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ORIGINAL SUBMISSION

JHeimbach LLC

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April 20, 2007

Robert L. Martin, Ph.D.
Deputy Director
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Premarket Approval
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Bob:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), Laboratorios Miret, S.A. (LAMIRSA), through me as its agent, herby provides notice of a claim that the food ingredient described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be generally recognized as safe (GRAS), based on scientific procedures, for addition to foods as described.

As required, three copies of the notification are provided. Since the foods to which the substance may be added include meat and poultry products regulated by the U.S. Department of Agriculture, a fourth copy is also provide for their use.

If you have any questions regarding this notification, please feel free to contact me at 202-237-8406 or jh@jheimbach.com. (After May 29, my telephone number will change to 804-742-5548 and my address to 923 Water Street, P.O. Box 66, Port Royal Virginia 22535. My e-mail address will not change.)

Sincerely,

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

Encl.

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1. GRAS EXEMPTION CLAIM

Laboratorios Miret, S.A. (LAMIRSA), through its agent JHEIMBACH LLC, hereby notifies the Food and Drug Administration (FDA) that the use of citric acid esters of mono- and diglycerides of edible fatty acids (citroglycerides) described below is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act (FDCA) because LAMIRSA has determined that such use is generally recognized as safe (GRAS) through scientific procedures.

James T. Heimbach, Ph.D., F.A.C.N.
President, JHEIMBACH LLC
U.S. Agent for LAMIRSA, VEDEQSA (Grupo LAMIRSA)

1.1. Name and Address of Notifier

Laboratorios Miret, S.A. (LAMIRSA)
Polig. Industrial Can Parellada
Géminis, 4
08228 Terrassa
Barcelona
Spain

Contact:
Telephone:
Facsimile:
E-mail:

1.2. Name of GRAS Substance

The substance (actually group of substances) that is the subject of this Generally Recognized as Safe (GRAS) determination is citric acid esters of mono- and diglycerides of edible fatty acids, referred to as citroglycerides (an alternate chemical name) in this document.

1.3. Basis for GRAS Determination

LAMIRSA's GRAS determination for the intended use of citroglycerides is based on scientific procedures as described under 21 CFR §170.30(b).

1.4. 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
Box 66, Port R
(b)

2. IDENTITY

2.1. Chemical Name

The name of the substance (actually group of substances) is citric acid esters of mono- and diglycerides of edible fatty acids. They 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, and citroglycerides. In this document they are referred to by the last of these terms, citroglycerides.

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 in the European Union (EU). The GSFA lists them under the general designation INS472 and the EU under E472. Specifically, citroglycerides are 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 will be 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

Citroglycerides have the common trade name of CITREM. In the EU they are labeled with that name as well as the additive number E472c.

2.3. CAS Registry Number

No registry number has been assigned to citroglycerides by the Chemical Abstracts Services (CAS); however, citroglycerides have 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 citroglycerides may include either one or two fatty acids, and since there is a variety of edible fatty acids with chain lengths ranging most commonly from 12 to18, there is no single molecular or structural formula for citroglycerides. The generic structural formula is shown below:

$$
\begin{array}{l}
CH_2\text{--}OR \\
| \\
CH\text{--}OR' \\
| \\
CH_2\text{--}OR''
\end{array}
$$

Where at least one of R, R′ or R′′ represents the citric acid moiety and at least one represents a fatty-acid moiety; the remainder may represent citric acid, a fatty acid, or hydrogen.

2.5. Production Process

Citroglycerides are formed by esterifying the hydroxyl groups of mono- and diglycerides of fatty acids under good manufacturing practices (GMP). 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.

2.6. Appearance and Physical Characteristics

Citroglycerides are 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. Citroglycerides are 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 current GMP. 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 citroglycerides are food-grade compounds meeting the specifications described in the Fifth Edition of the *Food Chemicals Codex* (IOM 2003).

2.8. Food-Grade Specifications of citroglycerides

No *Food Chemicals Codex* (IOM 1993) specifications exist for citroglycerides. However, specifications have been developed by both the Food and Agricultural Organization of the U.N. (FAO 1992) and the European Union (EU 1998), as shown below in Table 1. The two sets of specifications are not identical (for example, the FAO includes a specification for total fatty acids but not free fatty acids while the EU specifies free fatty acids but not total), but there are no conflicts between them and it would be expected that food-grade citroglycerides would meet both sets of specifications. Some citroglyceride products may contain small amounts of free fatty acids, free glycerol, free citric acid, and mono- and diglycerides.

Table 1. Specifications for Citroglycerides

Parameter	FAO Specification	EU Specification
Total citric acid	13-50%	13-50%
Total glycerol	8-33%	8-33%
Free glycerol	NMT[1] 4%	NMT 2%
Total fatty acids (as oleic acid)	37-81%	--[2]
Free fatty acids (as oleic acid)	--	NMT 3%
Sulfated ash	NMT 0 5%	NMT 0 5%
Arsenic	--	NMT 3 mg/kg
Heavy metals as Pb	--	NMT 10 mg/kg
Lead	NMT 2 mg/kg	NMT 5 mg/kg
Mercury	--	NMT 1 mg/kg
Cadmium	--	NMT 1 mg/kg

1 NMT = not more than
2. No specification is set for this parameter by this authority

3. Intended Technical Effect

The intended technical effect of citroglycerides is emulsification as described in 21 CFR §170.3(o)(8). Specifically, citroglycerides are intended to be combined with the GRAS antimicrobial substance lauramide arginine ethyl ester, also known as lauric arginate, to aid in preventing precipitation of this substance in food products to which it is added. The maximum intended use of citroglycerides is a 5:1 mixture with lauric arginate. (N.B. FDA responded to GRAS notice GRN 164 on September 1, 2005, indicating that the agency had no questions at that time regarding the GRAS status of the intended use of lauric arginate.)

4. INTENDED USE AND CONSUMER EXPOSURE

4.1. Intended Use of Citroglycerides

As noted in the previous section, citroglycerides are intended to be mixed with lauric arginate. While it is likely that the addition of citroglycerides will not be technologically advantageous in all permitted uses of lauric arginate, a conservative estimate of exposure is based on the possibility of universal usage. Lauric arginate is GRAS for direct addition to the food categories shown below in Table 2, and consequently the intended use of citroglycerides is for addition to these same food categories at the maximum concentrations shown, which are 5 times higher than the maximum GRAS addition levels of lauric arginate.

Table 2. Intended Uses of Citroglycerides

Food Category[1]	Maximum Concentration of Citroglycerides (ppm)
(3) Beverages and beverage bases, not including dairy products, soft drinks, or alcoholic beverages.	1125
(3) Carbonated beverages[2].	563
(5) Cheeses, including curd and whey cheeses, cream, natural, grating, processed, spread, dip, and miscellaneous cheeses.	1125
(7) Tea, including regular, decaffeinated, and instant types	1125
(8) Condiments and relishes, including plain seasoning sauces and spreads, olives, pickles, and relishes, but not spices or herbs.	1125
(11) Egg dishes including egg roll, egg foo young, egg salad, and frozen multicourse egg meals, but not fresh eggs.	1125
(12) Margarine and margarine-like table spreads, mayonnaise, and spoonable and pourable dressings for salads	1125
(13) Fish products, including all prepared main dishes, salads, appetizers, frozen multicourse meals, and spreads containing fish, shellfish, and other aquatic animals, but not fresh fish.	1125
(15) Fresh fish, including only fresh and frozen fish, shellfish, and other aquatic animals	1125
(17) Fresh meats, including only fresh or home-frozen beef or veal, pork, lamb or mutton and home-prepared fresh meat-containing dishes, salads, appetizers, or sandwich spreads made therefrom.	1125
(18) Fresh poultry, including only fresh or home-frozen poultry and game birds and home-prepared fresh poultry-containing dishes, salads, appetizers, or sandwich spreads made therefrom.	1125
(22) Pie fillings	1125
(24) Gravies and sauces, including all meat sauces and gravies, and tomato, milk, buttery, and specialty sauces.	1125

Food Category[1]	Maximum Concentration of Citroglycerides (ppm)
(29) Meat products, including all meats and meat-containing dishes, salads, appetizers, frozen multicourse meat meals, and sandwich ingredients prepared by commercial processing or using commercially processed meats with home preparation.	1125
(34) Poultry products, including all poultry and poultry-containing dishes, salads, appetizers, frozen multicourse poultry meals, and sandwich ingredients prepared by commercial processing or using commercially processed poultry with home preparation	1125
(35) Processed fruits and fruit juices excluding apple juice, including all commercially processed juices and juice punches, concentrates, dilutions, ades, and drink substitutes made therefrom, and dried fruits; also including strained fruits and fruit juices excluding apple juice as baby or toddler foods.	1125
(36) Processed vegetables and vegetable juices, including potato salads, raw vegetables, vegetable juices and blends, and tomato sauces	1125
(40) Soups and soup mixes, including commercially prepared meat, fish, poultry, vegetable, and combination soups and soup mixes.	1125
1. 21 CFR 170.3(n) 2. The category "carbonated beverages" was not listed in GRN 164 but later determined to be GRAS.	

4.2. Estimated Daily Intake of Citroglycerides

Consumption of foods in which citroglycerides combined with lauric arginate are intended for use in the U.S. was estimated based on data collected in the U.S. Department of Agriculture's 1994–96 Continuing Survey of Food Intakes by Individuals (CSFII) and its Supplemental Children's Survey (CSFII 1998), as provided on CD-ROM (USDA 2000). The CSFII 1994–96 was conducted between January 1994 and January 1997 with non-institutionalized individuals in the U.S. Twenty-four-hour diet recall data were collected through 2 in-person interviews conducted between 3 and 10 days apart from a nationally representative sample of individuals of all ages. The CSFII 1998 was designed as a supplement to CSFII 1994–96 to increase the sample size for children from birth through age 9 years. This survey employed the same methodology as CSFII 1994–96. In the merged surveys (designated CSFII 1994–96, 1998), 21,662 individuals provided dietary intake data on the first survey day, and 20,607 provided data on the second day.

Two-day average intakes of citroglycerides on a body-weight basis were calculated for CSFII 1994–96, 1998 respondents age 2 and older who provided two complete 24-hour diet recalls and who reported consumption of foods in the proposed citroglyceride use categories. All estimates were calculated with USDA sample weights to adjust for variable probabilities of selection, differential non-response rates, and possible deficiencies in the sampling frame.

Over 99% of the U.S. population age 2 and older reported consumption of foods in one or more of the intended citroglyceride use categories. Based on the assumption that citroglycerides are present at a concentration of 563 ppm in all carbonated beverages and at 1125 ppm in all other foods within all food categories for which lauric arginate is GRAS, the mean 2-day average citroglyceride intake for the U.S. population two years of age and older in the U.S. is estimated to be 15.2 mg/kg bw/day, while the estimated 90th percentile intake is 28.2 mg/kg bw/day, as shown in Table 3.

Table 3. Estimated Daily Intake of Citroglycerides

Age/Sex Group	Estimated Intake of Citroglycerides (mg/kg bw/day)	
	Mean	90th Percentile
U.S. population age 2+	15.2	28.2
Children age 2-5	31.5	55.2
Children age 6-10	21.8	37.6
Males age 11-19	18.5	30.9
Females age 11-19	15.3	26.2
Males age 20+	13.2	23.1
Females age 20+	12.3	20.6
Source of Data: 1994-96, 1998 CSFII (USDA 2000)		

5. Review Of Safety Data

5.1. Previous Expert Reviews

Both citroglycerides and related compounds (i.e., glycerol esters of acetic, lactic, and tartaric acids) have been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and by the EU's Scientific Committee on Foods (SCF). JECFA assigned all of these substances an ADI of "not limited," (FAO 1967) a term later replaced by "not specified." JECFA set the "not limited" ADI for citroglycerides 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 noted in its evaluation of citroglycerides 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 citroglycerides 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 citroglycerides, 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 citroglycerides 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 of to 7.5 g/l in ready-to-feed products made from dry powder and up to 9 g/l 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 citroglycerides to all foods for special medical purposes for infants and young children (SCF 2002)..

5.2. Current Uses of Citroglycerides

There are no current uses of citroglycerides in the U.S. However, as was noted earlier, citroglycerides and the other organic acid esters of glycerol are listed in the Codex Alimentarius GSFA (FAO/WHO 2006). The categories of foods in which listed additives may be used and their maximum addition levels are shown in Table 3 of the GSFA, which is displayed in the Appendix. The listing for "citric and fatty acid esters of glycerol," INS472c, includes 61 categories of foods to which citroglycerides may be added, including some very broad categories, such as "confectioneries," "bakery wares," "ready-to-eat savouries," and "cheese and analogues"; in all cases, the level of use is limited only by GMP (See Appendix).

Citroglycerides and the other organic acid/glycerol esters are permitted to be directly added to conventional foods in the EU with no restrictions other than GMP. In the EU, citroglycerides are most commonly used in margarine, meat sausages, mayonnaise, and salad dressings. Acetic acid esters are most typically used in topping powders, chewing gum base, cakes, and coatings; lactic acid esters in topping powders, non-dairy and dairy creams, cakes, and chocolate compounds; and mono- and diacetyl/tartaric acid esters in bread, beverage whiteners, dressings, and sauces. There is no record of any reports of adverse events as a result of this widespread use of these organic acid esters.

5.3. Biochemistry Data Regarding Citroglycerides and Other Organic Acid Esters

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

5.3.1. Citroglycerides

A report from Huntington Research Centre (1966) reviewed and reported by JECFA (FAO 1967) found that *in vitro* hydrolysis of citroglycerides 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 citroglycerides or the unesterified components, or lard at either 16.7% or 35.5%. These diets were calculated to be isocaloric. Fecal fat estimation and body fatty acid distributions showed no difference in digestibility of the ester and its unesterified components.

A report by Rosner (1959) reviewed and reported by JECFA (FAO 1967), noted that in 2 groups of 5 male and 5 female weanling rats that were fed diets containing either 0% or 2% citroglycerides 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%.

5.3.2. 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 better than that of the acetostearins, a finding fully in keeping 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.

5.3.3. 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.

5.3.4. 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. 1967).

5.4. Toxicity Data Regarding Citroglycerides 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 citroglycerides.

5.4.1. Citroglycerides

In the Huntingdon (1966) study cited earlier, gross and microscopic examination of major organs revealed only dystrophic lower-nephron calcification in animals receiving the highest level (37.5% dietary concentration) of either citroglycerides or the unesterified components, (FAO 1967). No effect was seen at the 23.1% level, 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).

5.4.2. 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 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.

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.

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 to an imbalance of the test diet with respect to vitamin E and essential fatty acids.

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).

5.4.3. 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.

5.4.4. Tartaric Acid Esters

Kieckebusch et al. (1967) 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. (1967) 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.

6. Safety Assessment and GRAS Determination

6.1. Introduction

Citroglycerides are safe and are GRAS for direct addition to foods at a maximum use level of 563 ppm in carbonated beverages and 1125 ppm in all other food categories listed in Table 2.

This safety assessment and GRAS determination included two steps. In the first step, the safety of citroglycerides under their intended conditions of use was demonstrated by showing that the EDI of citroglycerides under their intended conditions of use is well within safe levels established by consideration of the biochemical and toxicity studies of citroglycerides and similar organic acid esters of mono- and diglycerides. In the second step, citroglycerides were determined to be GRAS by demonstrating that the safety of citroglycerides under their 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 Citroglycerides for Their Intended Use

There are few data available on the toxicity of citroglycerides themselves, 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 citroglycerides and other organic acid esters to clearly demonstrate that these substances hydrolyze rapidly and completely in the GI tract, leaving only their constituent parts—citric acid (or other organic acid), fatty acid, and glycerol, all normal and harmless dietary components. 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 citroglycerides 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.

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

Finally, citroglycerides and the other organic acid esters have been available and widely used in Europe and 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 Citroglycerides

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.

The intended use of citroglycerides 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 first component of the common knowledge element of a GRAS determination. Determination of the safety and GRAS status of citroglycerides for addition to foods under their intended conditions of use has been made through the deliberations of an Expert Panel comprising Joseph F. Borzelleca, Ph.D., Robert J. Nicolosi, Ph.D., and John A. Thomas, Ph.D., who have prepared and signed the following statement.

"We, an Expert Panel of biomedical scientists, qualified by scientific training and experience to evaluate the safety of foods and substances intended to be added to food, have independently and collectively critically evaluated the available information on citroglycerides summarized in this document as well as other pertinent information available to us and conclude that:
Citric acid esters of mono- and diglycerides of edible fatty acids, when added with lauric arginate to foods listed in Table 2 at up to the levels shown to prevent precipitation of the lauric arginate, and meeting the specifications described in the GRAS monograph, are safe. It is also our opinion that other qualified and competent scientists reviewing the same available information would reach the same conclusion. Therefore, citroglycerides are safe and are GRAS, based on scientific procedures, when added with lauric arginate to foods listed in Table 2 at up to the levels shown to prevent precipitation of the lauric arginate."

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8. Appendix: GSFA Listing for Citric and Fatty Acid Esters of Glycerol

Citric and Fatty Acid Esters of Glycerol (472c)

Synonym(s)
CITREM
Citric acid esters of mono- and di-glycerides
Citroglycerides

Functional Classes
Antioxidant (antioxidant)
Emulsifier (emulsifier)
Emulsifying salt (sequestrant)
Stabilizer
Thickener

GSFA Table 3 Provisions

Citric and Fatty Acid Esters of Glycerol is a food additive that is included in Table 3, and as such may be used in the following foods under the conditions of good manufacturing practices (GMP) as outlined in the Preamble of the Codex GSFA. Note that food categories listed in the Annex to Table 3 were excluded accordingly.

Number	Food Category
01.1.2	Dairy-based drinks, flavoured and/or fermented (e.g., chocolate milk, cocoa, eggnog, drinking yoghurt, whey-based drinks)
01.3	Condensed milk and analogues (plain)
01.4.3	Clotted cream (plain)
01.4.4	Cream analogues
01.5	Milk powder and cream powder and powder analogues (plain)
01.6	Cheese and analogues
01.7	Dairy-based desserts (e.g., pudding, fruit or flavoured yoghurt)
01.8	Whey and whey products, excluding whey cheeses
02.2.1.2	Margarine and similar products
02.2.1.3	Blends of butter and margarine

Number	Food Category
02.2.2	Emulsions containing less than 80% fat
02.3	Fat emulsions maily of type oil-in-water, including mixed and/or flavoured products based on fat emulsions
02.4	Fat-based desserts excluding dairy-based dessert products of food category 01.7
03.0	Edible ices, including sherbet and sorbet
04.1.2	Processed fruit
04.2.2.2	Dried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds
04.2.2.3	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweeds in vinegar, oil, brine, or soy sauce
04.2.2.4	Canned or bottled (pasteurized) or retort pouch vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds
04.2.2.5	Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g., peanut butter)
04.2.2.6	Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed pulps and preparations (e.g., vegetable desserts and sauces, candied vegetables) other than food category 04.2.2.5
04.2.2.8	Cooked or fried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds
05.0	Confectionery
06.3	Breakfast cereals, including rolled oats
06.4.3	Pre-cooked pastas and noodles and like products
06.5	Cereal and starch based desserts (e.g., rice pudding, tapioca pudding)
06.6	Batters (e.g., for breading or batters for fish or poultry)
06.7	Pre-cooked or processed rice products, including rice cakes (Oriental type only)
06.8	Soybean products (excluding soybean products of food category 12.9 and fermented soybean products of food category 12.10)
07.0	Bakery wares

Number	Food Category
08.2	Processed meat, poultry, and game products in whole pieces or cuts
08.3	Processed comminuted meat, poultry, and game products
08.4	Edible casings (e.g., sausage casings)
09.3	Semi-preserved fish and fish products, including mollusks, crustaceans, and echinoderms
09.4	Fully preserved, including canned or fermented fish and fish products, including mollusks, crustaceans, and echinoderms
10.2.3	Dried and/or heat coagulated egg products
10.3	Preserved eggs, including alkaline, salted, and canned eggs
10.4	Egg-based desserts (e.g., custard)
11.6	Table-top sweeteners, including those containing high-intensity sweeteners
12.2.2	Seasonings and condiments
12.3	Vinegars
12.4	Mustards
12.5	Soups and broths
12.6	Sauces and like products
12.7	Salads (e.g., macaroni salad, potato salad) and sandwich spreads excluding cocoa- and nut-based spreads of food categories 04.2.2.5 and 05.1.3
12.8	Yeast and like products
12.9	Protein products
12.10	Fermented soybean products
13.3	Dietetic foods intended for special medical purposes (excluding products of food category 13.1)
13.4	Dietetic formulae for slimming purposes and weight reduction
13.5	Dietetic foods (e.g., supplementary foods for dietary use) excluding products of food categories 13.1 - 13.4 and 13.6
13.6	Food supplements

Number	Food Category
14.1.1.2	Table waters and soda waters
14.1.4	Water-based flavoured drinks, including "sport," "energy," or "electrolyte" drinks and particulated drinks
14.2.1	Beer and malt beverages
14.2.2	Cider and perry
14.2.4	Wines (other than grape)
14.2.5	Mead
14.2.6	Distilled spirituous beverages containing more than 15% alcohol
14.2.7	Aromatized alcoholic beverages (e.g., beer, wine and spirituous cooler-type beverages, low alcoholic refreshers)
15.0	Ready-to-eat savouries
16.0	Composite foods - foods that could not be placed in categories 01 - 15

Note: Unless otherwise specified, food additive provisions apply to the food category indicated (e.g. Dairy), as well as to all subcategories of that category (e.g. Cheese, Ripened Cheese, etc.).

SUBMISSION END