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April 4, 2014

Paulette Gaynor, Ph.D.
Senior Regulatory Project Manager
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Dr. Gaynor:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), Nestlé Nutrition U.S., through me as its agent, hereby provides notice of a claim that the use of citric acid esters of mono- and diglycerides (CITREM) in exempt infant formulas, as described in the enclosed notification document, is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because Nestlé Nutrition has determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

As required, three copies of the notification are provided. Each copy also includes the signed conclusion of the members of the GRAS expert panel.

If you have any questions regarding this notification, please feel free to contact me at 804-742-5548 or jh@jheimbach.com.

Sincerely,

(b) (6)

James T. Heimbach, Ph.D., F.A.C.N.
President

Encl.

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GRAS Determination for the Addition of Citric Acid Esters of Mono- and Diglycerides to Exempt Infant Formula As Antioxidants, Emulsifiers, Stabilizers and Thickeners

Prepared for
Nestlé Nutrition
Florham Park NJ

Prepared by
JHeimbach LLC
Port Royal VA

April
2014

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1. GRAS EXEMPTION CLAIM

Nestlé Nutrition U.S., through its agent JHEIMBACH LLC, hereby notifies the Food and Drug Administration that the addition of citric acid esters of mono- and diglycerides (CITREM) to exempt amino-acid based and extensively hydrolyzed infant formulas intended for term infants as described below is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Nestlé Nutrition has determined through scientific procedures that this use is generally recognized as safe (GRAS).

(b) (6)

James T. Heimbach, Ph.D., F.A.C.N. Date 4/4/14
President, JHEIMBACH LLC

1.1. Name and Address of Notifier

Nestlé Nutrition U.S.
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Telephone: 973-593-7607
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1.2. Name of GRAS Substance

The name of the group of substances reviewed in this GRAS determination is citric acid esters of mono- and diglycerides of edible fatty acids. These substances are also known as citrated mono- and diglycerides, citric and fatty acid esters of glycerol, mono- and diglycerides of fatty acids esterified with citric acid, citroglycerides, and CITREM. (This last term is an acronym for CITRic acid Esters of Mono- and diglycerides.)

In this document, these esters are referred to as CITREM. In the EU they are labeled with that name as well as the additive number E472c, and it is proposed that—for consistency with international usage—U.S. labeling use the same name, CITREM.

1.3. Intended Use and Consumer Exposure

CITREM is intended to be added to exempt amino-acid based and extensively hydrolyzed infant formula at a maximum level of 233 mg CITREM/100 ml formula as consumed in order to provide adequate emulsification to maintain consistent nutrient delivery.

The 90th percentile of intake of infant formula is about 207 ml/kg bw/day. Since CITREM is intended to be added at a maximum level of 233 mg per 100 ml formula as consumed, the 90th percentile of daily intake of CITREM is 233 mg x 2.07 = 482 mg/kg bw/day.

1.4. Basis for GRAS Determination

Nestlé Nutrition's GRAS determination for the intended use of CITREM is based on scientific procedures as described under 21 CFR §170.30(b). Determination of the safety and GRAS status of the intended addition of CITREM to infant formula was made through the deliberations of an Expert Panel consisting of individuals qualified by scientific training and experience to evaluate the safety of infant formula and ingredients. Panel members were Berthold V. Koletzko, M.D., Kelly Anne Tappenden, Ph.D., and John A. Thomas, Ph.D.

The Panel critically reviewed and evaluated the generally available information regarding the safety of ingestion of CITREM by infants and the potential exposure of infants to CITREM resulting from its intended use, and individually and collectively concluded that no evidence exists in the available information on CITREM or other organic acid esters that demonstrates or suggests reasonable grounds to suspect a hazard to infants under the intended conditions of use of CITREM.

It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data and related information would reach the same conclusion. Therefore, addition of CITREM to exempt amino-acid based and hydrolyzed infant formulas under the conditions described is GRAS by scientific procedures.

1.5. Availability of Information

The data and information that serve as the basis for the GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of James T. Heimbach, Ph.D., President, JHeimbach LLC, 923 Water Street, P.O. Box 66, Port Royal, Virginia 22535, telephone 804-742-5548 and e-mail jh@jheimbach.com.

2. IDENTITY

2.1. Chemical Name

The name of the group of substances reviewed in this GRAS determination is citric acid esters of mono- and diglycerides of edible fatty acids. These substances are also known as citrated mono- and diglycerides, citric and fatty acid esters of glycerol, mono- and diglycerides of fatty acids esterified with citric acid, citroglycerides, and CITREM. In this document they are referred to by the last of these terms, CITREM.

Mono- and diglycerides of fatty acids can be esterified with a number of organic acids and such esters are included in the Codex Alimentarius General Standards for Food Additives (GSFA; FAO/WHO 2006) and are added to foods (including infant formula) in the European Union (EU), Australia and New Zealand, and Canada. The GSFA lists them under the general designation INS472 and the EU under E472. Specifically, CITREM is designated INS472c and E472c; the other organic acid esters are as follows:

- INS472a and E472a = acetic acid esters
- INS472b and E472b = lactic acid esters
- INS472d and E472d = tartaric acid esters
- INS472e and E472e = mono- and diacetyl/tartaric acid esters
- INS472f and E472f = mixed acetic and tartaric acid esters

As is discussed in more detail below, these various organic acid esters share similar structural, functional, biochemical, and toxicological characteristics and may all be considered together in assessing safety.

2.2. Trade or Common Name

Citric acid esters of mono- and diglycerides are widely known by the acronym CITREM. In the EU they are labeled with that name as well as the additive number E472c, and it is proposed that—for consistency with international usage—U.S. labeling use the same name, CITREM.

2.3. CAS Registry Number

No registry number has been assigned to CITREM by the Chemical Abstracts Services (CAS); however, CITREM has been given the reference number 977093-28-9 by FDA's Center for Food Safety and Applied Nutrition.

2.4. Molecular and Structural Formula

Since the mono- or diglycerides in CITREM may include either one or two fatty acids, since there is a variety of edible fatty acids with chain lengths ranging most commonly from 12 to18, and since citric acid may be esterified with one, two, or three glycerols, there is no single molecular or structural formula for CITREM. The generic structural formulas are shown in Figure 1.

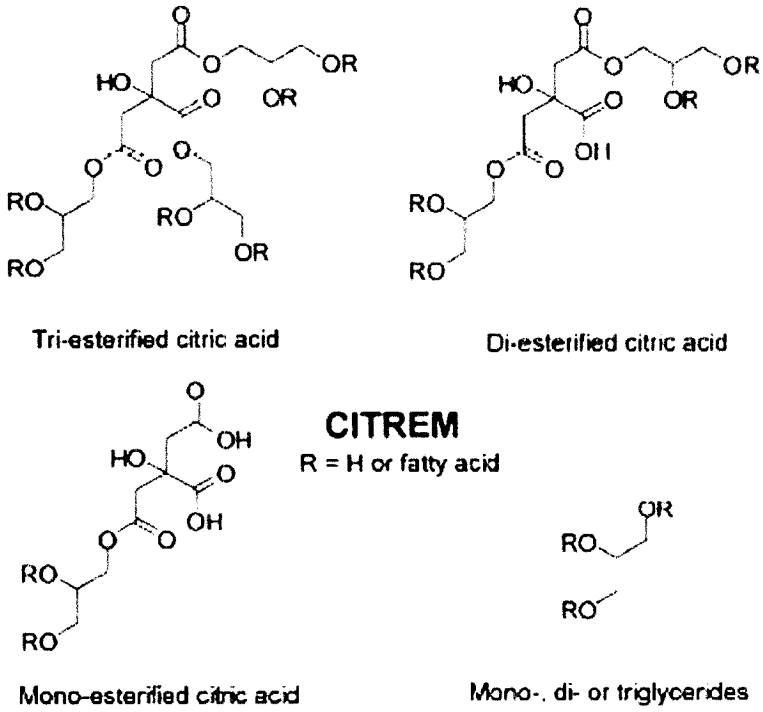

Figure 1. Generic Structural Formula for CITREM.

2.5. Production Process

CITREM is formed by esterifying the hydroxyl groups of mono- and diglycerides of fatty acids under current good manufacturing practice (cGMP). This may be accomplished either by the esterification of glycerol with both citric acid and edible fatty acids or by reaction of a mixture of mono- and diglycerides of edible fatty acids with citric acid. The fatty acid moiety may be any of a number of either saturated or unsaturated edible fatty acids as shown in 21 CFR §184.1505 with chain lengths most commonly of 12 to 18.

The manufacturing process is illustrated in the flow chart shown in Figure 2, which shows that food-grade vegetable oils (most commonly either sunflower oil or hydrogenated palm oil, depending on the desired consistency of the final product) are interesterified with food-grade glycerol and food-grade citric acid. The product is then filtered and optionally treated with activated carbon, quality-controlled, and packed.

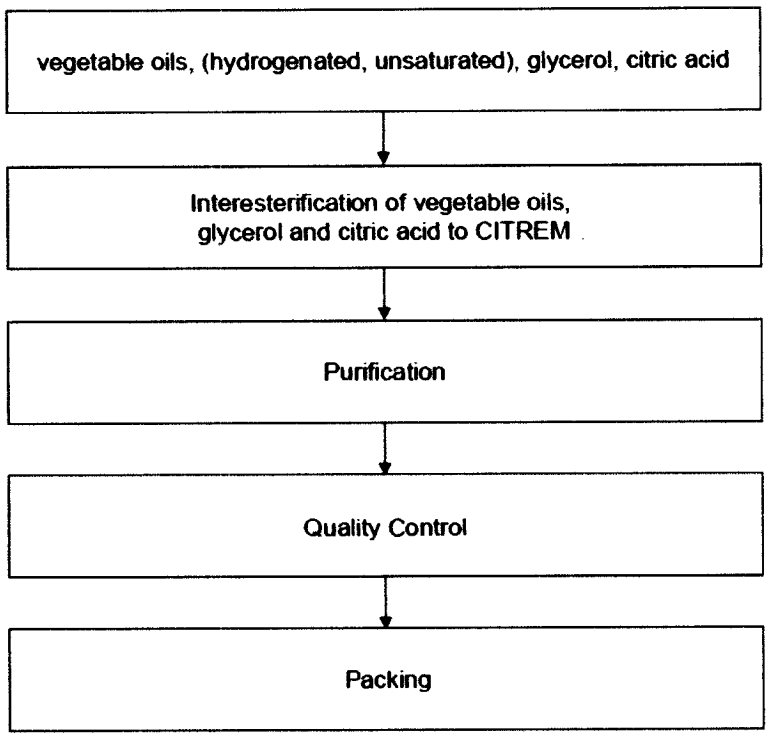

Figure 2. Manufacturing Flow Chart.

2.6. Appearance and Physical Characteristics

CITREM is white to ivory-colored, oily to waxy, of a consistency determined by the fatty acids. Those containing a preponderance of unsaturated fatty acids tend to be oily, while those containing a preponderance of saturated fatty acids are more solid. CITREM is typically dispersible in hot water, insoluble in cold water, and soluble in oils and fats.

2.7. Regulatory Status and Specifications of Source Materials

Both citric acid and mono- and diglycerides of fatty acids are affirmed as GRAS for direct addition to foods with no limitations other than cGMP. Citric acid is listed under 21 CFR §184.1033 while mono- and diglycerides are listed under §184.1505. Both the citric acid and the mono- and diglycerides that compose CITREM are food-grade compounds meeting the specifications described in the Seventh Edition of the *Food Chemicals Codex* (USP 2010).

Specifications for representative source materials—citric acid, glycerol, sunflower oil (representing a source of unsaturated fatty acids), and hydrogenated palm oil (representing a source of saturated fatty acids)—are shown in the following four tables (Table 1 through Table 4). These specifications, provided by suppliers, are intended only as representative of source materials—other seed and vegetable oils, or modified oils such as high-oleic-acid sunflower oil with up to 90% oleic acid—may be used.

Table 1. Specifications for Citric Acid.

Parameter	Specification
Appearance	Colorless crystals
Purity	NLT[1] 99.5%
Calcium	NMT[2] 50 mg/kg
Chloride	NMT 50 mg/kg
Heavy metals	NMT 5 mg/kg
Mercury	NMT 1 mg/kg
Lead	NMT 1 mg/kg
Arsenic	NMT 1 mg/kg
Iron	NMT 2 mg/kg
Aluminum	NMT 0.2 mg/kg
Sulfated ash	NMT 500 mg/kg
Oxalate	NMT 100 mg/kg
Water	NMT 0.15%
Bacterial endotoxins	NMT 0.5 IU/mg

1. NLT = not less than
2. NMT = not more than

Table 2. Specifications for Glycerol.

Parameter	Specification
Appearance	Clear, colorless viscous liquid
Odor	None
Purity	NLT[1] 98.0%
Density	NLT 1.2628
Chloride	NMT[2] 10 mg/kg
Heavy metals	NMT 5 mg/kg
Sulfated ash	NMT 100 mg/kg
Halogenes	NMT 35 mg/kg
Aldehydes	NMT 10 mg/kg
Water	NMT 2%

1. NLT = not less than
2. NMT = not more than

Table 3. Specifications for Sunflower Oil.

Parameter	Specification
Appearance	Clear, light yellow liquid
Density	~0.921
Refraction index	~1.474
Acid number (mg KOH/g)	NMT[1] 0.5
Peroxide value (meq O_2/kg)	NMT 10.0
Unsaponifiable matter	NMT 1.5%
Water	NMT 0.1%
Fatty acid composition	
Palmitic acid (16:0)	4.0 – 9.0%
Stearic acid (18:0)	1.0 – 7.0%
Oleic acid (18:1)	14.0 – 40.0%
Linoleic acid (18:2)	48.0 – 74.0%
1. NMT = not more than	

Table 4. Specifications for Hydrogenated Palm Oil.

Parameter	Specification
Iodine value (g I_2/100 g)	NMT[1] 2
Free fatty acids	NMT 0.8%
Trans-fatty acids	NMT 1.0%
Water	NMT 0.1%
Fatty acid composition	
Lauric acid (12:0)	0 – 1%
Aspartic acid (14:0)	1 – 2%
Palmitic acid (16:0)	54 – 62%
Stearic acid (18:0)	36 – 44%
1. NMT = not more than	

2.8. Food-Grade Specifications of CITREM

No *Food Chemicals Codex* (USP 2010) specifications exist for CITREM. However, specifications have been developed by both the Food and Agricultural Organization of the U.N. (FAO 1992) and the European Union (EU 2008; revised in EU 2012), as shown in Table 5. The two sets of specifications are not identical but there are no conflicts between them and it would generally be expected that food-grade CITREM would meet both sets of specifications. CITREM may contain small amounts of free fatty acids, free glycerol, free citric acid, and mono- and diglycerides.

Nestlé Nutrition has established specifications for CITREM consistent with the EU specifications with more stringent limits on acid value and heavy metals cadmium, arsenic, and mercury and the addition of new specifications for linoleic acid, iodine value, saponification value, alpha-tocopherol, ascorbyl palmitate, and microbiological purity (Table 5).

Table 5. Specifications for CITREM.

Parameter	FAO Specification[1]	EU Specification[2]	Nestlé Nutrition Specification
Total citric acid	13-50%	13-50%	13-50%
Total glycerol	8-33%	8-33%	8-33%
Free glycerol	NMT[3] 4%	NMT 2%	NMT 2%
Total fatty acids (as oleic acid)	37-81%	--[4]	37-81%
Linoleic acid	--	--	NMT 7.5%
Acid value	--	NMT 130	NLT[5] 20, NMT 40
Iodine value	--	--	~65
Saponification value	--	--	NLT 245, NMT 275
Sulfated ash	NMT 0.5%	NMT 0.5%	NMT 0.5%
Alpha-tocopherol (antioxidant)	--	--	NMT 200 mg/kg
Ascorbyl palmitate	--	--	NMT 200 mg/kg
Heavy metals			
Lead	NMT 2 mg/kg	NMT 2 mg/kg	NMT 1.5 mg/kg
Cadmium	--	--	NMT 0.1 mg/kg
Arsenic	--	--	NMT 0.2 mg/kg
Mercury	--	--	NMT 0.1 mg/kg
Microbiological purity			
Aerobic mesophiles	--	--	NMT 1,000 cfu[6]/g
Salmonella spp.	--	--	Not detected in 25 g
Enterobacteriaceae	--	--	NMT 10 cfu/g

1. Source: FAO 1992
2. Source: EU 2008, EU 2012
3. NMT = not more than.
4. No specification is set for this parameter by this authority.
5. NLT = not less than
6. cfu = colony-forming units

3. INTENDED TECHNICAL EFFECT

The intended technical effects of CITREM include those of antioxidant, emulsifier or emulsifying salt, stabilizer, and thickener as described in 21 CFR §170.3(o)(3), (8), and (28), respectively. CITREM is intended to be added to exempt amino-acid based and hydrolyzed infant formulas in order to provide adequate emulsification to maintain consistent nutrient delivery.

When reconstituted, formulas containing little intact protein are oil-in-water emulsions and satisfactory emulsification requires the use of substances such as CITREM with high hydrophilic-lipophilic balance values; other emulsifiers such as lecithin or mono- and diglycerides of fatty acids, with relatively low hydrophilic-lipophilic balance values, cannot provide adequate emulsification of these exempt amino-acid based and hydrolyzed infant formulas to maintain consistent nutrient dispersion and provide an organoleptically acceptable product.

4. INTENDED USE AND CONSUMER EXPOSURE

4.1. Intended Use of CITREM

CITREM is intended to be added to exempt amino-acid based and hydrolyzed infant formula at a maximum level of 233 mg CITREM/100 ml formula as consumed.

4.2. Estimated Daily Intake of CITREM

According to tables of daily energy intake by formula-fed infants provided by Fomon (1993), the subpopulation of infants with the highest intake per kg body weight is boys age 14–27 days. The mean energy intake by this group is 121.1 kcal/kg bw/day and the 90th percentile is 141.3 kcal/kg bw/day. Among girls, the highest energy intake is found in the same age group, 14–27 days, and is nearly as high as boys: the mean and 90th energy intake percentiles are 117.8 and 138.9 kcal/kg bw/day. Most term infant formulas contain 67.6 kcal/100 ml when ready to consume. Therefore, to obtain 141.3 kcal energy/kg bw, an infant boy must consume 209.0 ml formula/kg bw. To reach her 90th percentile of energy consumption, 138.9 kcal/kg bw/day, an infant girl must consume 205.5 ml formula/kg bw/day. The 90th percentile of formula intake for the two sexes combined is about 207 ml/kg bw/day.

Since CITREM is intended to be added to formula at a maximum level of 233 mg per 100 ml formula as consumed, the 90th percentile of daily intake of CITREM is 233 mg x 2.07 = 482 mg/kg bw/day.

5. REVIEW OF SAFETY DATA

5.1. Previous Expert Reviews

As discussed in the following paragraphs, CITREM has been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), by the EU's Scientific Committee on Foods (SCF), by the U.S. Food and Drug Administration (FDA), by Food Standards Australia New Zealand (FSANZ), and by Health Canada.

After reviewing evidence regarding the safety of CITREM and related compounds (i.e., glycerol esters of acetic, lactic, and tartaric acids), JECFA assigned all of these substances an ADI of "not limited," (FAO 1967; WHO 1974) a term later replaced by "not specified." JECFA set the "not limited" ADI for CITREM alone as well as for "the sum of total glycerol esters of fatty acids and acetic, citric, lactic, and tartaric acids," the latter provided that the intake of tartaric acid does not exceed 30 mg/kg bw/day.

According to JECFA, "ADI not specified" is:

> "A term applicable to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not, in the opinion of JECFA, represent a hazard to health. For that reason, and for reasons stated in individual evaluations, the establishment of an acceptable daily intake expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e., it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance" (WHO 1987).

JECFA concluded in its evaluation of CITREM that, "This substance is hydrolyzed completely in the intestinal tract into components which are normal constituents of the diet. Evaluation is based on knowledge of the metabolic fate and lack of toxicity of the constituent citric acid and fatty acid esters of glycerol" (FAO 1967). JECFA offered similar observations regarding the other organic acid esters of mono- and diglycerides and, as noted above, set a group ADI due to the similarity of the biochemical characteristics of the substances.

The SCF reviewed the biochemical and toxicological information regarding CITREM and the other esters and endorsed the JECFA conclusion of "ADI not specified" (SCF 1978). A decade later, the SCF again reviewed the safety of CITREM, this time for addition to weaning foods in biscuits, cereal-based, and baby foods, and determined that there were no safety concerns (SCF 1990). In 1997, the SCF further concluded that the use of CITREM was acceptable in products containing partially hydrolyzed proteins for infants and young children in good health and in foods for special medical purposes containing extensively hydrolyzed proteins or amino acids for infants and young children, at levels up to 750 mg/100 ml in ready-to-feed products made from dry powder and up to 900 mg/100 ml in ready-to-use ultra-high-temperature liquid formulas (SCF 1998). Finally, in 2002, the SCF saw no reason to object on safety grounds to the extension of the use of CITREM to all foods for special medical purposes for infants and young children (SCF 2002). EU Regulation No. 1129/2011 permitted the addition of CITREM at up to 750 mg/100 ml to infant formula sold in powder form and up to 900 mg/100 ml to infant formula containing partially hydrolyzed proteins, peptides, or amino acids sold in liquid form (EU 2011)..

Health Canada (2011) evaluated the safety of the intended use of CITREM in Canada; the evaluation "considered chemical, toxicological and nutritional aspects of the proposed uses of citric acid esters of mono- and diglycerides." Based on this review, Canadian food-additive regulations were amended to allow the use of CITREM at up to 0.155% in infant formula based on crystalline amino acids or protein hydrolysates as well as in unstandardized foods, with addition limited only by cGMP.

FDA received a GRAS notice for the use of CITREM (which in the notice was referred to as citroglycerides) as an emulsifier for the antimicrobial substance lauramide arginine ethyl ester and filed it on May 1, 2007, as GRAS Notice No. GRN 000222 (FDA 2007). The intended use of CITREM in this notice included addition to carbonated beverages at levels up to 563 mg/kg beverage and to other food categories, including meat and poultry products, at levels up to 1125 mg/kg food. In the GRAS notice, the estimated daily intake of CITREM (90th percentile users) was 28.2 mg/kg bw. In its response letter dated December 20, 2007, FDA stated that "the agency has no questions at this time regarding [the] conclusion that citroglycerides [CITREM] are GRAS under the intended conditions of use" (FDA 2007).

5.2. Biochemistry Data Regarding CITREM and Other Organic Acid Esters

Because CITREM and the other organic acid esters of glycerol share similar biochemical characteristics, it is appropriate to base conclusions regarding the biochemical behavior of CITREM on the totality of the research on these related substances.

5.2.1. CITREM

An earlier report from Huntingdon Research Centre (1966) reviewed and reported by JECFA (FAO 1967) found that *in vitro* hydrolysis of CITREM by pancreatic lipase and liver esterase produced the same yield of citric acid in 2 hours as spontaneous hydrolysis at pH 7.5-8.5. The same report presented a study in which groups of 20 male and 20 female rats were given a calorie-restricted diet for 10 days and fed 23.1% or 37.5% of either CITREM or the unesterified components, or lard at either 16.7% or 35.5%. These diets were calculated to be isocaloric. There were no differences in digestibility of the ester and its unesterified components based on fecal fat estimation and body fatty acid distributions.

In another earlier report by Rosner (1959) reviewed and reported by JECFA (FAO 1967), it was noted that in 2 groups of 5 male and 5 female weanling rats that were fed diets containing either 0% or 2% CITREM for 7 days there was no difference in feed intake or body-weight maintenance between the two groups. The digestibility of the ester was calculated to be 99%.

In an *in vitro* study, Amara et al. (2014) assessed the digestibility of CITREM under gastrointestinal conditions and its impact on fat digestion, confirming the findings of Huntingdon (1966) that fatty acids are released from CITREM by gastric lipase, pancreatic lipase, pancreatic-lipase-related protein 2, and carboxylester hydrolase. The lipolysis of CITREM was tested under *in vitro* conditions mimicking the physiological conditions found in the human GI tract, both in the stomach and the upper small intestine, in order to quantify the extent of CITREM hydrolysis.

Since CITREM is currently used in the composition of infant formula due to its ability to stabilize such complex food matrixes, both preterm and term infant/adult conditions were tested. Additionally, the lipolysis of a CITREM-containing infant formula was tested under the same *in vitro* conditions to assess the effects of CITREM on fat digestion. This part of the Amara et al. (2014) study was completed by testing the lipolysis of various fat emulsions containing the same amount of fat as infant formula, with and without CITREM.

Lipases tested included recombinant dog gastric lipase, an enzyme with properties similar to the human enzyme HGL in its activity on acylglycerols with various acyl chain lengths; recombinant human pancreatic lipase; pancreatic lipase-related protein 2; native porcine pancreatic lipase; and human pancreatic carboxyl ester hydrolase; as well as porcine pancreatic extract containing a number of enzymes.

CITREM was dispersed in an assay solution to which the selected lipase was added and the release of free fatty acids was measured. All the purified lipases tested as well as porcine pancreatic extract were found to be active on CITREM.

Prototype infant formulas were prepared containing 3.3% and 10% (w/w) fat and stabilized with CITREM or whey protein isolate and subjected to a two-step static *in vitro* digestion model including a gastric phase and a duodenal phase of lipolysis. For the term-infant model, the gastric pH was maintained at 5.5 and the duodenal pH at 6.25; for the preterm-infant model, both the gastric and duodenal pH were adjusted to 6.0. For the preterm-infant model, the concentrations of pancreatic lipase and bile salts were reduced to levels observed in duodenal aspirates of two preterm infants born at 35 weeks.

Overall, it was shown that fat digestion is not significantly changed by the presence of CITREM, and fatty acids contained in CITREM compounds are released to a large extent by lipases. Nevertheless, indigestible water-soluble compounds containing glycerol and citric acid units were identified, indicating that the ester bond between citric acid and glycerol is not fully hydrolyzed throughout the proposed digestion. The maximum hydrolysis level of CITREM in prototype infant formula reached 28.1% with term-infant conditions, whereas it was only 16.9% with preterm-infant conditions.

Amara et al. (2014) observed that the maximum hydrolysis levels obtained in these studies are consistent with data obtained in the course of 2 *in vivo* studies in which similar hydrolysis levels were recorded at the angle of Treitz with healthy volunteers fed solid-liquid test meal—27.7±6.8% (Carriere et al. 2001) and 28.1±13.5% (Carriere et al. 2005).

The two-step digestion model used by Amara et al. (2014) was therefore suitable for simulating lipid digestion in the upper part of the GI tract (stomach and duodenum), but was not well adapted for reproducing the entire digestion process of CITREM. Nevertheless, while further digestion of CITREM may occur in the jejunum and beyond, it appears likely that the complete hydrolysis of organic acid esters of mono- and diglycerides assumed by JECFA and others does not occur; rather, hydrolyzed components and surviving esters may reach an equilibrium in which small numbers of esters remain intact.

5.2.2. Acetic Acid Esters

Studies of acetoglycerides have found them to be resistant to changes in consistency, heat damage, and oxidative rancidity (Alfin-Slater et al. 1958; Ambrose and Robbins 1956b). In order

to compare the absorption of acetoglycerides of unsaturated fatty acids with those of saturated fatty acids, Ambrose and Robbins (1956b) prefasted groups of 10 mature male albino rats weighing 200-300 g for 48 hours, and then fed 1 of 2 acetooleins or 1 of 3 acetostearins as 20% of the diet for 4 hours. Control rats received either a fat-free diet or 20% partially hydrogenated vegetable shortening. Absorption of the acetooleins was greater than that of the acetostearins, a finding consistent with studies showing that unsaturated fatty acids are generally better absorbed than saturated fatty acids (Ambrose and Robbins 1956b). Overall absorption was 94% to 99%. Ambrose and Robbins (1956b) concluded that the various acetoglycerides are utilized by the rat "in much the same way as natural fats." Acetoglycerides cleared completely from the blood plasma within 30 minutes.

Coleman et al. (1963) gave groups of 10 male weanling rats diets containing 0% or 30% acetostearin for 20 weeks. Cholesterol levels in the plasma, liver, and adrenals were similar in the two groups.

5.2.3. Lactic Acid Esters

JECFA noted (FAO 1967) that glycerol lactopalmitate and lactostearate are frequently the predominant components of lactic acid esters of mono- and diglycerides, and that nearly all toxicity studies have used glycerol lactopalmitate (GLP).

An *in vitro* study of GLP hydrolysis in the presence of hog pancreatic lipase showed rapid production of glycerol, lactic acid, and palmitic acid as the sole reaction products (Treon et al. 1962).

When Treon et al. (1962) administered ^{14}C-labeled GLP orally to rats in different vehicles, the lactate moiety of GLP was metabolized as rapidly as and in a manner similar to lactic acid, which was largely absorbed, readily stored and oxidized by the liver, and randomly distributed throughout the body without high localization in any organ when equilibrium was attained. "This ester was easily hydrolyzed to compounds which are natural and accepted as safe, and since the lactate moiety of the ester was metabolized like free lactic acid, this glyceryl lactate palmitate is believed safe..." (Treon et al. 1962).

McKennis et al. (1958) gavaged dogs with GLP and reported the appearance of lactic acid in the thoracic duct lymph and blood, showing complete digestion of GLP. About 50% of the administered dose appeared as expired $^{14}CO_2$ over 48 hours. Additionally, no deleterious effect was observed on the *in vitro* hydrolysis of olive oil when GLP was added.

5.2.4. Mixed Acetic and Tartaric Acid Esters

In an aqueous medium the substance was spontaneously hydrolyzed into free tartaric acid and glycerol esters of acetic and fatty acids (Kieckebusch et al. 1968).

5.3. Toxicity Data Regarding CITREM and Other Organic Acid Esters

Based on the structural similarity of the various organic acid esters of glycerol, as well as their biochemical characteristics, it is appropriate to consider the totality of the toxicological information regarding all of these substances in evaluating the toxicity of CITREM.

5.3.1. CITREM

In the Huntingdon (1966) study cited earlier, in which 20 male and 20 female rats were fed diets with 23.1% or 37.5% CITREM for 10 days, gross and microscopic examination of major organs revealed some dystrophic lower-nephron calcification in animals receiving the highest level (37.5% dietary concentration, equivalent to 18,750 mg/kg bw/day) of either CITREM or the unesterified components (FAO 1967). No effect was seen at the 23.1% level (equivalent to 11,500 mg/kg bw/day), and the effect at the higher level was attributed to nutritional imbalance since no difference was seen between the esterified compounds and their unesterified components (FAO 1967).

The Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS) cited an acute oral toxicity study, reportedly performed in compliance with OECD Guideline 401, in which a single gavage administration of a limit dose of 2000 mg/kg bw of a mixture of citric and lactic acid esters of mono- and diglycerides of fatty acids to male and female rats produced no adverse effects (NICNAS 2001).

NICNAS (2001) also cited a reportedly OECD Guideline 471-compliant bacterial reverse mutation assay in which *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 were exposed to a mixture of citric and lactic acid esters of mono- and diglycerides of fatty acids at levels ranging from 50 to 5000 µg/plate with no significant increase in revertant colonies either with or without metabolic activation. This result indicates an absence of mutagenicity under the conditions of the test.

5.3.2. Acetic Acid Esters

Ambrose and Robbins (1956a) gavaged 20 mature rats, 10 males and 10 females, with a single dose of 4000 mg/kg bw of a mixture of acetostearin and acetoolein; no adverse effects were observed during the first 24 hours post-dosing or during the next 4 weeks.

Ambrose and Robbins (1956a) administered 80-100 mg (100 mg/kg bw) acetostearin intravenously to rabbits daily for 15 days. No adverse effects were reported and the viscera showed no pathological changes. A slight transitory effect was noted on clotting time. The acetostearin cleared from the plasma in 15-30 minutes.

Mattson et al. (1956) fed groups of 10 male weanling rats diets containing 25% of either stearin, olein, diacetostearin, or diacetoolein; additional groups received 50% olein or diacetoolein for 8 weeks or 15% acetoolein for 12 weeks. There were no differences between experimental and control groups in feed consumption or body weight gain. Feed utilization efficiencies were similar except that they were slightly higher in the group receiving diacetostearin. Blood chemistries and urinalyses were normal and similar across all groups.

Ambrose and Robbins (1956a) fed acetostearin to weanling female albino rats (5 per group) at 0%, 5%, 10%, or 20% of the diet for 14 weeks. Feed and water were available *ad libitum*. Feed consumption and body weights were recorded weekly and necropsies were performed at the end of the dosing period. The dietary intake of acetostearin among the rats on the 20% diet varied from approximately 23,000 mg/kg bw/day during the first week to approximately 10,000 mg/kg bw/day at 14 weeks. There were no significant differences in growth among the groups and no gross pathological effects were found at necropsy.

Ambrose and Robbins (1956a) fed diets containing 0%, 0.25%, 0.5%, 1.0%, 2.0%, or 4.0% acetostearin to groups of 5 weanling male albino rats for 57 weeks, while other groups of 5 weanling male albino rats received 0%, 0.25%, 0.5%, or 1.0% acetoolein for 57 weeks. Feed and water were available *ad libitum*. Feed intake and body weight were recorded weekly and blood was taken for hematological analysis at 4-month intervals. At study termination the liver, kidneys, testes, spleen, heart, and adrenals were removed and weighed; histopathological studies were performed on these organs as well as the thyroid, intestine, stomach, pancreas, and bladder.

Feed intake, body weight gain, and mortality did not differ between experimental groups and controls (Ambrose and Robbins 1956a). No toxic effects were seen in the hematology studies. Except for the testes there were no differences in absolute or relative organ weights, but decreased testicular weights were observed in the groups receiving acetoolein and the groups receiving 0.25% or 0.5% acetostearin. Again except for the testes, no gross or microscopic pathological differences were noted between the tissues of the experimental groups and the controls. Testicular hypoplasia and suppression of spermatogenesis of varying degrees were observed in all rats in both the experimental and control groups, with a tendency toward higher frequency in the test groups but without a clear dose-response relationship. These effects were small and within the normal range, and Ambrose and Robbins (1956a) attributed them to an insufficiency in vitamin E in the diet in the presence of stress rather than to the acetoglycerides. This attribution was based on the occurrence of similar findings in studies with "materials totally unrelated to the acetoglycerides" such as feather-meal protein and allethrin and supported by preliminary observations from Ambrose et al. (1958b), discussed below.

Ambrose et al. (1958a) fed weanling albino Slonaker rats weighing 41 to 52 g a basal diet and then randomized them into 16 groups, each including 10 males and 10 females. Each group received one of 3 acetostearins or one of 2 acetooleins at 0%, 5%, 10%, or 20% of the diet. Parental generation animals receiving 20% acetoglyceride were sacrificed after 57 weeks (along with 15 of the control animals); those receiving 5% or 10% of one of the acetostearins were sacrificed at 86 weeks; and those receiving 5% or 10% of the remaining acetostearins or the acetooleins were sacrificed at 101 weeks (along with the remaining control animals). All organs were examined for gross pathology; the liver, kidney, spleen, heart, and testes were weighed and subjected to microscopic histopathological study. The bladder, stomach, intestines, pancreas, adrenals, thyroid, brain, hypophysis, parathyroid, ovaries, and uterus were also studied for both gross and microscopic histopathology.

There were no differences in mortality up to 57 weeks (Ambrose et al. 1958a). The researchers reported that the experimental groups showed some increased mortality over controls after 86 weeks, but this difference was not statistically significant (controls 44.5% mortality, experimental groups 48.9% mortality, NS). There were no significant differences in feed consumption or in body weight gain. Reduced testicular weights were seen at the 20% level of all 3 acetostearins but not at the 5% or 10% level or with the acetooleins. Effects on liver weights at the 20% level were inconsistent—2 groups of males and 1 of females exhibited significantly enlarged livers, but 2 groups of females had significantly reduced liver sizes. No differences were seen at 5% or 10%. Liver glycogen was unaffected at any dose level. At the 20% level of acetostearins, fatty tissue changes resembling sclerema adiposum neonatorum were seen; these effects were not seen at lower doses. A variety of pathological changes were seen with individual acetoglycerides at varying dose levels, but these effects were not consistent or dose-dependent. Ambrose et al. (1958a) attributed the observed pathologies seen at 20% levels of acetostearins to

an imbalance of the test diet with respect to vitamin E and essential fatty acids; these attributions were based on discoloration of uteri, suggesting an increased vitamin E requirement, and crystalline deposits of "a high melting point fat."

Ambrose et al. (1958b) administered acetostearin at 0% or 10% of the diet to groups of 5 male and 10 female rats with or without supplementary vitamin E for 57 weeks. The group receiving acetostearin and vitamin E showed improved reproductive performance (increased litter numbers and pups per litter in 4 matings) compared with controls or animals receiving acetostearin alone.

JECFA regarded the findings of the toxicity studies as of little relevance due to the dietary perturbations and based its safety determination and setting the ADI for acetic acid esters of mono- and diglycerides as "no limit" on the biochemical and metabolic studies showing that the breakdown products of acetoglycerides are normal dietary constituents (FAO 1967).

5.3.3. Lactic Acid Esters

Two groups of 6 male rats were given a single dose by gavage of 5.75 or 8.65 g/kg bw of glycerol lactopalmitate in water. All animals survived without systemic effects; gross appearance of major organs was found to be normal after 14 days (Gongwer 1959, reviewed and reported by JECFA [FAO 1967]).

JECFA (FAO 1967) reported that a number of experiments using small numbers of rats for both short-term (Kaunitz 1958) and long-term (Fye and Katz 1953) studies revealed no toxic effects, but did not report the specific findings of these studies. JECFA again based its safety evaluation on biochemical and metabolic studies, which demonstrated that this substance is completely hydrolyzed in the GI tract to lactic acid, glycerol, and fatty acids, and that the metabolism of bound lactic acid is not different than that of free lactic acid.

5.3.4. Tartaric Acid Esters

Kieckebusch et al. (1968) found an acute oral LD_{50} in the mouse of 20,000 mg/kg bw of a product containing 16% tartaric-acid ester, equivalent to 3200 mg/kg bw of the ester itself.

Mosinger (1965, reviewed and reported by JECFA [FAO 1967]) fed 15 male and 15 female Wistar rats the tartaric-acid ester at a level of 0 or 5 g/kg bw/day in the diet for 24 months. JECFA reported that no effects were seen in feed consumption, body weight, external appearance, or mortality, and no test-material related differences were seen in the histological examination (FAO 1967). There was no evidence of carcinogenicity, and no effects were seen on reproduction or progeny over 2 generations.

Kieckebusch et al. (1968) fed 20 male and 20 female rats a product containing 16% tartaric-acid ester at levels of 0, 100, or 400 mg/kg bw/day for 28 months. These doses were equivalent to 0, 6, or 25 mg/kg bw/day of the tartaric-acid glycerol ester. No differences were found in feed consumption, feed utilization efficiency, body weight, external appearance, or mortality rate. No effects were evident in the histological examination and no reproductive effects were seen in 2 matings.

5.4. Pediatric Trials with CITREM-Containing Infant Formula

A number of clinical trials have been performed in which infants consumed formula containing CITREM. None of these studies had assessment of CITREM as a primary objective; rather, the goal was to evaluate the safety and nutritional value of amino-acid based or hydrolyzed infant formula

Vandenplas et al. (1993) enrolled 45 healthy neonates in a prospective, randomized, double-blind study comparing the nutritional value of a whey-predominant formula (n = 20) and a whey-hydrolysate formula (n = 25); the hydrolysate formula included 95 mg CITREM/100 ml formula as an emulsifier. The infants remained on their assigned formulas for 13 weeks and showed identical weight and length gain in the two groups. Blood sampled at 13 weeks and analyzed for hemoglobin, hematocrit, erythrocyte count, leukocyte count, lymphocytes, glycemia, protein, albumin, prealbumin, calcium, phosphorus, creatinine, iron, vitamins A and E, urea, zinc, and iron-binding capacity showed no significant differences between the two formula groups except for the last three measures, which were significantly higher in the hydrolysate group. The authors concluded that exclusive feeding of whey-hydrolysate formula to healthy infants from birth to 3 months of age results in adequate nutritional status. No adverse effects were reported due to the hydrolysate formula containing CITREM.

Forty-five 6-month-old infants (age range 5-7 months) with confirmed cow milk allergy were enrolled in a prospective, randomized study of the nutritional adequacy and growth-promoting efficacy of amino-acid based or protein hydrolysate formulas (Isolauri et al. 1995). Twenty-two infants received an hydrolyzed whey formula for 9 months, while 23 infants received an amino-acid based formula containing 126 mg CITREM/100 ml formula for the same period of time. Both formulas were clinically and biochemically tolerated. The mean concentration of essential amino acids in plasma among infants receiving the amino-acid based formula was significantly higher than values for breast-fed control infants, and growth was significantly greater among these infants than among those receiving hydrolyzed formula. No adverse effects were reported due to the amino-acid based formula containing CITREM. The authors concluded that "Extensively hydrolyzed formulas are safe and effective for most infants; an amino acid-derived formula may be preferable for infants with multiple food allergies."

In a prospective, randomized study (Mabin et al. 1995a), 85 children (median age 2.3 years, range 0.3 to 13.3 years) with refractory atopic dermatitis were randomly allocated to receive a few foods diet (eliminating all but five to eight foods) supplemented with either a whey hydrolysate (n = 27) or a casein hydrolysate formula (n = 32), or to remain on their usual diet and act as controls (n = 26), for a six week period. The whey hydrolysate formula contained CITREM at a level of 95 mg CITREM/100 ml formula. Although 27 children were withdrawn from the two hydrolysate groups prior to the first review appointment, most were due to noncompliance with the restricted foods diet. No patients were withdrawn because of intolerance to either hydrolysate formula, and there were no reported adverse effects attributable to the hydrolysates or to CITREM.

In another prospective, randomized trial of the few foods diet, Mabin et al. (1995b) assigned 24 children with atopic dermatitis (median age 1.8 years, range 0.6 to 8.9 years) to receive a few-foods diet supplemented with casein hydrolysate and 21 children (mediate age 2.8 years, range 0.6 to 11.5 years) to receive the same diet supplemented with whey hydrolysate containing 95 mg CITREM/100 ml formula. The children remained on their assigned diets for 6

days, during which their nutrient intake was measured. No adverse effects were reported, and the authors concluded that "Whey hydrolysate appears to be more palatable than casein hydrolysate."

Verwimp et al. (1995) assigned 79 infants ranging in age from 2 to 17 weeks, with diagnosed cow milk allergy, to receive one of 2 whey-hydrolysate formulas, one of which contained 95 mg CITREM/100 ml, for 10 weeks or longer, during which growth and allergy symptomatology were monitored. Infants in both groups showed normal growth and significantly reduced symptomatology, and no difference was seen in safety or efficacy between the 2 formulas.

Twenty-two infants (mean age 4.6 months) allergic to cow milk, who had been unable to tolerate hydrolyzed protein formula, were given an amino-acid based formula (containing 138 mg CITREM/100 ml) for 11.8±8.7 months (de Boissieu and Dupont 2000). The intervention was successful in helping the infants resume normal growth and there were no reported adverse effects.

In an open-label multicenter study, Giampietro et al. (2001) gave 32 children (median age 37 months) with proven cow milk allergy a hydrolyzed whey formula (with 95 mg CITREM/100 ml formula) for 7 days to compare their tolerance for this formula with that for 2 other hydrolysate products. Although 3% of the children proved unable to tolerate the formula (v. 6% and 36% for the other two formulas), there were no reported adverse effects and the authors concluded that the product "can be considered safe."

Niggemann et al. (2001) enrolled 73 infants (median age 5.7 months, range 1.6 to 9 months), with proven allergy to cow milk, in a 6-month prospective, randomized, multicenter trial to compare the effect of an amino-acid based formula containing 138 mg CITREM/100 ml (n = 42) with that of a hydrolyzed whey formula (n = 31). Dietary intake, growth (length and weight), and clinical symptoms of atopic dermatitis were monitored. No adverse effects were reported, and the authors concluded, "both an [amino-acid based formula] and [hydrolyzed formula] resulted in a significant clinical improvement in infants with an early onset of symptoms of cow's milk allergy/intolerance. Feeding an [amino-acid based formula] resulted in improved growth compared to feeding [hydrolyzed formula], despite similar dietary intakes, and may therefore be considered as a beneficial alternative in children with severe cow's milk allergy intolerance."

Fifty-two infants with a mean age of 5.3 months, with proven allergy to cow milk and who had been unable to tolerate hydrolyzed protein formula, were given an amino-acid based formula (containing 138 mg CITREM/100 ml) for an average of 11.4 months by de Boissieu and Dupont (2002). The study group included the cohort from de Boissieu and Dupont (2000) and 30 additional infants. Again, no adverse effects were reported and the authors concluded that "The amino acid-based formula proved to be safe, with infants exhibiting an overall gain in length and weight."

Milla et al. (2004) compared 2 amino-acid based infant formulas, both containing CITREM but differing in their content of long-chain n-3 polyunsaturated fatty acids, for use in infants with enteropathy and inflammation affecting the small intestine. Twenty infants aged 1 month to 2 years were randomly assigned to receive one of the 2 formulas for 6 weeks. Growth, stool output, and intestinal inflammation were monitored along with serum biochemistry, plasma

amino acids, and erythrocyte lipid profiles. The formulas did not differ significantly in alleviating intestinal inflammation, but both were well tolerated and no adverse effects were reported.

In a prospective, randomized, double-blind, placebo-controlled trial of the effects of probiotics on atopic dermatitis in infants, Gore et al. (2005) enrolled 137 infants aged 3-6 months with proven atopic dermatitis to receive a hydrolyzed formula (containing CITREM) with or without added probiotics for 3 months. While the probiotics failed to provide significant clinical benefit, no adverse effects were reported attributable to the consumption of the CITREM-containing formula.

In a prospective randomized trial, Evans et al. (2006) compared stepwise introduction of a high-energy infant formula (containing 162 mg CITREM/100 ml formula) to infants with faltering growth with administration of this same formula at full strength from day 1. Thirty infants aged 2-43 weeks and weighing 2.5-8 kg were randomly allocated to immediate v. step-wise introduction and maintained on formula for 2 weeks, during which feed intake, defecation habits, and vomiting were monitored. The formula was generally well tolerated with no adverse effects reported.

Clarke et al. (2007) compared the effectiveness of giving infants with faltering growth either an energy-supplemented formula or a nutrient-dense formula; the latter formula contained 162 mg CITREM/100 ml formula. In a randomized open-label study, 49 infants (median age 5 weeks, range 2-31 weeks) with faltering growth were assigned to receive the energy-supplemented formula (n = 23) or the nutrient-dense formula (n = 26) for 6 weeks. Both formulas were well tolerated, and no adverse effects were observed on blood biochemistry (albumin, CO_2, potassium, and urea) or urinary electrolytes, but the infants receiving nutrient-dense formula obtained significantly more protein, vitamins, and minerals.

In an open-label study, Vandenplas et al. (2010) enrolled 47 children aged 23±20 weeks hospitalized with functional gastrointestinal disorders to receive a hydrolyzed whey formula (containing CITREM as an emulsifier) for 4 weeks in order to evaluate its nutritional adequacy. Weight, length, and blood parameters were measured at enrollment and termination, and parents maintained records of formula intake, gastrointestinal symptoms, and stool characteristics. Twenty-two children withdrew from the study and there were 54 adverse events reported; the authors reported that "None of the [adverse events] was assessed to be related to the study formula." Based on the growth achieved, the authors concluded that the test formula "is safe and nutritionally adequate for pediatric patients with gastrointestinal disease."

Vandenplas et al. (2011) compared 2 hydrolyzed infant formulas (containing CITREM) for the management of children with cow milk intolerance in a prospective, randomized, multi-center study with 86 infants aged 0 to 6 months. A total of 75 patients were included in the Intent-to-Treat dataset, 35 and 40 receiving each formula for up to 8 months. Normal growth was observed in both groups with no significant differences in any of the growth measurements. The authors reported that "There were no adverse events related to the study formula in either of the groups" and concluded that "Both formulas were safe and well tolerated."

Harvey et al. (2013) carried out 2 studies to evaluate the effects of an amino-acid based formula with synbiotics on growth and tolerance in healthy infants and its hypoallergenicity in infants with IgE-mediated cow milk allergy. The formula contained 136 mg CITREM/100 ml. In

the first study, 115 healthy term infants aged 10.5±4.2 months (range 3-16 months) were assigned to receive either the test formula (n = 59) or a commercially available amino-acid based formula as a control (n = 55) for 16 weeks; measures of growth (weight, length, head circumference), stool characteristics, gastrointestinal symptoms, dietary intake, blood chemistries, and adverse events were recorded. Thirty-two infants in the test group and 38 infants in the control group completed the study; reasons for withdrawal did not differ between the groups. There were no significant differences in growth, feed intake, blood chemistries, or gastrointestinal symptoms, but infants receiving the synbiotic formula had significantly softer stools. The frequency of adverse events was significantly lower in the synbiotic-formula group that in the control group; most adverse events were regarded as unrelated to the formulas, while those that were considered possibly related—primarily gastrointestinal disorders such as constipation or flatulence—were more frequent among control infants than those receiving the synbiotic formula. The authors concluded that the data "demonstrate that an [amino-acid based formula] with synbiotics is safe and well tolerated and promotes normal growth when fed to healthy full-term infants as the sole source of nutrition."

In the second study (Harvey et al. 2013), 30 infants and children aged 17.3±10.8 months (range 3-47 months) with proven IgE-mediated cow milk allergy were given the synbiotic amino-acid based formula for 7 days. No formula-related adverse effects were observed.

In summary, CITREM has been a component of amino-acid based and hydrolyzed formulas given to both healthy infants and those suffering from cow milk allergy or atopic dermatitis in numerous clinical trials. Although none of these trials included the safety of CITREM as either a primary or secondary objective, most of them did include safety and tolerability of the infant formula as objectives. Clearly, demonstration that infant formula containing CITREM is safe is tantamount to demonstration that CITREM as used in these formulas is safe. Thus these studies provide evidence of the safety of the intended use of CITREM as an emulsifier in amino-acid based and hydrolyzed infant formula.

6. SAFETY ASSESSMENT AND GRAS DETERMINATION

6.1. Introduction

CITREM is safe and is GRAS for direct addition to exempt amino-acid based and hydrolyzed infant formula at up to 2330 mg/100 ml formula as consumed as an emulsifier to maintain nutrient availability.

This safety assessment and GRAS determination included two steps. In the first step, the safety of CITREM under its intended conditions of use was demonstrated by showing that the EDI of CITREM under its intended conditions of use is well within safe levels established by consideration of the biochemical and toxicity studies of CITREM and similar organic acid esters of mono- and diglycerides. In the second step, CITREM was determined to be GRAS by demonstrating that the safety of CITREM under its intended conditions of use is based on publicly available information and is generally recognized among experts qualified by scientific education and experience to evaluate the safety of substances added to food.

The regulatory framework for establishing whether a substance is GRAS, in accordance with Section 201(s) of the FDCA, is set forth under 21 CFR §170.30, which states that general recognition of safety based on scientific procedures may be based on the view of qualified experts as described in §170.30(b).

A GRAS determination based on scientific procedures requires the same quantity and quality of scientific evidence as is needed to obtain approval of the substance as a food additive; this is referred to as the "technical element." In addition, a GRAS determination requires that this scientific evidence of safety be generally known and accepted among qualified scientific experts. This "common knowledge" element of a GRAS determination has two components:

1. The data and information relied upon to establish safety must be generally available, although it may be supported by unpublished data; and
2. There must be agreement among qualified experts regarding the safety of the substance for its intended use.

6.2. Safety Assessment of CITREM for Its Intended Use

There are few data available on the toxicity of CITREM itself, but there have been numerous published and unpublished toxicity studies of other closely related organic acid esters, especially acetic acid esters, which have been administered to experimental animals for 57 weeks in 2 studies and for 101 weeks in one additional study. Further, there is sufficient information available from published and unpublished studies of the biochemical characteristics of CITREM and other organic acid esters to clearly demonstrate that these substances hydrolyze rapidly in the GI tract. It is notable that JECFA, despite the availability of relatively abundant toxicity data, particularly on the acetoglycerides, chose to base its evaluation of the safety of these substances on the biochemical data. Further, the chemical structural similarity of CITREM and the other organic acid esters, along with the similarity of their biochemical and metabolic behavior, indicates a high likelihood that their toxicological characteristics are also similar, a likelihood confirmed by the limited toxicity data that are available.

The recent *in vitro* research by Amara et al. (2014) indicates a likelihood that the earlier assumption of complete hydrolysis of organic acid esters of mono- and diglycerides may have

been in error, and some esters may remain intact at least through the duodenum. This possibility does not affect the overall biochemical conclusion reached by JECFA and others that organic acid esters of mono- and diglycerides are metabolized and utilized in a manner similar to that of triglycerides.

As noted earlier, both JECFA (FAO 1967) and SCF (SCF 1978) determined that the ADI for CITREM and the other organic acid esters of mono- and diglycerides are "not specified," indicating substances that present no hazard to health from expected consumption.

Numerous clinical trials of amino-acid based and hydrolyzed infant formulas containing CITREM to provide adequate emulsification and acceptable organoleptic characteristics, in both healthy and allergic infants and children, have consistently found that such infant formulas are safe and well tolerated.

CITREM and the other organic acid esters have been available and widely used worldwide for at least thirty years with no indication of adverse effects. During much of this time, they have been permitted for addition to infant and follow-on formulas and weaning foods intended for consumption by both healthy individuals and those requiring foods for special medical purposes, again with no reported adverse effects.

6.3. General Recognition of the Safety of CITREM

The JECFA and SCF evaluations of organic acid esters of mono- and diglycerides (FAO 1967; SCF 1978) were based primarily on published studies corroborated by unpublished reports. Further, the JECFA and SCF evaluations themselves have been generally available for four decades and three decades, respectively, and fulfill the GRAS requirement regarding the general availability of the information providing the basis for determining safety. Additionally, the safety assessment by Health Canada was published in 2011. The clinical trials of CITREM-containing infant formulas have all been published.

The intended use of CITREM has been determined to be safe through scientific procedures as set forth in 21 CFR §170.30(b). Because this safety evaluation was based on generally available information, it satisfies the common knowledge element of a GRAS determination. Determination of the safety and GRAS status of CITREM for addition to exempt amino-acid based and hydrolyzed infant formulas has been made through the deliberations of an Expert Panel comprising Berthold V. Koletzko, M.D., Kelly Anne Tappenden, Ph.D., and John A. Thomas, Ph.D.

6.4 Conclusion of the Expert Panel

We, the undersigned independent Expert Panel members, are qualified by scientific education and experience to evaluate the safety of substances intended to be added to infant formula. We have individually and collectively critically evaluated the information summarized above and other information deemed appropriate, and unanimously conclude that the intended use of citric acid esters of mono- and diglycerides of edible fatty acids (CITREM), produced in accordance with current Good Manufacturing Practice and meeting appropriate human food-grade specifications, to be added as emulsifiers to exempt amino-acid based and hydrolyzed infant formulas at a level not exceeding 233 mg/100 ml formula as reconstituted, is safe.

We further conclude that the intended use of citric acid esters of mono- and diglycerides of edible fatty acids (CITREM), produced in accordance with current Good Manufacturing Practice and meeting appropriate human food-grade specifications, to be added as emulsifiers to exempt amino-acid based and hydrolyzed infant formulas at a level not exceeding 233 mg/100 ml formula as reconstituted, is Generally Recognized as Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

(b) (6)

Signature: _____ Date: 3 April 2014
Berthold V. Koletzko, Dr med Dr med habil (M.D. Ph.D.)
Professor of Pediatrics
University of Munich
Munich, Germany

Signature: _____ Date: _____
Kelly Anne Tappenden, Ph.D., R.D.
Professor of Nutrition and GI Physiology
University of Illinois
Champaign, Illinois

Signature: _____ Date: _____
John A. Thomas, Ph.D.
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Indiana University School of Medicine
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Kelly Anne Tappenden, Ph.D., R.D.
Professor of Nutrition and GI Physiology
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