

GRAS Notice (GRN) No. 192

<http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm>

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ORIGINAL SUBMISSION

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**InFat™ (High 2-Palmitic Acid Vegetable Oil) GRAS
Notification**

**GENERALLY RECOGNIZED AS SAFE (GRAS)
EXEMPTION CLAIM**

Prepared for:

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

Prepared by:

Enzymotec Ltd
P.O. Box 6,
Migdal HaEmeq
Israel, 23106

January 31, 2006

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Enzymotec
Delivering Lipids

January 30, 2006

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

Re: GRAS Notification

Dear Sir or Madam:

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized As Safe (GRAS) determination] published in the Federal Register (62 FR 18939-18964), I am submitting in triplicate, as the notifier, Enzymotec, Ltd, P.O. Box 6, Migdal HaEmerq, Israel 23106 a GRAS notification of InFat™ for use in foods, a GRAS panel report setting forth the basis for the GRAS determination, and *curricula vitae* of the members of the GRAS panel for review by the agency.

Sincerely,

Ms. Iris Meiri-Bendek
Regulatory Affairs Manager

Enclosures

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INFAT™ NOTIFICATION

I GRAS Exemption Claim

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)]

InFat™ has been determined to be Generally Recognized As Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use in food, among experts qualified by scientific training and expertise. Therefore, the use of *InFat™* in food as described below is exempt from the requirement of premarket approval.

Signed,

Ms. Iris Meiri-Bendek
Regulatory Manager
Enzymotec, Ltd
P.O. Box 6, Migdal HaEmeq
Israel 23106

Jan. 31, 2006
Date

B. Name and Address of Notifier

Ms. Iris Meiri-Bendek
Regulatory Manager
Enzymotec, Ltd
P.O. Box 6, Migdal HaEmeq
Israel 23106

C. Common Name of the Notified Substance

High 2-palmitic acid vegetable oil

D. Conditions of Intended Use in Food

The individual proposed food-uses and use-levels for *InFat™* employed in the current intake analysis are summarized in Table 1. In general, *InFat™* will be used to replace vegetable oils in infant formulas for term and preterm infants up to a level of 70% and will be used to replace all added fat in baby/toddler foods and fat in generally processed food products.

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Table 1 Summary of the Individual Proposed Food-Uses and Use-Levels for InFat™ in the United States

Food Category	Proposed Food-Use	InFat™ Use-Level (%)*
Baked Goods and Baking Mixes	Cakes	0.2-26.0
	Cookies	<0.1-36.2
	Grain-Based Crackers	0.9-25.3
	French Toast, Pancakes, and Waffles	0.4-20.6
	Pastries	0.6-38.8
	Pies	5.3-38.9
	Quick Breads (Biscuits, Cornbread, Corn Muffins, Tortillas, Muffins, Popovers, and Other Quick Breads)	0.6-26.9
	Yeast Breads and Rolls	0.9-24.4
Breakfast Cereals	Ready-to-Eat Cereals	0.2-18.8
	Cereal, Baby Food	0.2-7.8
Dairy Product Analogs	Fluid Milk, Imitation	1.6-3.4
Fats and Oils	Margarine, and Margarine-Like Spreads	1.5-80.8
	Mayonnaise and Mayonnaise-Type Dressings	0.5-79.4
	Salad Dressings (regular and low calorie)	<0.1-61.7
Frozen Dairy Desserts and Mixes	Frozen Yogurt	0.1-16.4
	Ice Cream and Frozen Milk Desserts	0.1-22.7
Gelatins, Puddings, and Fillings	Puddings, Custards and Other Milk Desserts	<0.1-29.3
Grain Products and Pastas	Frozen Grain-Based Meals	1.0-14.6
	Grain Mixtures (Burritos, Enchiladas, Tacos, Tamales, Nachos, Pizza, Pasta Dishes)	<0.1-55.1
	Cereals Grains, Not Cooked	1.5-20.1
	Grain-Based Patties	5.3
Gravies and Sauces	White Sauces and Milk Gravies	6.1-10.7
Hard Candy	Hard Candy	0.2-21.3
Milk	Milk	0.1-10.7
Meat Products	Beef Baby Food	1.1-6.9
	Pork Baby Food	5.8-14.6
	Lamb or Veal Baby Food	4.7-4.8
Milk Products	Creams and Cream Substitutes	1.8-37.0
	Dry and Powdered Milk and Mixtures (not reconstituted)	0.1-28.0
	Evaporated and Condensed Milk	0.1-8.7
	Flavored Milk and Milk Drinks	0.1-15.2
	Infant Formulas	1.8-3.4
	Milk-Based Meal Replacements	0.3-28.0
	Milk Desserts, Baby Food	1.0-2.0
Sour Cream	3.0-21.0	

Food Category	Proposed Food-Use	InFat™ Use-Level (%)*
	Yogurt	0.2-3.2
Poultry Products	Poultry Baby Food	5.8-14.4
Processed Fruits and Fruit Juices	Fruits and Fruit Mixtures, Baby Food	<0.1-0.5
	Fruit Juices, Baby Food	<0.1-0.8
	Fruit Desserts and Fruit-flavored Puddings, Baby Food	0.1-1.6
Soups and Soup Mixes	Grain Based Soups	0.1-7.5
Soft Candy	Candies and Chocolate	0.3-46.0
Snack Foods	Grain-Based Salty Snacks	1.2-36.7
Processed Vegetables and Vegetable Juices	Vegetable and Vegetable Mixtures, Baby Food	0.1-14.3

* Use-levels for InFat™ were calculated as follows: 70% of total fat for infant formulas; and 100% of total fat for all other proposed food uses. Values for total fat calculated from the Survey Nutrient Database, which is based on the USDA Nutrient Database for Standard Reference.

The consumption of InFat™ from all proposed food uses was estimated using the United States Department of Agriculture (USDA) 1994-1996 Continuing Survey of Food Intakes by Individuals (USDA CSFII 1994-1996), and the 1998 Supplemental Children's Survey (USDA CSF II 1998) (USDA, 2000). On an all-user basis, the mean intake of InFat™ in young infants (0-5 months) that would be consuming infant formula was estimated to be 22.4g/person.day (3.4 g/kg body weight/day). The heavy consumer (90th percentile) all user intake of InFat™ from these infants was 36.4 g/person/day (5.9 g/kg body weight/day). On a body weight basis, infants were reported to have the greatest intakes of InFat™ of any population group.

On an all-user basis, the mean intake of InFat™ by the total population from all proposed food-uses was estimated to be 35.3 g/person/day (0.7 g/kg body weight/day). The heavy consumer (90th percentile) all-user intake of InFat™ by the total population from all proposed food-uses was 65.7 g/person/day (1.5 g/kg body weight/day). On an absolute basis (per person), male teenagers were determined to have the greatest mean and 90th percentile all-user intakes of InFat™ of 52.1 and 89.2 g/person/day, respectively.

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, InFat™ has been determined to be GRAS on the basis of scientific procedures. This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of InFat™ as a component of food. The safety of InFat™ is supported by a number of published studies on Betapol™, a high 2-palmitic acid functional fat that is similar in fatty acid composition and fatty acid distribution (e.g., palmitic acid at sn-2 position) to InFat™, including metabolic studies, short-term, subchronic and

INFAT™ NOTIFICATION

reproductive and developmental toxicity studies in experimental animals and clinical studies investigating the effects of *InFat*™. An unpublished comparative animal metabolic study between *InFat*™ and Betapol™ also supported their metabolic similarities. This determination is further supported by an expert panel evaluation of the health aspects of *InFat*™. (See Attached – EXPERT PANEL REPORT CONCERNING THE GENERALLY RECOGNIZED AS SAFE STATUS OF INFAT™ FOR USE IN FOODS).

As discussed, *InFat*™ is similar in fatty acid composition to Betapol™, a product that was previously self-affirmed as GRAS. In response to a GRAS Notice (GRN 000131), the FDA advised that they had no questions with respect to the conclusion that Betapol™ was GRAS, based on scientific procedures, for use in infant formula for both term and preterm infants at a level of up to 80% of total fat.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

Enzymotec, Ltd
P.O. Box 6, Migdal HaEmeq
Israel 23106

Should the U.S. Food and Drug Administration (FDA) have any questions or additional information requests regarding this notification, Enzymotec, Ltd. will supply these data and information.

II. Detailed Information About the Identity of the Substance

A. Identity

InFat™ is a functional fat enriched in palmitic acid at the sn-2 position similar to breast milk triglycerides. *InFat*™ is a colorless and flavorless product, that is a turbid liquid to pasty material at room temperature.

Common or Usual Name:	High 2-palmitic acid vegetable oil
Chemical Name:	Not applicable.
Chemical Abstracts Service (CAS) Number:	Not applicable.
Empirical Formula and Formula Weight:	Not applicable.
Molecular weight:	Not applicable.
Structural Formula:	Not applicable.

B. Method of Manufacture

The manufacturing process for the formation of **InFat™** is a 2-step process. The first step involves the randomization of palm stearin by treatment with the chemical catalyst, sodium methoxide. The mixture then undergoes processing similar to that used for traditional vegetable oils, including bleaching with bleaching earths.

The second step in the manufacturing process for the formation of **InFat™** involves a transesterification reaction between the triglycerides in the randomized palm stearin (palmitic) and the added oleic acid using a safe and suitable GRAS (GRN 000043) lipase preparation derived from *Aspergillus oryzae* carrying a gene encoding lipase from *Thermomyces lanuginosus*. The palmitic acid residues on the triglycerides are replaced by oleic acid residues at the sn-1 and sn-3 positions. Following reaction and separation of the catalyst, the mixture is distilled to remove excess free fatty acids (primarily oleic acid). The mixture then undergoes processing similar to that used for traditional vegetable oils, including bleaching and deodorization, and is then mixed with permitted antioxidants. All processing chemicals used in the manufacture of **InFat™** are appropriate for food-use. A schematic overview of the manufacturing process of **InFat™** is presented in Figures 1 and 2.

Figure 1 Schematic Overview of the Process for the Randomization of Palm Stearin

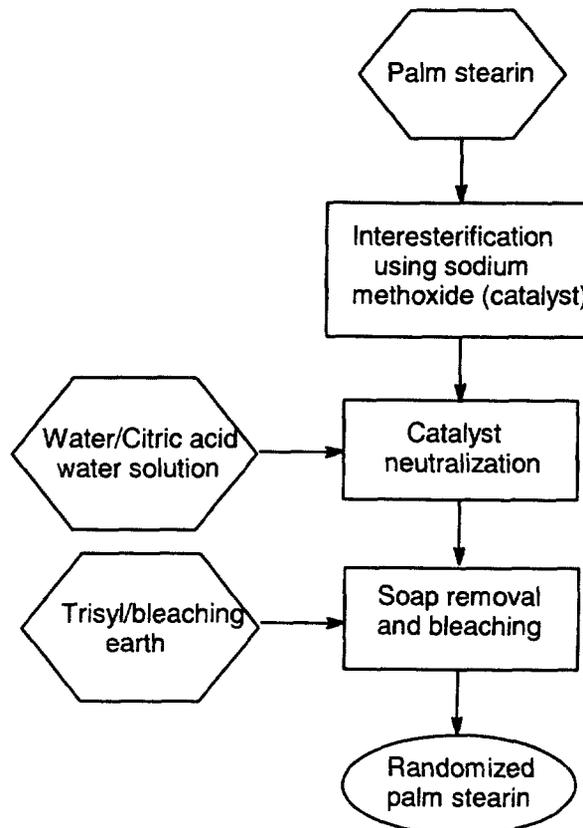
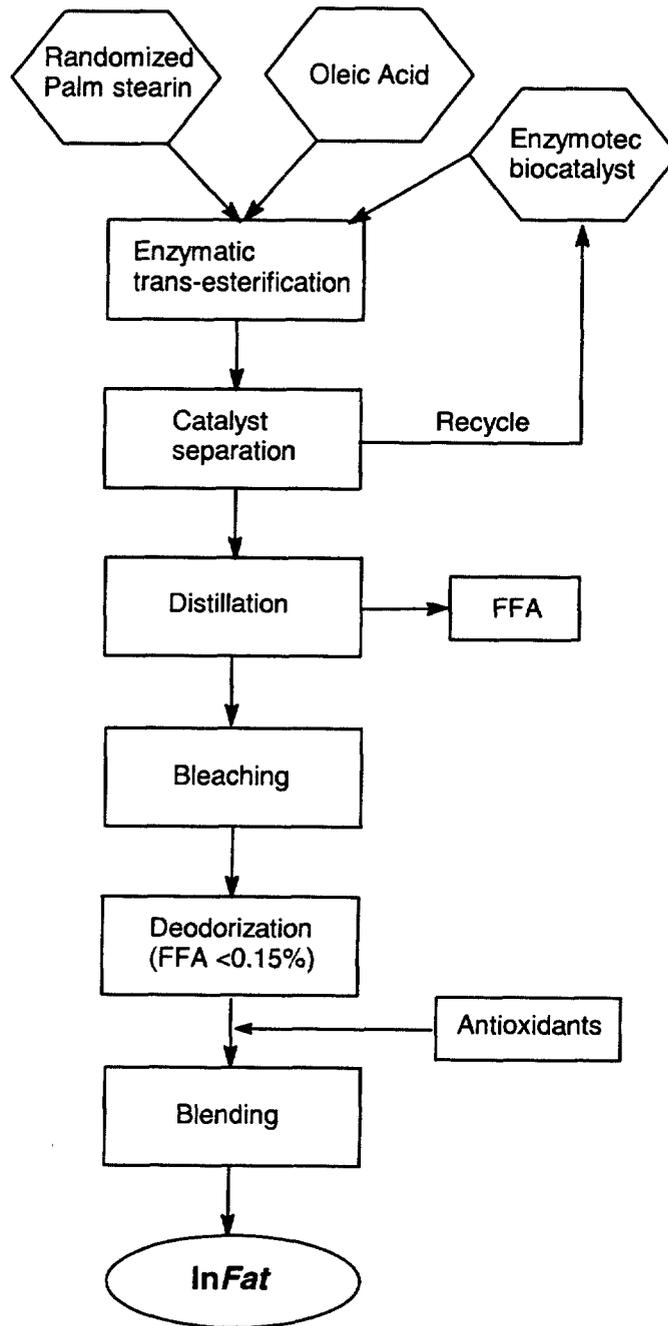


Figure 2 Schematic Overview of the Manufacturing Process for InFat™



C. Specifications for Food Grade Material

Table 2 Chemical Specifications for InFat™		
Specification Parameter	Specification	Method
Identification		
Consistency (60°C)	Clear liquid	
Consistency (25°C)	Turbid liquid to paste	
Purity		
Triglycerides (%)	>96	AOCS Cd 11d-96 (1999)
Diacylglycerides (%)	<4	AOCS Cd 11d-96 (1999)
Monoacylglycerides (%)	<1	AOCS Cd 11d-96 (1999)
Ash (%)	<0.05	AOCS Ca 11-55 (1999)
Water (%)	<0.1	AOCS Ca 2e-84 (1999)
Peroxide value (meq/kg)	≤5	AOCS Cd 8-53 (1999)
Acid value (mg KOH/g)	≤0.3	IUPAC 2.201
Total heavy metals (mg/kg)	<0.5	Arsenic: Karlshamns Method E2214-1 Cadmium: Karlshamns Method E2217-1 Ni, Fe, Cu - IUPAC 2.632
Lead (mg/kg)	<0.1	Pb - IUPAC 2.632
Fatty Acids (% total fatty acids)		
Hexadecanoic acid (Palmitic acid) (16:0)	25 to 42	IUPAC 2.302
Octadecanoic acid (Stearic acid) (18:0)	1 to 6	IUPAC 2.302
<i>cis</i> -9-Octadecenoyl acid (Oleic acid) (18:1)	42 to 60	IUPAC 2.302
di- <i>cis</i> -9,12-Octadecadienoyl acid (Linoleic acid) (18:2)	6 to 17	IUPAC 2.302
Total <i>trans</i> fatty acids (%)	<2	AOCS PP Ch2a-94
Hexadecanoic acid (Palmitic acid) (16:0) esterified at sn-2 position (% of total palmitic acid)	62 to 70	IUPAC 2.210

Table 3 Microbiological Specifications for InFat™		
Specification Parameter	Specification	Method
Total count (CFU/g)	<1000	Israel Standard SI 885 Part 3 (1999)
Molds (CFU/g)	<100	Israel Standard SI 885 Part 8 (1999)
Yeast (CFU/g)	<100	Israel Standard SI 885 Part 8 (1999)
Coliforms (CFU/g)	Negative	USP 61 (2000)
<i>Salmonella</i> (CFU/g)	Negative	Israel Standard SI 885 Part 7 (1999)
<i>Staphylococcus aureus</i> (CFU/g)	Negative	USP 61 (2000)

III. Self-Limiting Levels of Use

Balanced fat diets are required for infant formulas and considering the fatty acid composition of *InFat*™ with respect to essential fatty acids like linolenic acid (C18:3), *InFat*™ cannot be used to replace all fat in infant formula. Although *InFat*™ is also proposed for use in foods for older babies/toddlers, most of these types of foods do not contain significant amounts of added fat and thus the addition of *InFat*™ would not be technologically feasible. In other adult foods, the addition of *InFat*™ is restricted based on its technological properties such as melting temperatures.

IV. Basis for GRAS Determination

The determination that *InFat*™ is GRAS is on the basis of scientific procedures. (See Attached – EXPERT PANEL REPORT CONCERNING THE GENERALLY RECOGNIZED AS SAFE STATUS OF INFAT™ FOR USE IN FOODS).

Attachment I

EXPERT PANEL REPORT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF INFAT™ FOR USE IN FOODS

[November, 2005]

INTRODUCTION

At the request of Enzymotec Ltd., an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether, under the conditions of intended use as a nutrient in traditional foods, *InFat*™ would be "generally recognized as safe" (GRAS), based on scientific procedures. The Panel consisted of the below-signed qualified scientific experts: Dr. Joseph F. Borzelleca (Medical College of Virginia), and Dr. Robert J. Nicolosi, (University of Massachusetts Lowell). *Curricula vitae* evidencing the Panel members' qualifications for evaluating the safety of food ingredients are provided in Attachment 1.

The Panel, independently and collectively, critically examined a comprehensive package of publicly available scientific information and data compiled from the literature and other published sources through October, 2005 by CANTOX HEALTH SCIENCES INTERNATIONAL. In addition, the Panel evaluated other information deemed appropriate or necessary, including data and information provided by Enzymotec Ltd. The data evaluated by the 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 of *InFat*™ and its individual components.

Following independent, critical evaluation of such data and information, the Panel unanimously concluded that under the conditions of intended use in traditional foods described herein, *InFat*™, meeting appropriate food-grade specifications, and manufactured and used in accordance with current good manufacturing practice, is GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion, excluding confidential data and information, is provided below.

COMPOSITION, MANUFACTURING AND SPECIFICATIONS

InFat™ is a functional fat enriched in palmitic acid at the sn-2 position similar to breast milk triglycerides, intended to replace normal vegetable oils used in infant formulas for preterm and term infants, baby/toddler foods and traditional foods. The manufacturing process for the

formation of **InFat™** is a 2-step process. The first step involves the randomization of palm stearin by treatment with the chemical catalyst, sodium methoxide. The mixture then undergoes processing similar to that used for traditional vegetable oils, including bleaching with bleaching earths.

The second step in the manufacturing process for the formation of **InFat™** involves a transesterification reaction between the triglycerides in the randomized palm stearin (palmitic) and the added oleic acid using a safe and suitable GRAS (GRN 000043) lipase preparation derived from *Aspergillus oryzae* carrying a gene encoding lipase from *Thermomyces lanuginosus*. The palmitic acid residues on the triglycerides are replaced by oleic acid residues at the sn-1 and sn-3 positions. Following reaction and separation of the catalyst, the mixture is distilled to remove excess free fatty acids (primarily oleic acid). The mixture then undergoes processing similar to that used for traditional vegetable oils, including bleaching and deodorization, and is then mixed with permitted antioxidants.

All processing chemicals used in the manufacture of **InFat™** are appropriate for food-use. In order to ensure a consistent product, Enzymotec, Ltd. has established numerous chemical and microbiological specification parameters for **InFat™**, which are presented in Tables 1 and 2. Analyses of representative lots of **InFat™** demonstrate compliance with final product chemical and microbiological specifications. Potential residues from the manufacturing process including protein, sorbitan oleate, methanol and epichlorohydrin were considerably below acceptable levels. Additionally, analysis results of the long-term stability (16 weeks) of **InFat™** at room temperature and under normal food processing conditions (40°C) indicate conformity to product specifications.

INTENDED USE AND ESTIMATED EXPOSURE

The individual proposed food-uses and use-levels for **InFat™** employed in the current intake analysis are summarized in Table 3. In general, **InFat™** will be used to replace vegetable oils in infant formulas for preterm and term infants up to a level of 70% and will be used to replace all added fat in baby/toddler foods and fat in generally processed food products.

The consumption of **InFat™** from all proposed food uses, was estimated using the United States Department of Agriculture (USDA) 1994-1996 Continuing Survey of Food Intakes by Individuals (USDA CSFII 1994-1996), and the 1998 Supplemental Children's Survey (USDA CSF II 1998) (USDA, 2000). The estimated total daily consumption of **InFat™** from all proposed food uses is summarized in Tables 4 and 5 on a per person (mg/person/day) and per kilogram body weight basis (mg/kg body weight/day), respectively. On an all-user basis, the mean intake of **InFat™** in young infants (0-5 months) that would be consuming infant formula was estimated to be 22.4g/person.day (3.4 g/kg body weight/day). The heavy consumer (90th percentile) all user intake of **InFat™** from these infants was 36.4 g/person/day (5.9 g/kg body weight/day). On a

body weight basis, infants were reported to have the greatest intakes of **InFat™** of any population group.

On an all-user basis, the mean intake of **InFat™** by the total population from all proposed food-uses was estimated to be 35.3 g/person/day (0.7 g/kg body weight/day). The heavy consumer (90th percentile) all-user intake of **InFat™** by the total population from all proposed food-uses was 65.7 g/person/day (1.5 g/kg body weight/day). On an absolute basis (per person), male teenagers were determined to have the greatest mean and 90th percentile all-user intakes of **InFat™** of 52.1 and 89.2 g/person/day, respectively.

The type of intake methodology used to estimate intakes of **InFat™** is generally considered to be 'worst case' as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, overestimate consumption of food products that are consumed relatively infrequently.

DATA PERTAINING TO SAFETY

InFat™ is a functional fat enriched in palmitic acid at the sn-2 position to simulate human milk fat. Approximately 20 to 25% of the total human milk fatty acids are palmitic acid, of which 70% is esterified at the sn-2 position (Innis *et al.*, 1993a, 1994; Lien *et al.*, 1997). In contrast, the palmitic acid present in vegetable oil and other non-milk fats is primarily esterified at the sn-1 and sn-3 positions (Innis *et al.*, 1993a). Numerous studies have demonstrated that palmitic esterified at the sn-2 position is better absorbed than that at the sn-1 and sn-3 positions (Innis *et al.*, 1994).

The safety of **InFat™** is primarily based on a number of published studies on Betapol™, a high 2-palmitic acid functional fat that is similar in fatty acid composition to **InFat™**, as summarized in Table 6. In response to a GRAS Notice, the FDA advised that they had no questions with respect to the conclusion that Betapol™ was GRAS, based on scientific procedures, for use in infant formula for both term and preterm infants at a level of up to 80% of total fat (FDA, 2003).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION (ADME)

Approximately 95 to 98% of dietary fat is ingested as triacylglycerols (Linder, 1991). In general, ingested triacylglycerols are enzymatically hydrolyzed by pancreatic lipase in the upper small intestine (Linder, 1991, Linscheer and Vergroesen, 1994; Guyton and Hall, 1996; Nelson and Innis, 1999; IOM, 2002). Hydrolysis by pancreatic lipase is specific to the sn-1 and sn-3 positions of the triacylglycerol, and results in the release of free fatty acids and 2-

monoacylglycerol. These end products are then incorporated into bile acid micelles for diffusion to the interior of the intestinal epithelial cells (enterocytes). Within the enterocytes, the monoacylglycerols are reacylated, with no specificity to the glycerol 1 and 3 positions, leading to the formation of new triacylglycerols. Reconstituted triacylglycerols, together with phospholipids, cholesterol, and apoproteins, are then incorporated into chylomicrons (87% triacylglycerols, 9% phospholipids, 3% cholesterol, 1% apoprotein), which are released into the lymphatic duct and into the blood. As they pass through the capillaries of adipose tissue and the liver, chylomicrons are removed from the circulating blood by lipoprotein lipase, which hydrolyzes the contained triacylglycerols and phospholipids, releasing free fatty acids to the tissues for metabolism. The chylomicron remnants are taken up primarily by the liver *via* specific receptors and endocytosis (Linder, 1991; Krummel, 1996). Following cellular uptake, fatty acids are re-esterified into triacylglycerols and phospholipids for storage as a source of energy for the body or as structural components of cell membranes (Linder, 1991; Linscheer and Vergoesen, 1994; Krummel, 1996; IOM, 2002).

Monoacylglycerols are typically well absorbed, regardless of their constituent fatty acid (Lien *et al.*, 1997). In contrast, the absorption of free fatty acids is dependent upon their structure, with mono- and polyunsaturated fatty acids and saturated fatty acids with chain lengths of 12 carbons or less being better absorbed than long-chain saturated fatty acids (Lien *et al.*, 1997). In the small intestine, triacylglycerols esterified with palmitic acid at the sn-2 position are converted to free fatty acids and 2-monopalmitin (the 2-monoacylglycerol), which is readily absorbed (Lien *et al.*, 1997).

In a study comparing the absorption of palmitic acid (16:0) from human breast milk (21.0% 16:0, 54.2% of sn-2 esterified) or formula (22.3% 16:0, 4.8% of sn-2 esterified) in infants, Innis *et al.* (1994) reported that 16:0 esterified to the sn-2 position is absorbed intact by infants and reesterified to triacylglycerols in the chylomicrons. This conclusion was based on measurements of plasma triacylglycerols, which demonstrated a significantly higher level of 16:0 in plasma triacylglycerol sn-2 fatty acids in breast-fed infants (23.3% of sn-2 esterified) in comparison to formula-fed infants (7.4% of sn-2 esterified). Free fatty acids released during sn-1 and sn-3 lipolysis, however, often react with calcium once they are secreted into the intestine, resulting in an insoluble soapy fraction that is excreted, and which can result in decreased calcium absorption (Lien *et al.*, 1997).

Animal studies investigating the absorption of 16:0 from infant formulas containing 16:0 esterified at the sn-2 position (*e.g.*, Betapol™) have reported more efficient absorption of 16:0 in comparison to standard infant formulas (*i.e.*, formulas containing 16:0 esterified at the sn-1 and sn-3 positions) (Lien *et al.*, 1997). Male Sprague Dawley rats (10/group) were fed fat-free diets for 10 days, followed by a three-day period wherein they were fed diets containing 150 g fat/kg diet from either Betapol™ (27% 16:0, 71.3% sn-2 esterified) or a proprietary infant formula (13.2% 16:0, 8% sn-2 esterified) or one of two fat blends [blend A (29% 16:0, 8% sn-2 esterified) or blend B (19% 16:0, 9% sn-2 esterified)] in place of dextrose. After 3 days on the test diet, rats

were returned to the fat-free diet for three days. Fecal samples were collected for analysis during the final 6 days of the study, and food consumption was observed to calculate the total fatty acid intake. Control rats were fed the fat free diet for the duration of the study. In the first experiment, the absorption of Betapol™ was compared to that of the proprietary infant formula. Fecal excretion of saturated fatty acids, including 16:0, was significantly less in rats fed Betapol™ (0.07 mEq) compared to rats fed the infant formula (0.34 mEq), indicating that higher levels of sn-2 fatty acids in the diet result in higher absorption of saturated fatty acids. When compared to the two infant formula blends A and B, fecal excretion of 16:0 from Betapol™-fed rats was significantly less (1%) than rats on either blend A or blend B (18%). In another experiment, Betapol™ was mixed in varying concentrations (0, 25, 50, 75, or 100%) with blend C, a vegetable fat blend with the same fatty acid composition as Betapol™. These mixtures contained similar amounts of 16:0 (24 to 26%) but varying percentages of sn-2 esterified 16:0 (4.8 to 78.8%). Lien *et al.* (1997) reported that excretion of total fatty acids increased as the percentage of sn-2 esterified 16:0 decreased. A logarithmic relationship was observed between the percentage of sn-2 16:0 and the absorption of total fatty acids. In a final experiment, rats fed Betapol™ for 3 days were compared to those fed blend C alone (4.8% sn-2 esterified 16:0) or a 50/50 blend of Betapol™ and blend C (total of 41.8% sn-2 esterified 16:0). Analysis of the feces for calcium, soap fatty acids, and neutral fat revealed no significant differences between groups in fecal calcium content; however, total fat in the feces decreased as sn-2 palmitic acid in the diet increased. In addition, as the dietary level of sn-2 palmitic acid decreased, there was a significant increase in soapy fatty acid levels in the feces. Overall, the results of this study indicate that absorption of palmitic acid esterified at the sn-2 position is more efficiently absorbed than other forms, and has a positive effect on total fat absorption.

A comparative rat metabolism study with Betapol™ and InFat™ similar in design to the study of Lien *et al.*, 1997 confirmed that the sn-2 enriched palmitic acid structure resulted in better absorption of the saturated fatty acids and resulted in less fatty acid calcium soaps in the feces. No differences in growth or adverse effects were also noted between the two fat sources (Enzymotec, 2006).

TOXICOLOGICAL STUDIES

The safety of InFat™ is supported by a multigenerational study in rats in which Betapol™ was administered in the diet. Other studies that support the safety of InFat™ include short-term studies in piglets and clinical studies in infants and adults that have demonstrated that Betapol™, and therefore InFat™, is well-absorbed and supports growth and development, with no significant adverse effects.

Short-Term Studies

A number of studies have compared the absorption of sows' milk, Betapol™, a human milk fat equivalent primarily composed of 1,2-dipalmitoyl 3-oleoyl triglyceride and 1,3-dioleoyl 2-palmitoyl

triglyceride, similar to *InFat*TM, and other types of formulas in newborn piglets. Although these were nutritionally based studies rather than toxicological studies, some of the parameters examined included toxicological endpoints (e.g., body weight, serum chemistry). These studies, have demonstrated that dietary exposure of newborn piglets to approximately 18.4 g BetapolTM/kg body weight/day for periods of up to 18 days resulted in no significant effects on growth or development (Innis *et al.*, 1993a,b, 1995).

Subchronic and Chronic Studies

The potential toxicity of BetapolTM was investigated in a multigenerational study in groups of Specific Pathogen Free rats [CrI:CD(SD) BR VAF/Plus strain] (n=32/sex) fed diets containing 1.5, 7.5, or 15.0% BetapolTM (Spurgeon *et al.*, 2003). Based on published standard reference values for CrI:CD (SD) BR rats (Meingassner and Schmook, 1990), the dosages were calculated to range from 7.42 to 20.75 g/kg body weight/day in male rats and from 8.61 to 15.96 g/kg body weight/day in females. A comparative control group was fed a diet containing 15% food grade oil, while a negative control group was fed a standard laboratory animal diet (LAD). Rats in the F₀ generation were fed the test diets from the age of 6 weeks until weaning of the F₁ generation (approximately 10 weeks of exposure during the pre-mating period and a total of 5 months of exposure). Rats in the F₁ generation were fed the test diets from the age of 4 weeks until weaning of the F₂ generation (approximately 10 weeks of exposure during the pre-mating period and a total of 6 months of exposure). Body weight and food consumption was measured twice weekly, while water consumption was recorded daily over the first and final 2 weeks of the pre-mating period (F₀ generation) and during weeks 5, 6, 15, and 16 of the pre-mating period for the F₁ generation. Hematological and blood chemistry parameters were assessed at study termination. F₀ and F₁ rats were killed at the end of the study and organ weights were measured. Histopathological examination of a range of tissues was conducted in rats in the high-dose group (15% BetapolTM) and in rats from both control groups. Only tissues showing macroscopic changes were examined histopathologically for F₂ females.

Rats in all groups except the LAD group were reported to have pale fecal pellets, which was considered to be related to the high fat content of the diet. A number of deaths were reported in the comparative control (1 and 3 deaths in the F₀ and F₁ generations, respectively), BetapolTM (2 deaths in the 7.5% BetapolTM group in the F₀ generation, and 3, 2, and 1 deaths in the 1.5, 7.5, and 15% BetapolTM groups, respectively, in the F₁ generation) and LAD groups (1 death in the F₀ generation); however, the deaths were not considered to be compound-related. There were no significant differences in body weight and food and water consumption in groups fed BetapolTM compared to the comparative controls; however, values for these parameters were significantly higher in the LAD group in both the F₀ and F₁ generations. Examination of hematological parameters resulted in no significant differences between the BetapolTM and comparative control groups, with the exception of significantly increased clotting time in F₁ females in the 7.5 and 15% BetapolTM groups prior to mating, and in F₁ males in the 15% BetapolTM group at the end of the exposure period. Similarly, males and females in the LAD

group had significantly increased clotting time, both prior to mating and at the end of the exposure period. F₁ males in the 15% Betapol™ group also had significantly increased platelets. There were no other consistent patterns of intergroup differences in hematological parameters. The LAD group had significantly higher glutamic pyruvic transaminase (GPT) and lower cholesterol and lipid levels. There were no consistent differences in blood chemistry parameters between the comparative controls and the Betapol™ groups. With respect to organ weights, adult females of both the F₀ and F₁ generations that received 15% Betapol™ had significantly higher ovary weights compared to all other groups. The LAD group was reported to have significantly higher adrenal weight compared to all other groups; however, this was only apparent in the F₁ generation. There were no significant macroscopic or microscopic findings following examination of the tissues, with the exception of a dose-related increase in the incidence of vacuolation and fat deposition among F₀ females; however, these effects were not observed among F₁ females. The increase vacuolization and fat deposition was thought to be due to increased fat absorption. The results of this study indicate that Betapol™, a human milk fat equivalent that is similar to InFat™, did not produce any significant toxicological effects. The no-observed-adverse-effect level (NOAEL) was considered to be 15% in the diet, which is equivalent to 20.75 and 15.96 g/kg body weight/day in males and females, respectively.

Developmental and Reproductive Toxicity Studies

As discussed above, Spurgeon *et al.* (2003) also investigated the potential reproductive and developmental effects of Betapol™ in CrI:CD(SD) BR VAF/Plus rats. The authors reported no significant differences between the comparative controls and rats fed Betapol™ in reproductive performance in both the F₀ and F₁ generations. In addition, there were no significant developmental effects in any group in both the F₁ and F₂ generations. The no-observed-effect level (NOEL) for reproductive and developmental effects was therefore considered to be 15% in the diet, which is equivalent to 20.75 and 15.96 g/kg body weight/day in males and females, respectively.

Clinical Studies

A number of studies to assess the effects of Betapol™ have been conducted in both infants and adults. Clinical studies in infants have demonstrated no significant effects on growth or any significant adverse effects following consumption of approximately 2.27 to 7.8 g Betapol™/kg body weight/day for periods of 1 week to 4 months (Carnielli *et al.*, 1995a,b, 1996; Lucas *et al.*, 1997; Nelson and Innis, 1999). Likewise, studies in adults have demonstrated no significant effects following consumption of up to 1.26 g Betapol™/kg body weight/day for periods of up to 3 weeks (Zampelas, 1994; Zock *et al.*, 1996).

Potential Impact of Increased Exposure to Saturated Fatty Acids

The estimated mean and 90th percentile of **InFat**TM in the general population is 35.3 and 65.7 g/day. Considering that **InFat**TM consists of approximately 1/3 palmitic acid and 2/3 oleic acid, then the mean and 90th percentile consumption of palmitic acid would be 11.8 and 21.9 g/day. The current consumption of saturated fatty acids in the general population is 25.6 and 39.8 g/day (IOM, 2002). Among the saturated fatty acids, palmitic acid and stearic acid have less impact on cholesterol levels than lauric and myristic acids (Hunter, 2001). Thus, the consumption of **InFat**TM will not likely add to the current saturated fatty acid intake considering that **InFat**TM will replace other current sources of saturated fatty acids in the diet.

Although the use of **InFat**TM in infant formula increases the absorption of palmitic acid in infants, adults are thought to be capable of absorbing most dietary fatty acids efficiently regardless of whether there are in a non-esterified or monacyl form (Kubow, 1996). A comparison of the effects of BetapolTM (chemically identical to **InFat**TM) versus palm oil on cholesterol and lipoprotein levels in adults indicated little impact of the structural differences following 3 weeks of consumption (Zock *et al.*, 1995).

Although rabbit studies of native fats versus interesterified fat have not led to consistent effects on cholesterol or lipoprotein levels, fats bearing palmitic acid on the sn-2 position have been noted to be atherogenic in some animal species. For example, in a rabbit study of four synthetic fats, SOS, SSO, POP and PPO, all had similar effects on lipoprotein levels, PPO was significantly more atherogenic (Kritchevsky *et al.*, 2000). This effect appears to be specific to the rabbit since similar studies in the hamster indicated that randomization had neither effects on blood lipoprotein levels or atherogenicity (Hunter, 2001).

SUMMARY

Enzymotec, Ltd intends to market **InFat**TM, a functional fat enriched in palmitic acid at the sn-2 position similar to breast milk triglycerides, to replace normal vegetable oils used in infant formulas and traditional foods. The safety of **InFat**TM is primarily based on a number of published studies on BetapolTM, a high 2-palmitic acid functional fat that is similar in fatty acid composition to **InFat**TM and a comparative rat metabolism study of BetapolTM and **InFat**TM (Enzymotec, 2006) that confirmed that they were metabolized in the same fashion. In response to a GRAS Notice, the FDA advised that they had no questions with respect to the conclusion that BetapolTM was GRAS, based on scientific procedures, for use in infant formula for both term and preterm infants at a level of up to 80% of total fat (FDA, 2003). Under the conditions of intended use of **InFat**TM, the total population mean and 90th percentile all-user daily intakes of **InFat**TM are estimated to be 35.3 and 65.7 g/person/day, respectively. On a body weight basis, the average and 90th percentile intakes of **InFat**TM are estimated to be 0.7 and 1.5 g/kg body weight/day, respectively. Considering that **InFat**TM consists of approximately 1/3 palmitic acid and 2/3 oleic acid, then the mean and 90th percentile consumption of palmitic acid would be 11.8

and 21.9 g/day, which is less than the current consumption levels of saturated fatty acids in the general population cited by the Institute of Medicine (IOM) (25.6 and 39.8 g/day) (IOM, 2002). Thus, the consumption of **InFat™** will not likely add to the current saturated fatty acid intake considering that **InFat™** will replace other current sources of saturated fatty acids in the diet.

A multigenerational study in CrI:CD(SD) BR VAF/Plus rats revealed no significant toxicological, reproductive, or developmental effects following the administration of up to 15% Betapol™ in the diet (Spurgeon *et al.*, 2003), which is equivalent to up to 20.75 and 15.96 g/kg body weight/day in male and female rats, respectively. A number of studies in newborn piglets also have demonstrated that dietary exposure to approximately 18.4 g Betapol™/kg body weight/day for periods of up to 18 days resulted in no significant effects on growth or development (Innis *et al.*, 1993a,b, 1995). Clinical studies in infants have demonstrated no significant effects on growth or any significant adverse effects following consumption of approximately 2.27 to 7.8 g Betapol™/kg body weight/day for periods of 1 week to 4 months (Carnielli *et al.*, 1995a,b, 1996; Lucas *et al.*, 1997; Nelson and Innis, 1999). Similarly, studies in adults have demonstrated no significant effects following consumption of up to 1.26 g Betapol™/kg body weight/day for periods of up to 3 weeks (Zampelas *et al.*, 1994; Zock *et al.*, 1996). Although fats bearing palmitic acid on the sn-2 position have demonstrated atherogenic effects in rabbits (Kritchevsky *et al.*, 2000), such effects were not observed in hamsters (Hunter, 2001). Moreover, a clinical study by Zock *et al.* (1995) comparing the effects of Betapol™ and palm oil demonstrated that the structural differences in palmitic acid had little impact on cholesterol and lipoprotein levels.

The NOAEL of 15.96 g Betapol™/kg body weight/day in female rats provides a 22- and 10-fold margin of safety over the estimated average and 90th percentile intakes of **InFat™**, respectively.

Growth studies in which infants consumed up to 7.8 g Betapol™/kg body weight/day, doses that are up to 11- and 5.2-fold higher than the estimated average and 90th percentile intakes of **InFat™**, provide additional supporting evidence for the safety of **InFat™**. Considering that the material is well-absorbed and metabolized into normal body metabolites, that sn-2 esterified palmitic acid is a normal constituent of human breast milk, and that the intake of **InFat™** will not increase the current saturated fatty acid intake or significantly affect cholesterol or lipoprotein levels in humans, the available scientific evidence indicates that **InFat™**, meeting appropriated food grade specifications and manufactured in accordance with current good manufacturing practice, would be GRAS based on scientific procedures under the conditions of intended use in foods.

CONCLUSION

We, the Expert Panel, have, independently and collectively, critically evaluated the data and information summarized above and conclude that **InFaTM**, meeting appropriate food grade specifications and produced in according with current good manufacturing practice, is Generally Recognized As Safe (GRAS) based on scientific procedures under the conditions of intended use in foods specified herein.

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24 January 2006
Date

Robert J. Nicolosi, Ph.D., C.N.S.
University of Massachusetts Lowell

26 January 2006
Date

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Table 1 Chemical Specifications for InFa™		
Specification Parameter	Specification	Method
Identification		
Consistency (60°C)	Clear liquid	
Consistency (25°C)	Turbid liquid to paste	
Purity		
Triglycerides (%)	>96	AOCS Cd 11d-96 (1999)
Diacylglycerides (%)	<4	AOCS Cd 11d-96 (1999)
Monoacylglycerides (%)	<1	AOCS Cd 11d-96 (1999)
Ash (%)	<0.05	AOCS Ca 11-55 (1999)
Water (%)	<0.1	AOCS Ca 2e-84 (1999)
Peroxide value (meq/kg)	≤5	AOCS Cd 8-53 (1999)
Acid value (mg KOH/g)	≤0.3	IUPAC 2.201
Total heavy metals (mg/kg)	<0.5	Arsenic: Karlshamns Method E2214-1 Cadmium: Karlshamns Method E2217-1 Ni, Fe, Cu - IUPAC 2.632
Lead (mg/kg)	<0.1	Pb - IUPAC 2.632
Fatty Acids (% total fatty acids)		
Hexadecanoic acid (Palmitic acid) (16:0)	25 to 42	IUPAC 2.302
Octadecanoic acid (Stearic acid) (18:0)	1 to 6	IUPAC 2.302
<i>cis</i> -9-Octadecenoyl acid (Oleic acid) (18:1)	42 to 60	IUPAC 2.302
di- <i>cis</i> -9,12-Octadecadienoyl acid (Linoleic acid) (18:2)	6 to 17	IUPAC 2.302
Total <i>trans</i> fatty acids (%)	<2	AOCS PP Ch2a-94
Hexadecanoic acid (Palmitic acid) (16:0) esterified at sn-2 position (% of total palmitic acid)	62 to 70	IUPAC 2.210

Table 2 Microbiological Specifications for InFa™		
Specification Parameter	Specification	Method
Total count (CFU/g)	<1000	Israel Standard SI 885 Part 3 (1999)
Molds (CFU/g)	<100	Israel Standard SI 885 Part 8 (1999)
Yeast (CFU/g)	<100	Israel Standard SI 885 Part 8 (1999)
Coliforms (CFU/g)	Negative	USP 61 (2000)
<i>Salmonella</i> (CFU/g)	Negative	Israel Standard SI 885 Part 7 (1999)
<i>Staphylococcus aureus</i> (CFU/g)	Negative	USP 61 (2000)

Table 3 Summary of the Individual Proposed Food-Uses and Use-Levels for InFat™ in the United States

Food Category	Proposed Food-Use	InFat™ Use-Level (%)*
Baked Goods and Baking Mixes	Cakes	0.2-26.0
	Cookies	<0.1-36.2
	Grain-Based Crackers	0.9-25.3
	French Toast, Pancakes, and Waffles	0.4-20.6
	Pastries	0.6-38.8
	Pies	5.3-38.9
	Quick Breads (Biscuits, Cornbread, Corn Muffins, Tortillas, Muffins, Popovers, and Other Quick Breads)	0.6-26.9
	Yeast Breads and Rolls	0.9-24.4
Breakfast Cereals	Ready-to-Eat Cereals	0.2-18.8
	Cereal, Baby Food	0.2-7.8
Dairy Product Analogs	Fluid Milk, Imitation	1.6-3.4
Fats and Oils	Margarine, and Margarine-Like Spreads	1.5-80.8
	Mayonnaise and Mayonnaise-Type Dressings	0.5-79.4
	Salad Dressings (regular and low calorie)	<0.1-61.7
Frozen Dairy Desserts and Mixes	Frozen Yogurt	0.1-16.4
	Ice Cream and Frozen Milk Desserts	0.1-22.7
Gelatins, Puddings, and Fillings	Puddings, Custards and Other Milk Desserts	<0.1-29.3
Grain Products and Pastas	Frozen Grain-Based Meals	1.0-14.6
	Grain Mixtures (Burritos, Enchiladas, Tacos, Tamales, Nachos, Pizza, Pasta Dishes)	<0.1-55.1
	Cereals Grains, Not Cooked	1.5-20.1
	Grain-Based Patties	5.3
Gravies and Sauces	White Sauces and Milk Gravies	6.1-10.7
Hard Candy	Hard Candy	0.2-21.3
Milk	Milk	0.1-10.7
Meat Products	Beef Baby Food	1.1-6.9
	Pork Baby Food	5.8-14.6
	Lamb or Veal Baby Food	4.7-4.8
Milk Products	Creams and Cream Substitutes	1.8-37.0
	Dry and Powdered Milk and Mixtures (not reconstituted)	0.1-28.0
	Evaporated and Condensed Milk	0.1-8.7
	Flavored Milk and Milk Drinks	0.1-15.2
	Infant Formulas	1.8-3.4
	Milk-Based Meal Replacements	0.3-28.0
	Milk Desserts, Baby Food	1.0-2.0
Sour Cream	3.0-21.0	

Food Category	Proposed Food-Use	InFat™ Use-Level (%)*
	Yogurt	0.2-3.2
Poultry Products	Poultry Baby Food	5.8-14.4
Processed Fruits and Fruit Juices	Fruits and Fruit Mixtures, Baby Food	<0.1-0.5
	Fruit Juices, Baby Food	<0.1-0.8
	Fruit Desserts and Fruit-flavored Puddings, Baby Food	0.1-1.6
Soups and Soup Mixes	Grain Based Soups	0.1-7.5
Soft Candy	Candies and Chocolate	0.3-46.0
Snack Foods	Grain-Based Salty Snacks	1.2-36.7
Processed Vegetables and Vegetable Juices	Vegetable and Vegetable Mixtures, Baby Food	0.1-14.3

* Use-levels for InFat™ were calculated as follows: 70% of total fat for infant formulas; and 100% of total fat for all other proposed food uses. Values for total fat calculated from the Survey Nutrient Database, which is based on the USDA Nutrient Database for Standard Reference.

Population Group	% Users	Actual # of Total Users	All-Person Consumption		All-Users Consumption	
			Mean (g)	90 th Percentile (g)	Mean (g)	90 th Percentile (g)
Infants, 0 - 5 mos.	87.6	866	19.5	35.2	22.4	36.4
Infants, 6 - 11 mos.	99.6	495	21.5	33.7	21.6	33.8
Infants, 1 - 2 yrs.	100	2,096	27.1	44.8	27.1	44.8
Children, 3 - 5 yrs.	100	4,391	31.2	50.7	31.2	50.7
Children, 6 - 11 yrs.	100	1,913	37.8	61.2	37.8	61.2
Female Teenagers, 12 - 19 yrs.	100	702	36.3	64.4	36.3	64.4
Male Teenagers, 12 - 19 yrs.	100	696	52.1	89.2	52.1	89.2
Female Adults, 20 yrs. + up	99.9	4,567	28.7	53.0	28.8	53.0
Male Adults, 20 yrs. + up	99.8	4,744	40.6	77.2	40.7	77.2
Total Population, all ages	99.3	20,470	35.3	65.6	35.3	65.7

Table 5 Summary of the Estimated Daily Per Kilogram Body Weight Intake of InFat™ from All Proposed Food Categories in the United States by Population Group (1994-1996, 1998 USDA CSFII Data)

Population Group	% Users	Actual # of Total Users	All-Person Consumption		All-Users Consumption	
			Mean (g/kg)	90 th Percentile (g/kg)	Mean (g/kg)	90 th Percentile (g/kg)
Infants, 0-5 mos.	87.6	866	3.0	5.7	3.4	5.9
Infants, 6-11 mos.	99.6	495	2.5	4.1	2.5	4.1
Infants, 1-2 yrs.	100	2,096	2.2	3.6	2.2	3.6
Children, 3-5 yrs.	100	4,391	1.8	2.9	1.8	2.9
Children, 6-11 yrs.	100	1,913	1.3	2.2	1.3	2.2
Female Teenagers, 12-19 yrs.	100	702	0.7	1.2	0.7	1.2
Male Teenagers, 12-19 yrs.	100	696	0.8	1.4	0.8	1.4
Female Adults, 20 yrs. + up	99.9	4,567	0.4	0.8	0.4	0.8
Male Adults, 20 yrs. + up	99.8	4,744	0.5	0.9	0.5	0.9
Total Population, all ages	99.3	20,470	0.7	1.5	0.7	1.5

Table 6 Comparison of the Fatty Acid Composition of Human Milk, InFat™ and Betapol™

Fatty Acid	Human milk ¹		InFat™		Betapol™ ¹	
	% of Total Fatty Acids	% in sn-2 Position	% of Total Fatty Acids	% in sn-2 Position	% of Total Fatty Acids	% in sn-2 Position
14:0	6.6	57	<1.0	66-75	3.3	23.8
16:0	21.8	68	29-33	66-71	26.2	71.3
18:0	8.0	5	4-5	29-33	2.0	46.3
18:1	33.9	9	53-56	15-17.5	45.7	15.7
18:2	13.2	18	7.5-8.5	18.6-20.5	12.5	19.2
18:3	1.2	-	0.1-1.0	-	1.1	55.6

¹ Lien *et al.*, 1997

ATTACHMENT 1

CURRICULA VITAE OF EXPERT PANEL MEMBERS

000030

Pages 000031-000075 removed under Freedom of Information Act
Exemption 6.

SUBMISSION END

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AM



Fasano, Jeremiah

From: iris bendek [irisb@enzymotec.com]
Sent: Wednesday, May 24, 2006 1:06 PM
To: Fasano, Jeremiah
Subject: RE: GRAS Notice GRN 192 - Request for additional information
Attachments: InFat GRAS - Answers to FDA's questions 240506.doc

Dear Dr. Jeremiah Fasano,

It's been a pleasure speaking with you today.

Please find attached our answers to your questions regarding the InFat GRAS notification.

Following your recommendations I have made a few edits to the document so that the provided data will meet FDA requirements in terms of confidentiality. Only a few confidential details were removed while as much data as possible was maintained in order to provide a clear picture of the product's identity as well as its method of manufacture.

I hope you will find this data satisfactory. Should further clarifications are required or additional questions are raised during the evaluation process of our GRAS notification, please do not hesitate to contact me.

Sincerely,

Iris Meiri-Bendek
Regulatory Manager

Enzymotec - *Delivering Lipids*
P.O.Box 6, Migdal HaEmeq
Israel 23106
Tel: 972-4-6545112 (ex. 115)
Fax: 972-4-6443799
e-mail: irisb@enzymotec.com

000086

- You describe the common or usual name of the ingredient as "high 2-palmitic acid vegetable oil." The chemical name, Chemical Abstract Services Registration Number, empirical formula, formula weight, and structural formula are described as "not applicable." What are the predominant moieties in the ingredient? (e.g., 1,2-dipalmitoyl 3-oleyl triglyceride? 1,3-dioleoyl 2-palmitoyl triglyceride? Other minor triglyceride moieties?)

The predominant moieties in the ingredient are:

Chemical name	Chemical formula	CAS number
1,3-dioleoyl-2-palmitoylglycerol	$C_{55}H_{92}O_6$	1716-07-0
1,2-dipalmitoyl-3-oleoylglycerol	$C_{53}H_{90}O_6$	37179-82-1
Triolein	$C_{57}H_{94}O_6$	122-32-7

The following minor moieties may also be present:

Tripalmitin; 1-palmitoyl-2,3-dioleoylglycerol; 1,3-dipalmitoyl-2-oleoylglycerol; 1-linoleoyl-2-palmitoyl-3-oleoylglycerol; 1,2-dipalmitoyl-3-linoleoylglycerol; 1,2-dioleoyl-3-linoleoylglycerol; 1,3-dioleoyl-2-linoleoylglycerol; 1-stearoyl-2-palmitoyl-3-oleoylglycerol; 1,2-dipalmitoyl-3-stearoylglycerol; 1,2-dioleoyl-3-stearoylglycerol and 1,2-dioleoyl-2-stearoylglycerol.

In terms of the common name "high 2-palmitic acid vegetable oil", please also see the chemical analysis in Table 3.5-1 below that indicates that the sn-2 position of InFat™ contains between 59.7 and 66.1% palmitic acid hence the name "high 2-palmitic acid vegetable oil".

- You provide a comparison of the fatty acid composition of human milk, your ingredient, and the subject of GRN No. 000131 on page 17 of the notice. However, it is not clear if these values represent analyses of a single batch or of multiple lots of the ingredient. How many batches are represented by the values presented in Table 6? Are these means or single values? Have you assessed the ability of the method of manufacture to produce a consistent product?

The ranges presented in Table 6 were determined based on values obtained for 5 commercial representative lots. Table 3.5-1 below provides data on the total fatty acid composition, and composition of fatty acids esterified to the sn-2 position in each of these 5 lots. The ability of the method of manufacture to produce a consistent product is supported by both this table and Table 3.4.1-1 below.

Fatty acid content (% of total fatty acids)	Manufacturing Lot				
	Lot 593759-2	Lot 593759-3	Lot 593759-4	Lot 593759-5	Lot 593759-6
Octanoic acid (Caprylic acid) (8:0)	<0.05	0.1	0.1	0.1	<0.05
Decanoic acid (Capric acid) (10:0)	<0.05	0.1	0.1	<0.05	<0.05
Dodecanoic acid (Lauric acid) (12:0)	0.2	0.2	0.1	0.1	0.1
Tetradecanoic acid (Myristic acid) (14:0)	0.5	0.5	0.4	0.4	0.4
Hexadecanoic acid (Palmitic acid) (16:0)	29.4	29.6	32.6	32.2	30.6
9-Hexadecenoic acid (Palmitoleic Acid) (16:1)	0.1	0.1	0.1	0.1	0.1
Heptadecanoic acid (Margaric acid) (17:0)	0.1	0.1	0.1	0.1	0.1
Octadecanoic acid (Stearic acid) (18:0)	4.4	4.4	4	4.1	3.8
<i>Cis</i> -9-Octadecenoic acid (Oleic acid) (18:1)	55.9	55.5	53.1	53.4	55
<i>di-cis</i> -9,12-Octadecadienoic acid (Linoleic acid) (18:2)	7.8	8.2	8	7.9	8.4
Conjugated linoleic acid (18:2 conj)	<0.05	<0.05	0.1	0.1	0.1
9,12,15-Octadecatrienoic acid (<i>alpha</i> -Linolenic acid) (18:3)	0.2	0.3	0.3	0.3	0.5
Eicosanoic acid (Arachidic acid) (20:0)	0.3	<0.05	0.3	0.3	0.3
9-Eicosenoic acid (Gadoleic acid) (20:1)	0.1	0.2	0.2	0.2	0.3
Docosanoic acid (Behenic acid) (22:0)	<0.05	0.4	0.5	0.1	0.3
13-Docosenoic acid (Erucic acid) (22:1)	<0.05	<0.05	0.1	0.1	0.1
Tetracosanoic acid (Lignoceric acid) (24:0)	0.2	0.1	0.1	0.2	0.1
Fatty Acids Esterified at sn-2 Position (% total fatty acids)					
Octanoic acid (Caprylic acid) (8:0)	<0.05	0.2	0.2	0.1	<0.05
Decanoic acid (Capric acid) (10:0)	<0.05	0.1	0.1	-	<0.05
Dodecanoic acid (Lauric acid) (12:0)	0.5	0.4	0.2	0.2	0.2
Tetradecanoic acid (Myristic acid) (14:0)	1	1.1	0.8	0.8	0.9
Hexadecanoic acid (Palmitic acid) (16:0)	59.7	61.3	66.1	66	62.9
Octadecanoic acid (Stearic acid) (18:0)	4	4.1	3.7	3.6	3.8

Table 3.5-1 Fatty Acid Composition of InFat™					
Fatty acid content (% of total fatty acids)	Manufacturing Lot				
	Lot 593759-2	Lot 593759-3	Lot 593759-4	Lot 593759-5	Lot 593759-6
<i>cis</i> -9-Octadecenoic acid (Oleic acid) (18:1)	29.3	27	23.9	24.2	26.4
di- <i>cis</i> -9,12-Octadecadienoic acid (Linoleic acid) (18:2)	4.8	5	4.6	4.4	4.9
9,12,15-Octadecatrienoic acid (<i>alpha</i> -Linolenic acid) (18:3)	<0.05	0.3	<0.05	<0.05	<0.05
Eicosanoic acid (Arachidic acid) (20:0)	0.2	0.2	0.2	0.2	0.3
Docosanoic acid (Behenic acid) (22:0)	0.2	0.1	0.2	0.2	0.2
Fatty Acids Esterified at sn-2 Position (% total individual fatty acid)					
Octanoic acid (Caprylic acid) (8:0)	-	-	66.7	33.3	-
Decanoic acid (Capric acid) (10:0)	-	-	33.3	-	-
Dodecanoic acid (Lauric acid) (12:0)	83.3	66.7	66.7	66.7	66.7
Tetradecanoic acid (Myristic acid) (14:0)	66.7	73.3	66.7	66.7	75.0
Hexadecanoic acid (Palmitic acid) (16:0)	67.7	69.0	67.6	68.3	68.5
Octadecanoic acid (Stearic acid) (18:0)	30.3	31.1	30.8	29.3	33.3
<i>cis</i> -9-Octadecenoic acid (Oleic acid) (18:1)	17.5	16.2	15.0	15.1	16.0
di- <i>cis</i> -9,12-Octadecadienoic acid (Linoleic acid) (18:2)	20.5	20.3	19.2	18.6	19.4
Eicosanoic acid (Arachidic acid) (20:0)	22.2	-	22.2	22.2	33.3
Docosanoic acid (Behenic acid) (22:0)	-	8.3	13.3	66.7	22.2

Table 3.4.1-1 Summary of the Chemical Product Analysis for 5 Lots of InFat™						
Specification Parameter	Specification	Manufacturing Lot				
		Lot 593759-2	Lot 593759-3	Lot 593759-4	Lot 593759-5	Lot 593759-6
<u>Identification</u>						
Consistency (60°C)	Clear liquid	Conforms	Conforms	Conforms	Conforms	Conforms
Consistency (25°C)	Turbid liquid to paste	Conforms	Conforms	Conforms	Conforms	Conforms
<u>Purity</u>						
Triglycerides (%)	>96	98.9	98.7	99.2	99.2	99.2
Diacylglycerides (%)	<4	0.9	1.2	0.6	0.6	0.6
Monoacylglycerides (%)	<1	<0.1	<0.1	<0.1	<0.1	<0.1
Ash (%)	<0.05	<0.001	-	-	<0.001	<0.001
Water (%)	<0.1	0.04	-	-	<0.01	<0.01
Peroxide value (meq/kg)	≤5	<0.1	<0.1	<0.1	<0.1	<0.1
Acid value (mg KOH/g)	≤0.3	0.19	0.22	0.1	0.13	0.18
Total heavy metals (mg/kg)	<1	<0.5	<0.5	<0.5	<0.5	<0.5

Table 3.4.1-1 Summary of the Chemical Product Analysis for 5 Lots of InFat™						
Specification Parameter	Specification	Manufacturing Lot				
		Lot 593759-2	Lot 593759-3	Lot 593759-4	Lot 593759-5	Lot 593759-6
Lead (mg/kg)	<0.1	<0.01	<0.01	<0.01	<0.01	<0.01
Fatty Acids (% total fatty acids)						
Hexadecanoic acid (Palmitic acid) (16:0)	25 to 42	29.4	29.6	32.6	32.2	30.6
Octadecanoic acid (Stearic acid) (18:0)	1 to 6	4.4	4.4	4	4.1	3.8
<i>cis</i> -9-Octadecenoyl acid (Oleic acid) (18:1)	42 to 60	55.9	55.4	53.2	53.5	55
di- <i>cis</i> -9,12-Octadecadienoyl acid (Linoleic acid) (18:2)	6 to 17	7.8	8.2	8	7.9	8.3
Total <i>trans</i> fatty acids (%)	<2	1.4	1.1	0.9	0.9	1.2
Fatty Acids Esterified at sn-2 Position (% total fatty acids)						
Hexadecanoic acid (Palmitic acid) (16:0) esterified at sn-2 position (% of total palmitic acid)	62 to 70	67.7	69.0	67.6	68.3	68.5

- You do not describe the starting materials used in the manufacture of your ingredient. What is the source of the oleic acid? What is the composition of the palm stearin starting material? This information may have regulatory significance; for example, canola oil has been affirmed as GRAS by FDA with the specific exception of use in infant formula.

The source of oleic acids is either palm kernel oil or high oleic sunflower oil.

The palm stearin starting material is mainly composed of tripalmitin, and triglyceride moieties that are composed of two palmitic acids and one oleic acid (i.e. PPO, POP).

The fatty acid composition (as % of total fatty acids) of the palm stearin is:

C12:0 – 0.2-0.7

C14:0 – 1.3-1.4

C16:0 – 76.9-78.5

C17:0 – 0.1

C18:0 – 4.5-4.8

C18:1 – 12.1-13.1

C18:2 – 2.2-2.5

C20:0 – 0.3

Data represents 5 lots of palm stearin that were analyzed at our laboratory.

- Please describe the conditions of use of the enzyme. Is the enzyme preparation immobilized or is it added directly to the starting materials? Is it used in accordance with good manufacturing practices? Are all substances used in conjunction with the use of the (possibly immobilized) enzyme safe and otherwise in compliance with all applicable legal and regulatory requirements?

The enzyme preparation is immobilized onto the ionic exchange resin and is used as an immobilized enzyme in the reaction. The immobilization enables recycling of the enzyme.

The immobilization involves the use of the following raw materials and processing aids:

- Lipase - lipase derived from *Aspergillus oryzae* carrying a gene encoding lipase from *Thermomyces lanuginosus* is the subject of a current GRAS notification (GRN 000043) for use as a catalyst in the interesterification of glycerides and acydolysis between glycerides and fatty acids as the maximum level of 1 kg lipase per ton of triglycerides
- Resin - The resin is approved as a secondary direct additive under 21 CFR § 173.25.
- Buffer - the buffer components in the preparation of the immobilized lipase preparation meet food grade specifications if the FCC (2003).
- Sorbitan Monooleate - sorbitan monooleate is approved for use as a second direct food additive meeting the food grade specifications of 21 CFR § 173.75 and is used as an emulsifying agent to coat the immobilized enzyme. Current uses as a foaming agent in the manufacture of sugarcane or sugarbeet result in residual levels of 0.7 ppm in the sugar juice. The current group ADI is 0-25 mg/kg (JECFA, 1982).

The immobilization process (ionic binding) which is carried out in accordance with good manufacturing practices, involves pre-treatment for the resin according to the manufacturer's recommendations, immobilization of the lipase enzymes onto the resin in a buffer solution and in the presence of sorbitan monooleate. The immobilization step is followed by washings of the immobilized enzyme with water in order to remove excess protein and sorbitan monooleate, and drying. The solutions as well as the water used for the washings are all sterile and prepared from di-ionized water. Analytical tests such as microbial analysis and determination of protein residues are carried out at specific points during the process and on the final immobilized enzyme preparation in order to ensure proper quality. The immobilized enzyme is packed using packing materials approved for food and stored under controlled conditions.

- You state that "potential residues from the manufacturing process including protein, sorbitan oleate, methanol, and epichlorohydrin were considerably below acceptable levels."

- o What do you consider acceptable levels of these residues?

The acceptable level of protein residue in InFat™ was determined to be <100 ppm which is the level of detection in the analytical method (biuret reaction) employed to detect protein. Based on an exaggerated exposure to infants of 35 g/day from infant formula and 65 g/day to the total population as a whole from other proposed uses, the maximum exposure to protein would be <3.5 mg and <6.5 mg, respectively. Considering that *Aspergillus niger* is a common contaminant in food and used a source of many food processing enzymes, individuals have been exposed to these proteins for many centuries without the development of any adverse effects.

Acceptable levels of sorbitan monooleate are based on fact that it is an approved food additive with a relatively large ADI of 25 mg/kg bw/day. On a body weight basis, the heavy consumers will consume 5.9 g InFat/Kg BW/day. Based on the ADI of SMO, this means that the acceptable level of SMO in InFat is $25 \text{ mg} / 5.9 \text{ g} * 100 = 0.4\%$ while our levels are <0.01%.

Acceptable levels of methanol are based on the naturally occurring levels of methanol found in various fruit juices and other food products that can be considerable and result in background methanol exposures of 10-40 mg/day. In addition, risk assessment of methanol based on animal and human studies have indicated that 7.1-8.4 mg/kg body weight/day of methanol is considered safe (58 Fed Register 6088-6092, 1993). Thus the level from InFat™ (<0.5 ppm) would be considered to be trivial.

The deodorization process, aimed at removing excess free fatty acids (bp up to 330°C), ensures the complete removal of any possible methanol residues (bp of 65°C).

Acceptable levels of epichlorohydrin are based on risk assessment information published in the Federal Register (67 Fed Reg 42714-42717, 2002) (see tables and discussion in the points on sources of epichlorohydrin).

- o Are there specifications for these residues (e.g., methanol, epichlorohydrin)? We would expect specifications and/or a discussion of the ability of the method of manufacture to remove potential contaminants and to consistently produce a safe product.

Specifications for residual levels of protein, sorbitan monooleate, methanol and epichlorohydrin were not considered necessary because the levels of residues were below the levels of detection in several Lots of InFat™ and significantly below the levels that might be a health risk. In addition, the manufacturing process produces a consistent product in terms of potential residues.

- o What is the source of the epichlorohydrin? How is it produced during the manufacturing process? How is it removed?

The ion exchange resin used to immobilize the enzyme is chemically manufactured using epichlorohydrin which potentially could give rise to epichlorohydrin and 1,3-dichloro-2-propanol residues in InFat™. The potential residues from two Lots of the preconditioned resin using thermal desorption/purge and trap GC/MS indicated residues of epichlorohydrin in the solid resin of 1.2 to 2.4 ppm (Rohm and Haas Technical Report). The ratio of InFat™ to the resin is approximately 1:100 (w/w) and thus the level of epichlorohydrin would be 1/100th this level as summarized in the following Table 3.6-1. Assuming that all the epichlorohydrin reacted with water to form 1,3-dichloro-2-propanol and adjusting for the differences in molecular weight, the potential worst case levels of 1,3-dichloro-2-propanol in InFat™ can also be determined.

Chemical Residue	Residue Levels (ppm)	
	Lot 1 of resin	Lot 2 of resin
Epichlorohydrin	<0.012	<0.024
1,3-Dichloro-2-propanol	<0.0084	<0.017

These levels would be considered to be worst case residue levels since InFat™ undergoes a distillation process designed to remove fatty acids (boiling point 330°C at 760mmHg) from a level of 80% to 0.15%, and therefore, both the lower boiling residues (116°C, 174 °C at 760mmHg) will be removed as well. In addition, the supplied resins undergo a washing procedure prior to use.

Based on an estimated 90th percentile intake of InFat™ of 65.7 g/day, the exposures to epichlorohydrin and 1,3-dichloro-2-propanol from consumption of InFat™ can be determined and compared to permitted levels (Table 3.6.2). As calculated the potential residue levels are below what would be considered to have adverse health effects.

Chemical Residue	Potential Daily Residue Exposure		Permitted Exposure ¹ (µg/day)
	(µg/day) Lot 1 resin	Lot 2 resin	
Epichlorohydrin	0.79	1.57	22.11
1,3-Dichloro-2-propanol	0.55	1.11	1.75

¹ Permitted exposure to give risk of 1 in 1 x10⁶ (Federal Register, 2002).

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- You rely on safety studies with the subject of GRN No. 000131 to support the safety of your ingredient. You provide a comparison table, but we would also expect a discussion of the differences and similarities in composition between the two ingredients and the significance or insignificance of these differences with respect to the GRAS determination.

InFat™ and the product listed under GRN 000131 have similar overall proportions of fatty acids in terms of total fatty acids and similar distributions of fatty acids at sn-2 or sn-1 and sn-3 positions. In addition, the two materials are both manufactured from palm stearin and oleic acid although the reaction is catalysed by different GRAS enzymes. Thus, the two products should be digested and absorbed in a similar fashion and have similar nutritional attributes. The similar digestion was demonstrated in a comparative metabolism study of the two products (Enzymotec, 2006) in rats where they were absorbed to a similar degree and showed similar levels of excreted soaps in the feces.

The small differences in levels of some of the minor fatty acids (e.g. linoleic acid) has no safety consequences since these products are used with other fats in infant formula that supply the essential fatty acids, linoleic and linolenic acid.

In general terms, triglycerides would not be expected to have safety consequences unless they contained abnormal levels of specific fatty acids such as erucic acid or other unusual fatty acids. Also, the triglycerides are degraded to fatty acids that are considered to be normal body metabolites. Thus, potential differences in safety could only result from potential impurities in products resulting from manufacturing conditions. In both cases, potential impurities are strictly controlled and both products meet the specifications for other triglyceride oils found in the Food Chemicals Codex.

- You state that the use of your ingredient in adult foods is "restricted based on its technological properties such as melting temperature." Please explain these restrictions in more detail.

The use of different types of fats in various food products are highly dependent on technical properties such as "mouthfeel" and other properties such as solids content and stability all of which are related to the melting temperature of the fat. Since InFat™ is a semisolid fat at room temperature, it would only be used to replace other semisolid types of fat in adult foods. For example, fats used in baked goods are mainly hydrogenated fats that have good oxidative stability and have a higher solid fat index to create better texturized products. In addition, as a result of its more saturated fatty acid composition, it could be used in the manufacture of margarine that usually is a mixture of liquid and solid fats.

AM



Fasano, Jeremiah

From: iris bendek [irisb@enzymotec.com]
Sent: Monday, June 19, 2006 4:32 PM
To: Fasano, Jeremiah
Cc: 'Larry McGirr'
Subject: RE: GRAS Notice GRN 192 - Request for additional information
Attachments: Table 3.5-1.doc

Dear Dr. Fasano,

Thank you for your email.

I will respond to your questions regarding the distillation and deodorization processes tomorrow as I would like to confirm the technical details with our Process Engineering Manager before I answer. I hope this is OK.

You are absolutely correct in regards to table 3.5.-1. Please accept my apology for that. For your convenience, I enclosed the table again.

Best regards,

Iris Meiri-Bendek
Regulatory Affairs Manager

Enzymotec - *Delivering Lipids*
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e-mail: irisb@enzymotec.com

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Table 3.5-1 Fatty Acid Composition of InFat™

Fatty acid content (% of total fatty acids)	Manufacturing Lot				
	Lot 593759-2	Lot 593759-3	Lot 593759-4	Lot 593759-5	Lot 593759-6
Octanoic acid (Caprylic acid) (8:0)	<0.05	0.1	0.1	0.1	<0.05
Decanoic acid (Capric acid) (10:0)	<0.05	0.1	0.1	<0.05	<0.05
Dodecanoic acid (Lauric acid) (12:0)	0.2	0.2	0.1	0.1	0.1
Tetradecanoic acid (Myristic acid) (14:0)	0.5	0.5	0.4	0.4	0.4
Hexadecanoic acid (Palmitic acid) (16:0)	29.4	29.6	32.6	32.2	30.6
9-Hexadecenoic acid (Palmitoleic Acid) (16:1)	0.1	0.1	0.1	0.1	0.1
Heptadecanoic acid (Margaric acid) (17:0)	0.1	0.1	0.1	0.1	0.1
Octadecanoic acid (Stearic acid) (18:0)	4.4	4.4	4	4.1	3.8
<i>Cis</i> -9-Octadecenoic acid (Oleic acid) (18:1)	55.9	55.5	53.1	53.4	55
<i>di-cis</i> -9,12-Octadecadienoic acid (Linoleic acid) (18:2)	7.8	8.2	8	7.9	8.4
Conjugated linoleic acid (18:2 conj)	<0.05	<0.05	0.1	0.1	0.1
9,12,15-Octadecatrienoic acid (<i>alpha</i> -Linolenic acid) (18:3)	0.2	0.3	0.3	0.3	0.5
Eicosanoic acid (Arachidic acid) (20:0)	0.3	<0.05	0.3	0.3	0.3
9-Eicosenoic acid (Gadoleic acid) (20:1)	0.1	0.2	0.2	0.2	0.3
Docosanoic acid (Behenic acid) (22:0)	<0.05	0.4	0.5	0.1	0.3
13-Docosenoic acid (Erucic acid) (22:1)	<0.05	<0.05	0.1	0.1	0.1
Tetracosanoic acid (Lignoceric acid) (24:0)	0.2	0.1	0.1	0.2	0.1
Fatty Acids Esterified at sn-2 Position (% total fatty acids)					
Octanoic acid (Caprylic acid) (8:0)	<0.05	0.2	0.2	0.1	<0.05
Decanoic acid (Capric acid) (10:0)	<0.05	0.1	0.1	-	<0.05
Dodecanoic acid (Lauric acid) (12:0)	0.5	0.4	0.2	0.2	0.2
Tetradecanoic acid (Myristic acid) (14:0)	1	1.1	0.8	0.8	0.9
Hexadecanoic acid (Palmitic acid) (16:0)	59.7	61.3	66.1	66	62.9
Octadecanoic acid (Stearic acid) (18:0)	4	4.1	3.7	3.6	3.8
<i>cis</i> -9-Octadecenoic acid (Oleic acid) (18:1)	29.3	27	23.9	24.2	26.4
<i>di-cis</i> -9,12-Octadecadienoic acid (Linoleic acid) (18:2)	4.8	5	4.6	4.4	4.9
9,12,15-Octadecatrienoic acid (<i>alpha</i> -Linolenic acid) (18:3)	<0.05	0.3	<0.05	<0.05	<0.05
Eicosanoic acid (Arachidic acid) (20:0)	0.2	0.2	0.2	0.2	0.3
Docosanoic acid (Behenic acid) (22:0)	0.2	0.1	0.2	0.2	0.2
Fatty Acids Esterified at sn-2 Position (% total individual fatty acid)					
Octanoic acid (Caprylic acid) (8:0)	-	-	66.7	33.3	-
Decanoic acid (Capric acid) (10:0)	-	-	33.3	-	-
Dodecanoic acid (Lauric acid) (12:0)	83.3	66.7	66.7	66.7	66.7
Tetradecanoic acid (Myristic acid) (14:0)	66.7	73.3	66.7	66.7	75.0
Hexadecanoic acid (Palmitic acid) (16:0)	67.7	69.0	67.6	68.3	68.5
Octadecanoic acid (Stearic acid) (18:0)	30.3	31.1	30.8	29.3	33.3
<i>cis</i> -9-Octadecenoic acid (Oleic acid) (18:1)	17.5	16.2	15.0	15.1	16.0
<i>di-cis</i> -9,12-Octadecadienoic acid (Linoleic acid) (18:2)	20.5	20.3	19.2	18.6	19.4
Eicosanoic acid (Arachidic acid) (20:0)	22.2	-	22.2	22.2	33.3
Docosanoic acid (Behenic acid) (22:0)	-	8.3	13.3	66.7	22.2

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AM



Fasano, Jeremiah

From: iris bendek [irisb@enzymotec.com]
Sent: Tuesday, June 20, 2006 9:34 AM
To: Fasano, Jeremiah
Cc: 'Larry McGirr'
Subject: RE: GRAS Notice GRN 192 - Request for additional information

Dear Dr. Fasano,

To answer both your questions:

The production process of InFat involves two steps of distillation aimed at removal of the free fatty acids up to a level below 0.15%. The distillation step removes free fatty acids to a level of 1-2% and the deodorization step removes the free fatty acids from 1-2% to below 0.15%.

Both steps are carried out using a standard industrial procedure known as "steam-distillation". This technology is commonly used by the oils and fats industry for the very same purpose of removal of free fatty acids and other light components, specifically from edible oils. By adding steam, the boiling point of the compounds is depressed, allowing them to evaporate at lower temperatures

Typical conditions for both the distillation and the deodorization processes are - vacuum: 1-4 mbar; temperature: 190-230 degC. Following both the distillation and the deodorization steps conducted under these conditions, methanol residues are removed, as proved by the analytical results obtained for methanol residues in InFat (less than 0.5 ppm, where 0.5 ppm is the method's detection limit).

I hope you will find this data satisfactory.

Should you have further questions on this issue or in regards to other issues, please do not hesitate to contact me.

Best regards,

Iris Meiri-Bendek
Regulatory Affairs Manager

Enzymotec - *Delivering Lipids*
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Fax: 972-4-6443799
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**Fasano, Jeremiah**

From: Iris Bendek [irisb@enzymotec.com]
Sent: Wednesday, July 05, 2006 10:44 AM
To: Fasano, Jeremiah
Cc: Larry McGirr
Subject: RE: GRAS Notice GRN 192 - Request for additional information

Dear Dr. Fasano,

Thank you for your email and sorry for my delayed reply.

1. The detection limit for measurement of epichlorohydrin in the oil is 0.3 ppm, using a GC/MS-head space method that we developed for this specific case. It should be noted that there are no standard methods for measurement of epichlorohydrin in oil samples in contrast to the measurement of epichlorohydrin in water based matrices. Measurement of epichlorohydrin in InFat resulted in below detection limit level of epichlorohydrin which leads to residual levels below the predetermined risk assessment level (as indicated in our May 24, 2006 amendment). Since this analytical method did not seem to be sensitive enough, the potential worst case levels in InFat were also calculated based on the levels of epichlorohydrin that contaminated the dry resin and could potentially be washed off into the InFat during the manufacturing process. Resin epichlorohydrin residues were provided by the resin manufacturer. These levels would be considered worse case since the resin undergoes pre-treatment prior to use and is washed multiple times as part of the immobilization process. In addition, the final oil undergo distillation designed to remove free fatty acids with boiling point much higher than the boiling point of epichlorohydrin.

2. The bleaching earth (Bentonite) being used in the manufacture of InFat meet food grade specifications of the FCC (2003) and considered to be GRAS for use as processing aid in the processing of vegetable oils (21 CFR §184.1155).

I hope you will find the above information satisfactory. Should additional questions be raised regarding these matters or any other matter, please do not hesitate to contact me.

Best regards,

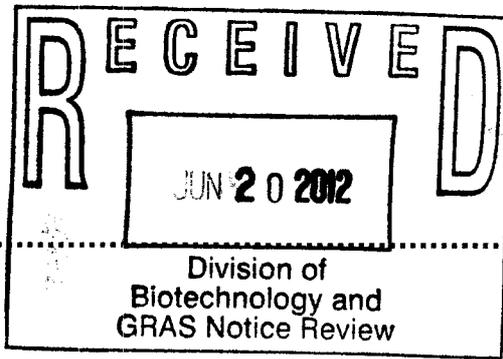
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Enzymotec
Delivering Lipids



SU



June 17, 2012

Antonia Mattia, Ph.D.
Division of Biotechnology and GRAS Notice Review, HFS-255
CFSAN/FDA
5100 Paint Branch Pkwy.
College Park, MD 20740-3835

RE: Amendment to GRAS Notice GRN 192 (High 2-palmitic vegetable oil)

Dear Dr. Mattia:

In January 2006, Enzymotec submitted a notice summarizing its position that high 2-palmitic acid vegetable oil (InFat™) is generally recognized as safe (GRAS), through scientific procedures, for use in term and pre-term infant formulas at levels up to 70% of total fat, as well as in a variety of other foods (GRN 192). On August 3, 2006, the agency responded that FDA had no questions regarding Enzymotec's conclusion that use of the product is GRAS under the conditions described in the notice (FDA letter to Iris Meiri-Bendek available at: <http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm154665.htm>). A copy of this letter is enclosed for your convenience.

Enzymotec is notifying the U.S. FDA of additional information to amend our GRAS Notification (GRN 000192) to change the specifications for several substances present in InFat™. By this letter, Enzymotec notifies FDA of Enzymotec's determination that the changes in the specifications for several substances in InFat™ is GRAS and will not result in any changes in the safety of InFat™ when used as described in GRN 192.

Enzymotec is making the changes in product specifications due to changes in the production of InFat™ and in order to meet market needs. Enzymotec has eliminated the randomization step in the new production process as it is no longer needed. The new production process also provides better products to satisfy our customer requirements. All of the substances for which Enzymotec are changing specifications are already present in many different food products at varying levels. Enzymotec has determined that the changes in specifications for these substances will not result in any significant changes in exposure to these substances. The catalyst and resin used are the same in both processes. Enzymotec has determined that no issues of concern arose with the new process.

The substances that were analyzed and reported in GRN 192 are listed on pages 10, 26, 88, 89, 90 and 99 [your page numbers]. Enzymotec has analyzed all batches of InFat™ produced using the revised production process. Listed below in Table I are

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the results of six representative batches for the substances that Enzymotec is proposing to change specifications.

Table I. Results from Analysis of Six Batches of InFat™

Specification Parameter	Batch No. 1064260	Batch No. 1064257	Batch No. 1064648	Batch No. 1067166	Batch No. 1068970	Batch No. 1069474
Lead (mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium (mg/g)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Arsenic (mg/g)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Mercury (mg/kg)	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Hexadecanoic acid (Palmitic acid) (16:0)	45.2	40	40.2	39.9	39.4	39.9
Octadecanoic acid (Stearic acid) (18:0)	6.1	5.1	5.7	5.6	5.1	5.2
Cis-9-Octadecenoyl acid (Oleic acid) (18:1)	41.1	46.5	45.8	45.9	47	46.6
di-cis-9,12-Octadecadienoyl acid (Linoleic acid) (18:2)	6.1	7.1	7.1	7.1	7.3	7.3
Hexadecanoic acid (Palmitic acid) (16:0) esterified at sn-2 position (% of total palmitic acid)	54.9	55.2	55.9	56.2	55.0	54.6

The substances for which Enzymotec has changed the specifications are listed in Table II below along with the current specifications in GRN 192. The specifications for all other substances, including stability parameters and residual levels of potential contaminants will remain the same.

Table II. Proposed Changes in Specifications for Several Substances in InFat™

Specification Parameter	Specification in Existing Notification	Proposed New Specification
Total heavy metals (mg/kg)	<0.5	
Lead (mg/kg)		<0.1
Cadmium (mg/g)		<0.02
Arsenic (mg/g)		<0.1
Mercury (mg/kg)		<0.2
Hexadecanoic acid (Palmitic acid) (16:0)	25 to 42	27 to 48
Octadecanoic acid (Stearic acid) (18:0)	1 to 6	3 to 8
Cis-9-Octadecenoyl acid (Oleic acid) (18:1)	42 to 60	40 to 58
di-cis-9,12-Octadecadienoyl acid (Linoleic acid) (18:2)	6 to 17	4 to 11
Hexadecanoic acid (Palmitic acid) (16:0) esterified at sn-2 position (% of total palmitic acid)	62 to 70	>52

The proposed change in specifications for heavy metals involves the definition of metal-specific limits instead of a single limit for total heavy metals. Metal-specific limits allow for better control of each of the four defined heavy metals. Enzymotec notes that nothing in the revised process would cause the heavy metals value to increase.

In conclusion, Enzymotec has determined that the changes in specifications for the substances listed in Table II above does not change Enzymotec's determination that InFat™ is GRAS based on scientific procedures and is consistent with the safety data contained in GRN 192. Enzymotec further concludes that other scientists competently qualified would reach the same conclusion.

Enzymotec agrees that the information in support of this supplement is based on generally available and accepted scientific data, information, methods and principles, as well as experience of these substances based on common use in foods. This information will be made available to FDA upon request by contacting the company at the address above. Therefore, Enzymotec has determined that the changes in specifications for InFat™, when used as indicated in GRN 192 is GRAS, and is exempt from the premarket approval requirements of the U.S. Food, Drug, and Cosmetic Act.



Enzymotec
Delivering Lipids

Thank you for your consideration of this request and your prompt response. Please contact me if you have any questions or require additional information.

Sincerely,

(b) (6)

Iris Meiri Bendek
Regulatory Affairs
Enzymotec Ltd.
Email: iris@enzymotec.com
Mobile: 972-54-5315999

Enclosure: Copy of Response Letter for GRN 192



Iris Meiri-Bendek
Enzymotec Ltd.
P.O. Box 6
Migdal HaEmeq
ISRAEL, 23106

AUG 3 2006

Re: GRAS Notice No. GRN 000192

Dear Ms. Meiri-Bendek:

The Food and Drug Administration (FDA) is responding to the notice, dated January 31, 2006, that you submitted in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received the notice on February 2, 2006, filed it on February 6, 2006, and designated it as GRAS Notice No. GRN 000192.

The subject of the notice is high 2-palmitic acid vegetable oil¹ (prepared by enzymatic modification of palm stearin). The notice informs FDA of the view of Enzymotec Ltd. (Enzymotec) that high 2-palmitic acid vegetable oil is GRAS, through scientific procedures, for use in term and pre-term infant formulas at levels up to 70% of total fat, to replace all added fat in baby and toddler foods (including meat and poultry products), and to replace all added fat in processed foods in general (excluding meat and poultry products).

As part of its notice, Enzymotec includes the report of a panel of individuals (Enzymotec's GRAS panel) who evaluated the data and information that are the basis for Enzymotec's GRAS determination. Enzymotec considers the members of its GRAS panel to be qualified by scientific training and experience to evaluate the safety of substances added to food. Enzymotec's GRAS panel discusses composition, manufacture, specifications, exposure, and safety data (including absorption, distribution, metabolism, and excretion studies; preclinical sub-chronic studies, chronic, and reproductive studies; and clinical studies). Enzymotec's GRAS panel considers that high 2-palmitic acid vegetable oil, meeting appropriate food-grade specifications and manufactured in accordance with good manufacturing practice, is GRAS based on scientific procedures under the conditions of its intended use in foods.

Our use of "high 2-palmitic vegetable oil" in this letter should not be considered an endorsement or recommendation of that term as an appropriate common or usual name for the purpose of declaring the substance in the ingredient statement of foods that contain that ingredient. 21 CFR 101.4 states that all ingredients must be declared by their common or usual name. In addition, 21 CFR 102.5 outlines general principles to use when establishing common or usual names for non-standardized

¹ Throughout the notice, Enzymotec refers to the ingredient that is the subject of GRN 000192 by the trade name "InFat™" and the common or usual name of "high 2-palmitic acid vegetable oil," referring to the content of palmitic acid at the *sn*-2 position of the glycerol backbone.

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foods. Issues associated with labeling and the appropriate common or usual name of a food are the responsibility of the Office of Nutritional Products, Labeling, and Dietary Supplements.

High 2-palmitic acid vegetable oil is prepared by the enzymatic modification of palm stearin (composed mainly of tripalmitin) to yield an ingredient that more closely resembles human milk triglycerides in its fatty acid positional distribution relative to traditional vegetable oils. High 2-palmitic acid vegetable oil is a colorless and flavorless product. Its consistency ranges from turbid liquid to paste at room temperature.

The manufacturing process for the formation of high 2-palmitic acid vegetable oil has two steps. The first step involves the randomization of palm stearin fatty acids by treatment with the chemical catalyst, sodium methoxide. The mixture then undergoes neutralization and a bleaching process similar to that used for traditional vegetable oils.

The second step in the manufacturing process is an enzymatic transesterification reaction. The high 2-palmitic acid vegetable oil is produced by enzymatic esterification of randomized palm stearin with fatty acids (primarily oleic acid) from either palm kernel oil or high oleic sunflower oil. The palmitic acid residues on the palm stearin triglycerides are replaced by oleic acid residues at the *sn*-1 and *sn*-3 positions, leaving the *sn*-2 fatty acids (primarily palmitic acid as well as some oleic acid) unaltered. Enzymotec states that this process uses a safe and suitable immobilized GRAS lipase enzyme preparation from *Aspergillus oryzae* carrying a gene encoding a lipase from *Thermomyces lanuginosus* (the subject of GRN 000043). The immobilization process is carried out in accordance with good manufacturing practice using materials approved for food use. Following reaction and separation of the catalyst, the oil is distilled to remove excess free fatty acids (primarily oleic acid). The oil next undergoes bleaching and steam distillation/deodorization processes. Enzymotec states that the oil is then mixed with permitted antioxidants.

Enzymotec states that potential residues from the manufacturing process include protein, sorbitan oleate, methanol, and epichlorhydrin, but that all residues were below the limits of detection. Enzymotec also states that residues of methanol, epichlorhydrin (used in the production of the immobilized enzyme), and 1,3-dichloro-2-propanol (formed by reaction of water with epichlorhydrin), if present, would be removed under the conditions (elevated temperature, vacuum) of the distillation and deodorization processes.

The predominant triacylglycerols in the high 2-palmitic acid vegetable oil are 1,3-dioleoyl-2-palmitoylglycerol; 1,2-dipalmitoyl-3-oleoylglycerol; and triolein. The fatty acid composition of the high 2-palmitic acid vegetable oil is predominantly oleic and palmitic acids, with lesser amounts of other fatty acids. Enzymotec provides specifications (expressed as a percentage of total fatty acids) for fatty acids: oleic acid (42 - 60%), palmitic acid (25 - 42%), stearic acid (1 - 6%), and linoleic acid (6 - 17%). In addition, Enzymotec specifies that 62 - 70% of the palmitic acid is esterified at the *sn*-2 position of the glycerol backbone. Enzymotec also provides the results of batch analyses of several lots to show that the product meets their specifications. According to these analyses, palmitic acid represents approximately 60 - 66% of all the fatty acids found at the *sn*-2 position.

High 2-palmitic acid vegetable oil is intended to replace vegetable oils in infant formula up to a level of 70% of total fat, to replace all added fat in baby and toddler foods (including meat and poultry products), and to replace all added fat in processed food products in general (excluding meat and poultry products). Enzymotec notes that high 2-palmitic acid vegetable oil cannot replace all the fat in infant formula since it is limited with respect to essential fatty acids like linolenic acid.

Use of high 2-palmitic acid vegetable oil is also limited in baby and toddler foods, because most of these types of foods do not contain significant amounts of added fat. In foods intended for general consumption, use of high 2-palmitic acid vegetable oil is self-limiting based on its technological properties such as melting temperature.

Enzymotec provides estimates of intake of high 2-palmitic acid vegetable oil from all proposed food uses, prepared using the United States Department of Agriculture (USDA) 1994-1996 Continuing Survey of Food Intakes by Individuals (USDA CSFII 1994-1996) and the 1998 Supplemental Children's Survey (USDA CSFII 1998). On an eaters-only basis, the estimated intake of high 2-palmitic acid vegetable oil for young infants (0-5 months; 87.6% eaters) that would be consuming infant formula is 22 grams (g)/person (p)/day(d) at the mean and 36 g/p/d at the 90th percentile. On an eaters-only basis, the estimated intake of high 2-palmitic acid vegetable oil by all ages (99.3% eaters) from all proposed food uses was 35 g/p/d at the mean and 66 g/p/d at the 90th percentile.

The notice contains a review of triacylglycerol biochemistry, as well as summaries of relevant studies. Enzymotec considers the safety of its high 2-palmitic acid vegetable oil to be supported by a number of published studies on another high 2-palmitic acid vegetable oil that is similar in fatty acid content and positional distribution. This ingredient was the subject of GRN 000131, submitted by Loders Croklaan B.V. (Loders Croklaan). GRN 000192 includes a comparison of the composition of these two high 2-palmitic acid vegetable oils to that of human milk, with respect to fatty acid content and positional distribution. GRN 000192 also cites a number of published studies with Loders Croklaan's high 2-palmitic acid vegetable oil, including absorption studies in Sprague-Dawley rats, a clinical study in infants comparing the absorption of palmitic acid from formula-based diets versus human milk, and other animal and human studies. Enzymotec conducted a comparative metabolic study in rats and reports that its high 2-palmitic acid vegetable oil exhibited absorptive and metabolic patterns similar to Loders Croklaan's high 2-palmitic acid vegetable oil.

Enzymotec's GRAS panel concludes that Enzymotec's high 2-palmitic acid vegetable oil is well absorbed and metabolized into normal body metabolites, that *sn*-2 esterified palmitic acid is a normal constituent of human milk, and that the intake of high 2-palmitic acid vegetable oil will not increase the current saturated fatty acid intake or significantly affect cholesterol or lipoprotein levels in humans.

In the notice, Enzymotec states its intention to use high 2-palmitic acid vegetable oil in several food categories, including foods for which standards of identity exist located in Title 21 of the Code of Federal Regulations. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

During its evaluation of GRN 000192, FDA consulted with the Labeling and Consumer Protection Staff of the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA). Under the Federal Meat Inspection Act and the Poultry Products Inspection Act, FSIS is responsible for determining the efficacy and suitability of food ingredients in meat and poultry products as well as prescribing safe conditions of use. Suitability relates to the effectiveness of the ingredient in performing the intended purpose of use and the assurance that the conditions of use will not result in an adulterated product, or one that misleads consumers. FSIS requested that FDA advise Enzymotec to seek regulatory guidance from FSIS, Labeling and Consumer Protection Staff, about the use of high 2-palmitic vegetable oil in meat and poultry products. Enzymotec should direct such an inquiry to Dr. Robert Post, Director, Labeling and Consumer Protection Staff,

Office of Policy, Program, and Employee Development, Food Safety and Inspection Service, 1400 Independence Ave., S.W., Suite 602, Annex, Washington, DC 20250-3700. The telephone number for that office is (202) 205-0279 and the telefax number is (202) 205-3625.

Under section 412 of the Federal Food, Drug, and Cosmetic Act (FFDCA), a manufacturer of a new infant formula must make a submission to FDA, providing required assurances about the formula, at least 90 days before the formula is marketed. As part of that submission, the manufacturer of the infant formula must provide a quantitative formulation that lists the amount of every ingredient used in the formula. Enzymotec should be aware that FDA's response to Enzymotec's GRAS notice does not alleviate the responsibility of any infant formula manufacturer who intends to market an infant formula that contains high 2-palmitic vegetable oil to make the submission required by section 412.

Based on the information provided by Enzymotec, as well as other information available to FDA, the agency has no questions at this time regarding Enzymotec's conclusion that high 2-palmitic vegetable oil is GRAS under the intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of high 2-palmitic vegetable oil. As always, it is the continuing responsibility of Enzymotec to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter responding to GRN 000192, as well as a copy of the information in this notice that conforms to the information in the proposed GRAS exemption claim (proposed 21 CFR 170.36(c)(1)), is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at <http://www.cfsan.fda.gov/~lrd/foodadd.html>).

Sincerely,
(b) (6)

Laura M. Tarantino, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

cc: Dr. Robert Post, Director
Labeling and Consumer Protection Staff
Office of Policy, Program, and Employee Development
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