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Article series: Safety of esterified propoxylated glycerol (EPG), a nonabsorbable fat replacer

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ABSTRACT

This article introduces a series of articles addressing the safety of esterified propoxylated glycerols (EPGs), 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. A general summary of the history, composition, metabolism, and safety of EPGs is provided.

1. Introduction

This article introduces a series of articles addressing the safety of esterified propoxylated glycerols (EPG or EPGs hereafter), 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.

EPGs have been studied extensively, but the majority of this information is not publicly available. As such, the present series provides an overview of EPGs, especially their safety. This introduction provides a general summary of the history, composition, metabolism, and safety of EPGs. The six articles that follow [mutation assays; 90-day study in rats; 90-day study in micropigs; one-generation study in rats; teratology study in rabbits; human 8-week study] discuss in more detail a subset of representative studies for assessment of the safety of EPGs when used as fat substitutes in foods.

2. History

EPGs were invented in the 1980s by ARCO Chemical Company and investigated 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, Bestfoods withdrew from the joint venture shortly thereafter, and ARCO was unable to continue the project. Activity on the project resumed a number of years ago 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.

When EPGs were first developed, it was expected that premarket approval *via* a food additive petition (FAP) to the U.S. FDA would be required to enable food uses. However, there is now a considerable data base of information on the safety and tolerability of fat substitutes, based on pre- and post-marketing experience with various products, including P&G's Olestra (Olean™) (see Table 1). It might therefore be possible to establish, based on scientific procedures including a discussion of expected adverse effects based on a history with similar substances, that the use of EPGs in certain foods is GRAS (generally recognized as safe). If the safety of EPGs is generally recognized by qualified experts, it would exempt EPGs from the definition of a food additive, and therefore from premarket approval requirements. A determination that the use of EPGs is GRAS requires the same quantity and quality of scientific evidence as is required for a FAP, but this evidence must be generally known and accepted (62 FR, 18939; April 17, 1997). The present series of articles provides evidence consistent with this *common knowledge* element of the GRAS standard.

3. Composition

Esterified propoxylated glycerols (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

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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 α-methyl group of the PGU sterically blocks access of lipase to the ester linkage thus impairing normal lipid digestion of EPGs. EPG oligomers in which not all glycerol hydroxyls are attached to at least one PGU are susceptible to lipase action and partial digestion. Fig. 1 provides a schematic representation of the composition of EPGs compared to conventional triglycerides.

The "crude" EPG produced after esterification is then refined, filtered, hydrogenated (if necessary) using standard edible oil refining methods, and fortified with a tocopherol mixture to improve the 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 vegetable-sourced fats and oils. These versions can be made to resemble and function like vegetable oils, semisolids 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 (Fig. 2), where EPG is followed by a number that represents the average total PGUs per glycerol, 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.

A core version was selected as the likely representative form for initial 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. In addition to the core EPG version, several other EPG variations were selectively investigated. These versions differed in the number of PGUs (up to 14) and fatty acid source, which also resulted in liquid versions. This "matrix" of test substances confirmed predictable characteristics with respect to digestibility and calories, as well as gastrointestinal tolerance. Use of a matrix approach to establish the safe use of a family of related compounds is not unusual. Table 2 lists some examples of substances approved for human food use based on a matrix approach.

EPG formulations are actually mixtures consisting of multiple oligomers, and the designations of EPG-05, EPG-08, EPG-14, *etc.* refer to the number of PGUs of the predominant oligomer in the mixture; 5, 8, and 14, respectively, in each of these. The graph in Fig. 3 shows the oligomer distribution in EPG-05, a form of EPG

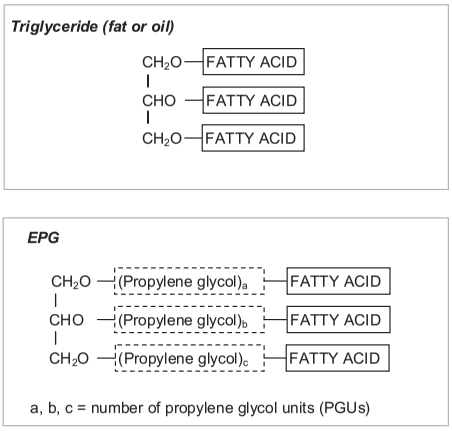

Fig. 1. Basic structure of triglycerides *vs.* EPG.

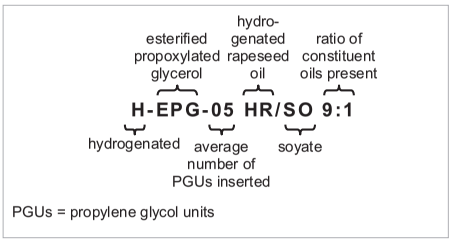

Fig. 2. EPG nomenclature. The following is the designated identification of the "core" version representative of the current commercial variants.

that has been extensively studied; Table 3 provides a representative fatty acid composition. The oligomer distribution is reproducible and tightly controlled by thermodynamic factors (Cooper and Polley, 1995).

4. Safety

Table 4 provides an overview of the studies related to the safety of EPG. Experimental animal studies showed that EPG was not associated with 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.

Ingested EPG is not metabolized or absorbed to any significant degree. The results of rat studies with radiolabeled EPG-08 oleate and EPG-14 oleate (^{14}C radiolabel on either the PGU or the fatty

Table 1
Summary of EPG and other fat substitutes.

Name	Company	Regulatory status	Caloric content	Form	Food product applications
Caprenin	P&G	GRAS (1992)	5	Solid	Candy bars
Salatrim	Nabisco/ Cultor	GRAS (1995)	5	Solid & liquid	Chocolate chips, granola bars, cookies
Olestra (Olean™)	P&G	FAP (1996) GRAS (2008, 2010)	0	Solid	Savory snacks, unpopped popcorn, prepackaged cookies; many food uses, including frostings/icings
EPGs	Chocofinesse	GRAS evaluation in progress	0.7	Solid	Chocolate-based products and ingredients
Sorbestrin	Cultor/ Danisco	Not filed	1.5	Liquid	Dressings, peanut butter, cooking oil

GRAS, generally recognized as safe; FAP, food additive petition.

Table 2
Food substances cleared by FDA using the matrix testing approach.

EXAMPLES:
- Modified Starches:
  - Hydroxypropyl distarch glycerol (up to 10% PO)
  - Hydroxypropyl distarch (up to 25% PO)
- Block polymers of EO and/or PO
- Polyglycerol fatty acid esters
- Rosins

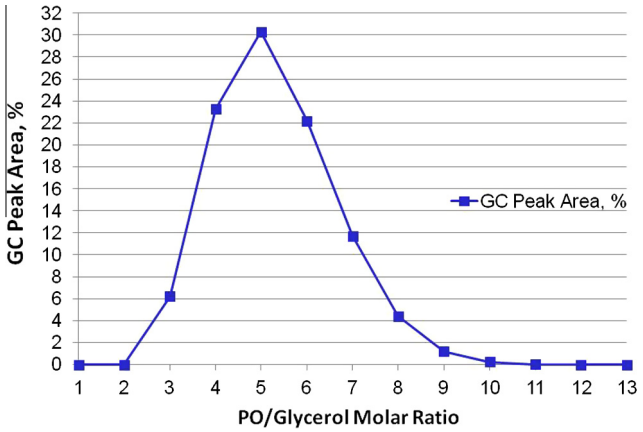

Fig. 3. Oligomer distribution of glycerol polyether polyols in EPG-05.

Table 3
Representative fatty acid composition of H-EPG-05 HR/SO 9:1.

Fatty acid	% (as oleic)
Palmitic, C16:0	1.5–12
Stearic, C18:0	47–70
Arachidic, C20:0	6–20
Behenic, C22:0	14–30
Arachidic + behenic	25–40
Oleic, C18:1	0–10
Linoleic, C18:2	0–6
Linolenic, C18:3	0–1
Total trans fat	<0.25*

* Once incorporated into the food product, trans fat levels would be diluted to trace amounts likely to be undetectable by present analytical methods.

Table 4
Overview of EPG safety studies.

Study type	Species
Tolerance	Dog, rat
Pharmacokinetics (ADME)	Rat
Genetic toxicity (mutagenicity and chromosome aberrations *in vitro* and *in vivo*)*	Bacterial and mammalian systems
Acute toxicity (oral; dermal & eye irritation)	Rat, rabbit
Dermal sensitization	Guinea pig
Subchronic toxicity (oral)*	Rat, mouse, dog, micropig
Chronic (1-year) toxicity	Rat, mouse, dog, micropig
Carcinogenicity (2-year)	Rat, mouse
Reproductive toxicity (1-generation, 3-generation)*	Rat
Developmental toxicity (teratology)*	Rabbit
Tolerance and effects on fat-soluble vitamins and other nutrients*	Human

* For more details, see other articles in this series.

acid component) showed poor absorption from the GI 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 the expired air, presumably as carbon dioxide; no accumulation in tissues has been observed. A greater number of PGUs inserted on the glycerol backbone was associated with greater resistance to degradation. Both forms of EPG investigated were liquid and contained a significant percentage of unsaturated fatty acids – factors that would tend to increase susceptibility to digestion by intestinal microflora. The core version for commercial applications is solid at body temperature and contains almost exclusively saturated fatty acids – factors increasing resistance to microfloral digestion and impairing caloric release (see Table 2).

As a substance that passes through the gastrointestinal tract largely unchanged, ingested EPG was not expected to result in systemic toxicity, which the safety data demonstrate. However, additional consideration was given to 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.

4.1. EPG and nutrient absorption

EPG is intended to replace fats and oils in food products, and some similar substances have been shown to interfere with the absorption of lipid-soluble nutrients from the gastrointestinal tract. For example, pigs fed olestra for 4–26 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).

Experimental animal studies of EPG have shown some treatment-related effects on the levels of some fat-soluble vitamins. 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 one of the articles in this series describes, in humans, consumption of EPG in spread, muffins, cookies, and biscuits at 10, 25, and 40 g/day (*vs.* margarine alone) for eight weeks was not associated with any effects on circulating retinol, α-tocopherol, or 25-OH D_2 (vitamin D, ergocalciferol). However, circulating β-carotene and phylloquinone (vitamin K_1) were lower in the EPG groups, and the levels of PIVKA-II (proteins induced in vitamin K absence) were higher; 25-OH D_3 (vitamin D, cholecalciferol) increased but to a lesser extent than the control. An association of these observations to EPG treatment could not be established with any certainty, because β-carotene showed no apparent relationship to EPG concentration (more severe at 10 and 40 g/day than at 25 g/day) and 25-OH D_3 rose unexpectedly in the control (margarine only) group. The decline in phylloquinone levels and corresponding rise in PIVKA-II 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, which is consistent with the experimental animal study results.

In assessing the possible affect of EPG on lipid-soluble nutrients, it is important to consider that EPG might act as a lipid "sink" or additional "compartment" for the distribution of lipid-soluble substances, including fat-soluble vitamins present in the gut lumen. 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. Accordingly, lipid-soluble substances partitioning into EPG are not strongly held and can readily repartition back into the aqueous phase, establishing an equilibrium state. While lipid-soluble nutrients are continuously absorbed from the gut lumen by the usual physiological processes, the solubility equilibrium would be expected to draw more of the nutrient from the EPG compartment. As the unabsorbable EPG progresses down the gastrointestinal tract, beyond the area of active nutrient absorption, it would still retain a certain amount of the lipid-soluble nutrient. The amount retained would be in part proportional to the mass of EPG present. This consideration may in great part account for the observed, generally small effects on fat-soluble nutrient levels in EPG studies, including those seen in the 8-week human study. Importantly, projected consumer exposure (90th percentile no greater than approximately 20 g EPG per day) from the anticipated initial food uses would not be expected to significantly affect normal absorption of lipid-soluble nutrients.

4.2. Gastrointestinal effects

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.

Early in its development, it became evident that less viscous (*i.e.*, liquid) versions of EPG resulted in passive oil leakage at lower dietary concentrations than semi-solid or solid versions. As such, the research has focused on semi-solid and solid forms. Administration of these forms of EPG to experimental animals and humans has not resulted in any adverse effects on gastrointestinal physiology.

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 lower-melting (99.3 °F) semi-solid variant of the core EPG version 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 (see article in this series).

5. Conclusions

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 nonclinical 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.

Clinical and nonclinical 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 have a Kow in excess of 40.

Taken as a whole, the extensive chemistry and safety data base provides a clear and strong basis to conclude that the consumption of EPG in the anticipated uses would entail a reasonable certainty that no harm would result.

Conflict of interest statement

The authors are unaware of any conflicts of interest.

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References

Cooper, C.F., Polley, C.W., 1995. The structures of low molecular weight glycerin propoxylates: an experimental, semi-empirical, and Monte Carlo study. J. Polym. Sci. Part A: Polym. Chem. 33, 31–47.

Schlagheck, T.G., Riccardi, K.A., Zorich, N.L., Torri, S.A., Dugan, L.D., Peters, J.C., 1997. Olestra dose response on fat-soluble and water-soluble nutrients in humans. J. Nutr. 127, 1646S–1665S.

Tulley, R.T., Vaidyanathan, J., Wilson, J.B., Rood, J.C., Lovejoy, J.C., Most, M.M., Volaufova, J., Peters, J.C., Bray, G.A., 2005. Daily intake of multivitamins during long-term intake of olestra in men prevents declines in serum vitamins A and E but not carotenoids. J. Nutr. 135, 1456–1461.