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# 90-Day dietary toxicity study with esterified propoxylated glycerol (EPG) in Micropigs

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ABSTRACT

The subchronic (90-day) toxicity of esterified propoxylated glycerol (EPG) was assessed in micropigs. Animals (5/sex/group) received feed containing 5%, 10%, and 17% EPG, mixed accordingly throughout the study to deliver 1.5, 3, and 5 g/kg bw/day of EPG, respectively. Corn oil served as the vehicle control (0 g/kg bw/day). Subsets of animals were evaluated at Week 6; the remainder between Weeks 12 and 14. With the exception of liver and serum vitamin levels, statistically significant difference between control and EPG groups were seen sporadically, and with no apparent connection to treatment and/or no consistency across time intervals. EPG intakes of 3 and 5 g/kg bw/day, but not at 1.5 g/kg bw/day were associated with significantly lower serum 25-OH vitamin D levels. Serum total vitamin D levels were significantly lower across all EPG groups. There were also trends toward lower levels of liver vitamins A and E among EPG-treated animals, but the effects were less consistent. The effects on vitamin levels observed in EPG-treated animals were not accompanied by any signs of vitamin deficiency (*e.g.*, effects on growth, clinical signs, or clinical pathology), and might have been related to the larger mass of EPG acting as a lipid "sink" during transit in the gastrointestinal tract.



## 1. Introduction

Esterified propoxylated glycerols (EPGs) represent a family of fat- and oil-like substances, resembling triglycerides in structure and appearance, but modified to prevent or limit their digestion when consumed in food. They consist of multiple propylene glycol units inserted between the glycerol and fatty acid moieties of fats and oils. Their poor absorption results in a low- to no-calorie profile when substituted for fat in the diet.

The present study examined safety of a hydrogenated version of EPG that is considered the "core" version (H-EPG-05-HR/SO 9:1)[2] in micropigs (*Sus scrofa*) following administration in the feed for up to 90 days (13 weeks). The micropig is considered an appropriate model species because like humans, they are true omnivores and share many important features of anatomy, physiology, and function of the gastrointestinal systems (Bode et al., 2010).

## 2. Materials and methods

This study was sponsored by ARCO Chemical Company, Newton Square, Pennsylvania and Best Foods, Somerset, New Jersey. It was conducted at T.P.S., Inc., Mt. Vernon, Indiana, from February to May, 1993 (in-life portion), in compliance with the principles of Good Laboratory Practice (GLP) regulations of the United States Food and Drug Administration (FDA).

### 2.1. Animals

Young healthy male and female Yucatan Micropigs® were obtained from Charles River Laboratories, Windham, ME. Twenty male micropigs weighing 12.808–18.189 kg and twenty female micropigs weighing 10.991–19.384 were started on the study following an acclimation period of approximately 5.5 months. Following the acclimation period, animals (8–10 months old; 5/sex/group) of adequate body weight and in good health

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[1] Now retired.

[2] The nomenclature used to identify specific EPG version is based on the total number of propylene glycol units attached to the glycerol back bone, the source or identity of the fatty acids attached to the propylene glycol units, and the hydrogenation status of the final product. For example; H-EPG-05 HR/SO 9:1 is an EPG in which 05 represents the mean number of propylene glycol units per glycerol, HR/SO represents high-erucic acid rapeseed oil/soybean oil in a 9:1 ratio. The initial "H" indicates that the product is hydrogenated converting all fatty acids into their fully saturated counterpart. For example, erucic acid is converted to behenic acid.

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(determined by a detailed physical and ophthalmic examination, including slit lamp biomicroscopy, urinalysis, and clinical pathology) were stratified by weight and assigned to each of the study groups (Table 1) based on a computer-generated randomization schedule.

### 2.2. Test article and dosing

The test material, esterified propoxylated glycerol [H-EPG-05 HR/SO 9:1; EPG (stabilized with tocopherols, including α-tocopherol), Lot Nos. 753485 and 850201], an off-white solid, was provided by the sponsor. Bulk (55-gallon drum containers) material was stored at −20 °C until thawed, repackaged (5-gallon), and stored at −25 to −3 °C. Prior to use, working aliquots were thawed and stored refrigerated (0–11 °C).

Animals received feed containing up to 5%, 10%, and 17% EPG, mixed accordingly throughout the study to deliver 1.5, 3, and 5 g/kg bw/day of EPG, respectively. The vehicle, corn oil, served as a control (0 g/kg bw/day).

The carrier was Certified Agway® Prolab® Minipig Diet Meal (Lots No. Jul 16 92 W2 and Dec 9 92 W1); corn oil (Mazola® 100% pure corn oil, Lot No. 2321) was added at 4% (w/w). The fat content in of the diets (with corn oil) was approximately 7% by weight; the remaining major components of the feed were approximately 17% protein, 13% crude fiber, 10% moisture, and 7% ash.

For each diet, the appropriate amount of EPG (corn oil only for control) was placed in a glass beaker with corn oil; the mixture was stored at 55 °C for at least 1 h, followed by stirring on a magnetic stir plate until dissolved. The EPG/corn oil blend and a fraction of the total feed was placed in a mixer (Univex M-20 or Hobart V-1401 mixer) for approximately 5 min; any residue remaining on the beaker and stir bar was rinsed into the mix with approximately 100 g of additional corn oil, followed by feed that was set aside. This mixture was blended in a ribbon mixer with the remaining feed for approximately 10 min.

Each diet was prepared the day before study initiation and weekly thereafter. Prepared diets were stored protected from light in plastic containers lined with food-grade plastic bags at 32–52 °C. Analysis of the (weekly) feed for EPG revealed the following range of concentrations for each of the 5%, 10%, and 17% diets, respectively: 4.5–5.1%; 8.4–9.9%; and 13.3–15%.

Animals were fed three times per day (morning, noon, and evening) for at least 90 days (13 weeks), with approximately three hours between feedings. The EPG portion of the diet was divided, weighed, and provided in the morning and evening feedings. Any supplemental feed (control feed) that might be needed to provide enough calories[3] was administered during the noon feeding. EPG-containing feed was weighed before and after the feeding period, unless none remained (*i.e.*, marked as "consumed").

### 2.3. Housing

Animals were housed individually in adjacent runs (approximately 3′ × 6′) with chain-link wire sides, epoxy coated floors, and hardwood chip bedding, in an isolated temperature – (54–81 °F) and humidity – (20–86%) controlled animal room with filtered air supply (10–15 changes/h) and cycled lighting (12-h light/12-h dark). Minimum and maximum room temperatures and humidity were recorded daily. The runs were cleaned daily and sanitized approximately every 2 weeks. Fresh tap water was available *ad libitum* from automatic water nipples. Concentrations of contaminants in the feed and drinking water were considered to be below levels capable of compromising the study.

**Table 1**
EPG concentration and group composition.

| Group | Treatment | | Number of animals/sex[b] |
|---|---|---|---|
| | EPG[*] g/kg/day | Dietary concentration[+] % (w/w) | |
| Control (AVI1)[**] | 0 | 0 | 5 |
| Low EPG (AVI2) | 1.5 | 5 | 5 |
| Mid EPG (AVI3) | 3 | 10 | 5 |
| High EPG (AVI4) | 5 | 17 | 5 |

[*] Approximate levels; animals received feed containing 5%, 10%, and 17% EPG, mixed accordingly throughout the study to deliver 1.5, 3, and 5 g/kg bw/day of EPG, respectively.
[**] The basic diet was supplemented with 4% (w/w) corn oil; test diets contained EPG and 4% (w/w) corn oil as vehicle.
[+] % (w/w) = weight of EPG per weight of basal diet including corn oil.

### 2.4. Observations

#### 2.4.1. Mortality and clinical signs

All animals were observed twice daily throughout the test period for mortality, moribundity, general health, physical appearance, and pharmacological, toxicological, or behavioral effects. This included visual inspection of the feces for appearance, consistency and any evidence of phase separation (*i.e.*, appearance of oily layers or deposits in the stools).

#### 2.4.2. Body weight and weight gain

Individual body weights were obtained on a single beam balance (Nordic Forge 4600) during pre-test, day-1, and weekly thereafter. Body weights used for calculating the relative organ weights at necropsy were obtained after an overnight fast immediately prior to necropsy.

#### 2.4.3. Feed consumption and EPG intake

Food consumption was recorded daily during the study. Feed efficiency [weight gain (g)/feed consumed (g)] was calculated on a weekly basis.

#### 2.4.4. Physical and ophthalmic examination

Each animal was subjected to a detailed physical examination by the attending veterinarian at pre-test and during Weeks 6 and 13; a general physical examination was conducted weekly otherwise by a technician. Animals were tested for the presence of fecal parasites at pre-test and during Weeks 7 and 14.

Ophthalmic examinations (including slit-lamp biomicroscopy) were performed by a Board-certified veterinary ophthalmologist prior to study initiation and during Weeks 6 and 13. Prior to examination, animals were sedated with 20–25 mg/kg of Ketamine HCl (Ketaset®, Aveco, Fort Dodge, IA, Lot Nos. 440164 and 440176,) *via* intramuscular (i.m.) injection. The eyes were dilated with Tropicamide Ophthalmic Solution, USP 1% (Schein, Port Washington, NY, Lot Nos. 91K410 and 92J800).

#### 2.4.5. Hematology, clinical chemistry, and urinalysis

Blood samples for hematology and serum chemistry were collected from the anterior vena cava after an overnight fast. Collections were made at pre-test and during Weeks 6 and 13. RBC count, hematocrit, MCV, MCH, MCHC, WBC count, hemoglobin, and platelet count were measured using a Cell-Dyn® 900 Hematology (Sequoia-Turner). ProT and APTT were measured using a BBL Fibrometer. Accustain™ (Sigma) was used on bone marrow smears

[3] Additional amounts of the control diet (containing 4% corn oil) were administered across all groups, based on an algorithm, to ensure each animal received approximately 500 to 600 g of feed per day. The levels ranged from 280 g/day in an animal weighing 9 kg to 15 g/day in a 19.5-kg pig; no supplementation would have been required once body weight reached 20 kg.