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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: Notice of a GRAS Exemption Claim
for Betapol™ (Structured Triglycerides)

To Whom It May Concern:

On behalf of Loders Croklaan B.V., we hereby provide the following information pursuant to proposed 21 CFR 170.36(c)(1) (62 Fed. Reg. 18938, 18961; April 17, 1997):

GRAS Exemption Claim: Loders Croklaan B.V. ("Loders Croklaan") hereby claims that Betapol™ (structured triglycerides), a triglyceride mixture composed of fatty acids present in edible oils and fats, including human milk, which is intended for use in infant formula for both term and preterm infants at levels of up to 80% total fat intake, is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Loders Croklaan has determined that it is generally recognized as safe ("GRAS") for such use, using scientific procedures.

(i) **Name and Address of Notifier:** Dr. Andreas Menzel
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(ii) **Common or Usual Name of the Substance:** Structured triglycerides.

(iii) **Applicable Conditions of Use:** As a source of fat in infant formula for both term and preterm infants at levels of up to 80% total fat intake

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Office of Food Additive Safety
May 28, 2003
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(iv) *Basis for the GRAS Determination:* Scientific procedures.

(v) *Statement of Availability:* The data and information that are the basis for Loders Croklaan's GRAS determination are available for FDA review and copying at reasonable times at the offices of the undersigned, and such data and information will be sent to FDA upon request.

Additional required information, including a comprehensive summary of the data relied on to establish safety, is enclosed.

Sincerely,

Daniel R. Dwyer 

Counsel to Loders Croklaan B.V.

enclosure

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GRAS NOTIFICATION

For

BETAPOL™ (Structured Triglycerides) For Use in Term and Preterm Infant Formula

Submitted by:

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May 28, 2003

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ABBREVIATIONS, ACRONYMS, AND TERMS

°C	degree Celsius
¹⁴ C	carbon-14 isotope
C8:0	octanoic acid (caprylic)
C10:0	decanoic acid (capric)
C12:0	dodecanoic acid (lauric)
C14:0	tetradecanoic acid (myristic)
C16:0	hexadecanoic acid (palmitic acid)
C16:1	cis-9-hexadecenoic acid (palmitoleic acid)
C18:0	octadecanoic acid (stearic acid)
C18:1	cis-9-octadecenoic acid (oleic acid)
C18:2	cis-9, cis-12-octadecadienoic acid (linoleic acid)
C18:3	cis-9, cis-12, cis-15-octadecatrienoic acid (linolenic acid)
C20:4	cis-6, cis-9, cis-12, cis-15-eicosatetraenoic acid (arachidonic acid)
d	day
ESPGAN	European Society of Paediatric Gastroenterology and Nutrition
F	female
FDA	Food and Drug Administration
g	gram
GRAS	Generally Recognized As Safe
kg	kilogram
l	liter
LSRO	Life Sciences Research Office
M	male
mg	milligram
ml	milliliter
MUFA	monounsaturated fatty acid
n	number of subjects
NAS	National Academy of Sciences
NOAEL	No-observed adverse effect level
O (C18:1)	octadecaenoic acid (oleic acid)
OPO	oleic-palmitic-oleic triglyceride
P (C16:0)	hexadecanoic acid (palmitic acid)
POs	palm oil stearine
ppm	parts per million
POO	palmitic-oleic-oleic triglyceride
PPO	palmitic-palmitic-oleic triglyceride
PPP	palmitic-palmitic-palmitic triglyceride (tripalmitin)
PUFA	polyunsaturated fatty acid
rDNA	recombinant DNA technology
SAFA	saturated fatty acid
sn-1,3, α	terminal glycerol carbon atoms in a glyceride molecule
sn-2, β	mid position glycerol carbon atom in a glyceride molecule
SP388	Code for Novo Nordisk's lipase preparation derived from <i>Rhizomucor miehei</i>

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I. INTRODUCTION

A. Regulatory Background

Pursuant to 62 Fed. Reg. 18938, 18960 (April 17, 1997) (proposed 21 CFR §170.36), Loders Croklaan B.V. ("Loders Croklaan") hereby claims that Betapol™ (structured triglycerides), a triglyceride mixture composed of fatty acids present in edible oils and fats, including human milk (described in detail in this document, and referred to as "Betapol™"), which is intended for use in infant formula for both term and preterm infants at levels of up to 80% total fat intake, is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Loders Croklaan has determined that it is generally recognized as safe ("GRAS") for such use, using scientific procedures.

This document accompanies the GRAS exemption claim required by proposed 21 CFR §170.36(c)(1) and provides the detailed information required by proposed 21 CFR §170.36(c)(2), (3), and (4). The following table correlates the requirements of the proposed regulation with the sections in this report where the information may be found:

<u>Requirements of the proposed rule</u>	<u>Section No(s).</u>
§ 170.36(c)(2): Detailed information about the identity of the notified substance, composition, method of manufacture, characteristic properties, and specifications	III, IV
§ 170.36(c)(3): Information on any self-limiting levels of use	V
§ 170.36(c)(4)(i)(A): Comprehensive discussion of, and citations to, generally available and accepted scientific data and information, including consideration of probable consumption	II, V, VI, VIII
§ 170.36(c)(4)(i)(B): Comprehensive discussion of any reports that may appear to be inconsistent with the GRAS determination	VI
§ 170.36(c)(4)(i)(C): The basis for concluding that there is a consensus of qualified experts that there is reasonable certainty that the substance is not harmful under the intended conditions of use	VII, VIII

This GRAS determination is based on generally available and accepted scientific data, information, methods and principles, and corroborated by unpublished information, including the lack of adverse effects reported in countries where Betapol™ is approved for use in infant formula and appropriate animal data. Loders Croklaan's conclusion is supported by the views of a panel of independent experts, including experts on food safety (Joseph F. Borzelleca, Ph.D., Walter H. Glinsmann, M.D.) and pediatrics, including the need for specific fatty acids during infancy (William Heird, M.D.). Accordingly, this GRAS determination meets the requirements

of § 201(s) of the Federal Food, Drug, and Cosmetic Act; 21 CFR §§170.3 and 170.30; and the amendments to these rules proposed at 62 Fed. Reg. 18960.

B. Fat in Infant Formula and Breast Milk

The diet of infants during the first months of life is critical to their ongoing growth and development. It is generally recognized that the best possible start in life is provided by the infant's mother's own milk, which provides vital nutrients and protective antibodies in a well balanced combination that is easily digested and absorbed, and is well tolerated by the infant. For these reasons, breast milk is often referred to as the "Gold Standard" against which artificial formulas are evaluated.

Infants need a high-fat diet that provides the energy required during the rapid growth phase following birth and to help in the delivery of numerous fat-soluble dietary components such as vitamins⁶. Breast milk contains more than 40% of its energy in the form of fat⁶. In many cases, however, the mother prefers not to, or is unable to, breast feed her baby. Further, mother's milk may not be adequate for preterm infants. In these cases, infants receive their nutrition in the form of infant formula. Although these formulas are increasingly sophisticated and complex, there are still clear differences between infant formula and human milk, including differences in the structure of fats. In fact, the importance of the fat structure in infant formula has only recently been recognized.

It has been known for some years that human milk contains a certain combination of fatty acids and that the structure of triglycerides – combinations of glycerol with three fatty acids – is not random. Indeed, the triglycerides of human milk have a very specific structure. In the last three decades it has become clear that triglyceride structure has significant effects on the digestion and absorption of fatty acids, and that vegetable fats are normally poor substitutes for human milk fats¹⁻⁵.

The reason lies not in the types of fatty acids but in the precise location of the fatty acids in the triglyceride⁸. Human milk fat contains a range of fatty acids, the most abundant of which are oleic acid (C18:1) and palmitic acid (C16:0)⁶. These normally comprise approximately 40% and 24% of total fatty acids, respectively⁶. In human milk fat, palmitic acid is attached predominantly to the middle carbon of the glycerol backbone of triglyceride (about 70% of total C16:0) and oleic acid is mainly positioned at the outer carbon atoms (about 90% of C18:1)^{2,5}. Thus, a main triglyceride in human milk would be 1,3-dioleoyl 2-palmitoyl triglyceride ("OPO"). Vegetable oils, on the other hand, have palmitic acid attached predominantly to the outer carbon atoms of the glycerol and oleic acid to the middle carbon atom⁷. So vegetable oils can be used to create a fat with similar amounts of palmitic and oleic acids as breast milk fat, but the positioning is reversed ("POP").

The structure of the fat determines its effectiveness in delivering nutrition. During digestion, the fatty acids attached to the outer carbon atoms are preferentially hydrolyzed. In the case of vegetable oils, palmitic acid is cleaved during digestion to yield free palmitic acid. This acid

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forms insoluble soaps with calcium, which is a major contributing factor in constipation^{3,9,10} that results in loss of both fatty acids and calcium. Indeed, it is clear that use of vegetable oils as the source of fat is a major cause of poor fat and mineral absorption and the constipation frequently associated with the use of infant formula^{3,9,10}. This is less likely to happen when oleic acid is attached to the terminal carbon atoms.

The structural characteristics of human milk fat have, until now, been impossible to mimic using the vegetable oil blends normally found in infant formula. Loders Croklaan has developed the technology to manufacture a structured vegetable oil-based fat in such a way that it brings infant formula a step closer to human breast milk. This technology, using the natural selectivity of enzymes, makes it possible to construct triglycerides which mimic the structure of human milk triglycerides and to deliver the benefits of improved fat and mineral absorption as well as softer stools which are normally associated with breast-feeding. The name given to the product of this technology is *Betapol*TM.

*Betapol*TM structured triglycerides are derived from the enzymatically catalyzed esterification of saturated vegetable fats (mainly palm stearine) with unsaturated fatty acids derived from suitable vegetable oils (e.g., sunflower) using a position-selective lipase. The predominant glyceride moiety in *Betapol*TM is 1,3-dioleoyl 2-palmitoyl triglyceride. This triglyceride has been shown to be present in human breast milk¹¹. The resulting triglyceride mixture can be blended with other vegetable oils to produce a fat which has a close resemblance to human breast milk fat in both fatty acid composition and, importantly, in positional distribution of palmitic and oleic acids.

C. Expert Panel Deliberations and Literature Searches

As noted above, an independent panel of recognized experts (hereinafter, "the Panel"), qualified by their scientific and/or medical training and international experience to evaluate the safety of food and food ingredients, was requested by Loders Croklaan to determine the GRAS status of *Betapol*TM. The Panel was comprised of Joseph F. Borzelleca, Ph.D.,ⁱ Walter H. Glinsmann, M.D.,ⁱⁱ and William C. Heird, M.D.ⁱⁱⁱ

A search of the relevant scientific literature for information on the uses, effects, and safety of *Betapol*TM and related materials was conducted by Loders Croklaan through June 1999 and made available to the Panel. Laboratory data, reprints, and abstracts were also provided. The members of the Panel independently and critically evaluated the materials provided by Loders Croklaan and other relevant scientific data, information, methods, and principles. In June 2000, the Panel

ⁱ Dr. Borzelleca is Emeritus Professor in the Department of Pharmacology and Toxicology at Virginia Commonwealth University's Medical College of Virginia, Richmond, VA. He is an expert in the evaluation of the safety of food ingredients.

ⁱⁱ Dr. Glinsmann is president of Glinsmann, Inc., Arlington, VA, adjunct fellow with the Center for Food and Nutrition Policy, Virginia Polytechnic Institute, and past Associate Director for Clinical Nutrition, FDA Center for Food Safety and Applied Nutrition. He is an expert in the evaluation of the safety of food ingredients.

ⁱⁱⁱ Dr. Heird is Professor of Pediatrics at Baylor College of Medicine and the USDA/ARS Children's Nutrition Research Center, Houston, TX. He is an expert in nutrition and has conducted extensive research on the need for specific fatty acids and amino acids during infancy.

finalized its report, which includes a critical evaluation of the pertinent information and the Panel's recommendations and conclusions.

Subsequent to June 2000, Loders Croklaan delayed submitting a GRAS Notification pending resolution of other issues relating to the project. At this time, the Panel's June 2000 conclusion continues to be current because (1) all of the information reviewed by the GRAS Panel (including the intended use, manufacturing process, specifications, safety data and exposure estimates) remains unchanged, and (2) Loders Croklaan has performed literature searches and has otherwise updated the generally available scientific information since June 2000. The literature searches, performed in November 2001 and January 2003, revealed a few new publications, all of which are consistent with the Panel's June 2000 conclusions and are incorporated in this document^{26,27,73,74,75}. Accordingly, the Panel's June 2000 conclusion provides an adequate basis for Loders Croklaan's determination that Betapol™ is GRAS for use in infant formula for both term and preterm infants at levels of up to 80% total fat intake.

D. Background on Betapol™

Betapol™ is a triglyceride mixture made specifically for use in infant formulas to closely mimic both the proportion of fatty acids in human breast milk and their arrangement on the glycerol backbone. Human breast milk is the reference against which infant formulas are compared. Triglycerides in human milk have around 70% of the palmitic acid located at the sn-2 (middle) position. Currently used triglycerides in infant formulas contain approximately the same proportions of fatty acids as those found in human milk, but their arrangement on the glycerol molecule is not the same. The palmitic acid is predominantly found in the sn-1 and -3 positions, which affects the absorption of the fatty acids, and reduces nutrient bioavailability.

Betapol™ is made using enzymatic rearrangement so that 45-80% of the palmitic acid is esterified at the sn-2 position. The remaining sn-1 and -3 positions are predominantly occupied by unsaturated fatty acids, in particular oleic acid. This is a common structure found in triglycerides in human milk and is considered to improve the absorption of fatty acids and minerals from the small intestine.

Betapol™ was developed for use in formula for both term and preterm infants. It will be blended with other safe and suitable vegetable oils to achieve the fatty acid profile required by the infant formula manufacturers to meet regulations and their own requirements.

Betapol™ is manufactured by enzymatic interesterification, the rearrangement of fatty acids on glycerol molecule. The process uses a lipase bound to an inert support. Currently, Betapol™ is manufactured using a lipase derived from *Rhizomucor miehei* lipase genes expressed in *Aspergillus oryzae*. The enzyme, free from microbial cells and DNA, is immobilized on Duolite 568 in a packed bed enzyme reactor. Duolite 568 is a phenol-formaldehyde anion exchange resin activated with triethylene tetramine. Food-grade fractionated palm oil is interesterified with food-grade oleic acid. The resulting mixture is then processed using conventional edible oil processing techniques to produce Betapol™ meeting appropriate specifications. While

Betapol™ is now manufactured using a lipase derived from *Rhizomucor miehei* lipase genes expressed in *Aspergillus oryzae*, Betapol™ can be manufacturing using any safe and suitable enzyme that accomplishes the interesterification.

E. Summary

Betapol™ is a triglyceride mixture composed of fatty acids present in edible oils and fats, including human milk. The predominant triglyceride structure is very similar to that of human breast milk fat. Betapol™ is produced by conventional methods used in the manufacture of edible oils and fats to meet a carefully controlled product specification. There is adequate support for the safety of specific elements of the enzymatic interesterification process particular to the current Betapol™ manufacturing process. The specifications for Betapol™ are suitable for use in infant formulas. No new components are being added to the diet. The safety of Betapol™ for use in infant formulas at levels up to 80% of total fat is supported by generally available and accepted scientific data, information, methods and principles, and corroborated by unpublished information, including the lack of adverse effects reported in countries where Betapol™ is approved for use in infant formula and appropriate animal data.^{iv}

^{iv} Information on lack of adverse events is based on lack of such events reported to Loders Croklaan by manufacturers of formulas in which Betapol™ is used. Formula manufacturers would become aware of any adverse events through (1) toll-free telephone numbers maintained to receive consumer complaints, (2) studies that have been conducted to evaluate consumer acceptance of products, and (3) interaction with hospitals and physicians who are familiar with the products. It is normal industry practice that any complaints received by formula manufacturers relating to an ingredient supplied by Loders Croklaan would be promptly conveyed to Loders Croklaan.

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II. REGULATORY STATUS

Although Betapol™ is a triglyceride mixture with a preferred structure, it is important to recognize that it nevertheless consists of triglycerides already present in food. It contains two of the most commonly occurring fatty acids to be found in human milk fat: oleic and palmitic. The technology used to produce Betapol™ has been widely used for the production of food fats since 1993.

Betapol™ is made from food-grade vegetable fats and oils, using a lipase enzyme for interesterification. The lipase gene was obtained from *Rhizomucor miehei* and is expressed in *Aspergillus oryzae*. (It should be noted that microbiologists have renamed *Mucor miehei* as *Rhizomucor miehei*. However, both names are still used. The names are used interchangeably in this document to match the reference which quotes the name of the organism.) The lipase enzyme is attached to a phenol-formaldehyde support, Duolite 568, activated with triethylene tetramine.

A. The United States

1. Status of triglyceride mixtures from vegetable sources

FDA has evaluated the GRAS status of a number of different triglycerides. For example:

- In general, FDA takes the position that vegetable oil is GRAS. 62 Fed. Reg. 18939 (April 17, 1997). This conclusion is based not only on the common use of vegetable oil in food but also on scientific procedures, in that FDA has evaluated the extensive safety literature on vegetable oils, linoleic acid, oleic acid, and other similar and related substances. (See e.g., FDA's 1973 review of the scientific literature on vegetable oils, oleic acid, and linoleic acid.)²⁵
- FDA affirmed sheanut oil as GRAS. 21 CFR §184.1702; 63 Fed. Reg. 28893 (May 27, 1998). In doing so, it noted that sheanut oil is similar in chemical composition to commonly used GRAS fats and oils, such as cocoa butter, cottonseed oil, soybean oil, corn oil, and palm oil. FDA stated that:
 - Sheanut oil is composed principally of triglycerides containing oleic acid in the 2-position and saturated fatty acids, usually stearic and palmitic acids, in the 1- and 3-positions.
 - The components of these triglycerides, glycerol, and oleic, stearic, and palmitic acids, as well as other fatty acids found as minor components, are naturally found as part of lipids and lipoproteins of both plants and animals; they are also the same fatty acids and glycerol components found in a broad range of edible fats and oils that are GRAS.

- The synthesis and metabolism of these substances are well understood and are documented in biochemistry textbooks (such as Lehninger, A. L., *Principles of Biochemistry*, Worth Publishers, Inc., New York, NY, 1982).
 - Sheanut oil has an overall composition that conforms to that of other edible oils in terms of its total glyceride content, fractions of tri-, di- and monoglycerides, and unsaponifiable matter.
- A number of GRAS affirmation and food additive regulations deal with the safe use of triglycerides and fatty acids, e.g.:
 - 21 CFR §172.860 (fatty acids)
 - 21 CFR §184.1555(c) (canola oil)
 - 21 CFR §172.861 (cocoa butter substitute)

The triglyceride components of Betapol™ are the same as those in common, GRAS vegetable oils, i.e., glycerol, and primarily palmitic and oleic acids. The only difference between Betapol™ and common vegetable oils is the arrangement of the fatty acids on the glycerol backbone (with Betapol™ having primarily palmitic acid attached to the middle carbon of the glycerol backbone, and vegetable oils having primarily oleic acid at this position).

When ingested, the component parts of Betapol™ are the same as those of vegetable oils and other triglycerides. That is, they are digested and absorbed into the body as a mixture of monoglycerides, glycerol, and free fatty acids. Thus, all of the components of Betapol™ are present as components of fats that are found in foods or that are generated in large amounts in the human digestive tract during the digestion of fat. The GRAS status of other fats and oils, then, provides a sound basis for concluding that Betapol™ is GRAS.

2. Status of the Enzymatic Interesterification Process

Betapol™ is made by enzymatic interesterification of fatty acids. Products of similar interesterification processes have been affirmed as GRAS by FDA. Examples are enzyme-modified fats (21 CFR §184.1287) and cocoa butter substitute (21 CFR §184.1259). Further, the fatty acids used in the interesterification process are commonly found in food and are approved for use in the manufacture of food components (21 CFR §172.860).

The use of enzyme preparations as processing aids is accepted by FDA so long as the source organisms are safe and suitable and potentially toxic byproducts are not produced. Betapol™ interesterification is accomplished by a suitable enzyme, at this time consisting of an esterase-lipase enzyme preparation expressed by *Aspergillus oryzae* through recombinant encoding of a gene from *Rhizomucor miehei*. The use of esterase-lipase from *Rhizomucor miehei* as a direct food ingredient is the subject of a GRAS affirmation petition by the manufacturer, Novo Nordisk, and accepted for filing by FDA (GRASP 7G0323, 54 Fed. Reg. 9565 (March 7, 1989)). The *Rhizomucor miehei* esterase-lipase expressed by *Aspergillus oryzae* and the carrier, Duolite 568, used to make Betapol™ are subjects of the same petition (amended in 1992). This petition

has not yet been acted on by FDA (either as a GRAS petition or GRAS notification). However, FDA has accepted a number of similar source organisms and enzymes, for example:

- Esterase-lipase derived from *Rhizomucor miehei* (originally named *Mucor miehei*) var. *Cooney et Emerson* (21 CFR §173.140).
- Milk-clotting enzyme derived from *R. miehei* var. *Cooney et Emerson* (21 CFR §173.150).
- Alpha-amylase derived from *Aspergillus oryzae* (21 CFR §137.105).
- Lipase enzyme preparation derived from *A. oryzae* (Response to GRAS Notice No. GRN 113).
- Esterase-lipase derived from *Rhizopus niveus* (21 CFR §184.1420).
- Lipase enzyme preparation derived from *Aspergillus niger* for use in a wide variety of applications, including edible fats and oils (Response to GRAS Notice No. GRN 111).
- Lipase enzyme preparation derived from *Candida rugosa* for use in fat/oil applications (Response to GRAS Notice No. GRN 081).
- Lipase enzyme preparation derived from *Penicillium camembertii* for use in the production of ingredients derived from fats and oils (Response to GRAS Notice No. GRN 068).

Recombinant DNA (“rDNA”) technology for producing an enzyme preparation has also been accepted by FDA. For example,

- Aspartic proteinase enzyme preparation derived from a recombinant strain of *Aspergillus oryzae* using a *Rhizomucor miehei* var. *Cooney et Emerson* gene has been approved for use in milk clotting (21 CFR §173.150 (a)(5)).
- Lipase enzyme preparation derived from *Aspergillus oryzae* carrying a gene encoding lipase from *Thermomyces lanuginosus* for use as a catalyst in the interesterification of glycerides, among other uses (Response to GRAS Notice No. GRN 043).
- Lipase enzyme preparation from *Aspergillus oryzae* carrying a recombinant gene encoding lipase from *Fusarium oxysporum* for use as a processing aid in the modification of fats and oils and in baking applications (Response to GRAS Notice No. GRN 075).
- Lipase enzyme preparation from *Aspergillus oryzae* carrying a recombinant gene constructed from a modified *Thermomyces lanuginosus* lipase gene and a portion of the *Fusarium oxysporum* lipase gene for use as a processing aid in the modification of fats and oils, among other applications (Response to GRAS Notice No. GRN 103).
- Glucose oxidase enzyme preparation from *Aspergillus oryzae* carrying the gene encoding glucose oxidase from *Aspergillus niger* for use in baking applications and certain beverages (Response to GRAS Notice No. GRN 106).
- Alpha-amylase enzyme preparation from *Bacillus licheniformis* carrying a gene constructed from a modified *Bacillus licheniformis* alpha-amylase gene and a portion of the *Bacillus amyloliquefaciens* alpha-amylase gene (Response to GRAS Notice No. GRN 079).

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The lipase currently used to make Betapol™ is immobilized on Duolite 568, a phenol-formaldehyde anion exchange resin. Phenol-formaldehyde resins activated with triethylene tetramine are approved for treatment of food by FDA (21 CFR §173.25(a)(7)). Studies conducted by Jensen *et al.* concluded that Duolite 568 is a safe carrier for lipase²⁸.

In conclusion, Betapol™ and its production process are consistent with the types of products and manufacturing processes that FDA has considered to be GRAS, in that:

- The components of Betapol™ are the same as the components of GRAS vegetable oils.
- Interesterification of fats by lipases has been accepted by FDA.
- The fatty acids used in the interesterification process are approved by FDA.
- The source organisms currently used in the manufacture of Betapol™, *Rhizomucor miehei* and *Aspergillus oryzae*, have been approved by FDA, as has a recombinant strain of *Aspergillus oryzae* with a *Rhizomucor miehei* gene.
- Lipases from microorganisms, including *Rhizomucor miehei*, have been approved by FDA for certain food applications.
- Duolite 568, a phenol-formaldehyde resin, and triethylene tetramine, a cross-linking agent, have been approved by FDA.

B. Other Countries

1. Canada. A lipase produced by *Aspergillus oryzae* has been approved for enzymatic interesterification for all food uses²². The Food & Drug Regulations (section B.25.062(2)(f)) allows infant formula to contain ingredients manufactured with food additives listed in Table V to section B.16.100. This Table contains lipases from *Aspergillus oryzae* and *Rhizomucor miehei* to modify triglycerides as long as they meet good manufacturing practice, and therefore covers the lipase used in the production of Betapol™.

2. European Union. Betapol™, made by the method outlined herein and supported by the safety data described below, has been approved for use in preterm infant formulas in the United Kingdom¹⁹ and has been cleared by the Dutch Health Authorities for use in all types of infant formula^{20,21}. As a result of these approvals, Betapol™ may be used as an infant formula ingredient throughout the European Union. Betapol™ is used in infant formulas for both term and preterm infants, with the predominant use being for term infant formula.

3. South Korea. Betapol™ (concentrate for further manufacture) is approved for use in infant formula in South Korea.

C. International Organizations

As further support of the use of source organisms mentioned above, The Joint Expert Committee on Food Additives ("JECFA") of the Food and Agriculture Organization and the World Health

Organization concluded that *Rhizomucor miehei* and *Aspergillus oryzae* are nonpathogenic and nontoxigenic to humans and, also, do not produce antibiotics. The enzyme preparation used to make Betapol™ is purified and standardized to meet the food-grade quality standards formulated by JECFA²³.

III. CHEMICAL IDENTITY AND COMPOSITION

A. Chemical Identity

“Betapol™” is the name given to an enzymatically modified, vegetable oil-based triglyceride mixture rich in palmitic acid (30-55% wt%). The main identifying chemical feature of Betapol™ is the distribution of palmitic acid such that approximately 45-80% of this fatty acid is esterified at the sn-2 position of the glycerol backbone (the mid position). This is similar to human milk fat in which approximately 70% of the palmitic acid is esterified to the sn-2 position^{2,29}. The sn-1-3 positions (the end positions of the glycerol backbone) are predominantly occupied by unsaturated fatty acids, primarily oleic acid (C18:1).

B. Chemical Structure and Composition

The predominant triglyceride moieties in Betapol™ are 1,2-dipalmitoyl 3-oleoyl triglyceride and 1,3-dioleoyl 2-palmitoyl triglyceride, sometimes referred to as PPO (palmitic-palmitic-oleic) and OPO (oleic-palmitic-oleic), respectively. The structural formula for OPO is presented in Figure 1. All triglyceride structures in Betapol™ have the same basic structure as the triglycerides found in natural foods.

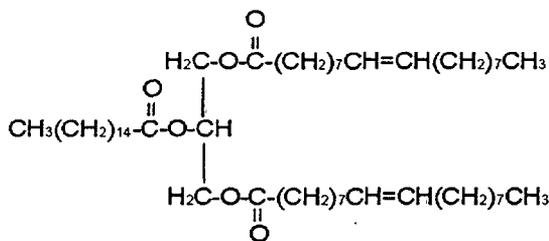


Figure 1: Structural formula for OPO

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C. Proposed Food-Grade Specifications

The composition of Betapol™ will always fall within the specification range presented in Table 1.

Table 1: Specification range of Betapol™.

Parameter	Value
Appearance (at 35 ° C)	light yellow liquid
Fatty acid composition (%)	
SAFA	30-60
MUFA	30-50
PUFA	0-20
% of total palmitic acid located on sn-2 position	≥ 43
Water & Insoluble matter (%)	< 0.05
Free Fatty Acids (%)	< 0.3
Peroxide Value (meq/kg)	≤ 2.0
Diglycerides (%)	< 6.0
Rancimat Induction Period (hrs at 120 °C)	≥ 3.0
Lead (ppm)	< 0.1

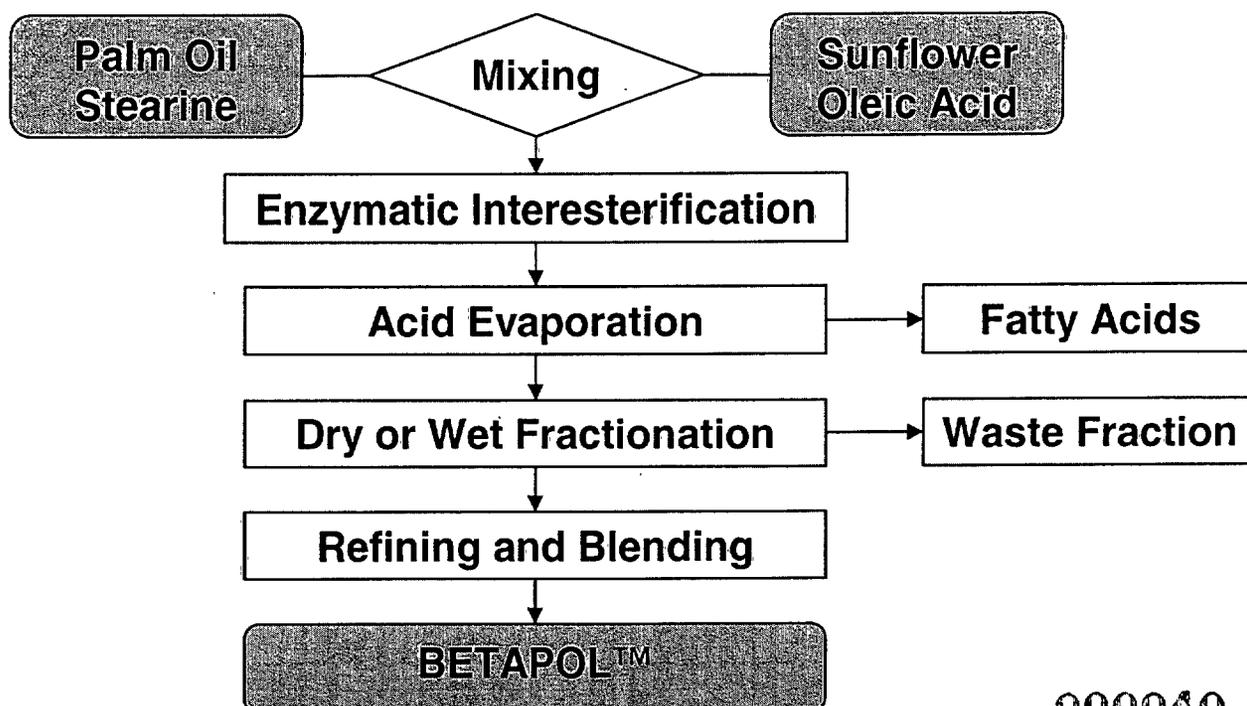
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IV. MANUFACTURE OF BETAPOL™

The manufacture of Betapol™ uses primarily traditional oil processing steps (e.g., solvent or dry fractionation, and refining). However, the key processing step in the manufacture of Betapol™ is the positional rearrangement of fatty acids on the glycerol molecule by enzymatic interesterification. Interesterification can be brought about either chemically or enzymatically. In chemical interesterification, the fatty acids are randomly distributed on the 1-, 2-, and 3-positions of the glycerol molecule. Rearrangement of the fatty acids takes place by heating the oil in the presence of a catalyst, which is subsequently removed. Research has shown that mixtures of vegetable oils are better absorbed by rats if the proportion of longer chain, saturated fatty acids in the sn-2 position is increased by random chemical interesterification³⁰. A limitation of chemical interesterification is that only one third of these fatty acids will be positioned at sn-2.

In order to improve upon this, industrial processes have been developed to selectively interesterify fats using enzymes. Although some enzymes are unselective and, as is the case with chemical interesterification, randomly redistribute the fatty acids on the glycerol molecule, most enzymes act selectively on the ester linkages at the 1- and 3- positions of the triglycerides, leaving the sn-2 position fatty acids attached to the glycerol molecule.

Betapol™ interesterification is accomplished by a suitable enzyme, at this time consisting of an esterase-lipase enzyme preparation expressed by *Aspergillus oryzae* through recombinant encoding of a gene from *Rhizomucor miehei*. The starting materials consist of a palm oil fraction rich in palmitate (C16:0) and free fatty acids from high oleic sunflower oil. The following is a summary of the manufacturing process:



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Information on fractions of saturated and unsaturated fat in Betapol™, as compared with fractions in the starting materials, is presented in Table 2.

Table 2: Fatty Acid Ratio in Starting Materials and Final Product of Betapol™ Production (typical range in %)

Fatty acid content	Palm oil stearine	Sunflower oleic acids	Betapol™
All Saturated	96-98	7-11	42-46
Palmitic Acid	87-91		21-25
All Unsaturated	2-4	89-93	54-58
Oleic Acid		78-84	39-44

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V. EXPOSURE UNDER INTENDED CONDITIONS OF USE

Betapol™ is a triglyceride mixture with a palmitate content of 30-55%, of which approximately 45-80% is esterified to the sn-2 position of the glycerol backbone. It has been developed specifically for use in infant formula. Betapol™ can be blended with other vegetable oils based upon the fatty acid composition requirements of the infant formula manufacturer in order to provide an appropriate balance of fatty acids and triglyceride structures suitable for an infant formula. Typically, Betapol™ will comprise 40-80% of this blend. Examples of the other safe and suitable vegetable oils are sunflower oil, high oleic sunflower oil, soy bean oil, palm kernel oil, and fractions of palm kernel oil.

A. Approach to Exposure Estimates

During the first months of life, all infants' diets consist solely of breast milk and/or formula. Therefore, potential exposure to Betapol™ can be estimated from breast milk and infant formula consumption figures. In the exposure calculations, 100% exposure to Betapol-based formula will be assumed.

Average daily breast milk or infant formula consumption of healthy infants born at term is 750 ml for the first six months (coefficient of variation approximately 12.5%)³². During the next six months, when complementary foods are given, the average daily consumption is 600 ml³².

B. Estimates of Expected Exposure

1. Dietary recommendations of fat intake via formula

No scientific data are available relating growth and development to a requirement for fat, other than the small amount needed to meet essential fatty acid requirements. However, it is unlikely that adequate energy can be provided with a daily fat intake below 36 kcal/kg (4 g/kg, approximately 30% of dietary energy). Therefore, the recommended intake of fat for enterally fed preterm and term infants is 40-50% of total dietary energy, which is the usual amount provided by human milk^{33,34}.

The National Academy of Sciences ("NAS") has specified an Adequate Intake ("AI") of total fat for infants based on the observed mean fat intake of infants principally fed human milk. This AI is 31 g/day (55% of total energy intake) for infants 0 to 6 months, and 30 g/day (40% of total energy intake) for infants 7 to 12 months. Infants 7 to 12 months consume foods other than human milk, and part of this 30 g/day average fat intake is attributable to these other foods. The average fat intake in this group attributable to human milk alone is 24 g/day. The NAS also

notes that, whereas human milk contains fat representing about 55% of total energy intake, conventional milk-based infant formulas contain approximately 48% of energy intake as fat²⁶.

Guidelines for infant formula feeding have been published in the United States by the Committee on Nutrition of the American Academy of Pediatrics, and by an expert panel of the Life Sciences Research Office ("LSRO")^{33,35-37}. Recently, the LSRO published nutrient requirements for preterm infant formulas²⁷. Fat provides about half the energy content of breast milk. LSRO has recommended a minimum fat content of infant formulas of 4.4 g/100 kcal (40% of total energy content)³³. Similar recommendations are published by the Canadian Pediatric Society and the European Society of Paediatric Gastroenterology and Nutrition ("ESPGAN")^{38,39}. Table 3 summarizes these recommendations for dietary fat and energy.

Table 3: Guidelines for energy and fat levels in infant formula feeding.

Reference	Daily intake		Formula composition	
	Energy (kcal/kg)	Fat (g/kg)	Energy (kcal/100ml)	Fat (g/100kcal)
Preterm				
USA ^{27, 35-37}	105-130	5-7	67-80	4.4-5.6
Canada ³⁸	105-135	4.5-6.8		4.4-6.1
Europe ³⁹	110-165	4.0-9.0	>65	3.6-7.0
Term				
USA ³³			63-71	4.4-6.4
USA ^{32,35-37,40}	100-135		67-70	3.3-6.0

The maximum intake of fat relates to the capacity of absorption. ESPGAN considers it unnecessary to give fat intakes in excess of 9 g/kg or to exceed a fat density of 7.0 g/100 kcal in a formula³⁹. LSRO has recommended a maximum fat content of infant formulas of 6.4 g/100 kcal (57.2% of total energy)³³.

2. Estimated exposure to Betapol™

As in human milk, the fat content of infant formula is usually about 3.5-4.5 g/100 ml, representing 40-50% of the energy content^{6,41}. Assuming a daily intake of 750 ml of formula in term infants, the total daily fat intake will generally be 26-34 g. As Betapol™ will comprise 40-80% of the fat phase, this means the daily intake of Betapol™ will not exceed 28 g.

In Table 4, an evaluation of exposure data for total fat is given for several hypothetical situations, based on a publication by Fomon *et al.*⁴².

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The results are based on the assumption that the percent of fat in the formula is 48.2% of calories (in general, protein = 15 g/l, fat = 36 g/l, carbohydrate = 72 g/l). Based on these data, a male infant at the 90th percentile of energy intake, weighing 5 kg, will consume 34.5 g fat per day. Given that Betapol™ will comprise 40-80% of the fat phase, daily intake of Betapol™ in infants with high energy intake will be 14-28 g.

Table 4: Fat intake from days 42-55 of life (mean weight about 5 kg)⁴².

Percentile of intake	Energy intake (kcal/kg/day)	Fat intake (kcal/kg/day)	Fat intake (g/kg/day)
Males			
10	94.3	45.5	5.1
50	109.2	52.6	5.8
90	129.0	62.2	6.9
Mean ± SD	110.5 ± 14.2	53.3 ± 6.8	5.9 ± 0.8
Females			
10	90.8	43.8	4.9
50	108.2	52.2	5.8
90	127.4	61.4	6.8
Mean ± SD	108.8 ± 14.2	52.4 ± 6.8	5.8 ± 0.8

3. Betapol™ exposure data from clinical trials

Both preterm infants and infants born at term have been fed Betapol-containing formula in published clinical trials (see Table 5)⁴³⁻⁴⁵. In all three studies, approximately 80% of the fat phase was Betapol™. The percentage of palmitic acid esterified in the sn-2 position of the Betapol™ was 70-80%.

Table 5: Daily intake of Betapol containing formula by preterm and term infants receiving Betapol™.^a

Birth status	Reference	n	Daily Intake	
			Formula (ml/kg)	Fat (g/kg)
Preterm	Lucas <i>et al.</i> ⁴³	8	185 ± 14	7.8 ± 0.6
Preterm	Carnielli <i>et al.</i> ⁴⁴	12	not reported	6.6 ± 0.7
Term	Carnielli <i>et al.</i> ⁴⁵	9	176 ± 19	6.3 ± 0.7

^a Approximately 80% of the fat phase as Betapol™.

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4. Conclusions on exposure to Betapol™

From the data presented it can be concluded that the daily exposure to Betapol™ at the 90th percentile of energy intake will be approximately 5.5 g/kg body weight, both in preterm and in term infants. The highest reported maximum fat intake recommendation, published by ESPGAN, is 9 g/kg³⁹. The maximum exposure to Betapol™ is within the recommendation and within general exposure to fats.

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VI. BIOLOGICAL STUDIES

A. Digestion, Absorption, and Metabolism

1. General fat absorption

About 50% of the dietary energy which an infant receives is provided in the form of lipids, predominantly triglycerides^{6,46}. Breast milk fatty acid composition varies widely between subjects, and is influenced by the mother's diet^{29,41,47-55}. The most abundant saturated fatty acid in human milk is palmitic acid (C16:0), which represents 20-25% of the total milk fatty acids and more than 10% of the infant's total energy intake.

After ingestion, the milk fat is emulsified. Enzymes (*e.g.*, pancreatic lipase) hydrolyze the triglycerides to release free fatty acids from the sn-1 and sn-3 positions (the two outer positions of the glycerol backbone). The free fatty acids and the sn-2 monoglycerides are then absorbed from the small intestine.

Not all free fatty acids are easily absorbed. Longer chain, saturated fatty acids (C12:0 to C18:0) are generally less well absorbed than medium chain (C6:0 to C10:0) and unsaturated fatty acids¹⁻³. The decreasing absorption with increasing chain length of saturated fatty acids is probably due to their inability to stay in solution, as long-chain saturated fatty acids have higher melting points (63°C for palmitic acid). They also readily form insoluble fatty acid-calcium soaps at the pH of the intestine^{4,10}.

2. Effects of Betapol™ on fat and calcium absorption

Animal studies

Studies in piglets and rats have shown that the fatty acid at the sn-2 position is preserved at this position throughout the digestive processes, and is absorbed as an sn-2 monoglyceride^{56,57}. A study in adult rats showed that palmitic acid was more rapidly absorbed from Betapol™, which has palmitic acid at the sn-2 position, than from the control diet, which has palmitic acid at the sn-1 and sn-3 positions. This finding was accompanied by more complete fat absorption overall⁵⁸.

Human studies

In breast-fed infants, it has also been demonstrated that palmitic acid is absorbed as sn-2 monoglyceride⁵⁹. In breast milk, approximately 70% of the palmitic acid is positioned at the sn-2 position of the glycerol backbone whereas in vegetable oils (the fat source of most current infant formulas) more than 80% of the palmitic acid is esterified to the outer positions^{29,60}. This means that in vegetable oil-based formula the palmitic acid is hydrolyzed from the glycerol backbone by the pancreatic lipase and, hence, may form insoluble and unabsorbable calcium soaps which are excreted with the feces, resulting in unnecessary loss of both dietary energy and calcium.

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Effects of Betapol™-containing formulas on fat absorption have been studied by Carnielli *et al.* both in premature and full-term infants^{44,45}. All fat blends studied contained 20-25% palmitic acid. The formulas containing Betapol™ had 66-76% of the palmitic acid at the sn-2 position (similar to human milk fat) whereas the control formulas had only 12.6% of the palmitic acid at the sn-2 position (*see* Table 6). Infants fed the Betapol™-containing formulas showed significantly better palmitic acid (C16:0) absorption, as well as stearic acid (C18:0) absorption. Total fat absorption was significantly better in term infants, but not in preterm infants.

Table 6: Absorption of fats enriched in sn-2 palmitic acid and calcium in preterm and term infants (% , mean values)^{44,45}.

	Preterm Infants		Term Infants	
	Control	Betapol™	Control	Betapol™
	n = 12	n = 12	n = 9	n = 9
Fat Blend C16:0				
Total C16:0 (%)	26	25	20	24
Proportion sn-2 C16:0 (%)	13	76	13	66
Fat Balance				
Fat absorption (%)	76	81	90	98 ^a
C12:0	95	97	76	96 ^a
C14:0	80	90 ^b	11 [*]	84 ^a
C16:0	51	73 ^b	78	97 ^a
C18:0	9 ^{**}	61 ^b	76	91 ^a
C18:1	88	82	96	99
C18:2	91	84	98	100
C18:3	95	96	99	100
Calcium Balance				
Calcium excretion (mg/kg/d)	82	59 ^a	53	33 ^a
Calcium absorption (%)	49	64	33	53 ^a
Calcium retention (%)	48	61	29	46

* SD = 36; ** SE = 12

^a P<0.05; ^b P<0.01 (control vs. Betapol™).

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Similar findings were demonstrated in a study by Lucas *et al.*⁴³. Preterm infants (n = 7) fed a Betapol™-containing formula (74% of palmitic acid at the sn-2 position) showed significantly better absorption of both palmitate (91% vs. 79% in controls) and stearate (81% vs. 68% in controls). Stearate is a relatively minor component of human milk fat (approximately 5%) and is concentrated at the sn-1 position. Reduction of the amount of free palmitic acid released when feeding Betapol™ apparently improves the absorption of the lower level of free saturated fatty acids generated by digestion. Significantly better calcium absorption (57% vs. 42% in controls) was also observed for Betapol™ compared to infants fed the control formula. Calcium

absorption was measured as fractional absorption by dual isotope technique. Total fat absorption did not differ significantly (94% vs. 91% in controls). It should be noted that absorption figures are derived from intake and excretion figures and therefore have greater variation than parameters that are direct measurements.

As previously mentioned, long-chain saturated free fatty acids may react with mineral ions to form insoluble soaps. Most of the non-absorbed fat is excreted, which, especially in the case of infants, leads to unnecessary losses of fat and energy^{9,10,61}. Likewise, the impact of soap formation can be significant on calcium absorption. One study with newborn, full-term infants taking high-palmitic acid formulas showed calcium absorption was only 6% of intake compared to 51% for breast-fed infants¹. Fecal calcium excretion was significantly lower in both preterm (59 vs. 82 mg/kg/d, $P < 0.05$) and full-term (43 vs. 60 mg/kg/d, $P < 0.05$) infants fed Betapol™-containing formula⁴⁴⁻⁴⁵. Reduced formation of insoluble calcium soaps in the stool of preterm infants was also demonstrated in the study by Lucas *et al.* (3.3% of milk-fat intake vs. 6.2% in controls)⁴³. Recently, it was demonstrated that feeding term infants a Betapol™-containing formula (20% palmitic acid, of which 50% in sn-2 position) versus a standard formula resulted not only in reduced fecal fatty acid soaps, but also higher whole-body bone mineral content⁶². Quinlan *et al.* showed that 90% of the excreted fats (30% of the stool dry weight) in formula-fed infants were in the form of soaps¹⁰. Breast-fed infants, on average, excreted feces containing only 3% of the dry weight as soaps. As a consequence, stool hardness was greater in the formula-fed infants. This is a major contributing factor to constipation and, in extreme cases, can lead to bowel obstruction. Use of Betapol™ in infant formula may reduce constipation and bowel problems.

3. Other effects of Betapol™

The outcomes of several studies in young animals and infants demonstrate that the Betapol™ groups typically are more related to the breast-fed groups than standard formula groups^{56,63-68}.

Animal studies

The post-absorptive metabolism of the dietary triglyceride blends used in the above formulas has been evaluated in a series of piglet studies that demonstrated that the positional distribution of palmitic acid can affect some aspects of plasma lipid metabolism^{56,63-66}. High plasma cholesteryl-palmitic acid levels, characteristic of sow-fed piglets, were replicated by feeding Betapol™ formula but not by feeding other formula. Furthermore, total- and high-density lipoprotein-cholesterol levels in the piglets fed Betapol™-containing formula were closer to levels in sow-fed piglets than in piglets fed the other formula⁶³. However, tissue fatty acid composition (adipose) and phospholipid composition (liver, bile, and platelets) was found to be more resistant to changes in dietary SAFA composition and was not affected by the positional distribution of the palmitic acid on dietary triglycerides⁶⁵⁻⁶⁸.

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Human studies

A study in preterm infants fed control and Betapol™ formulas found that the positional distribution of dietary palmitic acid on the triglyceride affected the proportion of plasma cholesteryl esters linked to palmitic acid. Those fed Betapol™ formula had proportions similar to breast-fed infants⁶⁹. These results are similar to those observed in piglets. A study in term infants concluded that dietary triglyceride fatty acid distribution may alter lipoprotein metabolism in the direction of findings in breast-fed infants⁷⁰.

Studies in adults using fat blends with comparable fatty acid compositions (total palmitic acid, 27-30%), and differing only in palmitic acid positional distribution (sn-2 palmitic acid levels of 6% vs. >67%) failed to show any effect on plasma lipoprotein fractions and levels or on postprandial lipemia^{71,72}.

4. Conclusions

The amount and position of palmitic acid in dietary triglycerides can influence digestive, absorptive, and metabolic processes in both animals and infants. Palmitic acid from unmodified vegetable oils occurs mainly in the sn-1,3 position. During digestion it is cleaved from the glycerol backbone and binds with mineral ions to form unabsorbable soaps. This reduces the absorption of both the fatty acid needed for energy and important minerals, such as calcium. Not only does this lead to losses of essential nutrients, but the infant may also suffer constipation.

Significant improvements in fat and calcium absorption were found when palmitic acid was predominantly esterified to the sn-2 vs. the sn-1,3 position of the dietary triglycerides. This reduction in fatty acid soap excretion was found to reduce stool hardness in term infants. Formulas containing palmitic acid at levels and positions similar to those found in human/sow milk generally resulted in tissue (piglets) and plasma lipid (piglets and infants) profiles that were closer to those observed in breast milk-fed piglets and infants.

The use of Betapol™ in infant formula has important advantages over traditionally used fats, especially considering the nutritional needs of preterm infants. There is sound biochemical and clinical evidence supporting the usefulness of incorporating palmitic acid at the sn-2 position of dietary triglycerides. These findings have been observed consistently in all animal and human studies conducted to date.

For ease of reference to the studies cited in this section, Table 7 presents a summary of the relevant clinical studies of Betapol™ in term and preterm infants.

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Table 7: Clinical studies of Betapol in preterm and term infants

Study	Design	Population	Palmitic acid content in triglycerides and relative distribution on <i>sn</i> -2 position	Duration	Results
Carnielli 1995a (Ref. 44)	Randomized crossover	N=12 Preterm infants, age 5 weeks at study begin	(1) Control: 26%; 12.7% (2) Betapol: 26%; 76.1%	1 week duration in each phase	Calcium fecal/urinary excretion compared to Control: significantly lower/higher. Absorption of saturated fatty acids in Betapol group compared to control: significantly higher
Carnielli 1995b (based on study Carnielli 1995a) (Ref. 69)	Randomized crossover	N=7 Preterm infants, age 5 weeks at study begin	(1) Control: 25.7%; 12.7% (2) Betapol: 25.4%; 76.1%	1 week duration in each phase	Plasma lipid composition compared to Control: Palmitic acid content in plasma sterol esters, triglycerides, and free fatty acids significantly higher, linoleic acid content in triglycerides significantly lower
Carnielli 1996 (Ref. 45)	Randomized, blinded three-arm parallel controlled study	N=27 Term newborn	(1) Control: 20%; 13% (2) Betapol: 24%; 66% (3) Intermediate: 24%; 39%	5 weeks	Absorption of saturated fatty acids in Betapol group compared to control: significantly higher Calcium fecal excretion in Betapol group compared to Control: significantly lower Intermediate group: Results for saturated fatty acid absorption and fecal calcium excretion are between Betapol and Control groups
Lucas 1997 (Ref. 43)	Randomized, double blind three-arm parallel controlled study	N=24 Premature infants, age below 10 days at study begin	(1) Control: 14.7%; 8.4% (2) Betapol: 23.9%; 73.9% (3) Intermediate: 23.9%; 27.8%	3 weeks	Palmitic and stearic acid absorption in Betapol group compared to Control and Intermediate: significantly higher Fatty acid soap excretion of Betapol group compared to Control and Intermediate: significantly and substantial lower. Calcium absorption in Control and Intermediate compared to Betapol group: lower

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Study	Design	Population	Palmitic acid content in triglycerides and relative distribution on <i>sn</i> -2 position	Duration	Results
Kennedy 1999 (Ref. 62)	Randomized, double blind Extra reference group of breast-fed infants	N=203 Term neonates N=120 Term infants, age 10 weeks at study begin (extra reference)	(1) Control: 19.6%; 12% (2) Betapol: 20.1%; 50%	12 weeks (2 weeks for extra reference)	Bone mineral content and density compared to Control: higher. Stool consistency compared to Control: softer Stool soap fatty acids compared to Control: lower Breast-fed infants had bone mineral density similar to Betapol group.
Nelson 1999 (Ref. 70)	Three-arm parallel controlled study Randomized to groups fed infant formula, Nonrandomized group breast fed infants	Healthy term neonates N=47 infants receiving formula N=40 infants breast fed	(1) Control: 27.2%, 6% (2) Betapol: 24.8%; 39% (3) Reference: 23.1%; 81%	120 days	More than 50% of palmitic acid on <i>sn</i> -2 pos is conserved through digestion, absorption, and chylomicron triglyceride synthesis in all groups Cholesterol and apolipoprotein compared to Control: lower HDL-cholesterol and apolipoprotein A-I and higher apolipoprotein B

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B. Toxicology of Betapol™ Components: Primarily Oleic and Palmitic Acids

The major components of Betapol™ are oleic and palmitic acids. Oleic acid, in esterified form, is found in many vegetable oils and animal fats, frequently constituting greater than 50% of the total fatty acid concentration. Oils rich in oleic acid include olive (80%), peanut (60%), tea seed (85%), and pecan (85%) oils. Very few fats contain less than 10% oleic acid. Palmitic acid is widely distributed, being found in practically all vegetable oils and animal fats at concentrations of at least 5%. Palmitic acid constitutes approximately 60% of saturated fatty acids consumed in the average American diet⁷⁶. Oleic and palmitic acids are used in direct and indirect food applications and are accepted as safe for use in food and in the manufacture of food-grade additives (see Part II, "Regulatory Status").

These fatty acids are part of the normal diet and are found in breast milk and infant formulas. Vegetable oils in general, and the fatty acids found in Betapol™, have been the subject of continuing and extensive evaluation in the scientific literature relevant to safety, including analysis of the role of these substances in human nutrition and studies evaluating their safety as part of the diet²⁵. The safety of oleic and palmitic acids for use in cosmetics was also reviewed as part of the ongoing Cosmetic Ingredient Review⁷⁷. While the focus of the review was on skin applications, there were also some data on oral exposure.

Acute Oral Toxicity

Oleic and palmitic acids were tested for acute oral toxicity in rats. Administration of doses up to 21.5 ml/kg (approximately 19.2 g/kg body weight) of oleic acid and up to 10 g/kg of palmitic acid by gavage to albino rats resulted in no deaths and no significant gross lesions at necropsy⁷⁷.

Subchronic and Chronic Oral Toxicity

Feeding of 5% oleic acid to chicks for 4 weeks resulted in no adverse effects. Rats fed diets providing 15% oleic acid (approximately 10 g/kg body weight/d) for up to 16 weeks showed no adverse effects on growth or general health. Rats developed hyperlipemia when fed diets providing 4.6 g/kg body weight/d palmitic acid for 6 weeks⁷⁷.

Carcinogenicity

Oleic and palmitic acids have mainly been tested for carcinogenic potential using the subcutaneous route of administration⁷⁷. As subcutaneous injections are of doubtful relevance to the assessment of the carcinogenic potential of a dietary ingredient, particularly if the material is mildly irritating (like free fatty acids), these studies are not considered here.

The few oral studies that have been done are of questionable design: using mixtures of various vegetable oils as vehicles, using inappropriate controls, or testing in the presence of other fatty acids. Increased numbers of lung and gastric (forestomach and pyloric) tumors were found in

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T.M. mice receiving free oleic and linoleic acids (1.5% in refined corn oil) incorporated into the diet⁷⁷. A second study of similar design also produced increased gastric tumors⁷⁷.

Statistical techniques were used to determine possible associations between dietary fatty acids in triglycerides and the incidence of spontaneous mammary tumors in C3H mice. Palmitic acid had little effect on tumor incidence or the time needed for tumors to appear. Oleic acid produced no significant effect on tumor incidence⁷⁷.

C57BL/1 strain mice were fed 1.5% oleic acid in corn oil dispersed in feed for up to 24 months. Colon adenocarcinomas were found in 8% of the mice. Control animals were from a different supplier, making comparisons questionable⁷⁷.

Reproductive and Developmental Toxicity

No studies were found in the literature on the potential for reproductive and developmental toxicity of oleic and palmitic acids⁷⁷.

Conclusion

The primary components of Betapol™, oleic and palmitic acids, appear to be innocuous as typically eaten in substantial amounts as a component of the human omnivorous diet.

C. Safety Support for the Betapol™ Production Process

Betapol™ production utilizes methodology similar to that used for the production of enzymatically interesterified vegetable fats for use in confectionery applications, such as cocoa butter substitute (*see* 21 CFR §184.1259).

1. Safety support for the use of enzyme technology to interesterify fat

Loders Croklaan's manufacturing process for enzymatically produced Betapol™ has been subjected to rigorous control consistent with requirements for good manufacturing practice (GMP). The specifications of the Betapol™ starting materials, processing aids, and process conditions are consistent with conventional procedures for edible oil production. The currently utilized enzyme has been extensively tested for safety.

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The safety support for the enzymatic interesterification process was initially based on lipase derived from *Mucor miehei* (not using recombinant DNA technology) published by Stavnsbjerg *et al.*¹⁶. The micro-organism is a weak pathogen to mice but there is no evidence of pathogenic potential in healthy persons. No evidence of genotoxic potential was observed either for the bacterial gene mutation assay or the *in vivo* cytogenetic tests. The enzyme preparation was nontoxic to rats after acute oral administration of up to 10 g/kg body weight. Subchronic toxicity studies with the enzyme preparation showed no-effect levels of 0.9 g/kg body weight/d for rats and 4.3 g/kg body weight/d for dogs. No evidence was found of teratogenic potential or for any effect on fertility and general reproductive performance. The authors concluded that the enzyme

preparation could be considered safe and suitable for the production of fats and edible oils for human consumption whether it is used in liquid or in the immobilized form¹⁶.

The lipase is immobilized on an ion exchange resin, Duolite. The results of studies conducted on the resin immobilization support have been published by Jensen *et al.*²⁸. These studies concluded that the carrier is safe for the purpose of fixing a lipase from *Mucor miehei* for interesterification of fats and oils. Jensen *et al.*²⁸ also concluded that the immobilized enzyme is safe for use for interesterification of fats and oils.

2. Safety support for the lipase produced by rDNA technology

For technical reasons, the currently preferred enzyme in the production of Betapol™ is a lipase derived using rDNA technology. This lipase is produced by splicing its gene from *Rhizomucor miehei* into *Aspergillus oryzae* to increase the yield of enzyme. The protein is identical to that naturally occurring in *Rhizomucor miehei* because the gene spliced into *Aspergillus oryzae* has not been altered. (In published studies, this enzyme has been referred to as "SP388," and the discussion below uses this term for consistency.)

As the enzyme is unaltered, the safety conclusions reached in Stavnsbjerg *et al.*¹⁶ apply to the preparation from *Aspergillus oryzae*. Further, *Aspergillus oryzae* is the source for a number of enzymes approved for food use. Under normal circumstances it is not a mycotoxin or antibiotic producer. Nonetheless, following gene splicing, it was necessary to ensure that no toxic material would result from this *Aspergillus* during fermentation for lipase production. For this reason the following safety studies were conducted on the enzyme preparation designated SP388:

- Bacterial gene mutation assay (Ames test)
- Mouse lymphoma cell mutation test
- Human lymphocyte chromosomal damage test
- 28-day rat feeding study
- 90-day rat feeding study.

The results from the above studies were published and confirm the absence of toxicological effects with SP388¹⁷. Some minor effects were reported for the group of animals receiving the highest dose (40,000 mg enzyme preparation/kg diet) in the 90-day feeding study. The summary of these studies states:

The *Rhizomucor miehei* lipase enzyme expressed in *Aspergillus oryzae*, is used in the production of speciality fats, the production of existing fats from new raw materials, or new fats with improved nutritional or functional qualities. It is produced by *A. oryzae* containing the structural gene for the precursor of *R. miehei* triglyceride lipase. It was subjected to a series of toxicological tests to document the safety in use. The enzyme preparation was not found to be mutagenic either in bacterial cultures (Ames test) or in the mammalian cell cultures (mouse lymphoma assay), nor did it cause chromosomal damage (human lymphocyte assay). Dietary concentrations of

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up to 1600 mg/kg diet for up to 13 weeks caused no adverse effects in rats. At higher concentrations there were effects upon food intake, possibly arising from some irritant property of the enzyme preparation in the diet at such high levels, with consequential effects upon body weight and energy metabolism. A minor effect upon renal function was indicated by increased kidney weight and changes in the urine. At 40,000 mg/kg diet the enzyme was considered to have exacerbated the onset of normally occurring chronic myocarditis in male Sprague-Dawley rats.

The authors conclude that the NOAEL was 1600 mg SP388/kg diet, or approximately 115 mg/kg bodyweight/d. Based on this value, and assuming that all ingested triglycerides were treated with the lipase, the authors estimate a safety factor of more than 10,000 for the enzyme. The interesterification process used to produce Betapol™ uses immobilized lipase on an inert support in a reaction column. None is anticipated to leach out and none is detected in the finished product. Further, any enzyme that might leach out would be denatured and removed during the subsequent refining process.

In conclusion, a range of safety studies supports the use of the *Rhizomucor miehei* lipase produced by recombinant *Aspergillus oryzae* as a processing aid in the production of Betapol™. In addition to review of this enzyme by the Expert Panel, Loders Croklaan also sought the opinion of a recognized expert in the field of enzyme safety, Michael W. Pariza, Ph.D., Professor in the Department of Food Microbiology and Toxicology, Director of the Food Research Institute, and Wisconsin Distinguished Professor at the University of Wisconsin–Madison. Dr. Pariza reviewed information on the enzyme and relevant information in the published literature, and concluded that the enzyme is GRAS for use in the manufacture of Betapol™.⁵

D. Safety Evaluation of Fat Blends Containing Betapol™

The safety of Betapol™, especially in developing and growing animals, was established before clinical trials were conducted in humans:

1. Single generation rat feeding study

The purpose of this study was to evaluate the effect of feeding Betapol™ in the diet of sexually mature male and female rats for four weeks prior to mating, through mating, gestation, and lactation, and to selected offspring through six weeks of age¹⁸.

Groups of rats were fed either control oil (15% wt/wt in the diet) or different concentrations of Betapol™ (5, 10, or 15% wt/wt) equivalent to approximately 3, 6, and 8 g Betapol™/kg body weight/day in the F0 generation. The caloric density of the Betapol™ diets was not balanced in this study, as the intention was to evaluate whether the test animals would tolerate up to 15% inclusion of fat in the diet.

⁵ Letter from Michael W. Pariza, Ph.D. to Richard W. Lane, Ph.D., April 3, 2003.

Rats were monitored daily for clinical signs related to treatment. Food consumption and body weight were measured weekly for both sexes of the F0 generation and for selected animals of the F1 generation. For the F0 generation, pregnancy rate, mating performance, and length of gestation period were evaluated. The uterus of each female giving birth was visually inspected for implantation sites. Uteri of apparently non-pregnant females were examined for evidence of implantation. The testes of males that failed to induce pregnancy in their female partner were weighed and preserved. Macroscopic post mortem examination was performed for all surviving F1 animals.

Results F0 generation

None of the F0 generation died. Pale fecal pellets were observed in all groups during the pre-mating period, returning to normal from week 4. No other signs of treatment were noted.

Within the Betapol™-treated groups, there was a concentration-related trend in mean body weight gain for both sexes during the pre-mating period. Animals (males and females) fed 15% reference oil gained slightly less weight than those treated with 15% Betapol™. The body weight gains of males during the mating phase were similar for all treatment groups whereas the females maintained the difference in body weight established prior to mating. The increase in body weight gain with increasing Betapol™ concentration in the diet continued throughout the pregnancy and lactation periods. Females fed 5% Betapol™ showed a lower weight gain during lactation with retarded gain to weaning of selected F1 females at this inclusion level. None of the differences in body weight gain referred to above were statistically significant.

At 5 and 10% Betapol™, mean food intake for both sexes during the first 4 weeks of treatment generally showed a concentration-related increase compared with pre-treatment values. Intake at 15% Betapol™ remained essentially constant throughout the pre-mating period with values marginally higher than for concurrent controls. Food utilization for both sexes showed an improved efficiency with increasing Betapol™ concentration.

Mating performance was not affected by treatment. Most animals conceived within the first estrus cycle after pairing. Pregnancy rate and mean duration of gestation were similar for all groups. Mean pup weight at birth was similar for all groups. From day 4 through weaning, there was a slight concentration-related trend of increasing pup growth in the Betapol™ treatment groups. There were no intergroup differences in implantation rates and pup survival, either pre- or post-cull, related to treatment. There also were no treatment-related effects on sex-ratio.

Results F1 generation

There were no deaths among the selected F1 offspring. Pale fecal pellets were observed in all groups throughout the time period recorded (weeks 5 and 6).

Female rats fed 5% Betapol™ showed a statistically significant reduction in body weight gain up to day 21 but, thereafter, females in all groups were similar to the reference fat control group. Male rats fed 5% Betapol™ exhibited body weight gains which were comparable to control through termination. Food consumption was increased in both sexes fed 5% Betapol™ during

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weeks 5 and 6. Food consumption of the remaining groups was similar. Food utilization improved in both sexes with increasing Betapol™ concentration.

In conclusion, Betapol™ supported reproduction and development without adverse effects. Growth of adult rats and offspring was slightly greater in the 10% and 15% Betapol™ groups compared to the reference oil group. Rats fed 5% Betapol™ did slightly less well than the 15% fat reference oil group, though this was considered to be due to the reduced energy content of the Betapol™ diet compared to the reference diet. Occasional macroscopic changes observed at terminal autopsy were considered to be background findings unrelated to treatment. The no-observed-effect level for Betapol™ was 15% of the diet (equivalent to 8 g/kg body weight/day), the highest level fed. This study has been accepted for publication.

2. Two-generation rat feeding study

In this study, rats were fed Betapol™ prior to mating and during gestation and lactation over two generations²⁴. The effects of Betapol™ on growth and reproductive performance were studied. Diets were analytically characterized to ensure a suitable nutritional profile and absence of potential toxic components.

Betapol™ was incorporated into a purified diet at fixed inclusion levels of 0 (control), 1.5, 7.5, or 15% (equivalent to approximately 0, 1, 5, or 10 g Betapol™/kg body weight/day, respectively). Where appropriate, each Betapol™ group was supplemented with reference oil (an identical balanced diet in terms of fatty acid composition, with the only difference being the position of the fatty acids on the glycerol backbone) to give a total of 15% fat. A standard control commercial diet (LAD2), routinely used in the laboratory, was used to provide a comparison with the reference oil group.

At termination, all animals were subjected to macroscopic post mortem examination. Specified organ weights were recorded for all F0 and F1 adults and selected weanlings. Microscopic examination of a range of tissues from the reproductive tract and the target organ (liver) was conducted for all F0 and F1 adults.

Findings related to high oil-based diets in general

Compared to the LAD2 control group, several differences were noted for adult animals that were considered to be due to the higher fat content of the diet (whether oil and/or Betapol™). These were: pale fecal pellets, lower absolute body weight and food consumption, increased efficiency of food utilization, lower water consumption, and higher plasma cholesterol and phospholipid levels. Among adult males and offspring of both generations, changes in the macroscopic appearance of the liver (pallor and/or accentuated lobular markings) were often noted but were considered associated with the high proportion of dietary fat in a semi-purified diet and were not considered to be of toxicological importance.

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Findings related to Betapol™ in the diet

The only consistent observation among adults of both generations was a small, dose-related increase in ovarian weights in all Betapol™-treated groups, although statistical significance was only attained at the 15% level. Significant differences in ovarian weights, most noticeable in the 15% Betapol™ group, occurred in absolute weights (F0 and F1 adults, F1 weanlings) and the organ-to-body weight ratio for F0 weanlings. Such an increase in weight is possibly linked to improved fat absorption in the Betapol™ groups. However, no microscopic changes were associated with this finding. In the absence of microscopic changes and without associated changes in fertility, this increase in ovarian weight was concluded not to be of toxicological importance.

In comparison with the 15% reference oil control group, an increased incidence of fat deposition in periportal hepatocytes was seen in the liver of female rats from the 7.5% and 15% Betapol™ groups of the F0 generation and in the 15% Betapol™ group females of the F1 generation. An associated increase in periportal hepatocyte vacuolization was seen in F0 generation female rats fed 15% Betapol™, but not in the F1 generation rats. These changes were consistent with a physiological response to high levels of dietary fats fed in semi-purified diets and were therefore not considered to be of toxicological importance. No change in the incidence of fat deposition was detected between male rats from the 15% Betapol™ group and the 15% reference oil group in either the F0 or F1 generation.

There were no obvious or consistent adverse effects of Betapol™ treatment at any level on F0, F1, or F2 (where appropriate) adult survival or well being (as assessed by overall body weight gain, food intake and utilization, water consumption, hematological and biochemical parameters), reproductive capacity, male organ weights, and macroscopic pathology, or on F0 and F1 pup survival and development to weaning.

The results of this study demonstrate the ability of Betapol™ to support the growth and general reproductive performance of the rat. The no-observed-effect level was 15% Betapol™ in the diet (equivalent to approximately 10 g Betapol™/kg body weight /day). This study has been accepted for publication.

3. The comparative metabolic fate of 1-, and 2-palmitoyl triglycerides in the rat. Part 1. The male weanling rat

The metabolic fate of 1-palmitoyl dioleoyl triglyceride ("POO") and 2-palmitoyl dioleoyl triglyceride ("OPO") was studied in the male weanling rat⁷³. Carbon radiolabelled derivatives were prepared as either [¹⁴C]POO or O[¹⁴C]PO and administered in a dietary slurry by gavage.

The excretion pattern of the radiolabel was followed over 96 hours in expired air, urine and feces. Test animals were also prepared for whole body autoradiography at intervals during the 96 hours post dosing.

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The metabolism of [^{14}C]POO was rapid over the first few hours, but ^{14}C was still being recovered at 96 hours. The majority of ^{14}C was expired as $^{14}\text{CO}_2$ (approximately 75%), with 41% collected during the first three hours and 65% at 24 hours. From 72-96 hours approximately 2% was recovered as $^{14}\text{CO}_2$. Just over 1% of the ^{14}C was recovered in the urine and 6% in the feces.

The metabolism of O[^{14}C]PO was similar to [^{14}C]POO. The majority of the ^{14}C was expired as $^{14}\text{CO}_2$ (approximately 72%), with 37% collected during the first three hours and 61% at 24 hours. From 72-96 hours approximately 2% was recovered as $^{14}\text{CO}_2$. Just over 1% of the ^{14}C was recovered in the urine and 2% in the feces.

Thin layer chromatography of the fecal extracts showed no trace of either parent material.

The distribution of ^{14}C in the body, as determined by whole body autoradiography, after dosing with [^{14}C]POO and O[^{14}C]PO was almost identical. The brown fat deposits became labeled very quickly, with the activity moving to the white fat and persisting to 96 hours. Other tissues with high levels of radioactivity were the liver, stomach, and intestinal mucosa.

In conclusion, there is no apparent difference in the rate of metabolism and distribution between [^{14}C]POO and O[^{14}C]PO in the male weanling rat. Slightly higher fecal levels of ^{14}C are found in rats dosed with [^{14}C]POO. This study has been published⁷³.

4. The comparative metabolic fate of 1- and 2-palmitoyl triglycerides in the rat. Part 2. The suckling rat

Three litters of 12 suckling rat pups (13 days of age) were divided into two littermate treatment groups⁷³. The treatment groups received either test or control compounds as used in the previous metabolism study (*see above*). Dosing was by gavage in a skimmed milk preparation. At 2, 4, 8, 24, and 72 hours after dosing, one rat pup was from each treatment group was taken for sacrifice. Selected organs and the carcass were weighed, solubilized, and assayed for radioactivity. At 8 hours, one pup was taken for whole body autoradiography.

The metabolism of [^{14}C]POO and O[^{14}C]PO was shown to be rapid, with a peak ^{14}C level in the brown fat deposits at 4 hours post dosing. All of the tissues taken showed the presence of ^{14}C at 2 hours through 72 hours after dosing. The highest levels of ^{14}C activity per gram of tissue were found in the gastro-intestinal tract and the brown fat deposits. Whole body autoradiography showed the disposition of ^{14}C in the two treatment groups to be similar, although there was more ^{14}C in the stomach of the rats dosed with [^{14}C]POO. This was probably due to individual variation in the rate of emptying of the stomach. The ^{14}C levels in the blood and brain were very low in both treatment groups.

It is concluded that the metabolism and disposition of [^{14}C]POO and O[^{14}C]PO are very similar in the suckling rat. This work, while not as detailed as that carried out on weanling rats, supports those studies and extends our knowledge to younger animals. This study has been published⁷³.

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E. Safety Summary Statement

The safety support for the production process and the interesterified product Betapol™ has been described. Assessment of the processing aids used in the production process confirms these to be safe and suitable for use and the overall process to be acceptable for production of safe and wholesome fats and oils for human consumption.

Safety studies also have been carried out with Betapol™. In a two-generation study, rats were fed diets containing high levels of Betapol™ and no adverse effects were noted. The NOAEL from this study was approximately equivalent to 10 g/kg body weight of Betapol™ per day, the highest dose tested. This is roughly twice the estimated 90th percentile daily intake from infant formula.

The results of published clinical trials of Betapol™ fed to preterm and term infants confirmed the absence of adverse effects. In addition, benefits of improved calcium absorption, bone mineral content, and stool texture were found in infants fed Betapol™.

It is concluded that Betapol™ is safe for use in infant formula.

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VII. FINDINGS OF THE EXPERT PANEL

Betapol™ is a triglyceride mixture made specifically for use in infant formulas to closely mimic both the proportion of fatty acids in human breast milk and their arrangement on the glycerol backbone. Human breast milk is the reference against which infant formulas are compared. Triglycerides in human milk have around 70% of the palmitic acid located at the sn-2 (middle) position. Currently used triglycerides in infant formulas contain approximately the same proportions of fatty acids as those found in human milk, but their arrangement on the glycerol molecule is not the same. The palmitic acid is predominantly found in the sn-1 and -3 positions, which affects the absorption of the fatty acids, and reduces nutrient bioavailability.

Betapol™ is made using enzymatic rearrangement so that 45-80% of the palmitic acid is esterified at the sn-2 position. The remaining sn-1 and -3 positions are predominantly occupied by unsaturated fatty acids, in particular oleic acid. This is a common structure found in triglycerides in human milk and is considered to improve the absorption of fatty acids and minerals from the small intestine.

Betapol™ was developed for use in formula for both term and preterm infants. It will be blended with other safe and suitable vegetable oils to achieve the fatty acid profile required by the infant formula manufacturers to meet regulations and their own requirements.

Betapol™ is manufactured by enzymatic interesterification, the rearrangement of fatty acids on the glycerol molecule. The process uses a lipase bound to an inert support. The lipase is derived from *Rhizomucor miehei* lipase genes expressed in *Aspergillus oryzae*. The enzyme, free from microbial cells and DNA, is immobilized on Duolite 568 in a packed bed enzyme reactor. Duolite 568 is a phenol-formaldehyde anion exchange resin activated with triethylene tetramine. Food-grade fractionated palm oil is interesterified with food-grade oleic acid. The resulting mixture is then processed using conventional edible oil processing techniques to produce Betapol™ meeting appropriate specifications.

The general manufacturing method (enzymatic interesterification) is accepted for the production of oils and fats for specific uses by the U.S. Food and Drug Administration (FDA) and other regulatory authorities around the world. The lipase manufacturer has filed a GRAS petition with FDA for the enzyme and immobilization support; extensive supporting safety data have been published in peer-reviewed journals. Phenol-formaldehyde resins activated with triethylene tetramine are approved by FDA for processing of food. The enzyme and support are approved for enzymatic interesterification of fats for all food uses in Canada. Thus, the materials and processes used to make Betapol™ are considered safe and suitable for use in foods and conform with existing regulations.

An estimate of the likely intake of Betapol™ can be made from fat intake studies in infants and from clinical studies with infant formula containing Betapol™. If Betapol™ comprises 80% of total fat intake, the expected typical daily intake of Betapol™, assuming a total daily fat intake of 6.9 g/kg body weight, will be approximately 5.5 g/kg body weight.

Infant formulas containing Betapol™ have been fed to premature and full-term infants in clinical studies and compared with control fat blends containing the same amount of palmitic acid but not enriched in the sn-2 position. These published studies conducted in Europe and North America show that Betapol™-fed infants generally have improved fat and calcium absorption, with no adverse effects being reported. Furthermore, since the approval of Betapol™ for use in infant formula in Europe, no adverse events have been reported by the manufacturers of formulas containing Betapol™.

The principal fatty acids in Betapol™ (oleic and palmitic acids) are consumed as components of fat in infant formulas in the USA. The metabolic fate of ¹⁴C-labeled palmitic acid esterified to glycerol in the sn-1 and -3 or sn-2 positions were compared in suckling and weanling rats. No apparent differences were noted in the rate of metabolism or distribution of radioactivity in the body between rats dosed with palmitic acid in either the sn-1 and -3, or sn-2 positions.

Single generation and two-generation rat feeding studies with Betapol™ have been conducted. Betapol™ was incorporated into the diet of the test animals at levels of 0, 1.5, 7.5, and 15% (wt/wt), such that total fat was maintained at 15%. Betapol™ supported reproduction and the development of offspring without adverse effect. The no-observed adverse effect level (NOAEL) in the two-generation rat feeding study was 15% Betapol™, the highest level fed, which is equivalent to 10 g/kg/d (considering adults of both sexes in both generations).

Betapol™ has been approved for use in formulas for both term and preterm infants in the Netherlands. It has also been approved for use in formulas for low-birth-weight infants in the United Kingdom. The above approvals were based upon a critical evaluation of the safety data from animal and clinical studies.

Betapol™ is a triglyceride mixture composed of fatty acids present in edible oils and fats, including human milk. The predominant triglyceride structure is very similar to that of human breast milk fat. Betapol™ is produced by conventional methods used in the manufacture of edible oils and fats to meet a carefully controlled product specification. There is adequate support for the safety of specific elements of the enzymatic interesterification process particular to Betapol™ manufacture. The specifications for Betapol™ are suitable for use in infant formulas. No new components are being added to the diet. The safety of Betapol™ for use in infant formulas at levels up to 80% of total fat is supported by generally available and accepted scientific data, information, methods and principles, and corroborated by unpublished information, including the lack of adverse effects reported in countries where Betapol™ is approved for use in infant formula and appropriate animal studies.

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Based on a critical evaluation of the information summarized in this report, the Expert Panel concludes that the use of Betapol™, meeting appropriate food-grade specifications and produced by current good manufacturing practice (21 CFR §182.1(b)), is safe for use as an ingredient in infant formulas in amounts not to exceed 80% of total fat. Furthermore, it is the Panel's opinion that qualified experts in the field would generally recognize that Betapol™ is safe for this use, i.e., that Betapol™ is generally recognized as safe (GRAS) using scientific procedures.

Joseph F. Borzelleca, Ph.D.

Walter Glinsmann, M.D.

William Heird, M.D.

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VIII. BASIS FOR CONCLUDING THAT THERE IS A CONSENSUS AMONG QUALIFIED EXPERTS THAT THERE IS REASONABLE CERTAINTY THAT THE SUBSTANCE WILL NOT BE HARMFUL UNDER THE INTENDED CONDITIONS OF USE

GRAS status is based on common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances added to food. A GRAS evaluation through scientific procedures is based on "generally available and accepted scientific data, information, methods, or principles, which ordinarily are published and may be corroborated by unpublished scientific data, information, or methods." Proposed 21 CFR §170.30(b); 62 Fed. Reg. 18960 (April 17, 1997). *See also* 21 CFR §§170.3(h); 170.30(a), (b). There must be a "consensus among qualified experts about the safety of the substance for its intended use." 62 Fed. Reg. 18940. This section summarizes why there is a basis for concluding that there is a consensus among qualified experts that there is reasonable certainty that Betapol™ will not be harmful under the intended conditions of use.

A. The GRAS Determination is Based on Generally Available Information, and Corroborated by Unpublished Information

This GRAS notification, particularly Section VI, sets forth the scientific data and information that is published or otherwise generally available on the safety of Betapol™ and related compounds in humans and animals, and also refers to unpublished corroborative information. The following is a summary of the general availability of this data and information:

- Vegetable oils in general, as well as the fatty acids in Betapol™, have been the subject of extensive discussion in the scientific literature relevant to safety, including analysis of the role of these substances in human nutrition and studies evaluating their safety as part of the diet²⁵.
- Vegetable oil is GRAS. 62 Fed. Reg. 18939 (April 17, 1997). When ingested, the component parts of Betapol™ are the same as those of vegetable oils and other triglycerides, that is, they are digested and absorbed into the body as a mixture of monoglycerides, glycerol, and free fatty acids. Thus, all of the components of Betapol™ are present as components of fats found in foods or are generated in large amounts in the human digestive tract during the digestion of fat. The proposed use of Betapol™ will not result in a material increase in exposure to these substances. Thus, the general availability of information on Betapol™, and its GRAS status, are based on the similarity between Betapol™ and the basic components of fats and oils in the human diet. (This analysis is similar to that used by FDA in affirming the GRAS status of glyceryl palmitostearate. 60 Fed. Reg. 63620 (Dec. 12, 1995).)

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- Several studies evaluated infants fed Betapol™ formulas. These studies show that Betapol™-fed infants generally have improved fat and calcium absorption, with no adverse effects being reported. This work has been published^{43-45, 69}. Furthermore, since the approval of Betapol™ for use in infant formula in Europe, no adverse events have been reported by the manufacturers of formulas containing Betapol™.
- The major components of Betapol™, oleic and palmitic acids, have been the subject of acute oral, subchronic, and chronic oral toxicity testing. This work has been published⁷⁷.
- The metabolic fate of ¹⁴C-labeled palmitic acid esterified to glycerol in the sn-1 and -3 or sn-2 positions were compared in suckling and weanling rats. This work has been published⁷³.
- Single generation and two-generation rat feeding studies with Betapol™ have been conducted. Betapol™ supported reproduction and the development of offspring without adverse effect. This work has been accepted for publication^{18, 24}.
- With respect to the currently used lipase enzyme:
 - The safety support for the enzymatic interesterification process was initially based on lipase derived from *Mucor miehei* (not using recombinant DNA technology). The authors concluded that the enzyme preparation could be considered safe and suitable for the production of fats and edible oils for human consumption whether it is used in liquid or in the immobilized form. This work has been published¹⁶.
 - Studies on the enzyme preparation following gene splicing confirm the absence of toxicological effects. This work has been published¹⁷.
 - The lipase is immobilized on an ion exchange resin, Duolite. Studies conducted on the resin immobilization support concluded that the carrier is safe for the purpose of fixing a lipase from *Mucor miehei* for interesterification of fats and oils and that the immobilized enzyme is safe for use for interesterification of fats and oils. This work has been published²⁸.

Conclusion on General Availability

Loders Croklaan concludes that its GRAS determination, which is based on the weight of all of the available scientific information, is grounded on generally available scientific data and information, with additional corroborating data.

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B. The GRAS Determination is Based on a Consensus Among Qualified Experts

Based on a critical evaluation of the information summarized in this notification, an independent Panel of qualified experts convened by Loders Croklaan unanimously concluded that the use of Betapol™, meeting appropriate specifications and produced by current good manufacturing practice, is safe for use as an ingredient in infant formulas not exceeding 80% of total fat. It is also the Panel's opinion that qualified experts in the field would generally recognize that Betapol™ is safe for this use. In summary, Betapol™ is generally recognized as safe using scientific procedures.

As discussed above, the Expert Panel that has provided its advice to Loders Croklaan consists of experts on food safety (Joseph F. Borzelleca, Ph.D., Walter H. Glinsmann, M.D.) and pediatrics, including the needs for specific fatty acids and amino acids during infancy (William Heird, M.D.). These individuals are recognized as pre-eminent in their fields (additional information on the qualifications of these individuals is available on request). As a result, the individual and collective opinions of these experts provide a strong basis for concluding that Betapol™ is generally recognized as safe by experts qualified by scientific training and experience to evaluate its safety, as required by section 201(s) of the Federal Food, Drug, and Cosmetic Act.

Accordingly, Loders Croklaan concludes that its GRAS determination is based on a consensus among qualified experts that there is reasonable certainty that the substance will not be harmful under the intended conditions of use.

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IX. GRAS DETERMINATION

Based on the information summarized in this notification, Loders Croklaan determines that Betapol™, intended for use in infant formula for both term and preterm infants at levels of up to 80% total fat intake, is generally recognized as safe within the meaning of § 201(s) of the Federal Food, Drug, and Cosmetic Act; 21 CFR §§170.3 and 170.30; and the proposed rules described at 62 Fed. Reg. 18960.

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SUBMISSION END

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