Regulatory Toxicology and Pharmacology 70 (2014) S114–S122

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

One-generation reproduction study of esterified propoxylated glycerol (EPG) administered in the feed to CD® (Sprague-Dawley) rats

Rochelle W. Tyl [a,1], David H. Bechtel [b,2,*]

[a] RTI International, 3040 E. Cornwallis Road, Research Triangle Park, NC, PO Box 12194, United States
[b] Best Foods, Division of CPC International, 150 Pierce St., Somerset, NJ 08873, United States

ARTICLE INFO

Article history:
Available online 10 December 2014

Keywords:
Esterified propoxylated glycerol
EPG
Fat substitute
Reproductive toxicity
One-generation

ABSTRACT

This one-generation study assessed the potential of esterified propoxylated glycerol (EPG) to affect reproduction and offspring development in rats. Male and female Crl:CD(SD)BR rats (30/sex/group) were exposed to EPG at 0, 0.5, 1, and 2 g/kg bw/day or at 5% (w/w) in the diet prior to (13 weeks), during, and after two consecutive matings. For dams, exposure continued through gestation and lactation; F_{1a} and F_{1b} pups were weaned to the respective diet (for up to 91 days). No consistent treatment-related effects were observed in: body weights/gains; feed consumption; clinical observations; mating indices; survival, growth and development of litters, litter sizes, body weights, sex ratios (lower % males/litter at 1 and 2 g/kg bw/day), acquisition of developmental landmarks, behavioral indices, or histology of selected organs. Lower serum vitamin D, liver vitamin A, and liver vitamin E levels were seen in some EPG-treated groups. None of the reductions were judged to be biologically significant. A/G ratio was greater among males receiving 2 g/kg bw/day and 5%. In the absence of any other related effects, the biological significance of this finding is doubtful.

1. Introduction

Esterified propoxylated glycerols (EPGs) represent a family of modified fat- and oil-like products, 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.

A one-generation reproduction study was performed in Crl:CD(SD)BR rats to evaluate the potential of a version of EPG that is considered the "core" version (H-EPG-05 HR/SO 9:1) to elicit alterations in reproductive ability, including effects during lactation, growth and development of the offspring. Vitamin status and neurobehavioral parameters were also evaluated.

2. Materials and methods

This study was sponsored by ARCO Chemical Company, Newton Square, Pennsylvania, and conducted at Research Triangle Institute (RTI), Research Triangle Park, North Carolina, from September 24, 1992 to August 10, 1993, in compliance with the Principles of Good Laboratory Practice (GLP) regulations, of the United States Food and Drug Administration (FDA).

2.1. Animals

Sprague-Dawley-derived outbred albino (Crl:CD(SD)BR rats (age: 28 days) were obtained from Charles River Laboratories, Inc. (Raleigh, NC) and allowed to acclimate to the laboratory environment for approximately 2 weeks before exposure to the test diets. At the end of the acclimation period, animals were stratified based on body weight and allocated randomly within weight classes, to five treatment groups, each consisting of 30 animals per sex per group.

2.2. Mating

Thirty (30) animals/sex/group assigned were designated as F_0 parental animals. One male to one female from each group was selected at random and housed together continuously for up to 21 days until insemination of the female was confirmed. Females presumed pregnant were observed twice daily during gestation for evidence of littering. The first litters were designated as F_{1a}. The F_0 females were allowed to rear their young to postnatal day

* Corresponding author at: Intertek Scientific & Regulatory Consultancy, 1011 US Highway 22, Suite 200, Bridgewater, NJ 08807, United States. Fax: +1 908 429 9260.
E-mail address: david.bechtel@intertek.com (D.H. Bechtel).
[1] Now retired.
[2] Now with Intertek Scientific & Regulatory Consultancy, Bridgewater, NJ, United States.

http://dx.doi.org/10.1016/j.yrtph.2014.11.018
0273-2300/

(PND) 21, after which the dams were removed and the litters were weaned. As the last F_{1a} litter was weaned, F_0 females were allowed to rest for at least 10 days before a second mating with different males within the same treatment group. The second mating followed the same procedure; litters from this mating were designated as F_{1b}.

2.3. Satellite animals

Prior to weaning of the first F_{1a} and F_{1b} litters, ten (10) litters per dose group were randomly selected to create satellite groups, A, B, and C. Group A and B pups were sacrificed on PND 21 and PND 49, respectively, and subjected to necropsy, clinical pathology, vitamin status and tissue EPG. Group C pups were sacrificed on PND 91 and subjected to these procedures and to motor activity and functional observational battery on PNDs 21, 42, 63, and 84.

2.4. Housing

F_0 females were housed individually in solid bottom polycarbonate cages with stainless steel wire lids, except during mating, when each cage held one male and one female; females were housed individually during gestation and after littering until litters were weaned (PND 21). Individual weanlings were housed singly. Environmental controls for the animal room were set to maintain 68–75 °F, a relative humidity of 40–70%, and a 12-hour light/12-hour dark cycle. Variations from these conditions were documented and none were considered to have any effect on the outcome of the study. Food and water were provided *ad libitum*. There were no known contaminants in the food or water that would have interfered with this study.

2.5. Test material

The test material, esterified propoxylated glycerol [H-EPG HR/SO 9:1; EPG (stabilized with tocopherols, including α-tocopherol), lot # 753489], was provided by the sponsor. The test article was a white-colored, odorless solid received and stored frozen (−20 ± 5 °C) in the original containers at RTI.

The vehicle, Mazola® corn oil, was received from Best Foods, a Division of CPC international, Inc. 1120 Commerce Avenue, Union, NJ 07083. According to the supplier, all cases came from the same lot (Lot No. 2321) of material. The vehicle was received and stored frozen (−20 ± 5 °C). The characterization data were provided by the supplier (100% total fatty acids by GC analysis as methyl esters) and the purity was assumed to be 100% for the purpose of concentration calculations.

The carrier was Modifed NIH-07 Certified Mouse/Rat Diet No. 7722 [omitting soy oil (2.5%), decreasing corn oil by 3.5%, and factoring each ingredient level by 0.94 to allow for the addition of 6% corn oil] manufactured by Harlan Teklad Permier Lab Diets (Madison, WI), milled in Winfield, IA. The feed was stored in temperature- and humidity-regulated rooms (18 °C; 50% relative humidity).

2.6. Feed formulation

Test diets were formulated weekly in a 2-ft^3 V-shell blender with an intensifier bar (Patterson-Kelley Co., East Stroudsburg, PA). Corn oil was used as is for the control feed; EPG was blended with corn oil (40–60 °C, 20 min) at the appropriate levels and the mixture was combined with the basic feed for 0.75 to 1.25 h, depending on the size of the batch.

Samples collected showed that the feed was homogeneous and stable for at least 30 days when refrigerated (1–7 °C) and for 49 days when frozen (−12 to −18 °C). Formulations were also stable under ambient conditions (in a feed jar in a cage; exposed to air, normal room lighting, and temperatures of 22–26 °C) for at least 9 days.

2.7. Dietary level selection and study design

EPG was incorporated in the diet at levels providing 0 (vehicle control), 0.5, 1, or 2 g/kg bw/day; one group received EPG at a constant level of 5% (w/w) in the feed. The dietary concentration of 5% EPG was consistent with what is generally considered the maximum that can be given to animals without interfering with their nutrition status and caloric needs. Other EPG dose levels were administered on a g/kg bw/day/day basis to achieve constant exposure levels throughout the study. During the last week of lactation, females with litters were exposed to one-half of the target dietary EPG level to prevent potential overdosing of self-feeding pups. After weaning of their litters, F_0 females went back to the target EPG dietary levels until necropsy. F_0 males were maintained on the target EPG dietary level until necropsy. Litters were weaned (PND 21) to the respective parental diet, adjusted based on body weight.

2.8. Mortality and clinical signs

Cage-side observations for mortality and general condition were made twice daily (morning and afternoon). Any animal judged to be in moribund condition was euthanized by carbon dioxide asphyxiation and then necropsied. Any animal found dead was subjected to a complete gross pathological examination. Cage-side observations included examination for general condition, appearance, behavior, movement within the cage, availability of feed and water, presence of excreta, and appearance and consistency of the stools. Pregnant animals were examined for dystocia (difficulty in delivery); any dam showing signs of imminent abortion or premature delivery was sacrificed on the day such signs were observed. Any abnormality in nesting and nursing behaviors of the dams was recorded.

Detailed clinical examinations were conducted daily. Observations included, but were not limited to, changes in: skin and fur, eyes and mucous membranes, excretion, respiratory system, circulatory system, autonomic and central nervous system, somatomotor activity, and behavior pattern.

2.9. Body weights, feed consumption, and EPG intake

F_0 male body weights were determined and recorded at initiation of treatment and weekly thereafter until scheduled sacrifice; F_0 female body weights were recorded in the same manner except as follows. During gestation, females were weighed on gestational days (GD) 0, 7, 14, and 20. Dams producing litters were weighed on lactational days (PND) 0, 4, 7, 14, and 21. For non-pregnant females showing either no signs of mating or signs of mating but no litters, and for pregnant females with all dead pups on PND 0 or with complete loss of the litter during lactation, body weights were recorded at least weekly until scheduled sacrifice. Individual body weights of live pups were measured at the time of parturition (PND 0) and were recorded for days PND 4, 7, 14 and at weaning (PND 21). All animals were weighed at sacrifice. Individual body weight gains were computed.

Feed consumption was recorded weekly for all F_0 parental animals throughout the pre-mating treatment periods until cohabitation of the sexes. For the male rats, weekly measurement of feed consumption was resumed on the Monday following the return to individual housing. During pregnancy, feed consumption of the F_0 females was recorded for GD 0–7, 7–14 and 14–20. During lactation of the F_{1a} and F_{1b} litters, maternal feed consumption was measured for PND 0–4, 4–7, and 7–14. Weekly measurement