GRAS Opinion Statement Concerning the Generally Recognized as Safe (GRAS) Status of Esterified Propoxylated Glycerol for Use in Additional Conventional Food Categories

29 April 2020

INTRODUCTION

Choco Finesse (now Epogee, LLC) previously submitted 3 notifications of Generally Recognized as Safe (GRAS) status to the United States (U.S.) Food and Drug Administration (FDA) for the use of esterified propoxylated glycerol (EPOGEE, formerly EPG) (i) as a fat replacer at levels up to 34.5% (w/w) in confectionary applications (GRN No. 583); (ii) as a fat replacer at levels up to 38% (w/w expressed on a fat basis) in spreadable and baked goods (GRN No. 640); and (iii) as a fry oil in commercial French fries and doughnut production (GRN No. 761). The U.S. FDA reviewed these GRAS notifications with "no resulting questions" (U.S. FDA, 2015, 2016, 2018). Epogee, LLC now seeks to add use in selected additional food categories.

At the request of Epogee, LLC, a panel of independent scientists (the "GRAS Panel"), qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, and who had participated in the previous GRAS evaluations of EPOGEE, was convened to conduct a critical and comprehensive evaluation of the available pertinent data and information and to determine whether, for the additional conditions of intended use in non-milk-based protein drinks, non-milk-based meal replacement beverages, specialty coffee drinks, non-dairy milk and cream, protein drinks (milk-based), meat analogs, chicken nuggets, corn-based savory snacks, and potato chips, EPOGEE would be GRAS, based on scientific procedures.

The GRAS Panel consisted of the below-signed qualified scientific experts: Dr. John A. Thomas (consultant) and scientific advisor, Dr. David H. Bechtel (Bechtel Consulting, Inc). The GRAS Panel was selected and convened in accordance with the U.S. FDA's guidance for industry on *Best Practices for Convening a GRAS Panel* (U.S. FDA, 2017). Epogee, LLC ensured that all reasonable efforts were made to identify and select a balanced GRAS Panel with expertise in food safety and toxicology. Efforts were placed on identifying conflicts of interest or relevant "appearance issues" that could potentially bias the outcome of the deliberations of the GRAS Panel; no such conflicts of interest or "appearance issues" were identified. The GRAS Panel received a reasonable honorarium as compensation for their time; the honoraria provided to the Panel were not contingent upon the outcome of their deliberations.

The GRAS Panel, independently and collectively, critically examined a comprehensive package of publicly available scientific information and data compiled from the literature and other published sources based on searches of the published scientific literature conducted through April 2020. In addition, the GRAS Panel evaluated other information deemed appropriate or necessary, including data and information provided by Epogee, LLC, as well as information contained in the 3 previous GRAS Notifications. The data evaluated by the GRAS Panel included information pertaining to the method of manufacture and product specifications, analytical data, intended use levels in specified food products, consumption estimates for all intended uses, and comprehensive literature on the safety EPOGEE.

Following independent, critical evaluation of such data and information, the GRAS Panel unanimously concluded that for the additional conditions of intended use in non-milk-based protein drinks, non-milk-based meal replacement beverages, specialty coffee drinks, non-dairy milk and cream, protein drinks (milk-based), meat analogs, chicken nuggets, corn-based savory snacks, and potato chips, described herein, EPOGEE, meeting appropriate food-grade specifications and manufactured and used in accordance with current Good Manufacturing Practice (cGMP), is GRAS based on scientific procedures. A summary of the basis for the GRAS Panel's conclusion, excluding confidential data and information, is provided below.

COMPOSITION, MANUFACTURING, AND SPECIFICATIONS

EPOGEE is a family of fat- and oil-like substances that resembles triglycerides in structure and appearance but 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 EPOGEE can be produced through modification of the fatty acid moieties of the triglyceride and the extent of the propoxylation of the glycerol. However, all food use versions conform to the specifications in Table A-1, provided in Appendix A.

EPOGEE is manufactured in compliance with cGMP regulations. The production of EPOGEE consists of 2 basic processes: (i) propoxylation of glycerol; and (ii) 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 EPOGEE by molecular distillation. Batch analysis data demonstrate that this manufacturing process produces a consistent product that meets specifications. EPOGEE has been shown to resist oxidation and thermal decomposition as well as or better than current edible fats, oils, and shortenings as measured by the oxidative stability index and the smoke and flash point. Decomposition products are similar to those seen with other unsaturated fatty acids.

INTENDED USE AND ESTIMATED EXPOSURE

EPOGEE (formerly EPG) has been notified to be GRAS for use in confectionary coatings (GRN No. 583), as a fat replacer (GRN No. 640), and as a fry oil (GRN No. 761) (Choco Finesse, LLC, 2015, 2016, 2018). EPOGEE is now proposed for additional uses, for use as a fat replacer, as summarized in Table 1 below.

Table 1 Summary of the Individual Proposed Food Uses and Use Levels for Esterified Propoxylated Glycerol in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2019)	Food Uses[a]	EPOGEE Fat Replacement Use Levels (%)
Beverages and Beverage Bases	Protein drinks (non-milk-based)	50
	Non-milk-based meal replacement beverages	75
Coffee and Tea	Specialty coffee drinks (lattes, cappuccinos, mochas)	71
Dairy Product Analogs	Non-dairy milk and cream	75
Milk Products	Protein drinks (milk-based)	50
Plant Protein Products	Meat analogs	50
Snack Foods	Chicken nuggets	60
	Corn-based savory snacks	75
	Potato chips	75

Table 1 Summary of the Individual Proposed Food Uses and Use Levels for Esterified Propoxylated Glycerol in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2019)	Food Uses[a]	EPOGEE Fat Replacement Use Levels (%)

CFR = Code of Federal Regulations; EPOGEE = esterified propoxylated glycerol; U.S. = United States.
[a] EPOGEE is intended for use in unstandardized products when standards of identity, as established under 21 CFR §130 to 169, do not permit its addition.

Epogee, LLC estimated the *per capita* and consumer-only intakes of EPOGEE for specific demographic groups and for the total U.S. population using consumption data from *the National Health and Nutrition Examination Survey (NHANES): 2015-2016* (CDC, 2018) and information pertaining to the individual proposed food uses of EPOGEE are summarized in Table 2. Estimates for the total daily intakes of EPOGEE from proposed food uses are provided in Tables 3 and 4 on an absolute (g/person/day) and per body weight basis (mg/kg body weight/day), respectively. The consumer-only intakes are more applicable to the assessment of safety as they are more likely to represent exposure in the target populations. Consequently, only the consumer-only intake results will be discussed below. On a consumer-only basis, the resulting mean and 90th percentile intakes of EPOGEE by the total U.S. population from all proposed food uses, were estimated to be 5.8 and 13.2 g/person/day, respectively. Of the individual population groups, male teenagers were determined to have the greatest mean consumer-only intakes of EPOGEE on an absolute basis, at 7.7 g/person/day. The highest 90th percentile intake was observed in male adults, at 14.7 g/person/day. Infants had the lowest mean and 90th percentile consumer-only intakes 3.8 and 7.6 g/person/day, respectively.

Table 2 Summary of the Estimated Daily Intake of Esterified Propoxylated Glycerol from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 3	1.8	5.9	47.3	304	3.8	7.6
Children	4 to 11	4.1	9.9	70.0	742	5.9	11.7
Female Teenagers	12 to 19	5.0	12.2	72.8	332	6.9	14.6
Male Teenagers	12 to 19	4.8	11.7	62.9	317	7.7	14.4
Female Adults	20 and up	3.3	9.0	67.6	1,412	4.8	10.5
Male Adults	20 and up	4.1	11.9	61.7	1,156	6.6	14.7
Total Population	All ages	3.8	10.3	64.7	4,263	5.8	13.2

n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

On a body weight basis, the total population (all ages) mean and 90th percentile consumer-only intakes of EPOGEE were determined to be 99 and 226 mg/kg body weight/day, respectively. Among the individual population groups, infants were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 278 and 541 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 66 and 151 mg/kg body weight/day, respectively (Table 3).

Table 3 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Esterified Propoxylated Glycerol from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 3	131	423	47.3	302	278	541
Children	4 to 11	149	375	70.1	741	212	438
Female Teenagers	12 to 19	90	221	72.8	326	123	251
Male Teenagers	12 to 19	75	184	62.8	316	120	237
Female Adults	20 and up	45	118	67.6	1,404	66	151
Male Adults	20 and up	46	133	61.7	1,143	75	162
Total Population	All ages	64	172	64.7	4,232	99	226

bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

A cumulative intake assessment was conducted encompassing these uses, as well as the current commercial applications included in the previous GRAS Notices. As shown in Tables 4 and 5, the cumulative mean and 90th percentile consumer-only intakes of EPOGEE were estimated to be 14.5 and 29.8 g/person/day, respectively, equivalent to intakes of 258 and 581 mg/kg body weight/day on a body weight basis.

Table 4 Summary of the Estimated Cumulative Daily Intake of Esterified Propoxylated Glycerol from All GRAS Notified and Proposed Applications in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)					
		Mean	90th Percentile	%	n	Mean	*Mean (Net Change from 2017 Cumulative Assessment)*[a]	90th Percentile	*90th Percentile (Net Change from 2017 Cumulative Assessment)*[a]
Infants and Young Children	0 to 3	7.3	16.9	75.9	516	9.7	*+2.0*	18.6	*+3.2*
Children	4 to 11	16.3	31.5	96.4	1,027	16.9	*+3.8*	31.6	*+5.7*
Female Teenagers	12 to 19	15.6	32.0	97.1	456	16.1	*+4.3*	33.0	*+8.6*
Male Teenagers	12 to 19	16.6	33.8	91.7	447	18.1	*+4.0*	36.9	*+9.8*
Female Adults	20 and up	12.4	27.6	95.0	2,068	13.1	*+2.5*	27.9	*+3.6*
Male Adults	20 and up	14.0	30.1	92.1	1,807	15.1	*+3.3*	31.0	*+5.2*
Total Population	All ages	13.5	29.0	93.1	6,321	14.5	*+3.1*	29.8	*+4.8*

GRAS = Generally Recognized as Safe; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
[a] The 2017 cumulative assessment was originally conducted using the NHANES 2011-2012. However, recently, this cumulative assessment has been updated using the latest NHANES 2015-2016 and the net change is calculated from the results provided in Table B-1 of Appendix B.

Table 5 Summary of the Estimated Cumulative Daily Per Kilogram Body Weight Intake of Esterified Propoxylated Glycerol from All GRAS Notified and Proposed Applications in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)					
		Mean	90th Percentile	%	n	Mean	*Mean (Net Change from 2017 Assessment)*[a]	90th Percentile	*90th Percentile (Net Change from 2017 Assessment)*[a]
Infants and Young Children	0 to 3	538	1,263	75.7	511	710	*+144*	1,466	*+258*
Children	4 to 11	596	1,163	96.4	1,025	618	*+137*	1,170	*+195*
Female Teenagers	12 to 19	275	549	97.0	448	283	*+78*	553	*+134*
Male Teenagers	12 to 19	262	550	91.6	446	286	*+61*	571	*+120*
Female Adults	20 and up	170	374	95.1	2,055	179	*+34*	381	*+46*
Male Adults	20 and up	163	353	92.3	1,784	176	*+37*	365	*+49*
Total Population	All ages	240	562	93.2	6,269	258	*+52*	581	*+99*

bw = body weight; GRAS = Generally Recognized as Safe; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

[a] The 2017 cumulative assessment was originally conducted using the NHANES 2011-2012. However, recently, this cumulative assessment has been updated using the latest NHANES 2015-2016 and the net change is calculated from the results provided in Table B-2 of Appendix B.

Tocopherol Exposure

Using the above cumulative exposure estimates, Epogee, LLC calculates that if tocopherols is present in a final food product at the maximum specified level of 1,300 mg/kg EPOGEE, the estimated mean and 90th percentile exposures to tocopherol for all-users would be 18.9 mg/person/day and 38.7 mg/person/day, respectively, for the total U.S. population. These values represent approximately 6% and 12% of the upper intake levels (ULs) for vitamin E (as d-α-tocopherol) of 300 mg/day, set by the Scientific Committee on Food (SCF, 2003).

DATA PERTAINING TO SAFETY

The safety of EPOGEE is based on published subchronic studies in rats and micropigs, a 1-generation reproductive toxicity study in rats, a developmental toxicity evaluation in rabbits, and genotoxicity studies of EPG.[1] Unpublished studies (*i.e.*, absorption, distribution, metabolism, and excretion studies, subchronic toxicity studies in mice and dogs, 2-year combined chronic dietary safety study and carcinogenicity studies in rats and mice, a 1-year chronic safety studies micropigs, a 3-generation reproduction study [with a teratology phase] in rats, and irritation and sensitization) were available and reviewed by the panel, and

[1] Within this section, "EPG" will be used to maintain consistency with the nomenclature in the published studies referenced herein.

providing corroborative evidence of safety. All of these studies have been detailed in the previous GRAS Notifications; key studies are reiterated here for completeness.

Absorption, Distribution, Metabolism, and Excretion (ADME)

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

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

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

In the 2 studies where the propoxylated glycerol units were radiolabeled, small amounts of radiolabel were detected in the liver and other metabolically active tissues, indicating that a small portion of this material was assimilated into normal body constituents during the oxidation process. This ADME pattern for EPG was considered predictable and similar to that which would be expected from normal triglycerides. When given intravenously in fine liposome emulsion, the 2 versions of EPGs tested were rapidly oxidized to fatty acids and glycerol containing propoxylated glycerol units. The disposition pattern was similar to that *via* the oral route, except that larger portions of the metabolites of the EPGs were deposited in the liver and lungs. The route by which the metabolites of the various versions of EPGs were excreted appeared to be governed by their molecular weights. Namely, the greater the molecular weight, the more of the metabolites of the EPGs excreted into the feces, and the less into the urine (See additional supporting documentation in GRAS Notice 583, 640, and 761).

Toxicological Studies

Preclinical studies were conducted with H-EPG-05 HR/SO 9:1 (Mettler dropping point 106.9°F) unless otherwise stated. EPG-05 HR/ST 45:55 (Mettler dropping point 104.3°F) is a softer version at average normal body temperature and was selectively investigated in safety studies. It is worthwhile to note that, unlike olestra, EPG is not strongly hydrophobic and exhibits far less interaction with fat-soluble

substances including fat-soluble vitamins. As such, vitamin fortification of animal diets was not required in any of the EPG preclinical safety studies including lifetime studies in rats and mice as well as up to 3 generations in reproductive and development studies. This differed from studies conducted with olestra, which required vitamin fortification. It is also important to note that residues of intact EPG were not found in any tissues from any animals placed on study, indicating efficient clearance and absence of accumulation even following lifetime administration. In addition, the stability and homogeneity of prepared diets for the safety studies was established.

Subchronic and Chronic Studies

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

Results of the above 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-observed-effect level (NOEL). The possible effect of EPG on vitamin levels in the absence of any clinical signs of deficiency was not considered "adverse" *per se*. As such, the adjusted concentration of 2 g/kg and the fixed intake of 5% EPG (equivalent to an average EPG intake of approximately 6 g/kg body weight/day in the beginning of the study and declining to approximately 2 g/kg body weight/day) were considered to represent no-observed-adverse-effect levels (NOAELs).

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

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

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

effects. Chemical analysis of liver, kidney, spleen, and adipose tissue yielded negative data for EPG residue. Gross necropsy and histopathology examinations indicated no treatment-related effects.

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

Reproductive and Developmental Studies

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

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

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

Based on the results of this study in rats, the NOAEL was 5.0% EPG. Also, in the absence of any effects on behavioral development, immunologic status, and blood clotting, and with group 4 animals tolerating a

fixed dietary EPG percentage, it was recommended that the 3-generation study with 2 litters per generation utilize fixed dietary percentages with the highest concentration 5.0% EPG, and endpoints examined not include behavioral, immunologic, or coagulation assessments.

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

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

Genotoxicity Studies

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

Clinical Studies

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

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

and tolerability. Tolerability was assessed by the incidence of 14 specific gastrointestinal adverse events: passing gas; gas with discharge; abdominal bloat/cramp; heartburn; diarrhea; constipation; urgency of bowel movement; fecal incontinence; oily spotting; oily evacuation; oily stool; liquid stool; soft stool; and hard stool.

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

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, significant decreases in mean α-tocopherol levels from baseline were see 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 ($p=0.0166$) and Day 42 ($p=0.0030$) compared to the placebo group. No other statistically significant differences in the change from baseline were noted between the EPG groups and the placebo group at Days 14, 28, 42, and 56. The end point analysis using the last observation carried forward was similar to the Day 56 results for each treatment group.

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

EPG intake was associated with a slight decline in phylloquinone levels across all groups. However, the declines did not exceed 0.1 ng/mL and were not statistically significant within any of the individual groups. Small statistically significant declines were 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. Importantly, 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 some gastrointestinal discomfort, all adverse events reported 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 gastrointestinal symptoms correlated with EPG dietary concentration. The results suggest 10 g/day of EPG was reasonably well tolerated.

SUMMARY

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

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

Studies examining the influence of physical state, consumed mass, and solubility properties of EPOGEE 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 (i) 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 EPOGEE) and moderation of consumption; and (ii) 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 EPOGEE (log Kow of approximately 3.2 to 3.4), and in strong contrast to the interaction of fat mimetics, such as olestra which have a log Kow in excess of 40.

CONCLUSION

We, the undersigned independent qualified members of the Generally Recognized as Safe (GRAS) Panel, have independently and collectively, critically evaluated the data and information summarized above that is pertinent to the safety of the proposed use of EPOGEE (formerly EPG). We unanimously conclude that EPOGEE, meeting appropriate food-grade specifications and produced in accordance with Current Good Manufacturing Practice (cGMP), is GRAS based on scientific procedures under the conditions of intended use in the additional foods specified herein.

It is our professional opinion that other qualified experts would also concur with this conclusion.

John A. Thomas

5/5/2020

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

Date

David H. Bechtel, Ph.D., DABT
President,
Bechtel Consulting, Inc.
Monroe, NJ

Date

CONCLUSION

We, the undersigned independent qualified members of the Generally Recognized as Safe (GRAS) Panel, have independently and collectively, critically evaluated the data and information summarized above that is pertinent to the safety of the proposed use of EPOGEE (formerly EPG). We unanimously conclude that EPOGEE, meeting appropriate food-grade specifications and produced in accordance with Current Good Manufacturing Practice (cGMP), is GRAS based on scientific procedures under the conditions of intended use in the additional foods specified herein.

It is our professional opinion that other qualified experts would also concur with this conclusion.

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

Date

David H Bechtel

David H. Bechtel, Ph.D., DABT
President,
Bechtel Consulting, Inc.
Monroe, NJ

May 01, 2020
Date

REFERENCES

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

CDC (2018). *National Health and Nutrition Examination Survey (NHANES): 2015-2016*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/ContinuousNhanes/Default.aspx?BeginYear=2015.

Choco Finesse, LLC (2015). *Summary of Data Supporting a Determination That the Use of Esterified Propoxylated Glycerol (EPG) in Confectionary Foods is Generally Recognized as Safe (GRAS)*. (Submitted as U.S. FDA, 2015 - GRN 583). Prepared by Indianapolis (IN): Choco Finesse, LLC for submission to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=583 [FDA had no questions – Nov. 12, 2015].

Choco Finesse, LLC (2016). *Summary of Data Supporting a Determination That the Use of Esterified Propoxylated Glycerol (EPG) in Spreadable and Baked Goods is Generally Recognized as Safe*. (Submitted as U.S. FDA, 2016 - GRN 640). Prepared by Indianapolis (IN): Choco Finesse, LLC for submission to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=640 [FDA had no questions – Sep. 21, 2016].

Choco Finesse, LLC (2018). *GRAS Notice for Esterified Propoxylated Glycerol (EPG) for Use in Select Commercial Frying Applications.* (Submitted as U.S. FDA, 2018 – GRN 761). Prepared by Indianapolis (IN): Choco Finesse, LLC for submission to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://wwwn.cdc.gov/nchs/nhanes/ContinuousNhanes/Default.aspx?BeginYear=2015 [FDA had no questions – May 25, 2018]

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

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

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

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

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

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

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

U.S. FDA (2017). *Best Practices for Convening a GRAS Panel: Guidance for Industry*. (Draft Guidance – Docket No: FDA-2017-D-0085). U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN) & Center for Veterinary Medicine (CVM). Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/draft-guidance-industry-best-practices-convening-gras-panel [Content current as of 11/16/2017].

U.S. FDA (2018). *Agency Response Letter GRAS Notice No. GRN 761 [Esterified propoxylated glycerol, Indianapolis (IN); Choco Finesse, LLC].* College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=761 [May 25, 2018 - FDA response - no questions].

U.S. FDA (2019). Part 170—Secondary direct food additives permitted in food for human consumption. Section §170.30—Eligibility for classification as generally recognized as safe (GRAS). In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs*. (U.S. Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/2019/title21.

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

APPENDIX A
Master Specifications for EPOGEE

Table A-1 Master Chemical Specifications for EPG-05

Attribute	Specification	Range	Method
Identity			
Appearance (<30°C)	Solid, white	Solid, white	Visual (internal procedure)
Color, Lovibond (@ 50°C)	Red	≤4	AOCS Cc 13j-97
Taste	Sensory	Flavorless	Taste (internal procedure)
Texture	Sensory	Waxy	Taste (internal procedure)
Chemical Specifications			
Purity (%)	%EPG – (%FFA+%TOC)	>99.5	Calculation
Melting Point (°C)	Mettler Dropping Point	38 to 41	AOCS Cc 18-80
Free Fatty Acid (%)	% FFA as oleic	<0.5	AOCS Ca 5a-40
Peroxide Value	Meq.peroxide/1,000 g	0 to 1	AOCS Cd 8-53
Anisidine Value	p-Anisidine	1 to 10	AOCS Cd 18-90
Hydroxyl Value	mg KOH/1 g	<7	ASTM D4274
Trans Fat (%)	Total "trans"	0 to 1.5[a]	AOCS Ce 1h-05
Tocopherols (ppm)	Alpha	70 to 230	AOCS Ce 8-89
	Beta	5 to 30	
	Gamma	500 to 800	
	Delta	150 to 300	
	Total	800 to 1,300	
Fatty Acid Composition (% as oleic)	Palmitic, C16:0	1 to 12	AOCS Ce 1a-13
	Stearic, C18:0	5 to 40	
	Arachidic, C20:0	2 to 50	
	Behenic, C22:0	10 to 45	
	Oleic, C18:1	0 to 25	
	Linoleic, C18:2	0 to 3	
	Linolenic, C18:3	0 to 1	
	Myristic, C14:0	0 to 6	
	Lauric, C12:0	0 to 12	
Solid Fat Content (%)	@ 10°C	71 to 98	Cd16b-93
	@ 20°C	59 to 97	
	@ 25°C	44 to 95	
	@ 30°C	30 to 92	
	@ 35°C	15 to 53	
	@40°C	0 to 2	
Heavy Metals			
Arsenic (ppm)		<0.05	ICP-MS/AOCS 993.14
Lead (ppm)		<0.05	ICP-MS/AOCS 993.14
Microbiological Specifications			
Aerobic Plate Count	Petrifilm	<10 CFU/1 g	AOAC 990.12
Coliform	Petrifilm	<10 CFU/1 g	AOAC 991.14
Escherichia coli	Petrifilm	<10 CFU/1 g	AOAC 991.14
Salmonella spp.	ELFA	Negative/25 g	AOAC 2004.03
Yeast		<10 CFU/1 g	FDA-BAM, 7th ed.
Mold		<10 CFU/1 g	FDA-BAM, 7th ed.

Table A-1 Master Chemical Specifications for EPG-05

Attribute	Specification	Range	Method

AOAC = Association of Official Agricultural Chemists; AOCS = American Oil Chemists' Society; ASTM = American Society for Testing and Materials; CFU = colony forming unit; ELFA = Enzyme Linked Fluorescent Assay; EPG = esterified propoxylated glycerol; FDA-BAM = ; ICP-MS =; ppm = parts per million; TOC = total organic carbon.

[a] Trans contributed by unsaturated fatty acids. Less than 8% of the amount trans present is bioavailable which corresponds to less than 0.05% that may actually be absorbed from EPG.

*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.

APPENDIX B
Summary of the Estimated Daily Intake of Esterified Propoxylated Glycerol from All GRAS Notified Applications

Epogee, LLC
29 April 2020

Table B-1 Summary of the Estimated Daily Intake of Esterified Propoxylated Glycerol from All GRAS Notified Applications in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 3	5.5	14.4	71.9	487	7.7	15.4
Children	4 to 11	12.1	25.8	92.9	972	13.1	25.9
Female Teenagers	12 to 19	10.6	23.7	90.2	419	11.8	24.4
Male Teenagers	12 to 19	11.7	26.0	83.2	398	14.1	27.1
Female Adults	20 and up	9.1	23.2	86.2	1,856	10.6	24.3
Male Adults	20 and up	9.9	24.3	83.7	1,607	11.8	25.8
Total Population	All ages	9.7	23.7	85.3	5,739	11.4	25.0

GRAS = Generally Recognized as Safe; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

Table B-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Esterified Propoxylated Glycerol from All GRAS Notified Applications in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 3	406	1,054	71.7	482	566	1,208
Children	4 to 11	447	955	93.0	970	481	975
Female Teenagers	12 to 19	185	411	90.4	412	205	419
Male Teenagers	12 to 19	187	414	83.1	397	225	451
Female Adults	20 and up	125	308	86.3	1,845	145	335
Male Adults	20 and up	116	284	83.8	1,584	139	316
Total Population	All ages	176	444	85.4	5,690	206	482

bw = body weight; GRAS = Generally Recognized as Safe; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.