, diet
53124100	Cake, zucchini, NS as to icing
53124110	Cake, zucchini, without icing
53124120	Cake, zucchini, with icing
53420000	Cream puff, eclair, custard or cream filled, NS as to icing
53420100	Cream puff, eclair, custard or cream filled, not iced
53420200	Cream puff, eclair, custard or cream filled, iced

53420210	Cream puff, eclair, custard or cream filled, iced, reduced fat
53420250	Cream puff, no filling or icing
53440000	Strudel, apple
53440300	Strudel, berry
53440500	Strudel, cherry
53440600	Strudel, cheese
53440700	Strudel, peach
53440750	Strudel, pineapple
53440800	Strudel, cheese and fruit
53450000	Turnover or dumpling, apple
53450300	Turnover or dumpling, berry
53450500	Turnover or dumpling, cherry
53450800	Turnover or dumpling, lemon
53451000	Turnover or dumpling, peach
53451500	Turnover, guava
53451750	Turnover, pumpkin
53452400	Pastry, puff
53452420	Pastry, puff, custard or cream filled, iced or not iced
53500100	Breakfast pastry, NFS
53510000	Danish pastry, plain or spice
53510100	Danish pastry, with fruit
53510200	Danish pastry, with nuts
53511000	Danish pastry, with cheese
53511500	Danish pastry, with cheese, fat free, cholesterol free
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, oriental
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
53521250	Doughnut, wheat
53521300	Doughnut, wheat, chocolate covered
53530000	Breakfast tart
53530010	Breakfast tart, lowfat
55201000	Waffle, plain
55202000	Waffle, wheat, bran, or multigrain
55203000	Waffle, fruit
55203500	Waffle, nut and honey
55203600	Waffle, chocolate chip
55204000	Waffle, cornmeal
55205000	Waffle, 100% whole wheat or 100% whole grain
55206000	Waffle, oat bran
55207000	Waffle, multi-bran
55211000	Waffle, plain, fat free
55211050	Waffle, plain, lowfat

Ice Cream Inclusion
[Coating Inclusion Rate] = 10%
[EPG] = 30%

Mixed Foods containing Ice Cream Inclusion
Adjusted for Ice Cream Inclusion content of 5.7 to 58.7%

Food Code	Food Name
11461250	Yogurt, frozen, cone, chocolate
11461260	Yogurt, frozen, cone, flavors other than chocolate
11461270	Yogurt, frozen, cone, flavors other than chocolate, lowfat milk
11461280	Yogurt, frozen, cone, chocolate, lowfat milk
13120700	Ice cream cone with nuts, flavors other than chocolate
13120710	Ice cream cone, chocolate covered, with nuts, flavors other than chocolate
13120720	Ice cream cone, chocolate covered or dipped, flavors other than chocolate
13120730	Ice cream cone, no topping, flavors other than chocolate
13120750	Ice cream cone with nuts, chocolate ice cream
13120760	Ice cream cone, chocolate covered or dipped, chocolate ice cream
13120770	Ice cream cone, no topping, chocolate ice cream
13120780	Ice cream cone, chocolate covered, with nuts, chocolate ice cream
13121300	Ice cream sundae, chocolate or fudge topping, with whipped cream
13121500	Ice cream sundae, fudge topping, with cake, with whipped cream
13130620	Light ice cream, soft serve cone, flavors other than chocolate (formerly ice milk)
13130630	Light ice cream, soft serve cone, chocolate (formerly ice milk)
13130640	Light ice cream, soft serve cone, NS as to flavor (formerly ice milk)

13130700	Light ice cream, soft serve, blended with candy or cookies
13140450	Light ice cream, cone, NFS (formerly ice milk)
13140500	Light ice cream, cone, flavors other than chocolate (formerly ice milk)
13140550	Light ice cream, cone, chocolate (formerly ice milk)
13140600	Light ice cream, sundae, soft serve, chocolate or fudge topping, with whipped cream (formerly ice milk)
13140630	Light ice cream, sundae, soft serve, fruit topping, with whipped cream (formerly ice milk)
13140660	Light ice cream, sundae, soft serve, chocolate or fudge topping (without whipped cream) (formerly ice milk)
13140670	Light ice cream, sundae, soft serve, fruit topping (without whipped cream) (formerly ice milk)

Frozen Dairy Desserts and Mixes

Ice Cream Novelty
[Coating Inclusion Rate] = 40%
[EPG] = 34.5%

Food Code	Food Name
13120050	Ice cream bar or stick, not chocolate covered or cake covered
13120100	Ice cream bar or stick, chocolate covered
13120110	Ice cream bar or stick, chocolate or caramel covered, with nuts
13120120	Ice cream bar or stick, rich chocolate ice cream, thick chocolate covering
13120121	Ice cream bar or stick, rich ice cream, thick chocolate covering
13120130	Ice cream bar or stick, rich ice cream, chocolate covered, with nuts
13120140	Ice cream bar or stick, chocolate ice cream, chocolate covered
13120300	Ice cream bar, cake covered
13120400	Ice cream bar or stick with fruit
13120500	Ice cream sandwich
13120550	Ice cream cookie sandwich
13121200	Ice cream sundae, prepackaged type, flavors other than chocolate
13122100	Ice cream pie, no crust
13122500	Ice cream pie, with cookie crust, fudge topping, and whipped cream
13135000	Ice cream sandwich, made with light ice cream, flavors other than chocolate
13135010	Ice cream sandwich, made with light chocolate ice cream
13136000	Ice cream sandwich, made with light, no sugar added ice cream
13140100	Light ice cream, bar or stick, chocolate-coated (formerly ice milk)
13140110	Light ice cream, bar or stick, chocolate covered, with nuts (formerly ice milk)
13140700	Light ice cream, creamsicle or dreamsicle (formerly ice milk)
13140900	Light ice cream, fudgesicle (formerly ice milk)
13142000	Milk dessert bar or stick, frozen, with coconut
13161000	Milk dessert bar, frozen, made from lowfat milk
13161500	Milk dessert sandwich bar, frozen, made from lowfat milk

13161520	Milk dessert sandwich bar, frozen, with low-calorie sweetener, made from lowfat milk
13161600	Milk dessert bar, frozen, made from lowfat milk and low calorie sweetener
13161630	Light ice cream, bar or stick, with low-calorie sweetener, chocolate-coated (formerly ice milk)

Grain Products and Pasta

Snack and Meal Replacement Bars
[Coating Inclusion Rate] = 30%
[EPG] = 34.5%

Food Code	Food Name
53540000	Breakfast bar, NFS
53540200	Breakfast bar, cereal crust with fruit filling, lowfat
53540300	Fiber One Chewy Bar
53540400	Kellogg's Nutri-Grain Cereal Bar
53540402	Kellogg's Nutri-Grain Yogurt Bar
53540404	Kellogg's Nutri-Grain Fruit and Nut Bar
53540500	Breakfast bar, date, with yogurt coating
53540600	Milk 'n Cereal bar
53540700	Kellogg's Special K bar
53540800	Kashi GOLEAN Chewy Bars
53540802	Kashi TLC Chewy Granola Bar
53540804	Kashi GOLEAN Crunchy Bars
53540806	Kashi TLC Crunchy Granola Bar
53540900	Nature Valley Chewy Trail Mix Granola Bar
53540902	Nature Valley Chewy Granola Bar with Yogurt Coating
53540904	Nature Valley Sweet and Salty Nut Granola Bar
53540906	Nature Valley Crunchy Granola Bar
53541000	Quaker Chewy Granola Bar
53541002	Quaker Chewy 90 Calorie Granola Bar
53541004	Quaker Chewy 25% Less Sugar Granola Bar
53541006	Quaker Chewy Dipps Granola Bar
53541200	Meal replacement bar
53541300	Slim Fast Original Meal Bar
53542000	Snack bar, oatmeal
53542100	Granola bar, NFS
53542200	Granola bar, lowfat, NFS
53542210	Granola bar, nonfat
53543000	Granola bar, reduced sugar, NFS
53543100	Granola bar, peanuts, oats, sugar, wheat germ
53544200	Granola bar, chocolate-coated, NFS

53544210	Granola bar, with coconut, chocolate-coated
53544220	Granola bar with nuts, chocolate-coated
53544230	Granola bar, oats, nuts, coated with non-chocolate coating
53544250	Granola bar, coated with non-chocolate coating
53544300	Granola bar, high fiber, coated with non-chocolate yogurt coating
53544400	Granola bar, with rice cereal
53544410	Quaker Granola Bites
53544450	PowerBar (fortified high energy bar)
91780010	Snickers Marathon Energy bar
91781010	Snickers Marathon Protein bar

Hard Candy and Cough Drops

Panned Confectionery Items
[Coating Inclusion Rate] = 70%
[EPG] = 34.5%

Mixed Foods containing Panned Confectionery Items
Adjusted for Panned Confectionery Items content of 35 to 90%

Food Code	Food Name
54403110	Popcorn, sugar syrup or caramel-coated
54403120	Popcorn, sugar syrup or caramel-coated, with nuts
54403150	Popcorn, sugar syrup or caramel-coated, fat free
91701010	Almonds, chocolate covered
91703040	Caramel candy, chocolate covered
91703060	Caramel with nuts, chocolate covered
91703500	Nuts, carob-coated
91703600	Espresso coffee beans, chocolate-covered
91706000	Coconut candy, chocolate covered
91709000	Gumdrops, chocolate covered
91713010	Fudge, chocolate, chocolate-coated
91713020	Fudge, chocolate, chocolate-coated, with nuts
91715000	Fudge, caramel and nut, chocolate-coated candy
91716110	Halvah, chocolate covered
91718050	Honey-combed hard candy with peanut butter, chocolate covered
91723010	Marshmallow, chocolate covered
91726410	Nougat, chocolate covered
91727010	Nuts, chocolate covered, not almonds or peanuts
91731000	Peanuts, chocolate covered
91734000	Peanut butter, chocolate covered
91760100	Toffee, chocolate covered

91760200	Toffee, chocolate-coated, with nuts
91770030	Dietetic or low calorie candy, chocolate covered

Sugar Shelled Confectionery Pieces
[Coating Inclusion Rate] = 70%
[EPG] = 30%

Mixed Foods containing Sugar Shelled Confectionery Pieces
Adjusted for Sugar Shelled Confectionery Pieces content of 45 to 80%

Food Code	Food Name
91701020	Almonds, sugar-coated
91701030	Almonds, yogurt-covered
91723020	Marshmallow, candy-coated
91723050	Marshmallow, coconut-coated
91728500	Sugared pecans (sugar and egg white coating)
91731010	M&M's Peanut Chocolate Candies
91731060	M&M's Peanut Butter Chocolate Candies
91731100	Peanuts, sugar-coated
91731150	Peanuts, yogurt covered
91746010	Sugar-coated chocolate discs
91746100	M&M's Milk Chocolate Candies (formerly M&M's Plain Chocolate Candies)

Processed Fruits and Juices

Panned Fruit Pieces
[Coating Inclusion Rate] = 60%
[EPG] = 34.5%

Food Code	Food Name
62101000	Fruit, dried, NFS (assume uncooked)
62101050	Fruit mixture, dried (mixture includes three or more of the following: apples, apricots, dates, papaya, peaches, pears, pineappl
62101100	Apple, dried, uncooked
62101150	Apple, dried, uncooked, low sodium
62101300	Apple chips
62104100	Apricot, dried, uncooked
62107200	Banana chips
62108100	Currants, dried
62109100	Cranberries, dried
62114050	Mango, dried
62114110	Papaya, dried
62116100	Peach, dried, uncooked

62119100	Pear, dried, uncooked
62120100	Pineapple, dried
62125100	Raisins
62125110	Raisins, cooked
91708020	Soft fruit confections
91736000	Pineapple candy, Puerto Rican style

Mixed Foods containing Panned Fruit Pieces
Adjusted for Panned Fruit Pieces content of 58 to 79%

Food Code	Food Name
63401070	Fruit, chocolate covered
63401990	Banana, chocolate-covered with nuts
91708150	Yogurt covered fruit snacks candy, with added vitamin C
91708160	Yogurt covered fruit snacks candy rolls, with high vitamin C
91739010	Raisins, chocolate covered
91739510	Raisins, carob covered
91739600	Raisins, yogurt covered

Snack Foods

Snack Items
[Coating Inclusion Rate] = 70%
[EPG] = 34.5%

Food Code	Food Name
54408000	Pretzels, NFS
54408010	Pretzels, hard
54408020	Pretzels, soft
54408030	Pretzel, hard, unsalted
54408040	Pretzels, soft, unsalted
54408050	Pretzel, oatbran, hard
54408070	Pretzel, hard, multigrain
54408200	Pretzel, hard, chocolate-coated
54408250	Pretzel, yogurt-covered
54408300	Pretzels, cheese-filled

Mixed Foods containing Snack Items
Adjusted for Snack Items content of 15.7 to 19.2%

Food Code	Food Name
54402200	Salty snack mixture, mostly corn or cornmeal based, with pretzels, without nuts
54420010	Multigrain mixture, pretzels, cereal and/or crackers, nuts
54420200	Multigrain mixture, bread sticks, sesame nuggets, pretzels, rye chips

Trail mix
[Coating Inclusion Rate] = 30%
[EPG] = 30%

Food Code	Food Name
42501000	Nut mixture with dried fruit and seeds
42502000	Nut mixture with seeds
54420100	Oriental party mix, with peanuts, sesame sticks, chili rice crackers and fried green peas

SUBMISSION END