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LIPID NUTRITION

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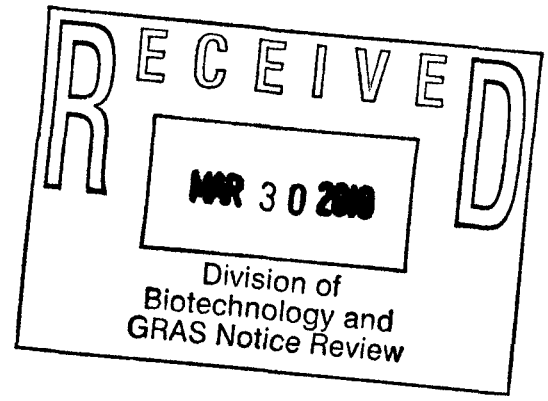

25th March 2010

Re: GRAS Notice for Refined Pine Nut Oil

Dear Dr. Martin,

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized As Safe (GRAS) determination] published in the Federal Register [62 FR 18938 (17 April 1997)], I am submitting in triplicate, as the notifier [*Lipid Nutrition B.V., Hogeweg 1, 1521 AZ Wormerveer, The Netherlands*], a Notice of the determination, on the basis of scientific procedures, that Refined Pine Nut Oil, produced by Lipid Nutrition B.V., as defined in the enclosed documents, is GRAS under specific conditions of use as a food ingredient in various foods, and therefore, is exempt from the premarket approval requirements of the Federal, Food, Drug and Cosmetic Act. Information setting forth the basis for the GRAS determination, which includes a comprehensive summary of the data available and reviewed by an independent panel of experts (the Expert Panel) qualified by scientific training and experience to evaluate the safety of Refined Pine Nut Oil in food.

I trust that the enclosed Notice is acceptable. Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

(b) (6)

Jaap D. Kluifhooft, M.Sc.
Director Regulatory Affairs

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GRAS EXEMPTION CLAIM FOR REFINED PINE NUT OIL

Summary of Data Concerning the Safety and GRAS Determination of Refined Pine Nut Oil for Use as a Food Ingredient

Submitted to:

Robert L. Martin, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835
U.S.A.

Submitted by:

Lipid Nutrition B.V.
Hogeweg 1
1521 AZ Wormerveer
The Netherlands

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MAR 30 2010
Division of
Biotechnology and
GRAS Notice Review

March 24, 2010

GRAS EXEMPTION CLAIM FOR REFINED PINE NUT OIL

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GRAS EXEMPTION CLAIM FOR REFINED PINE NUT OIL

I. GRAS EXEMPTION CLAIM

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

As defined herein, Refined Pine Nut Oil has been determined by Lipid Nutrition B.V. (hereafter Lipid Nutrition) to be Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, and on the consensus opinion of an independent panel of experts qualified by scientific training and expertise to evaluate the safety of Refined Pine Nut Oil under the conditions of intended use in food. Therefore, the use of Lipid Nutrition's Refined Pine Nut Oil in food as described below is exempt from the requirement of premarket approval (Section 409 of the *Federal Food, Drug and Cosmetic Act*).

Signed,

(b) (6)

24/03/2010

Date

Mr. Jaap D. Kluifhooft
Director Regulatory Affairs
Lipid Nutrition B.V.

I.B Name and Address of Notifier

Mr. Jaap D. Kluifhooft
Director Regulatory Affairs

Lipid Nutrition B.V.
Hogeweg 1
1521 AZ Wormerveer
The Netherlands
Telephone: +31-756292432
Facsimile: +31-756292817
Email: jaap.kluifhooft@lipidnutrition.com

I.C Common Name of the Notified Substance

The common name of the notified substance is Refined Pine Nut Oil.

I.D Conditions of Intended Use in Food

Lipid Nutrition intends to market Refined Pine Nut Oil as a food ingredient in the United States under the proposed food uses as described in Table I.D-1 at a use level of 3.0 g/serving. Refined Pine Nut Oil is intended to be used as a replacement for other oils and is not intended to be used in any meat or meat-containing products.

Table I.D-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Refined Pine Nut Oil in the U.S.

Food Category	Proposed Food-Use	Level (g/RACC†)	Use-Level (%)
Baked Goods and Baking Mixes	Cookies (excluding low fat and dietetic)	3 0	10.0
Beverages and Beverage Bases	Meal Replacement Beverages	3 0	1.25
	Sports, Energy, and Isotonic Drinks	3.0	1 25
Breakfast Cereals	Oatmeal-based Instant and Regular Hot Cereals	3.0	7.5 5 45
Dairy Product Analogs	Imitation and Soy Milks	3.0	1.25
Fats and Oils	Salad Dressings (excluding mayonnaise, fat-free and low-fat types)	3 0	10.0
Grain Products and Pasta	Cereal and Granola Bars (excluding low-fat types)	3.0	7 5
	Energy, Meal Replacement, and Fortified Bars	3.0	7.5
Milk and Milk Products	RTD Flavored Milk and Milk Drinks (excluding fat-free types)	3.0	1 25
	Meal Replacement Beverages (milk-based)	3.0	1 25
	Yogurt Beverages‡	3.0	1.5
	Yogurt (excluding fat-free types)	3.0	1.33
Nuts and Nut Products	Nut Snacks and Mixed Nuts	3.0	10.0
Processed Fruits and Fruit Juices	Fruit Flavored Drinks	3.0	1.25
	Fruit Juice	3.0	1.25
	Fruit Smoothie Drinks	3 0	1.25
Processed Vegetables and Vegetable Juices	Vegetable Juice	3.0	1 25
Snack Foods	Chips, Pretzels and Other Savory Snacks	3.0	10 0
Soft Candy	Nut-based Chocolate Confections	3.0	7 5
	Soft Candies	3 0	7 5
Soups and Soup Mixes	Dehydrated and Powdered Soup Mixes	3.0	1 22 38.13

† RACC = Reference Amounts Customarily Consumed per Eating Occasion (21 CFR §101.12 – U.S. FDA, 2009) When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC.
‡ Food codes representative of yogurt beverages were not identified, thus codes representing milk containing acidophilus were used as surrogates.

I.E Basis for the GRAS Determination

Pursuant to 21 CFR §170.30, Refined Pine Nut Oil has been determined by Lipid Nutrition to be GRAS on the basis of scientific procedures (U.S. FDA, 2009). This determination is based on data generally available in the public domain pertaining to the safety of Refined Pine Nut Oil for use in food, as discussed herein and in the accompanying documents, and on a consensus among a panel of experts[1] who are qualified by scientific training and experience to evaluate the safety of Refined Pine Nut Oil as a component of food [see Appendix A, **EXPERT PANEL REPORT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF REFINED PINE NUT OIL FOR USE IN FOOD**].

I.F Availability of Information

The detailed data and information that serve as a basis for this GRAS determination will be provided to the United States Food and Drug Administration (FDA) upon request, or are available for the FDA's review and copying during reasonable business hours at the offices of:

Mr. Jaap D. Kluifhooft
Director Regulatory Affairs

Lipid Nutrition B.V.
Hogeweg 1
1521 AZ Wormerveer
The Netherlands

Should the FDA have any questions or additional information requests regarding this notification, Lipid Nutrition will supply these data and information.

II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

II.A Identity

Lipid Nutrition's Refined Pine Nut Oil is an edible oil obtained from Korean pine (*Pinus koraiensis*) consisting of a mixture of triglycerides of which the major fatty acid components are oleic (min. 22%), linoleic (min. 44%) and pinolenic acids (min. 14%) as shown in Figure II.A-1. Refined Pine Nut Oil is a pale yellow clear liquid at ambient temperature.

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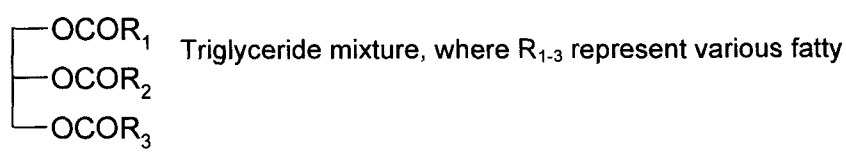
[1] The Panel consisted of the following qualified scientific experts: Professor Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Professor Robert J Nicolosi, Ph.D. (University of Massachusetts Lowell), and Professor John A. Thomas, Ph.D. (Indiana University School of Medicine).

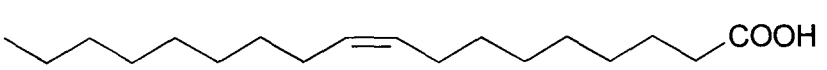
Common or Usual Name:	Refined Pine Nut Oil
Chemical Name:	Not applicable
Chemical Abstracts Service (CAS) Number:	84988-87-4
Empirical Formula and Formula Weight:	Not applicable
Molecular Weight:	Not applicable
Structural Formula:	See Figure II.A-1 below.

The chemical structures of the major fatty acid components of Refined Pine Nut Oil (*i.e.*, oleic, linoleic, and pinolenic acids) are presented in Figure II.A-1. A detailed summary of the fatty acid composition of Refined Pine Nut Oil is presented in Table II.A-1.

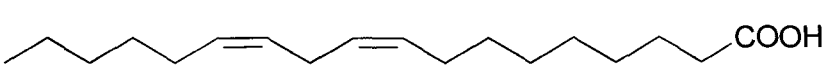
Figure II.A-1 Major Fatty Acid Components of Refined Pine Nut Oil (as Triglycerides)



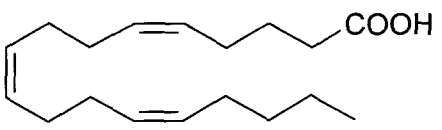
Triglyceride mixture, where R_{1-3} represent various fatty acids including:



Oleic acid; 9-*cis*-octadenoic acid; C18:1 (n-9)



Linoleic acid; 9-*cis*,12-*cis*-octadecadienoic acid; C18:2 (n-6)



Pinolenic acid; 5-*cis*,9-*cis*,12-*cis*-octadecatrienoic acid; C18:3 *c*5,*c*9,*c*12

Table II.A-1 Fatty Acid Composition of 3 Commercial Batches of Refined Pine Nut Oil

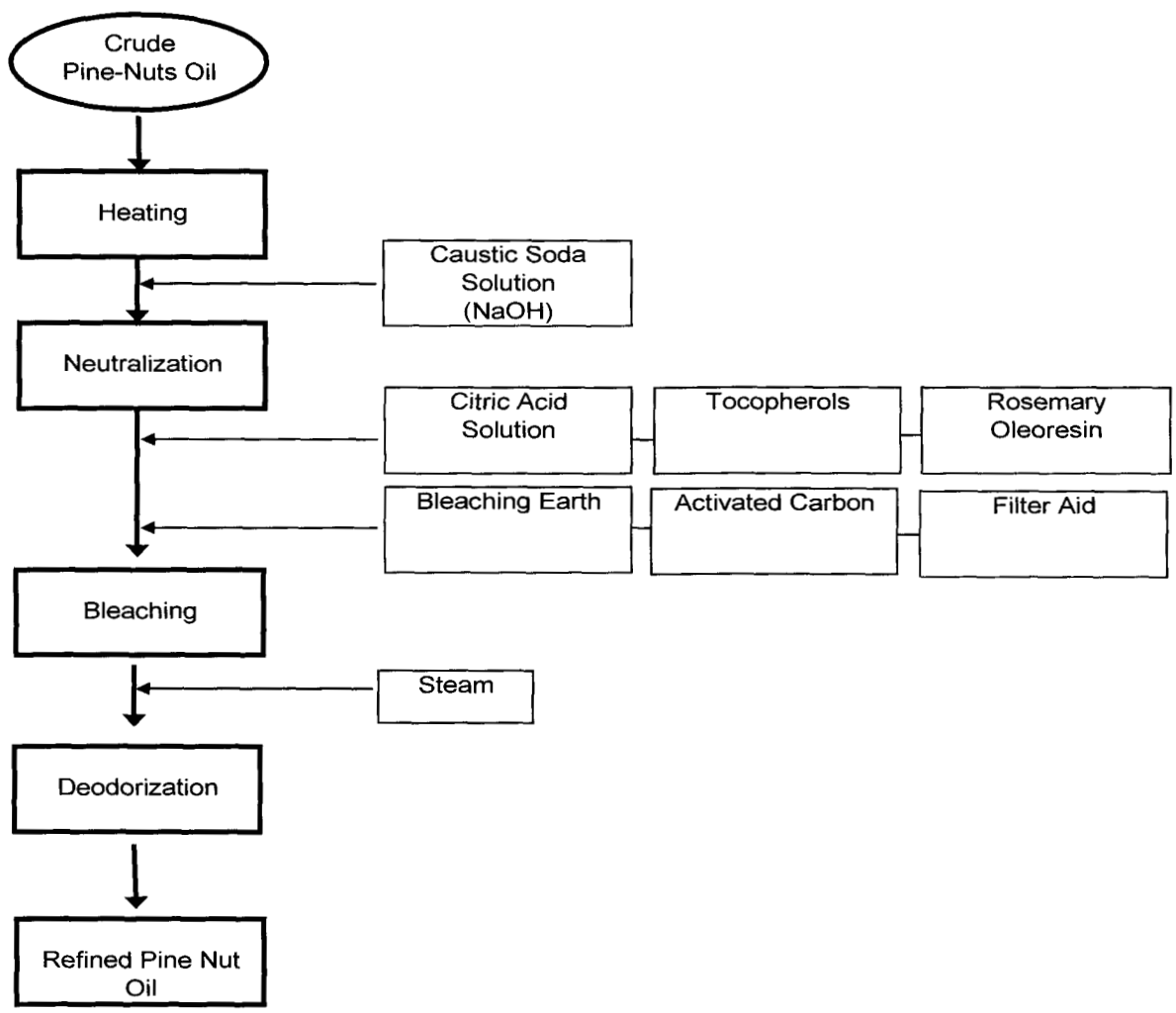
Fatty Acid	Crude oil (two batches)	Fatty Acid Composition (% by wt)		
		Batch 7239	Batch 7334	Batch 7365
Palmitic acid; C16.0	4 5 // 4.5	4.4	4.4	4.3
Palmitoleic acid; C16:1	0 2 // 0.1	0.2	0.2	0.1
Heptadecanoic acid; C17·0	0.1 // 0.0	0.1	0.1	0
Stearic acid; C18:0	2.1 // 2.2	2.1	2 2	2 1
Oleic acid; C18·1 (n-9)	24.8 // 25.5	24.8	24.7	25.5
Linoleic acid; C18:2 (n-6)	45.0 // 45.3	45.0	44.6	45.1
Taxoleic acid; C18:2 *c*5,*c*9	2.1 // 2.2	2.2	2.2	2 2
Linolenic acid, C18:3	0 3 // 0.2-	0.3	0.3	0.2
Pinolenic acid; C18·3 c5,c9,c12	15.8 // 15.3	15.8	15.4	15.0
Arachidic acid; C20:0	0 5 // 0 2	0.5	0.4	0.6
Eicosatrienoic acid; C20:3	1.5 // 1.4	1.5	1.4	1.4
Eicosenoic acid; C20:1	1.2 //1.3	1 2	1 2	1.3
Eicosadienoic acid; C20·2	0 8 //0.-	0 8	0.8	0.9
Behenic acid; C22:0	0 0 // 0.0	0	0	0.1
Other Fatty Acids	0.8 // 0.7	1.0	1.7	1.0
Total Saturated Fatty Acids	7.2 // 7.0	7	7	7.1
Total Monounsaturated Fatty Acids	26.2 // 26.9	26 2	26.1	26.9
Total Polyunsaturated Fatty Acids	65 8 // 65.5	65.8	65.2	65.0
Total C18 trans Fatty Acids	0.2 // 0.2	0 2	0.4	0.3

II.B Method of Manufacture

Refined Pine Nut Oil is obtained from crude cold-pressed pine nut oil. The pine nuts are slightly toasted and crushed and are then pressed mechanically by a stone mill without the application of heat in order to obtain a cold pressed oil (as defined by Codex Stan, 1999). The crude pine nut oil is then used directly in the manufacture of Refined Pine Nut Oil. The crude oil is heated and the free fatty acids are removed by adding caustic soda. After precipitation of the free fatty acids the pine nut oil is neutralized by a citric acid solution. The oil is stabilized by the addition of safe and suitable antioxidants that are added in accordance with U.S. Food and Drug Administration (FDA) regulations. After addition of the antioxidants, the oil passes through a mixture of bleaching earth, activated carbon, and filter aid in order to remove any minor traces of impurities and improve the physical characteristics (*e.g.*, color, organoleptic properties) of the oil. The resultant elute is then deodorized by conventional steam stripping procedures. Refined Pine Nut Oil is packed in suitable containers that minimize oxidation of the product during storage under an inert (nitrogen) atmosphere.

The processes involved in the manufacture of Refined Pine Nut Oil are conventional techniques employed by the fats and oils industry to improve the quality of the oil and does not result in any chemical modification of the crude pine nut oil. The manufacturing process is consistent with current Good Manufacturing Practices (cGMP). A schematic diagram of the manufacturing process for Refined Pine Nut Oil is provided in Figure II.B-1.

Figure II.B-1 Schematic Overview of the Manufacturing Process for Refined Pine Nut Oil



All processing aids and antioxidants used in the manufacture of Refined Pine Nut Oil are used in compliance with appropriate federal regulations, as indicated in Table II.B-1.

Table II.B-1 List of Processing Aids and Antioxidants Used in the Manufacture of Refined Pine Nut Oil

Processing Aids Used in the Manufacture of Refined Pine Nut Oil	Function/Manufacturing Step(s) at which Processing Aid is Used	Reference to Appropriate Use in Food Method of Analysis
Sodium hydroxide (NaOH)	pH control agent	21 CFR §184.1763
Citric Acid	pH control agent	21 CFR §184.1033
Bleaching Earth	Purification and deodorization	21 CFR §184.1155
Activated carbon	Purification, deodorization, and decolorization	No specific regulations pertaining to activated carbon; however, it is an accepted processing aid in food oil manufacture (GRAS Notice No. GRN 000138 [U.S. FDA, 2004])
Filters (Cellulose)	Various separation steps throughout processing	21 CFR §177.2260
Tocopherols	Antioxidant	21 CFR §182.3890
Rosemary Extract	Antioxidant	21 CFR §182 20

CFR = Code of Federal Regulation; GRAS = Generally Recognized as Safe (U.S. FDA, 2009)

II.C Specifications for Food Grade Material

II.C.1 Product Specifications and Analysis

Refined Pine Nut Oil is produced in accordance with cGMP, and in order to ensure consistent, safe products, Lipid Nutrition has established food-grade specification parameters for the ingredient. These parameters comprise specifications for the physical appearance, purity, fatty acid components, as well as specifications for potential chemical and microbiological impurities and contaminants. The chemical and microbiological specifications for Refined Pine Nut Oil are presented in Table II.C.1-1. Analyses of three representative, non-consecutive lots confirm that the material produced by the manufacturing process is consistent and complies with these product specifications (see Appendix B for batch analysis results).

Table II.C.1-1 Chemical and Microbiological Specifications for Refined Pine Nut Oil

Parameter	Specification	Analytical Method
Appearance	Pale Yellow Liquid	Visual
Free Fatty Acids (as % oleic acid)	Max. 1.0%	Titration method / ISO 660
Peroxide Value	Max. 5.0 meqO_2/kg	AOCS Cd 8b-90 (97)
Total Saturated Fatty Acids	Max 10%	High resolution capillary gas chromatography
Total Monosaturated Fatty Acids	Min. 24%	High resolution capillary gas chromatography
Oleic Acid (C18.1, n-9)	Min 22%	High resolution capillary gas chromatography
Total Polyunsaturated Fatty Acids	Min. 64%	High resolution capillary gas chromatography
Linoleic Acid (C18:2, n-6)	Min. 44%	High resolution capillary gas chromatography
Pinolenic Acid (C18:3, c5,c9,c12)	Min. 14%	High resolution capillary gas chromatography
Trans fatty acids	Max. 1%	High resolution capillary gas chromatography
Lead (Pb)	Max. 0.1 mg/kg	DIN EN 13805 / 14083 / Atomic absorption method
Arsenic (As)	Max 0.1 mg/kg	DIN EN 13805 / 14083 / Atomic absorption method
Total aerobic count	<1,000 cfu/g	ISO 4833
Yeasts	<10 cfu/g	ISO 7954
Moulds	<10 cfu/g	ISO 7954
Escherichia coli	< 10 cfu/g	ISO 16649-2
Salmonella	Absent in 25 g	ISO 6579
Enterobacteriaceae	<10 cfu/g	ISO 21528-2

AOCS = American Oil Chemists Society; cfu = colony-forming units, ISO = International Standards Organization

II.C.2 Stability

Refined Pine Nut Oil is stable for a minimum of 18 months from the date of production when stored unopened in dry conditions under an inert atmosphere (nitrogen) between temperatures of 10 to 20°C (50 to 60°F). Full details are provided in Appendix C.

III. SELF-LIMITING LEVELS OF USE

The use of Refined Pine Nut Oil is self-limiting based on the taste characteristics of the oil.

IV. BASIS FOR GRAS DETERMINATION

IV.A Introduction

The determination that Refined Pine Nut Oil is GRAS is on the basis of scientific procedures. The safety of Refined Pine Nut Oil is based on the natural background occurrence of pine nuts in the diet and an estimate of the probable consumption of Refined Pine Nut Oil as calculated

using the most recent publicly-available survey of U.S. food consumption, as well as a database of published toxicological and human studies, including studies conducted with Lipid Nutrition's Refined Pine Nut Oil product. In a 13-week rat toxicity study, the highest level studied for Lipid Nutrition's Refined Pine Nut Oil product, 15% mixed into the diet, was established as the no-observed-adverse-effect level (NOAEL). This NOAEL corresponded to a daily intake of approximately 8.9 or 10.2 g Refined Pine Nut Oil/kg body weight/day for female and male rats, respectively.

The information in the human studies corroborates the safety information provided from animal studies. Human studies in which subjects were supplemented with a single dose of 2 to 6 g of Refined Pine Nut Oil revealed that the intervention was well-tolerated, with no significant adverse effects or changes in blood glucose and insulin, and serum triglycerides (Hughes *et al.*, 2008; Pasman *et al.*, 2008).

These data were reviewed by a panel of experts, qualified by scientific training and experience to evaluate the safety of Refined Pine Nut Oil as a component of food, who concluded that the proposed uses of Refined Pine Nut Oil are safe and suitable and would be GRAS based on scientific procedures [see Appendix A, **EXPERT PANEL REPORT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF REFINED PINE NUT OIL FOR USE IN FOOD**] and that other qualified experts would concur with these conclusion. It also is Lipid Nutrition's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. A summary of these data is presented herein.

IV.B Probable Consumption of Refined Pine Nut Oil

IV.B.1 Estimated Intake of Refined Pine Nut Oil Under the Intended Conditions of Use in Foods

As mentioned, Refined Pine Nut Oil is intended for use in a variety of food products, including baked goods and baking mixes, beverages and beverage bases, breakfast cereals, dairy product analogs, fats and oils, grain products and pasta, milk and milk products, nuts and nut products, processed fruit and fruit juices, processed vegetables and vegetable juices, snack foods, soft candy, and soups and soup mixes, at a level of 3.0 g/serving. Refined Pine Nut Oil is intended to be used as a replacement for other oils.

The consumption of Refined Pine Nut Oil from all intended uses and use levels was estimated using the National Center for Health Statistics' (NCHS) 2005-2006 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2009). Under the intended food uses, 94.6% of the total U.S. population was identified as potential consumers of Refined Pine Nut Oil (7,043 actual users identified). On an all-user basis, the mean intake of Refined Pine Nut Oil by the total U.S. population from all proposed food-uses was estimated to be 8.9 g/person/day or 150 mg/kg body weight/day. The heavy consumer (90th percentile) all-user intake of Refined

Pine Nut Oil by the total U.S. population from all proposed food-uses was estimated to be 17.8 g/person/day or 327 mg/kg body weight/day. Under the intended conditions of uses the estimated intakes of Refined Pine Nut Oil for all population groups are presented in Tables IV.B.1-1 and IV.B.1-2 on a per person and per kilogram body weight basis, respectively.

Table IV.B.1-1 Summary of the Estimated Daily Intake of Refined Pine Nut Oil from All Proposed Food-Uses in the U.S. by Population Group (2005-2006 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
				Mean (g)	90th Percentile (g)	Mean (g)	90th Percentile (g)
Children	3 to 11	98.7	1,427	8.7	16.0	8.9	16.0
Female Teenagers	12 to 19	96.1	956	7.8	15.1	8.3	15.4
Male Teenagers	12 to 19	95.2	896	9.7	20.0	10.4	20.3
Female Adults	20 and Up	93.7	2,016	7.4	15.7	7.9	16.3
Male Adults	20 and Up	91.4	1,748	9 2	19.6	10.0	20.4
Total Population	All Ages	94.6	7,043	8.4	17.2	8.9	17.8

Table IV.B.1-2 Summary of the Estimated Daily per Kilogram Body Weight Intake of Refined Pine Nut Oil from All Proposed Food-Uses in the U.S. by Population Group (2005-2006 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-User Consumption	
				Mean (mg/kg)	90th Percentile (mg/kg)	Mean (mg/kg)	90th Percentile (mg/kg)
Children	3 to 11	98.7	1,423	343	661	350	665
Female Teenagers	12 to 19	96 1	950	133	271	142	276
Male Teenagers	12 to 19	95.2	894	148	312	159	314
Female Adults	20 and Up	93.7	1,997	105	228	111	234
Male Adults	20 and Up	91.4	1,722	109	228	118	237
Total Population	All Ages	94.6	6,986	141	314	150	327

As mentioned, the estimated intakes of Refined Pine Nut Oil under the intended conditions of use were calculated using recently published U.S. dietary consumption surveys (*i.e.*, 2005-2006 NHANES) (CDC, 2006; USDA, 2009), which include two 24-hour dietary recalls administered on 2 non-consecutive days. The surveys provide the most appropriate data for evaluating food-use and food-consumption patterns in the United States; however, it is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys overestimate consumption over longer time periods (Anderson, 1988). Moreover, the calculations assume that all food products within a food category contain the ingredient at

the maximum specified level of use. Therefore, the estimated intakes of Refined Pine Nut Oil are over-estimates of anticipated actual consumption.

IV.B.2 Occurrence of Refined Pine Nut Oil in the Diet and Background Dietary Intakes

The crude pine nut oil used as the starting material for the manufacture of Refined Pine Nut Oil is obtained from the edible seeds (pine nuts) of the pine tree *Pinus koraiensis.* Commonly referred to as the Korean cedar, *Pinus koraiensis* is found in Korea, Japan, Siberia, and Manchuria (north of China).

Pine nuts are traditional foods in Europe, Asia, and America consumed both on their own and in a range of vegetable, meat and fish dishes and in desserts (FAO, 1995). For example, they are a key ingredient in the popular Italian sauce, pesto, and are used to produce a nutty flavored coffee in New Mexico (U.S.). In Asia and the Near East, *Pinus gerardinia*, *Pinus koraiensis*, *Pinus pumila,* and *Pinus sibirica* are important pine producing species. The nuts of *Pinus sibirica* and *Pinus koraiensis* are pressed commercially for the production of cooking oil. Specific data on world production of pine nuts are not available. In the south western United States, annual crops are estimated to average between 454 and 900 tons. During the years 1976 to 1980, the United States imported an average of $800,000 of pine nuts annually (FAO, 1995). In 2003, there was a reported $100 million in annual pine nut sales in the United States (Sharashkin and Gold, 2004). The most important pine nut imported was derived from *Pinus pinea*, which accounted for 68% of the imports. Spain and Portugal were the major sources of nuts of this pine species. The remainder of the imported pine nuts were derived from *Pinus koraiensis* imported from China. Globally, China is the largest producer and exporter of seeds of *Pinus koraiensis* and *Pinus sibirica*. The nuts of these species also are harvested in Siberia, Russia, where there also is a high domestic demand.

IV.C Absorption, Distribution, Metabolism, and Elimination

No specific absorption, distribution, metabolism, and elimination (ADME) studies have been conducted on Lipid Nutrition's Refined Pine Nut Oil; however, data are available on the absorption, distribution and metabolism of the component fatty acids from studies in literature. The general ADME properties of pine nut oil are comparable to that of any triglyceride.

The effect of feeding maritime pine nut oil (MPNO) on the fatty acid profile of mothers' milk and brain tissues of developing fetuses and pups was studied in pregnant and lactating Wistar rats to determine whether Δ5-PMI-PUFA [a polymethylene-interrupted (PMI) double bond system with a double bond at the Δ5 position] altered levels of n-3 or n-6 long chain polyunsaturated fatty acids (PUFA) (Pasquier *et al.,* 2001). MPNO is an edible oil extracted from *Pinus pinaster* containing the fatty acids, pinolenic acid and sciadonic acid, which have Δ5-PMI-PUFA bonds (Asset *et al.,* 2001). Two groups of 10 female Wistar rats were fed semi-purified diets containing 10 g of a blend of high-oleic sunflower, sunflower, and canola oils/100 g diet, or 10 g

MPNO/100 g diet. The MPNO consisted of 6.6% pinolenic acid. The rats were allowed to mate after 16 days on the experimental diets. On Day 17 of pregnancy, 5 rats from each group were euthanized and blood, liver, and breast tissues from the mothers, and fetus brains and bodies were collected for lipid analysis. The remaining animals were allowed to give birth, and the number of pups and birth weights were recorded. A milk sample was collected from the mothers on Day 21 following delivery, and all animals were killed on Day 22. Blood, liver, brain, and breast tissues were collected.

Fatty acid analysis of tissues from 17-day pregnant and lactating mother rats fed MPNO demonstrated that the Δ5-PMI-PUFA were incorporated into blood, liver and breast tissues, while the none of these fatty acids were detected in the control rats. Pinolenic and sciadonic acid were the major Δ5-PMI-PUFA detected in all tissues. There were no differences in the n-3 or n-6 long chain PUFA in blood and tissues between the dietary groups. The phospholipid and triacylglyceride fractions of the liver had similar proportions of total Δ5-PMI-PUFA; however, the phospholipid fraction contained mostly sciadonic acid, and the triacylglyceride fraction primarily contained pinolenic acid. Similar to the results of the 17-day pregnant and lactating rats, Δ5-PMI-PUFA were detected in the blood and brain samples from 17-day fetuses and 22-day-old pups. As seen in the pregnant and lactating mothers, pinolenic and sciadonic acid were the primary fatty acids detected. The mother's milk drawn on Day 21 of lactation contained all of the Δ5-PMI-PUFA. Additionally, all of the Δ5-PMI-PUFA were detected in blood of the pups on Day 22, but the pup's brains contained very little Δ5-PMI-PUFA. Mothers' milk and the brains of fetuses and pups of rats fed the maritime pine oil contained normal levels of the n-3 and n-6 fatty acids, demonstrating that pine oil had no negative effect on the levels of these fatty acids during rat brain development.

Fatty acids also can be metabolized partly or completely in the β-oxidation pathway, or modified by elongation and desaturation pathways. When oxidized in the β-oxidation pathway, fatty acids are bound to fatty acid binding protein (FABP). Activation occurs by conversion of the free fatty acid to its coenzyme A derivative, after which it can be transported to the mitochondrion or the peroxisome. The mitochondria oxidize short, medium, and most long chain fatty acids, while peroxisomes oxidize some long chain, but mostly very long chain fatty acids. Certain fatty acids, before or after modification, are used for physiological purposes such as incorporation into the phospholipids of cell membranes. The modification of fatty acids for these purposes takes place via a number of elongation and desaturation steps, sometimes following partial β-oxidation. The chain elongation system catalyzes the addition of 2-carbon units to the growing fatty acid. The desaturation pathway catalyzes the introduction of a double bond. Many different fatty acids can be formed in this way, of which the functions have not all been elucidated (Zapsalis and Beck, 1985).

Tanaka *et al.* (1998) performed a number of metabolic investigations to determine the metabolic pathway of pinolenic acid. In the first experiment, the effect of a pine nut oil, consisting of 15%

pinolenic acid, on the fatty acid composition of rat liver was examined. The C-2 elongation metabolite of pinolenic acid was not found in the rat liver and the pinolenic acid concentrations in phosphatidylcholine, phosphatidylethanolamine, and triglyceride fractions were relatively low. The authors hypothesized that the Δ5-PMI *cis* double bond structure in pinolenic acid is not recognized by the fatty acid chain elongation system (FACES).

This hypothesis was tested in a second study in rat liver microsomes from male Sprague-Dawley rats using the FACES assay and condensing enzyme assay (Tanaka *et al.*, 1998). The conversion rate of pinolenic acid to its carbon 2-unit chain-elongated metabolite was 31% in the total elongation assay as compared to *gamma*-linolenic acid, a structurally similar fatty acid. In the condensing enzyme assay, the conversion rate of pinolenic acid to its corresponding C20 *beta*-keto fatty acid was reported to be 19% as compared to *gamma*-linolenic acid. However, these results have not been confirmed in *in vivo* experiments. From previous results and the results of this investigation, the authors proposed that the C-2 elongated metabolite is rapidly transferred to the mitochondria and subjected to *beta*-oxidation without accumulation (Tanaka *et al.,* 1998).

In a third study by Tanaka *et al.* (1999), the effect of pinolenic acid on the arachidonic acid (AA) concentration in the phosphatidylinositol (PI) fraction in human hepatoma HepG2 cells was investigated *in vitro*. HepG2 cells were incubated for 48 hours with or without 100 μM pinolenic acid, and the AA concentration in the PI fraction was observed to be decreased from the baseline value of 15.9 to 7.0%. A 2-C elongated metabolite of pinolenic acid was detected in the PI fraction at a concentration of 19.3%. This metabolite, but not pinolenic acid, was substituted for AA in the PI fraction. The authors proposed that the 2-carbon-unit-chain-elongated metabolite of pinolenic acid may only accumulate in *in vitro* cell culture systems.

In several *in vitro* assays it was demonstrated that pinolenic acid is a better substrate for carnitine palmitoyltransferase than *alpha*-linolenate, while no significant differences were measured in glycerol 3-phosphate acyltransferase and diacylglycerol acyltransferase activity (Ide *et al.,* 1995). Thus pinolenic acid is rapidly metabolized by the β-oxidation pathway.

In summary, all studies indicate that Refined Pine Nut Oil is absorbed, distributed, metabolized, and excreted in a similar fashion as other food oils. Pinolenic acid is rapidly metabolized by the β-oxidation pathway, does not accumulate in tissues, and has no negative effect on the levels of n-3 and n-6 fatty acids during rat brain development.

IV.D Toxicological Studies

IV.D.1 Repeated Dose Studies

A 13-week study was conducted in male and female Wistar rats to assess effects of dietary administration of Lipid Nutrition's Refined Pine Nut Oil (containing 15.3% pinolenic acid)

(Speijers *et al.*, 2009). The study was conducted in accordance with the Organization for Economic Cooperation and Development (OECD) guideline 408 and Good Laboratory Practices (GLP) (OECD, 1997). Following a 5-day acclimatization period, rats (approximately 6 weeks of age) were randomized to 1 of 4 groups (10 animals/sex/group) and were fed diets supplemented with 0 (control, 15% corn oil), 1, 5, or 15% Refined Pine Nut Oil. These dietary concentrations were reported to result in mean intakes of Refined Pine Nut Oil of 0, 579, 2,930, and 8,866 mg/kg body weight/day for males, respectively, and 0, 671, 3,330, and 10,242 mg/kg body weight/day for females, respectively.

No mortality or clinical signs of toxicity were observed in any animals over the study period. Pale feces were observed for all groups and the finding was considered to be due to the high fat content of the diet. Feed consumption, mean body weight, and body weight gain of treated animals were not significantly different from the control group. One male rat in the 15% dose group had significantly lower body weight gain, but since all other animals in this group grew normally, this finding was not considered to be related to treatment.

All functional observation tests were normal in all animals with the exception of a variation in motor activity that was not considered to be related to treatment. There were no ophthalmoscopic findings at pre-dose or Week 13. Changes in hematological parameters included decreased white blood cell counts (WBC) and prothrombin times in males at 15%, higher relative neutrophil and lower relative lymphocyte counts in females at 1 and 5%, and lower hemoglobin levels in females at 1%. All of these findings were statistically significant, but occurred in the absence of treatment-related distribution and therefore, were not considered to be toxicologically relevant. Clinical biochemistry parameters revealed a number of statistically significant effects; however, for most parameters, there were no clear dose-response relationships or consistency of effects between the sexes. In males, decreases in serum protein occurred at the 5% dose level, in total bilirubin at the 5 and 15% dose levels, in calcium levels at the 1, 5, and 15% dose levels, and a decrease in cholesterol and inorganic phosphate at the 15% dose level. In females, there were decreases in albumin (1 and 5% dose groups) and inorganic phosphate (1% dose group), significant increases in the creatine and sodium levels in the 5% dose group, a significant increase in alkaline phosphatase (ALP) at the 15% dose level, a significant decrease in total protein in the 1, 5, and 15% dose levels, significant decrease in cholesterol at the 1 and 15% dose level, significant decreases in potassium at the 1 and 5% dose levels, and significant decrease in calcium levels in the 1, 5, and 15% dose groups. Although some small decreases in biochemical parameters were observed it is important to note that compared to normal rats on a normal fat diet, the control values were relatively high. As a consequence, the "decreased" values were similar to those observed in rats on a normal diet.

Macroscopic examination revealed an accentuated lobular pattern of the liver in 4 out of 10 male rats in each of the 5 and 15% dose groups, in 3 out of 10 male rats in the 1% dose group, and 3 out of 10 male rats in the control group. In the 1% group, 2 males were reported to have

a pale discoloration of the liver or a tan focus on the right lateral lobe of the liver. These findings were accompanied by minimal to moderate periportal vacuolation in the liver occurring in males in all dose levels. Since these changes occurred in all treated groups at similar incidence and severity, the high fat content of the diet was considered to be responsible for the observed effects. There were no significant changes in absolute and relative organ weights, with the exception of absolute and relative spleen weights, which were significantly decreased in females in the 15% dose group. Minimal to moderate tubular mineralization of the kidneys was reported to occur in females in the control and 15% dose level groups, and was considered to be unrelated to the treatment. No toxicologically significant changes in hematology were reported. The reported changes in the liver, spleen, and kidneys described above were considered to be of no toxicological significance given that there was no treatment-related distribution and that they were within the range of background pathology seen among Wistar rats of this age.

Treatment with Refined Pine Nut Oil up to 15% of the diet was well-tolerated by rats and no treatment-related clinical signs of toxicity, abnormalities in functional observation tests, adverse findings in ophthalmologic examinations, body weight, food intake, hematology, clinical chemistry, macroscopic examinations, and histopathological evaluations occurred. The NOAEL for Refined Pine Nut Oil was the highest concentration tested, 15% of the diet, which corresponds to a mean intake of 8,866 and 10,242 mg/kg body weight/day for males and females, respectively.

IV.D.2 Reproductive and Developmental Toxicity Studies

No studies specific to Lipid Nutrition's Refined Pine Nut Oil were conducted; however, as described in detail in Section IV.B above, Pasquier *et al.* (2001) assessed the effect of feeding a blend of high-oleic sunflower, sunflower, and canola oils or MPNO during pregnancy and lactation to Wistar rats. The adult female rats grew normally, and there were no significant differences in body weights, liver weight to total body weight, daily food intake, litter size, or pup brain weights in rats fed pine nut oil compared to rats fed other oils. Macroscopic examination at necropsy revealed no tissue or organ abnormalities in the mothers or pups. Lactating mothers fed MPNO had a significantly higher leukocyte and platelet count (7.42±1.86 $x10^9$/L and 7.12±0.29 x 10^9/L, respectively) than the control group; however, these values were within the normal range for adult females, and the control group had unusually low leukocyte and platelet counts (3.95±1.17 $x10^9$/L and 6.98±0.25 $x10^9$/L, respectively). No significant differences in other hematological parameters were observed. The authors concluded that the consumption of MPNO had no adverse effects on the mothers' and pups' health, or the levels of n-6 and n-3 long chain PUFAs in rat brain during development.

IV.D.3 Genotoxicity Studies

The mutagenic potential of Refined Pine Nut Oil (PinnoThin™ TG) was assessed in a reverse mutation test in *Salmonella typhimurium* strains TA1535, TA1573, TA98, and TA100 and in *Escherichia coli* strain WP2uvrA (Speijers *et al.*, 2009). The study was performed in compliance with OECD guideline 471 and GLP Guidelines. In an initial dose-range finding test, concentrations of up to 5,000 µg/plate of Refined Pine Nut Oil were tested in strains TA100 and WP2uvrA in the absence and presence of metabolic activation. There were no biologically relevant decreases in the number of revertants and Refined Pine Nut Oil precipitated at doses of 3,300 and 5,000 µg/plate. In the main test, concentrations of 33 to 3,300 µg/plate were tested in strains TA1535, TA1573, TA98, TA100, and WP2uvrA with or without metabolic activation. Refined Pine Nut Oil did not cause any increase in the number of revertants, and, therefore was concluded by the authors to not be mutagenic to *S. typhimurium* and *E. coli* in the reverse mutation assay.

IV.D.4 Other Studies

Other studies conducted with different pine nut oil preparations provide additional support for the safety of Refined Pine Nut Oil; however, these studies were not traditional toxicology studies. In these studies, dietary feeding of up to 20% pine nut oil for periods of 2 weeks to 4 months in mice and rats was well-tolerated and no adverse effects were reported (Sugano *et al.,* 1992, 1994; Matsuo *et al.,* 1996; Asset *et al.,* 1999a,b, 2000, 2001; Lee *et al.,* 2004).

IV.E Human Studies

A limited number of studies examining oral Refined Pine Nut Oil supplementation in humans were identified in the scientific literature (Hughes *et al.*, 2008; Pasman *et al.*, 2008). The majority of the studies did not include assessment of safety-related endpoints, but rather focused on the effects of acute or short-term intake of Refined Pine Nut Oil; however, the information in the human studies corroborates the safety information provided from animal studies.

The effect of Korean pine nut oil on appetite sensations and appetite-regulating hormones was investigated in 18 overweight post-menopausal women (average age of 55 ± 1 years, average body weight of 76.7 ± 1.6 kg, and average Body Mass Index (BMI) of 27.1 ± 0.4 kg/m^2) (Pasman *et al.,* 2008). In a double-blind, placebo controlled study, subjects were randomized (6/group) to receive a single dose of either 3 g of Korean pine nut FFA (free fatty acids), Korean pine nut triglyceride (TG), or olive oil (placebo) (containing 15.2, 15.3, and 0.0% pinolenic acid, respectively) to be taken with a fixed load breakfast. Blood samples were obtained at 0, 30, 60, 90, 120, 180, and 240 minutes after supplementation and the samples were analyzed for satiety hormones, glucose, insulin, FFA, and TG. These procedures were followed for each test substance on 3 separate test days, with a wash out period of one week between treatments.

The satiety hormones cholecystokinin (CCK), glucagon-like peptide-1 (GLP), and peptide YY (PYY) were all increased by Korean pine nut FFA and TG, when compared to olive oil. However, levels of the satiety hormone gherlin were similar among all three treatments. Blood glucose and insulin, and serum FFA demonstrated marginal differences among treatment groups. There were no significant differences in serum TG levels between the three dietary groups. The authors reported that no adverse events were caused by the test substances.

The impact of Refined Pine Nut Oil on food intake and appetite suppression was assessed in a double-blind, placebo-controlled randomized crossover trial (Hughes *et al.,* 2008). Forty-five healthy women aged 18 to 64 (average age 33.8 ± 15.6 years; average weight 73.9 ± 7.0 kg; and average BMI of 27.4 ± 1.3 kg/m^2) were enrolled in the study; however, 3 did not finish the protocol: 2 due to illness not related to the study, and 1 due to child minding difficulties. Subjects ate a fixed load breakfast and dinner at the test facility. The test substance or placebo was given to subjects before lunch, in the form of 6 capsules to be taken at once. The capsules contained 0, 2, 4, or 6 g of refined pine nut oil with 6, 4, 2, or 0 g of olive oil added, respectively. An additional group of capsules was included which contained 2 g of Refined Pine Nut Oil FFA and 4 g of olive oil. The protocol was repeated for each experimental condition with a one-week washout period separating each procedure. The amount of food and water consumed was measured before and after meals and visual analogue scales were used to rate hunger, fullness, satisfaction, desire to eat, thirst, and nausea.

Food intake was decreased in groups consuming Refined Pine Nut Oil FFA when compared to placebo. There were no significant differences among groups in the subjects' rating in the visual analogue scales among the experimental conditions. There were no signs or symptoms of serious adverse events during the 5-week study period. Four days after the last treatment, one participant reported blotchiness of the hands, but after un-blinding it was revealed the participant was in the placebo group and the effect was not related to the test substances. The authors concluded that Refined Pine Nut Oil in the FFA and TG form was well-tolerated.

A traditional Korean herbal formula (KH), containing *P. koraiensis* nut was given to 22 obese children (8 female and 14 male; average age of 11 ± 2.6 years; and average weight of 53.37 ± 17.29 kg) daily for a 30-day period in a weight loss study (Yoo *et al.*, 2005). *P. koraiensis* was included in the formula at a level of 8 g out of a total of 76 g, and 152 g of this formula was boiled and concentrated to a 350 mL decoction and administered to the children 3 times a day. There was no control group included in this study on the basis that it was a pilot study, and instead each subject acted as their own control, with the results obtained after 30 days of treatment compared to the initial values recorded at baseline. The subjects were children admitted to the hospital for treatment of obesity with relative body weight of 20% or more. Anthropometric parameters, blood pressure, pulse rate, EKG, chest radiography, and serum liver and kidney were evaluated prior to KH administration, after 15 days of treatment, and after 30 days of treatment. Additionally, blood samples were taken at these time points and serum

lipid, insulin, glucose, and leptin levels were measured. The study originally contained 31 subjects, but 8 dropped out for personal reasons, and 1 patient from nasal bleeding. Minor effects including loss of appetite, increased urinary frequency, insomnia, and nasal bleeding were reported to occur. Loss of appetite and urinary frequency was reported to return to normal after 1 week, and the insomnia symptoms were absent with a decrease in the dose. The authors stated that these effects were thought to be caused by another component of KH and not due to Korean pine. Changes in anthropometric parameters included significant increase in height, decrease in the average weight, and reductions in skin-fold thickness over the 4-week study period. Serum cholesterol and atherogenic index significantly decreased, and no significant effect on pulse rate or blood pressure was observed. Aspartate aminotransferase levels decreased significantly after treatment, when compared to baseline values, but no changes were observed in the kidney function tests. The authors concluded the Korean herbal mixture was safe at 456 g/day when administered to children for weight loss for 30 days.

IV.F Summary and Basis for GRAS Conclusion

The GRAS determination for the use of Refined Pine Nut Oil as a food ingredient is based on scientific procedures. Refined Pine Nut Oil is proposed for use as an ingredient in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, dairy product analogs, fats and oils, grain products and pasta, milk and milk products, nuts and nut products, processed fruit and fruit juices, processed vegetables and vegetable juices, snack foods, soft candy, and soups and soup mixes, at a level of 3.0 g/serving. Under the intended conditions of use, the estimated all-user mean and 90th percentile intakes of supplemental Refined Pine Nut Oil in the total population are 8.9 and 17.8 g/day, respectively (150 and 327 mg/kg body weight/day, respectively).

Refined Pine Nut Oil is produced in accordance with cGMP and meets appropriate food-grade specifications. Refined Pine Nut Oil is produced from cold-pressed pine nut oil that is subjected to refining through deacidification, purification, deodorization, and decolorization steps. The resulting product consists of a mixture of triglycerides of which the major fatty acid components are oleic, linoleic and pinolenic acid. Lipid Nutrition has established chemical and microbiological specifications consistent with other food-grade materials. Lot samples are routinely evaluated to verify compliance with the specifications.

The safety of Refined Pine Nut Oil under the intended conditions of use is supported by the available toxicological and human studies, including studies conducted with Lipid Nutrition's Refined Pine Nut Oil product (detailed in Sections IV.C through IV.E). In a 13-week rat toxicity study, the highest level studied for Lipid Nutrition's Refined Pine Nut Oil product, 15% mixed into the diet, was established as the NOAEL. This NOAEL corresponded to a daily intake of 8.9 and 10.2 g Refined Pine Nut Oil/kg body weight/day for female and male rats, respectively.

The information in the human studies corroborates the safety information provided from animal studies. Human studies in which subjects were supplemented with a single dose of 2 to 6 g of Refined Pine Nut Oil revealed that the intervention was well-tolerated, with no significant adverse effects or changes in blood glucose and insulin, and serum TG (Hughes *et al.*, 2008; Pasman *et al.*, 2008).

The data provided support the conclusion that the consumption of Refined Pine Nut Oil under the intended conditions of use would not be expected to produce adverse effect in consumers.

The Expert Panel convened on behalf of Lipid Nutrition, independently and collectively, critically evaluated the data and information summarized above and concluded that the proposed uses of Refined Pine Nut Oil, produced consistently with cGMP and meeting appropriate food grade specifications described herein, are safe and suitable. Furthermore, the Expert Panel unanimously concluded that the intended uses of Refined Pine Nut Oil are Generally Recognized as Safe (GRAS) based on scientific procedures. It is also Lipid Nutrition's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion.

Based on scientific procedures, Refined Pine Nut Oil is GRAS under the intended conditions of use as a food ingredient described herein and therefore, Refined Pine Nut Oil is exempt from the definition of a food additive and thus may be marketed and sold for the uses designated above in the U.S. without the promulgation of a food additive regulation under 21 CFR.

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Table of CFR Sections Referenced (Title 21—Food and Drugs)		
Part	**Section §**	**Section Title**
101—Food labeling	101.12	Reference amounts customarily consumed per eating occasion
170—Food Additives	170.30	Eligibility for classification as generally recognized as safe (GRAS).
177—Indirect food additives: Polymers	177.2260	Filters, resin-bonded
182—Substances generally recognized as safe	182.20	Essential oils, oleoresins (solvent-free), and natural extractives (including distillates)
	182.3890	Tocopherols
184—Direct food substances affirmed as generally recognized as safe	184 1033	Citric acid
	184.1155	Bentonite
	184.1763	Sodium hydroxide

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APPENDIX A

EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF REFINED PINE NUT OIL FOR USE AS AN INGREDIENT IN FOOD

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Refined Pine Nut Oil for Use in Food

December 17, 2009

INTRODUCTION

At the request of Lipid Nutrition B.V. (Lipid Nutrition), an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether the intended uses of refined pine nut oil as a food ingredient are safe and suitable and would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Panel consisted of: Professor Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Professor Robert J. Nicolosi, Ph.D. (University of Massachusetts Lowell), and Professor John A. Thomas, Ph.D. (Indiana University School of Medicine).

The Panel, independently and collectively, critically examined a comprehensive package of scientific information and data on refined pine nut oil from the literature and other published sources through October 2009. This information was presented in a dossier [Documentation Supporting the Evaluation of Refined Pine Nut Oil as Generally Recognized as Safe (GRAS) for Use in Food] that was submitted by Lipid Nutrition to the Panel. In addition, the Panel evaluated other information deemed appropriate or necessary. The information evaluated by the Panel included details pertaining to the method of manufacture and product specifications, supporting analytical data, intended use-levels in specified food products, consumption estimates for all intended uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of refined pine nut oil.

Following independent, critical evaluation of such data and information, the Panel convened on December 17, 2009 and unanimously concluded that the intended uses in traditional foods described herein for refined pine nut oil, meeting appropriate food-grade specifications as described in the supporting dossier and manufactured according to current Good Manufacturing Practice (cGMP), are safe and suitable and GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

SUMMARY

Refined Pine Nut Oil, trade named PinnoThin™ TG, is obtained from Korean pine (*Pinus koraiensis*) and consists of a mixture of triglycerides of which the major fatty acid components are oleic, linoleic and pinolenic acid. The levels of oleic and linoleic acid in Refined Pine Nut Oil are comparable to the range of oleic and linoleic acid levels reported in the CODEX standards for several vegetable oils (*e.g.,* canola, olive oil, high oleic safflower oil, and high oleic sunflower oil). Since these food oils are widely consumed in the diet under uses that are comparable to the intended uses of the refined pine nut oil, adverse toxicological effects are not expected.

Refined pine nut oil is intended for use as a food ingredient at a level of 3.0 g/serving in selected baked goods and baking mixes, beverages and beverage bases, breakfast cereals, dairy product analogs, fats and oils, grain products and pasta, milk and milk products, nuts and nut products, processed fruits and fruit juices, processed vegetables and vegetable juices, snack foods, soft candy, and soup and soup mixes. Estimates of the intake of refined pine nut oil were based on the proposed food-uses and use-levels in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2005-2006 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2009). Under the conditions of intended use, mean and 90th percentile total population all-user intakes of 8.9 and 17.8 g/day were estimated. On a body weight basis, these intakes correspond to 150 and 327 mg/kg body weight/day, respectively. Historical use of pine nut oil in foods such as traditional pesto sauce at levels up to 7.5 g pine nut oil per day is not associated with any history of adverse events. Since pine nuts are often used in a variety of foods, including bread, ice cream, and cookies, exposure might exceed these estimates.

A 13-week dietary study conducted in Wistar rats was considered pivotal to the safety of refined pine nut oil (Speijers *et al.*, 2009). When administered at a concentration of up to 15% in the diet, refined pine nut oil was well tolerated, with no clinical signs of toxicity, nor any test article-related abnormalities in functional observation tests, ophthalmologic examinations, body weight, food intake, hematological and clinical chemistry parameters, and macroscopic and histopathological analysis. The NOAEL for refined pine nut oil was the highest level tested, 15% of the diet, which corresponds to a mean intake of 8,866 and 10,242 mg/kg body weight/day for males and females, respectively. In addition, refined pine nut oil was not mutagenic in a reverse mutation assay (Speijers *et al.*, 2009). In human studies in which subjects received a single dose of 2 to 6 g of refined pine nut oil, no significant adverse effects or changes in blood glucose and insulin, and serum triglycerides were reported (Hughes *et al.*, 2008; Pasman *et al.*, 2008).

The scientific evidence examined by the Panel demonstrates that under the conditions of intended use, refined pine nut oil would not produce any adverse health effects in consumers.

CONCLUSION

We, the Expert Panel, have, independently and collectively, critically evaluated the data and information summarized above and conclude that the intended uses of refined pine nut oil, meeting appropriate food-grade specifications presented in the supporting dossier [Documentation Supporting the Evaluation of Refined Pine Nut Oil as Generally Recognized as Safe (GRAS) for Use in Food] and produced consistent with current Good Manufacturing Practices (GMP), are safe and suitable.

We further conclude that the intended uses of refined pine nut oil, meeting appropriate food-grade specifications presented in the supporting dossier and produced consistent with current GMP, are Generally Recognized as Safe (GRAS) based on scientific procedures.

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

(b) (6)

Professor Joseph F. Borzelleca, Ph.D.
Virginia Commonwealth University School of Medicine

20 January 2010
Date

(b) (6)

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

01, Jan 18, 2010
Date

(b) (6)

Professor John A. Thomas, Ph.D.
Indiana University School of Medicine

Jan 19, 2010
Date

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APPENDIX B

BATCH ANALYSES

Chemical and Microbiological Specifications: Analytical Data				
Parameter	**Specification**	**Analytical Results**		
		Batch 7293	**Batch 7334**	**Batch 7365**
Appearance	Pale Yellow Liquid	Conforms	Conforms	Conforms
Free Fatty Acids (as % oleic acid)	Max. 1.0%	0.26	0.18	0.16
Peroxide Value	Max. 5.0 meqO_2/kg	0.2	0.4	0.1
Total Saturated Fatty Acids	Max. 10%	7.0	7.0	7.1
Total Monosaturated Fatty Acids	Min. 24%	26.2	26.1	26.9
Oleic Acid (C18:1, n-9)	Min 22%	24.8	24.7	25.5
Total Polyunsaturated Fatty Acids	Min. 64%	65.8	65.2	65.0
Linoleic Acid (C18:2, n-6)	Min. 44%	45.0	44.6	45.1
Pinolenic Acid (C18:3, *c*5,*c*9,*c*12)	Min. 14%	15.8	15.4	15.0
Trans fatty acids	Max. 1%	0.2	0.4	0.3
Lead (Pb)	Max. 0.1 mg/kg	<0.06	<0.05	<0.05
Arsenic (As)	Max. 0.1 mg/kg	<0.10	<0.10	<0.10
Total aerobic count	<1,000 cfu/g	70	<10	<10
Yeasts	<10 cfu/g	<10	<10	<10
Moulds	<10 cfu/g	<10	<10	<10
Escherichia coli	< 10 cfu/g	<10	<10	<10
Salmonella	Absent in 25 g	ND	ND	ND
Enterobacteriaceae	<10 cfu/g	<10	<10	<10

cfu = colony-forming units; ND = not detected

APPENDIX C

STABILITY DATA

LIPID NUTRITION

MEMO

Wormerveer, 3 May 2007

From: Patrick van der Waal Extension: 2240

Ref: PWa

To: CRa, AH, EM

Stability data PinnoThin TG

PRELIMINARY DATA

Introduction

On lab scale a batch Pinnothin TG was produced with an anti-oxidant system consisting of 1000 ppm mixed tocopherols and 2500 ppm Rosemary extract (AOX system 2).

The stability of this batch is investigated by following the PV development at 40°C under air and by measuring Rancimat induction point at 100°C. The results are compared with PinnoThin TG with an anti-oxidant system consisting of 250 ppm mixed tocopherols (AOX system 1).

The shelf life of PinnoThin TG with 250 ppm mixed tocopherols was already investigated at 25 and 40°C under nitrogen.

Results

The PV development at 40°C under air of PinnoThin TG with AOX system 1 and 2 is given in figure 1. The Rancimat data of these batches is given in figure 2. From these figures it is clear that the PinnoThin TG with 1000 ppm mixed tocopherols and 2500 ppm Rosemary extract is more stable than the PinnoThin with only 250 ppm mixed tocopherols.

In figures 3 to 6 the results of the shelf life study of 3 batches PinnoThin TG with AOX system 1 is given. The stability is followed for 15 months at 25 (normal) and 40°C (accelerated) under nitrogen. Under these conditions only a small increase of the PV is noticed and the increase is not depending on the temperature. The AV is not increased during 15 months. The pinolenic acid content is stable under the tested conditions and the colour of the product did not change.

Conclusions

Based on these data we concluded that the PinnoThin TG with 1000 ppm mixed tocopherols and 2500 ppm Rosemary extract (AOX system 2) will be stable for at least 18 months stored at 10-20°C packed under nitrogen.

LIPID NUTRITION

Figure 1: PV development of PinnoThin TG at 40°C under air

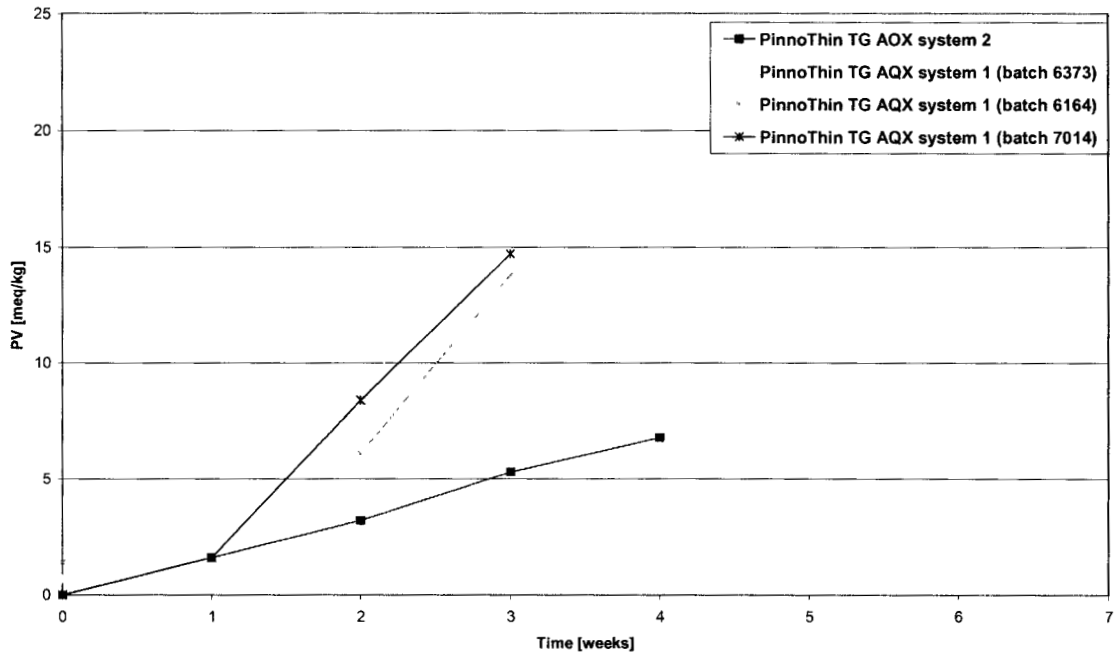


Figure 2: Rancimat stability measured at 100°C

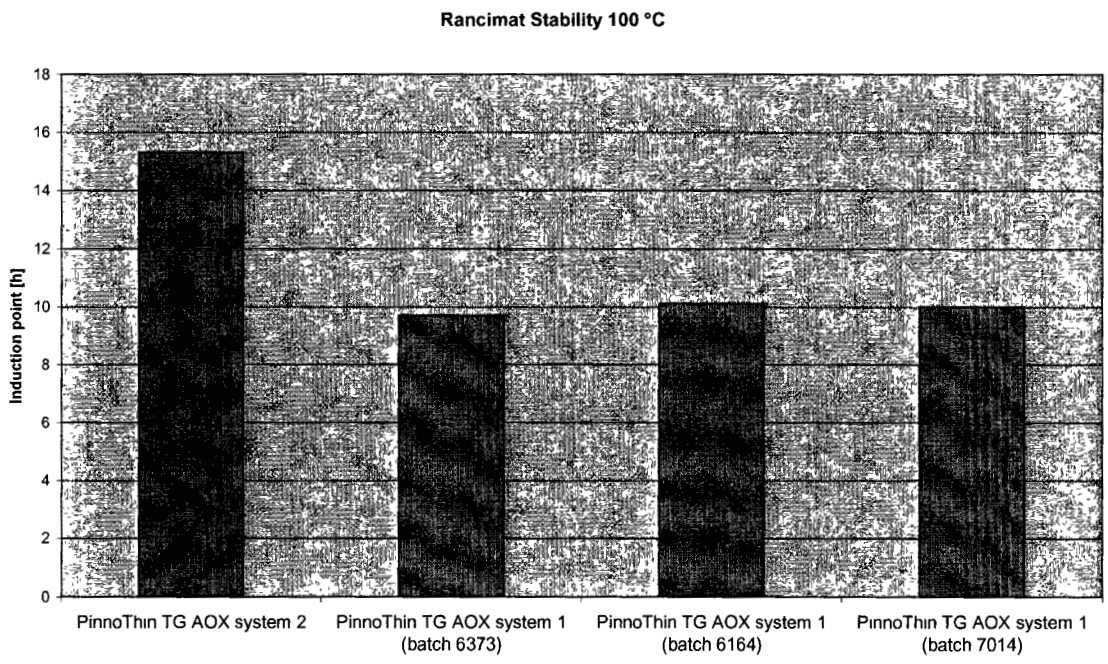


Figure 3: PV development of PinnoThin TG AOX system 1 at 25 and 40°C under nitrogen.

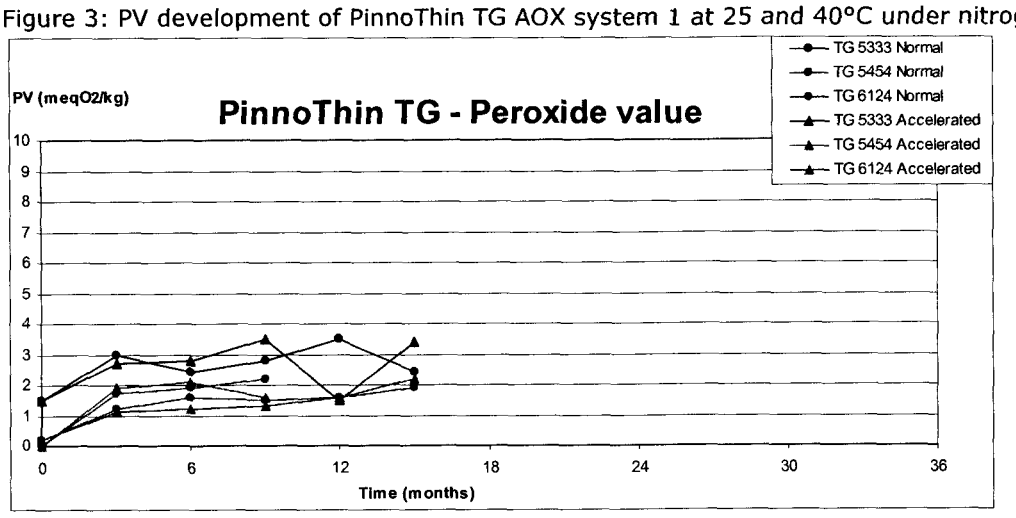


Figure 4: AV development of PinnoThin TG AOX system 1 at 25 and 40°C under nitrogen.

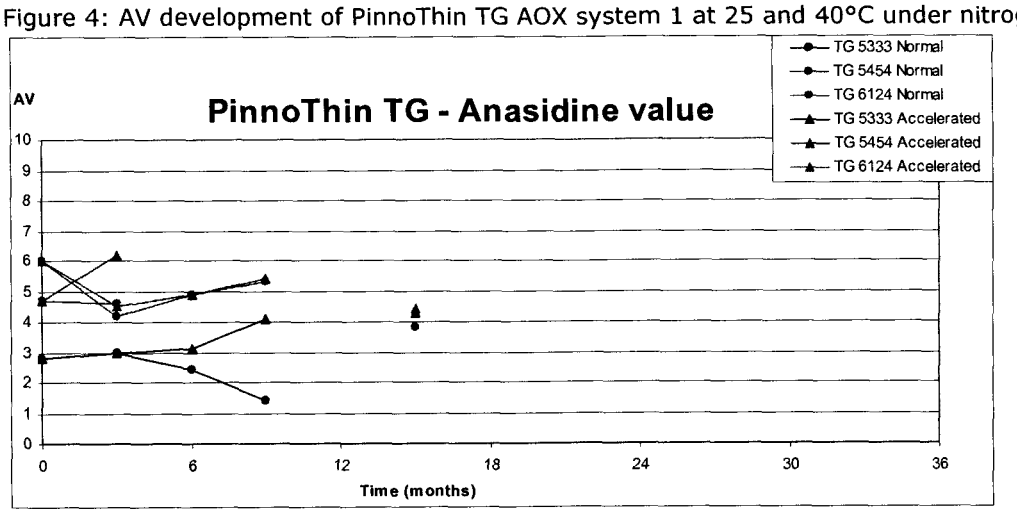
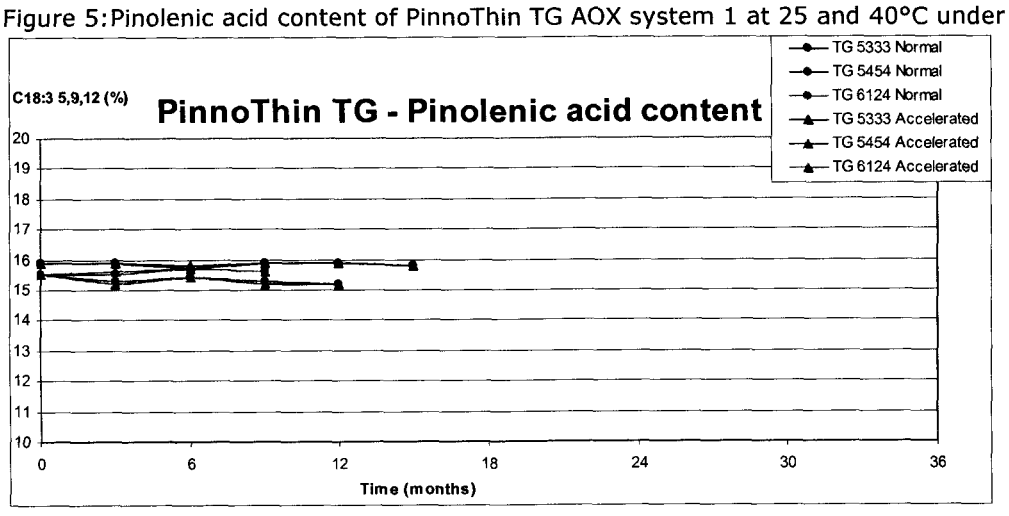
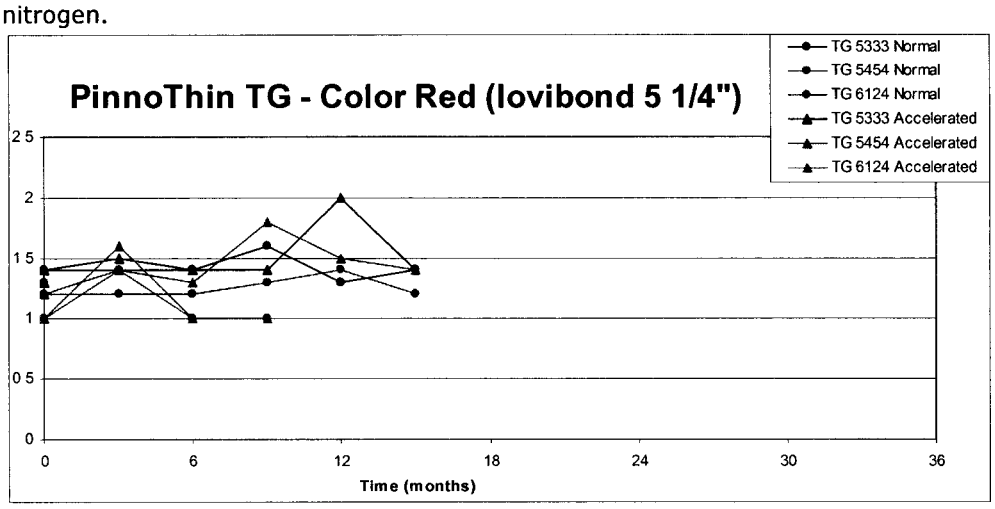


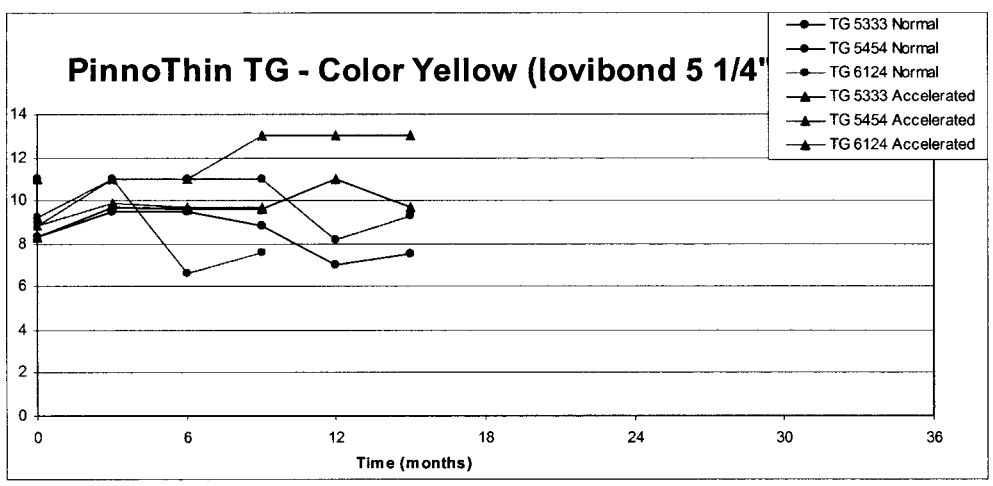
Figure 5:Pinolenic acid content of PinnoThin TG AOX system 1 at 25 and 40°C under nitrogen.



LIPID NUTRITION

Figure 6A and B: Colour changes of PinnoThin TG AOX system 1 at 25 and 40°C under nitrogen.





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