ORIGINAL SUBMISSION

GRN 000494

AIBMR
Life Sciences, Inc.

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BY:

Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
5100 Paint Branch Parkway (HFS-200)
College Park, MD 20740-3835

December 16, 2013

ATTN: Dr. Antonia Mattia, PhD

Our Reference: GRAS Notification and Exemption Claim for Provinal™, a blend of purified edible fatty acid esters

Dear Dr. Mattia,

AIBMR Life Sciences, Inc. has been retained as an agent by Tersus Pharmaceuticals, LLC. ('the Notifier') to submit a GRAS notification to the FDA for Provinal™, a blend of purified edible fatty acid esters, derived from anchovy or menhaden oil, standardized to approximately 50% palmitoleic acid (C16:1) and not more than 1% palmitic acid, intended for use as an ingredient in food. Provinal™ is therefore exempt from the requirement of pre-market approval, consistent with section 201 (s) of the Federal Food, Drug and Cosmetic Act.

The GRAS determination is based on scientific procedures. The basis for the determination relies on the GRAS status and worldwide consumption profiles of the edible fatty acids, which comprise Provinal™. The fatty acids in Provinal™ are ubiquitous in the diet, and undergo catabolism in the body via well-known beta-oxidation reactions.

Please find enclosed one hard copy of the notification: *Notice to US Food and Drug Administration that the use of* Provinal™ *is Generally Recognized as Safe.* On the enclosed disc, you will find an e-copy of the notification plus all cited references. As stated in the exemption claim, the data and the information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Tersus Pharmaceuticals, LLC (387 Golf View Lane, Suite 201, Cleveland, OH 44143) or will be sent to FDA upon request. Please do not hesitate to contact us with any questions.

Yours sincerely,

(b) (6)

John R. Endres
Chief Scientific Officer
AIBMR Life Sciences, Inc.

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Notice to US Food and Drug Administration that the use of Provinal™ is Generally Recognized as Safe

Submitted by the Notifier:

Tersus Pharmaceuticals, LLC
387 Golf View Lane, Suite 201
Cleveland, OH 44143

Prepared by the Agent of the Notifier:

AIBMR Life Sciences, Inc.
4117 S Meridian
Puyallup WA 98373

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GRAS Exemption Claim

Tersus Pharmaceuticals, LLC (the notifier) has determined that Provinal™ is Generally Recognized as Safe (GRAS) for its intended use, consistent with section 201(s) of the *Federal Food, Drug and Cosmetic Act*. The determination has been made based on scientific procedures, and, therefore, the use of Provinal™ for its intended purpose is exempt from the requirement of pre-market approval.

(b) (6)

[redacted] 11/25/13

Lochlainn O' hAimhirgin
President
Tersus Pharmaceuticals, LLC

Date

Name and Address of the Notifier

Notifier

Tersus Pharmaceuticals, LLC
387 Golf View Lane, Suite 201
Cleveland, OH 44143

Agent of the Notifier

John R. Endres, ND
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Common or Usual Name

Blend of purified edible fatty acid esters, derived from anchovy or menhaden oil, standardized to approximately 50% palmitoleic acid (C16:1) and not more than 1% palmitic acid.

Conditions of Use

Provinal™ is intended for use as a replacement source of edible fatty acids in food categories found in 21 CFR 170.3 (n), consistent with current Good Manufacturing Practices (cGMPs), where standards of identity allow. Provinal™ is not intended for use in infant formula, meat, egg, catfish or any products that would require additional regulatory review by USDA.

Basis for GRAS determination

Scientific procedures are the basis for the conclusion that the intended use of Provinal™ as an ingredient in food is GRAS.

Data/Information Availability Statement

The data and the information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Tersus Pharmaceuticals, LLC (387 Golf View Lane, Suite 201, Cleveland, OH 44143) or will be sent to FDA upon request.

Characterization

The subject of this GRAS determination is Provinal™, sold by Tersus Pharmaceuticals, LLC (387 Golf View Lane, Suite 201, Cleveland, OH 44143). Provinal™ is a blend of purified edible fatty acid esters, derived from anchovy or menhaden oil, with the predominant fatty acid as palmitoleic acid (C16:1), a monounsaturated omega-7 fatty acid. Provinal™ is standardized to its palmitoleic acid content at a 50:1 ratio of palmitoleic acid to palmitic acid. Provinal™ has little to no eicosapentanoic (EPA) or docosahexaenoic (DHA) acid. A typical fatty acid profile of Provinal™ is represented in Table 1. The fatty acid percentages may vary slightly depending on the crude oil source (anchovy versus menhaden), however, the palmitoleic and palmitic acid concentrations are controlled.

Table 1. Typical Fatty Acid Profile of Provinal™

Typical Fatty Acid Profile of Provinal™	
C14:0 Myristic	1.59%
C15:0 Pentadecanoic	0.33%
C15:1 Pentadecanoic	0.13%
C16:0 Palmitic	0.44%
C16:1n7 Palmitoleic	53.04%
C17:0 Margaric	0.27%
C17:1 Margaroleic	0.42%
C18:0 Stearic	0.04%
C18:1 Oleic	13.49%
C20:1 Gadoleic	0.36%
C18:2n6 Linoleic	2.19%
C18:3n3 Alpha-Linolenic	1.06%
C18:3n6 Gamma-Linolenic	0.44%
C18:4n3 Stearidonic	3.28%
C20:2n6 Auricolic	0.26%
C20:5n3 Eicosapentanoic	0.16%
C22:6n3 Docosahexaenoic	0.00%
Other Fatty acids	22.51%

Palmitoleic acid

Palmitoleic acid (9-hexadecenoic acid) is a monounsaturated sixteen-carbon fatty acid (C16:1Δ9) discovered after its isolation from cod liver oil and herring oil in 1906.[1] It is considered an omega-7 (n-7) fatty acid, indicating where the double bond is located counting from the terminal methyl end of the fatty acid carbon chain. Both cis and trans isomers of palmitoleic acid (*cis*-C16:1n-7 and *trans*-C16:1n-7) are naturally occurring although only the *cis* form is found in Provinal™ (Figure 1).

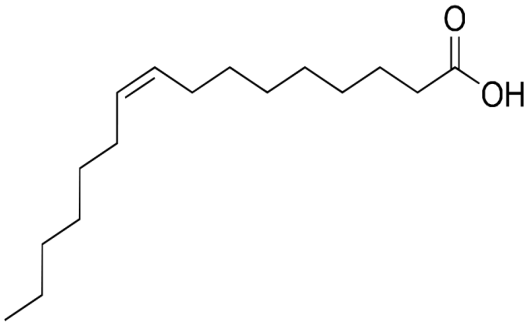


Figure 1. Chemical structure of *cis*-palmitoleic acid free fatty acid

Provinal™ contains ethyl ester forms of fatty acids isolated from anchovy or menhaden oil. Fats and oils from natural sources are predominantly found in the triglyceride form. Triglycerides, or triacylglycerols, found in both animal and plant-derived fats and oils, are comprised of three fatty acids (which can be saturated, monounsaturated, or polyunsaturated) esterified to a glycerol backbone. Fatty acid ethyl esters, on the other hand, are formed when fatty acids are trans-esterified, whereby the glycerol backbone of a triglyceride is removed and substituted with ethanol (see Manufacturing Overview).

Endogenous Palmitoleic acid

Palmitoleic acid is an important product of endogenous lipogenesis within the human body. Stearoyl-coenzyme-A desaturase (SCD) enzymes are involved in the synthesis of monounsaturated fatty acids such as palmitoleic (16:1) and oleic (18:1) acids, which are derived from the saturated fatty acid SCD substrates, palmitic (16:0) and stearic acid (18:0), respectively. These unsaturated fatty acid SCD products become substrates for the formation of triglycerides.[2]

The desaturase enzymes that make palmitoleic acid have a weak affinity for the precursor palmitic acid and preferential affinity for stearic acid; thus, only small amounts of palmitoleic acid are generally found in the body.[3, 4] Often, palmitoleic acid is converted into several other members of the omega-7 family, such as

palmitolinoleic acid (16:2n7) and rumenic acid (18:2n7) and is, therefore, considered a "parent" omega-7 fatty acid.[5]

Palmitoleic acid can be found in the plasma, lipids, red blood cell phospholipid bilayer, adipose tissue, brain, retina and viscera of most, if not all, mammals.[3, 5-10] It is also found in human breastmilk where it is estimated to make up 2% of the total fatty acid composition.[11] Palmitoleic acid is primarily synthesized in the liver. There it is used in the formation of triglycerides, packaged in very low-density lipoprotein (VLDL) and secreted into the blood.[2] Adipose and liver tissue exhibit the highest concentration of palmitoleic acid found in the human body, and it appears to be a highly regulated fatty acid in adipose tissue, affecting systemic metabolism.[12] Circulating palmitoleic acid is thought to be a fatty acid regulator and/or lipokine (lipid hormone) that is directly involved in the regulation of lipid and glucose metabolism.[7, 12]

ADME

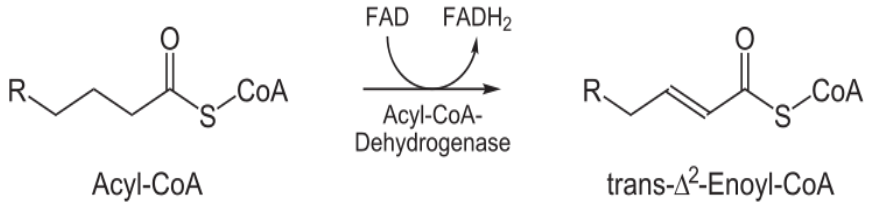
Fatty acid ethyl esters, when ingested, are emulsified by peristalsis and bile salts during digestion. They are also hydrolyzed by pancreatic lipase. This hydrolysis frees the free fatty acids and yields ethanol. The ethanol released in this process is considered insignificant. One study reported a theoretical yield of 1.3 grams of ethanol from the ingestions of 8 grams of ethyl oleic acid, a similar percentage as that found in common foods such as vanilla ice cream and orange juice.[13] The free fatty acids, once released from ethanol, must then obtain a glycerol molecule within the enterocyte to become transformed into a triglyceride.

Triglycerides are then packaged into chylomicrons, fat carrier molecules that transport the triglycerides into the lymphatic channel and subsequently into the blood. It has been demonstrated that although the hydrolysis of ester forms of fatty acids may occur at a slower rate than hydrolysis of triglyceride forms, the uptake of fatty acids into chylomicrons is similar when the fatty acids are given in either form.[14, 15]

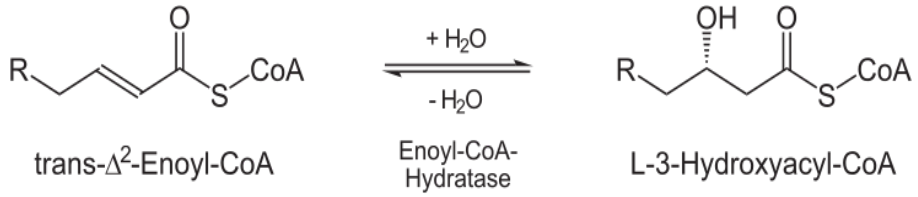
From the bloodstream, the fates of fatty acids are several. These include oxidation, conversion into other fatty acids, incorporation into cell membranes as part of phospholipids, or storage.[16]

Fatty acids are further catabolized in the mitochondria by a process called beta-oxidation, which is the sequential removal of two-carbon units by oxidation at the β-carbon.[17] The end result is the formation of acetyl-CoA, which is then used in the citric acid cycle to form ATP. The β-oxidation process for saturated fats begins with the linkage of the fatty acid to coenzyme A to form an acyl-CoA fatty acid. This is followed by a recurring sequence of four reactions, which are listed below. These four reactions lead to the removal of two carbon atoms and the generation of $FADH_2$, NADH, and acetyl-CoA.

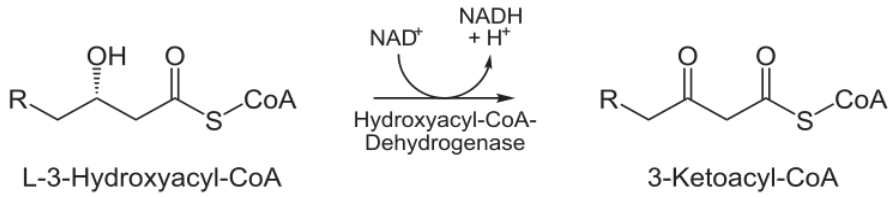
1. Oxidation by flavin adenine dinucleotide (FAD). A fatty acyl-CoA is oxidized by acyl-CoA dehydrogenase. This leads to the formation of an enoyl CoA with a *trans* double bond between C2 and C3 (*trans*-Δ2-enoyl CoA) and the release of $FADH_2$.



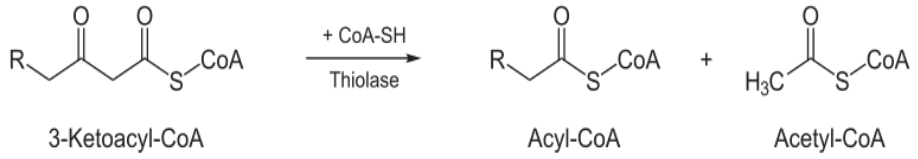
2. Hydration of the double bond of *trans*-Δ2-enoyl CoA between C2 and C3 by enoyl-CoA hydratase, resulting in L-β-3-hydroxyacyl-CoA.



3. Oxidation (by NAD^+) by hydroxyacyl-CoA dehydrogenase, which converts the hydroxyl group at C3 into a keto group (β-ketoacyl-CoA) and the release of NADH.



4. Cleavage (thiolysis) (catalyzed by acyl-CoA acetyl-transferase), which leads to the separation of the 3-ketoacyl CoA by the thiol group of a second CoA molecule (CoA-SH) to yield acyl-CoA.



Unsaturated fatty acids, such as palmitoleic acid (C16:1) and oleic acid (C18:1), undergo the same reactions described above until the double bond is reached (step 2). At that point, depending on the original location of the double bond (at odd or even carbon from terminal methyl end), either an isomerase reaction or a reductase and an isomerase reaction are required.

Odd chain fatty acids undergo the same oxidation reactions as even chain fatty acids until the formation of propionyl CoA; at which point propionyl CoA is converted to succinyl CoA which enters the citric acid cycle; this reaction requires vitamin B12 as a cofactor.

Palmitoleic acid undergoes three cycles of beta-oxidation to yield Δ3-*cis* enoyl CoA, a 10-carbon compound. Because the double bond is *cis,* the enoyl-CoA-hydratase (step 2) cannot act upon it. Therefore, an isomerase changes the Δ3-*cis* double bond to a Δ2-*trans* double bond. Beta-oxidation of the fatty acid then continues as it would with a saturated fatty acid.

Palmitoleic acid is thought to be readily absorbed, and consuming foods rich in palmitoleic acid causes a significant increase in plasma palmitoleic acid. One study indicated that a 4% increase of energy consumed (calories) in dietary palmitoleic acid content equated to a 60% increase in plasma palmitoleic acid.[18]

In a study in humans, consumption of a bolus of palmitic acid (11.9 g) resulted in a small but rapid conversion to palmitoleic (<2%) and stearic acids (6%). Consumption of 789 mg palmitoleic acid at breakfast resulted in "enrichment" above baseline of plasma phosphatidylcholine palmitoleic acid levels in men and women, which remained elevated for 21 days. The highest concentrations were seen from 24–72 hours after ingestion. Beta-oxidation (catabolism) of alpha linolenic acid (18:3n-3—an omega-3 fatty acid) also results in the formation of palmitoleic acid in humans.[3]

History of Consumption

Provinal™ is derived from the oil of menhaden or anchovies, which have a long history of use in the human diet.

Typical total daily intake of fat/oil (and hence edible fatty acids) by humans is approximately 72 grams, of which approximately 26 grams is oleic acid.[13] Some have suggested that typical intakes of oleic acid are up to 35 g/day.[19] Oleic acid is the most widely distributed fatty acid in nature. Levels of oleic acid in animal and vegetable oils are in the 30–50% range, and are as high as 80% in olive oil. A 1997 meta-analysis demonstrated the wealth of data on the use and safety of edible oils in a review of 36 studies, containing over 500 subjects that consumed an average of 10–12 grams per day of edible fatty acids for a mean duration of 7–10 weeks. The most common test article was a high EPA fish oil and the most common placebo was olive oil, however, other oils consumed included corn, safflower, linseed, etc.; oils that contain many similar fatty acids as Provinal™. No adverse events were reported.[19]

Palmitoleic acid is found in a variety of food products, oils derived from animal products and agricultural products, including marine and tropical oils, as well as nuts and seeds. Therefore, it has a significant history of consumption in the human diet. It is considered the second most common *cis*-configured monounsaturated fatty acid in daily nutrition.[2 20]

Macadamia nuts and their oil contain one of the highest percentages of palmitoleic acid.[21] From one serving size of macadamia nuts (10–12 nuts or 28.35 g) an individual would consume approximately 3.7 g of palmitoleic acid.[22] Based

on National Health and Nutrition Examination Surveys, approximately one third of Americans have been reported to consume nuts on a daily basis (averaging 21 g/d and up to 57 g/day).[23] Fish and fish oils also contain a broad spectrum of fatty acids, including omega-7 fatty acids. Herring, mackerel, mullet, anchovies and salmon are examples of fish known to contain the highest levels of palmitoleic acid. One serving of anchovies (3 oz) contains 0.34 g of palmitoleic acid.[22] However, this quantity is small compared to a serving of macadamia nuts. Palmitoleic acid is also found in plants of the *Brassica* family at levels ranging from 0.7% to 0.31% of fatty acid composition.[5] Sea buckthorn oil (*Hippophae rhamnoides*), a common Chinese herbal medicine, whose medicinal use dates back to 618 AD, is also known to be high in palmitoleic acid.[24] Sea buckthorn pulp oil can contain up to 39% palmitoleic acid.[25, 26] Palmitoleic acid concentrations are also highly significant in certain yeasts, algae and cyanobacteria that are consumed by humans, representing up to 56.9% of the total fatty acid content in these organisms.[27, 28]

Many other edible oils contain palmitoleic and oleic acids, the predominant fatty acids in Provinal™ products (Table 2). Of the edible oils, macadamia nut oil is closest in its concentration of palmitoleic acid to Provinal™, although it has a higher oleic acid concentration. Several studies have indicated that macadamia nuts can positively affect lipid levels by lowering cholesterol, particularly in comparison with a diet higher in saturated fats.[21, 29-32] Tree nuts, including macadamia nuts, have been associated with a wide range of health benefits, and in 2003, the FDA released an accepted qualified health claim for nuts: "Scientific evidence suggests but does not prove that eating 1.5 ounces per day of most nuts [such as *name of specific nut*], as a part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. [See nutrition information for fat content]." In general, consumption of oils rich in monounsaturated fatty acids, such as oleic and palmitoleic acids, are thought to offer greater health benefits when compared to other dietary fatty acids. The American Heart Association (AHA) and USDA Dietary Guidelines for Americans recommend that the majority of fatty acids in the diet should be monounsaturated or polyunsaturated.

Table 2. Approximate levels of Palmitoleic in Edible Oils*

Provinal™	50%
Macadamia nut oil	17%
Herring oil	9.6%
Cod liver oil	8%
Salmon oil	5%
Olive oil	1%

*Data from USDA Nutrient Database, Maguire et al. 2004,[33] Nestel et al. 2009,[18] and Shahidi and Miraliakbari 2005.[34]

Manufacturing and Production

Company Overview

Tersus Pharmaceuticals is focused on the development of "ultrapurified" fatty acid ester blends, with special interest in palmitoleic acid. Tersus was the recipient of a Qualifying Therapeutic Discovery Project (QTDP) Grant from the NIH/HHS in January 2011 supporting further research and development of this product.

Raw Materials

The palmitoleic acid (omega-7) ethyl ester is derived from either anchovy or menhaden oil.

Manufacturing Overview

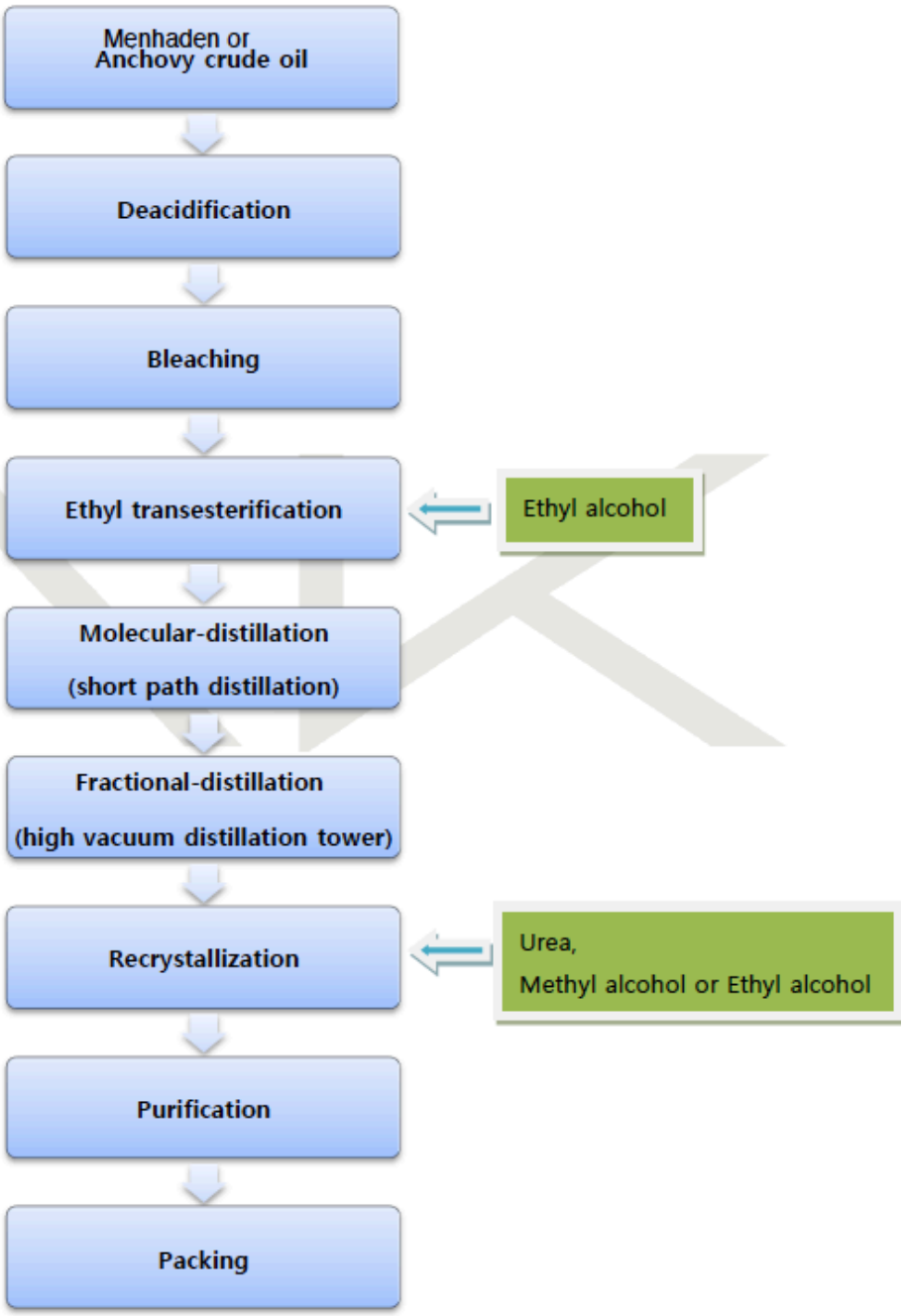


Figure 2. Manufacturing flow chart

Good Manufacturing Practice

Provinal™ Ethyl Ester (EE) products from Tersus Pharmaceuticals are produced under strict adherence to Good Manufacturing Practice (GMP) requirements (as specified in 21 CFR 110 for its intended use as an ingredient added to food) and its production facility in Ulsan, Korea has been certified by the Korean Food and Drug Administration.

Specifications

Table 3. Specifications of Provinal™

	Method	Provinal™ Specifications
Appearance:	Visual	Clear liquid
Odor:	Sensory	Very light odor
Color	Gardner	NMT 6
Palmitoleic EE Concentration (C16:1n7)	AOCS Ce 1b-89	NLT 500 mg/g
Palmitic acid ethyl ester (C16:0)	AOCS Ce 1b-89	NMT 10 mg/g
Acid Value	AOCS Cd 3d-63	NMT 2.0 mg KOH/g
Peroxide Value	AOCS Cd 8-53	NMT 5.0 meq/kg
p-Anisidine	AOCS Cd 18-90	NMT 15.0
TOTOX	Calculation	NMT 25.0
Moisture	Karl Fisher	NMT 0.1%
Heavy Metals		
Lead (Pb)	ICP-MS	NMT 0.1 ppm
Arsenic (As)	ICP-MS	NMT 0.1 ppm
Mercury (Hg)	ICP-MS	NMT 0.1 ppm
Cadmium (Cd)	ICP-MS	NMT 0.1 ppm
Contaminants		

PCBs	HRGC-HRMS	NMT 0.09 ppm (mg/kg)
PCDDs and PCDFs	HRGC-HRMS	NMT 2 ppt (pg/g)
Dioxin-like PCBs	HRGC-HRMS	NMT 3 ppt (pg/g)
Solvent Residue		
Methanol	USP <467>	NMT 100 ppm
Microbiology		
Total Plate Count	ICP-MS	NMT 100 CFU/ml
Yeasts and molds	ICP-MS	Negative
Escherichia coli	ICP-MS	Negative
Salmonella spp.	ICP-MS	Negative
Staphlococcus aureus	ICP-MS	Negative

Shelf-life Stability

Formal shelf-life studies on Provinal™ products have not yet been completed; however, these studies are currently underway. Similar oils produced from this source using the same process have been proven to have a shelf life in excess of two years.

Self-limiting Levels of Use

As is true for other ingredients manufactured from fish oils, organoleptic properties of Provinal™ such as a mild fishy odor and taste, as well as the lipid nature of the product, will cause there to be some self-limitation on the addition levels per serving that will be added to finished food products. In addition, the cost of this specialty functional ingredient will have an additional self-limiting effect on its use in foods.

Safety Assessment

Toxicology Studies on Edible Fatty Acids

Toxicological studies on Provinal™, the finished product, have not been conducted, and toxicological studies on pure palmitoleic acid were not found in the public domain. The absence of such studies is most likely due to the fact that this fatty acid, as well as the other fatty acids that constitute Provinal™, are considered "edible" and have an extensive history of consumption in foods.

Although no toxicological studies have been conducted on Provinal™ or palmitoleic acid, there have been a few studies conducted on similar ingredients or products, including oleic acid, the second most prevalent fatty acid in Provinal™, and medium and long-chain triglyceride oil, as described below.

Bacterial Reverse Mutation Assay

An Ames mutagenicity test on palmitoleic acid was not found in the public domain; however, Hayatsu and colleagues reported that unsaturated fatty acids in the range of C16-24 (including palmitoleic acid) showed inhibition of mutagenicity by the food pyrolysate mutagen, Trp-P-1 (1.5 nmols per plate). Palmitoleic acid specifically required a concentration of 0.06 μmol/plate to achieve 50% inhibition, and 0.20 μmol/plate to achieve >95% inhibition.[35]

In another study, medium chain triacylglycerol oil was found to have no genotoxic potential in an Ames assay when tested at levels up to 5000 μg/plate.[36]

Acute and Subchronic Animal Feeding Studies

In 2006, the safety of medium (triglyceride esters of saturated fatty acids with chain lengths of 8-10 carbons) and long-chain (both saturated and unsaturated fatty acids of a chain length >12 carbons) triacylglycerol oil (MLCT) produced from edible vegetable oil was evaluated in a series of toxicological studies.[36] Although these studies were conducted primarily to demonstrate the safety of medium chain triacylglycerols (MCT), the use of long chain triacylglycerols (LCT) as a control additionally demonstrates the safety of these fatty acids. These studies are briefly outlined below.

In a 14-day, acute oral exposure study, two groups of five-week-old Wistar rats (5/sex/group) were administered either 5000 mg/kg bw mixed rapeseed and soybean oils, or MLCT by gavage daily. Body weights were measured on days 0, 1, 2, 3, 4, 7, and 14 after test administration. Gross autopsy was conducted after the 14th day. No abnormal clinical signs or gross pathological abnormalities were noted in any organ or tissue examined. The LD_{50} value was determined to be greater than 5000 mg/kg bw for MLCT.

A subchronic oral exposure study was conducted on six-week-old male Wistar rats (20/group), with the rats administered 7% of the diet (approximately 3500 mg/kg/day) of either rapeseed oil (LCT) or MLCT for six weeks. Adipose tissue weights surrounding the epididymes and kidneys, and mesenteric adipose tissue were measured, and liver weight, subcutaneous fat weight, and carcass protein were determined for each rat. No adverse effects were noted and no overt signs of toxicity were noted. No significant differences in liver and renal function values, urinalysis, or hematology parameters were found between or within groups. The NOAEL was determined to be 3500 mg/kg/day, the highest dose tested.

These studies on MLCT and LCT, while not equivalent to Provinal™, corroborate the high level of safety of triacylglycerol oils containing fatty acids of different chain lengths and the esters of these oils.

Human Clinical Studies

Although the human clinical studies discussed below are not conducted on palmitoleic acid itself, they do demonstrate the innate safety of edible fatty acids and both triglyceride and ethyl ester forms.

A 12-week, double-blind, randomized, parallel, placebo-controlled trial in normal healthy subjects (n=234; aged 18–65) was conducted to evaluate the safety of oleic acid ethyl esters.[13] Two hundred and thirty-five subjects were randomized to 5 groups according to body weight and body mass index (BMI). The subjects were administered either 8 g (single dose at breakfast in a milk-based beverage) or 16 g/day (8 grams at breakfast and mid-afternoon) of oleic acid ethyl ester. Three control groups were included: the milk-based beverage given once daily, twice daily, and a triacylglycerol control of 8 grams high oleic safflower oil given once daily in the beverage. The subjects voluntarily reported adverse events and clinically significant adverse events were followed to resolution by the principle investigator. Evaluations are described in the table below:

Evaluation	Time of evaluation
ECG	Screening
Physical exam	Screening
Weight	Screening
Vital signs	Screening
Complete blood count	Screening, 2, 4, 8 and 12 weeks
Differential count	Screening, 2, 4, 8 and 12 weeks
Serum chemistry profile	Screening, 2, 4, 8 and 12 weeks
Lipid profile	Screening, 2, 4, 8 and 12 weeks
Total free fatty acid profile	Screening, 2, 4, 8 and 12 weeks
Thyroid stimulating hormone (TSH)	Screening, 12 weeks
Thyroxin (T4)	Screening, 12 weeks
Hemoglobin A1c (HgbA1c)	Screening, 12 weeks
Urinalysis	Screening, 12 weeks
Pregnancy test (les)	Screening, 4, 8 and 12 weeks

Average exposure to the oleic acid products was 77.4 days for the 8 g group and 73.9 days for the 16 g group. In this human safety study, no clinically significant negative effects were found from consuming oleic acid ethyl ester at levels of up to 16 g/day (approx. 0.2 g/kg) Two-hundred and thirteen subjects completed the study, 13 unable to meet protocol requirements, 4 were lost to follow-up, and 5 withdrew due to adverse events. No clinically significant differences were found between groups for CBC, differential, serum chemistry parameters, TSH, T4, HgbA1c, urinalysis or lipid parameters. The total number of adverse events was comparable across all groups and most common were gastrointestinal and mild

in nature, including abdominal pain, constipation, flatulence and nausea. No clinically relevant adverse events were reported.

Matulka et al. evaluated the safety MLCT and LCT in humans in a placebo-controlled, double-blind study in 40 subjects for 21 days.[36] Each group, including 20 healthy Japanese men and women (aged 21–39 years), consumed bread containing either LCT or MLCT. The amount of oil consumed via the bread source was 42 grams per day. Body measurements, hematological analysis and urinalysis were conducted at study initialization and completion. No significant changes were found in liver and renal function, urinalysis parameters, or hematology parameters. Additionally, no adverse effects were reported.

Additional Scientific Studies on Palmitoleic Acid

In a recent study, thirty (sex not reported), six-week old KK-A^y strain mice (a mouse strain susceptible to obesity, fatty liver and diabetes) were randomized into three groups and administered 1.5% (w/w) polyglycerol ester (vehicle), 300 mg/kg palmitic acid solution or 300 mg/kg palmitoleic acid solution by gavage daily for four weeks.[37] The mice were evaluated as follows:

Evaluations	Time of examinations
Food Intake	Every 3-4 days
Body Weight	Weekly
Plasma Glucose	Weekly
Insulin Sensitivity (via insulin tolerance test)	Day 22 and Day 28
Plasma triglycerides, total cholesterol, free fatty acids, insulin concentrations	Day 28
Organ weight	Day 28
Mesenteric adipose tissue and liver total RNA	Day 28

A significant decrease was noted in both body weight and food intake ($p<0.05$) in the palmitoleic acid group compared to the control group. Additionally, significant decrease in liver weight ($p<0.05$), as well as a marked increase ($p<0.05$) in pancreas weight was found after administration of palmitoleic acid to KK-A^y mice compared to the palmitic acid or control groups; these results can be interpreted as beneficial in this particular mouse population. The authors suggest that the increase in pancreas weight can be attributed to the prevention of beta cell apoptosis by palmitoleic acid, an effect demonstrated in in vitro trials. Mesenteric white adipose, brown adipose and skeletal muscle tissue did not differ significantly between the three groups. Plasma glucose, insulin, and plasma and liver triglyceride levels were significantly decreased in the palmitoleic acid group compared to control animals ($p<0.05$). Palmitoleic acid also improved insulin sensitivity compared to the control group. Mesenteric TNFα and resistin mRNA levels were markedly suppressed in the palmitoleic group. Additionally, lipogenic mRNA levels in the liver were reduced. In this 28-day study, 300 mg/kg of palmitoleic acid given daily to KK-A^y strain mice for four weeks was without reported signs of toxicity.

In a series of trials on fatty acid-induced satiety, nine-week-old male Sprague Dawley rats were randomly divided into groups of 8–10 animals and administered (by gavage) a one-time dose of 50, 150 or 500 mg/kg of palmitoleic acid. Administration by gavage of up to 500 mg/kg bw of palmitoleic acid in rats suppressed food consumption in a dose-dependent manner 1 hour postprandially (a common finding with fatty acid administration), and no adverse events were reported.[38]

In human clinical trials, macadamia nut-based diets, containing up to 50% of energy intake as fat from macadamia nuts, have demonstrated no significant adverse effects. In one pilot study, 70 free-living subjects were given 90 g or 45 g of macadamia nuts for one month (approximately 11.68 g or 5.84 g of palmitoleic acid per day, respectively). No adverse events were reported.[29] In a subsequent feeding study, a macadamia nut based diet (37% energy from fat) for 30 days also produced no adverse events. In the pilot study, reports of minor gastrointestinal tract discomfort often seen with major changes in daily consumption of fats were noted; however, they were temporary.[29] As a component of macadamia nuts or oil, humans have consumed up to 4% palmitoleic acid in the diet (or approximately 10.51 g/day) for up to four weeks, without serious adverse events.[18, 39] Lastly, as a component of Sea buckthorn oil, up to 1.25 g of palmitoleic acid were consumed by humans for 4 months without signs of toxicity.[26, 40]

Allergenicity

No reports of allergies to palmitoleic acid were found in the public domain.

History of Consumption

Edible fats and oils from plants and animals have been consumed since ancient times, are currently widely consumed and are considered GRAS. Salad and other cooking oil consumption reached 52.5 pounds per capita per year in 2010, according to the USDA Oil Crops Yearbook.[41]

The United States is also the world's largest consumer of macadamia nuts, one of the richest sources of palmitoleic acid. In 2001–2002, domestic consumption was expected to reach 44,069 tons.[42] According to the U.S. Department of Agriculture, macadamia nut consumption per capita in the United States has risen from 0.07 lbs in 1980–1981 to 0.13 lbs in 2006–2007.[43] A serving of macadamia nuts (10–12 nuts; 1 oz.; 28.35 g) contains approximately 3.7 grams of palmitoleic acid, and one tablespoon (14 g) of macadamia nut oil contains approximately 2.38 g of palmitoleic acid.[22, 34] Despite this widespread use, there is no history of adverse events (with the exception of a rare allergy) associated with macadamia nut or macadamia nut oil and, therefore, palmitoleic acid consumption in humans.

According to the USDA, fats and oils should constitute a maximum of 35% of daily caloric intake for those 4 years old or older. In a 2000 kcal diet, this equates to a maximum of 78 g of fat per day. The American Heart Association (AHA) recommends that the majority of the fatty acids in the diet should be monounsaturated or polyunsaturated (www.heart.org). If one were to eat all of this fat as commercially available macadamia nut oil, which is rich in unsaturated fats, it is possible to consume 13.26 g of palmitoleic acid in one day.

Previous Sales and Reported Adverse Events

Tersus Pharmaceuticals, LLC has sold 1964 kg of Provinal™ to date, representing 4,676,190 servings at the suggested daily intake of 420 mg (210 mg palmitoleic acid). No reportable adverse events have occurred.

Similar Products in the Marketplace

Fish (including anchovies and menhaden), macadamia nuts, and their oils are widely available in commerce and commonly consumed. A Google search for "macadamia nut oil recipes" results in numerous recipes, suggesting that consumption of this oil is widespread throughout the World.

Current Regulatory Status

A number of GRAS affirmations and food additive regulations confirm the safe use of fatty acid containing oils, including those containing palmitoleic acid:

- The FDA considers vegetable oil to be GRAS and exempt from a food additive petition (Federal Register Volume 62, No. 74, pp. 18938–18964 April 17, 1997).

- As per *21 CFR 172.860*, "any mixture of the following straight-chain monobasic carboxylic acids **and their associated fatty acids** manufactured from **fats and oils derived from edible sources**: capric acid, caprylic acid, lauric acid, myristic acid, oleic acid, palmitic acid and stearic acid" are considered a food additives permitted for direct addition to food for human consumption, subject to specifications (unsaponifiable matter does not exceed 2%, and it is free from chick-edema factor) and the intended uses are as follows:

  (1) In foods as a lubricant, binder, and as a defoaming agent in accordance with good manufacturing practice.

  (2) As a component in the manufacture of other food-grade additives.

- Sheanut oil is GRAS per *21 CFR 184.1702* and identified GRAS in the Federal Register Volume 63, No. 101, 1998 pp. 28893–28895. In this document the FDA states that sheanut oil is composed of "oleic, stearic and palmitic acids, as well as other fatty acids found as minor components…the same fatty acids found in a broad range of edible fats and oils that are GRAS". FDA also states that sheanut oil is "similar in chemical composition to commonly used GRAS fats and oils, such as cocoa butter, cottonseed oil, soybean oil, corn oil and palm oil".
- Rapeseed oil (0.21% Palmitoleic acid) is considered GRAS as per *21 CFR 184.1555*
- A 1975 Select Committee on GRAS Substances (SCOGS) Opinion states: "Coconut oil, peanut oil, oleic acid and linoleic acid have been used as

foods or as food components by man for many years. None of the available biological information indicates that these substances are hazardous to man or animals even when consumed at levels that are orders of magnitude greater than could result from their use for the purposes covered in this report."

- Oleic acid is mentioned in *21 CFR 172.862* as an approved food additive. The additive, consisting of purified oleic acid separated from refined tall oil fatty acids, is limited to uses 1) as a lubricant, binder, and defoaming agent in accordance with good manufacturing practice and 2) as a component in the manufacture of other food-grade additives.
- Mono- and diglycerides, consisting of a mixture of glyceryl mono- and diesters, and minor amounts of triesters, that are prepared from fats or oils derived from edible sources (most prevalent lauric, linoleic, myristic, oleic, palmitic and stearic) are considered GRAS as per 21 CFR 184.1505.
- Menhaden oil (10.48% Palmitoleic acid), prepared from fish of the genus *Brevoortia,* is GRAS as per *21 CFR 184.1472.* "This ingredient may be used in food only within the following specific limitations to ensure that total intake of eicosapentaenoic acid and docosahexaenoic acid does not exceed 3.0 g/person/day". Furthermore, in the Federal Registrar 62 Issue 74, FDA states: "Specifications for the notified (GRAS) substance may be the same as, or similar to, specifications for an oil that is substantially similar to hydrogenated and partially hydrogenated menhaden oil, which FDA has affirmed as GRAS for use as an edible fat or oil, could be based on the specifications in *21 CFR 184.1472*.
- Anchovies (oil is 9.42% palmitoleic acid), are considered "food for human consumption" by FDA and defined in *21 CFR 123.3*—"fish means fresh or saltwater finfish, crustaceans, other forms of aquatic life (including but not limited to, alligator, frog, aquatic turtle, jellyfish, sea cucumber, and sea urchin and the roe of such animals) other than birds or mammals, and all mollusks, where such animal life is intended for human consumption"

Relevant GRAS Notifications

There are several GRAS notifications in the FDA's GRAS Notice Inventory database that were filed by FDA without question and attest to the safety of ingredients containing palmitoleic acid (Table 4).

Table 4. GRAS Notifications

	GRN 105	GRN 131	GRN 146	GRN 332	GRN 371
Subject	Fish Oil Concentrate (4% palmitoleic acid)	Betapol™, a triglyceride mixture composed of fatty acids present in edible oils and fats, primarily oleic and palmitic	Salmon oil	Refined Pine Nut oil (palmitoleic acid 0.2%; oleic acid NLT 22%)	Krill oil (4.9% palmitoleic acid; 12.1% oleic acid)
Uses	Baked goods and baking mixes, plant protein products, cereals, fats and oils (not including infant formula), cheese products, milk products, cheese products, frozen dairy products, dairy product analogs, condiments, meat products, egg products, fish products, condiments, soup mixes, snack foods, nut products, gravies and sauces, processed vegetable drinks, hard candy, soft candy,	Use in infant formula for both term and preterm infants at levels of up to 80% total fat intake	Baked goods and baking mixes, nonalcoholic beverages, cereals, cheese products, chewing gum, condiments, confections and frosting, dairy product analogs, egg products, fats and oils (not including infant formula), fish products, frozen dairy products/desserts, gelatins and puddings, pastas, gravies, sauces, hard candy, jams and jellies, meat products, milk	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, soups and soup mixes	Breakfast cereals, cheese, beverages (nonalcoholic), fruit juices, frozen dairy desserts, milk products, and medical foods

	nonalcoholic beverages, pastas, poultry products, processed fruit juices, white granulated sugar, sugar substitutes, chewing gum, gelatins and puddings, confections and frostings, sweet sauces, toppings, and syrups		products, nut products, plant protein products, poultry products, processed vegetable juices, snack foods, soft candy, soup mixes, white granulated sugar, sugar substitutes, sweet sauces, toppings and syrups		
Intended Level of Use	57% of levels listed in menhaden oil (Levels not to exceed EPA+DHA 3.0 g/day)	80% total fat intake	Levels not to exceed EPA+DHA 3.0 g/day	3.0 g per serving	500 mg per serving
Outcome	FDA has no questions	FDA has no questions	FDA has no questions	FDA has no questions	FDA has no questions
Date of Closure	October 15, 2002	December 4, 2003	December 4, 2002	September 24, 2010	July 22, 2011

Intended Use and Estimated Exposure

For the purpose of this GRAS notification, Tersus' Provinal™, manufactured in accordance with GMP, is intended for use as a replacement source of edible fatty acids in food categories found in 21 CFR 170.3 (n), where standards of identity allow. Provinal™ is not intended for use in infant formula, meat, egg, catfish or any products that would require additional regulatory review by USDA.

Provinal™ is expected to replace some of the edible fats normally found in food products, and has the potential to decrease the ratio of saturated to mono- and polyunsaturated fats in processed foods by the addition of monounsaturated fatty acids (including palmitoleic and oleic acids). Replacement of saturated fats with monounsaturated (and polyunsaturated) fats in the diet is recommended for supporting health by many organizations including the American Heart Association and USDA, as discussed previously in this notification.

USDA published an April 2011 report on the per capita consumption of fats and oils in food products.[41] The authors used data from the Economic Research Services Oil Crops Yearbook, March 2011,[44] and reported that 80.9 pounds of fats

and oils were consumed per person in 2010, equivalent to approximately 100 g per person per day. This includes consumption of fats from salad and cooking oils, baking and frying oils, margarine, butter, tallow, lard, and other similar sources, which are typical fats and oils that Provinal™ is intended to partially replace. FDA has stated in guidance for industry on estimating dietary intake of substances in food that examination of food frequency and other food-consumption surveys conducted in the US show that consumption at the 90th percentile for most commonly-consumed foods is approximately 2 times the mean consumption for that food, which would be equivalent to approximately 200 g of fats and oils per day.

While Provinal™ is intended to replace fats and oils in a variety of food products, the ingredient's mild organoleptic properties due to its derivation from fish will have a self-limiting effect related to its level of use in finished food products. The higher cost of this specialty branded ingredient and the fraction of the market share that will be available for Provinal™ sales will also limit its distribution in commerce. For the purpose of an exposure estimation with regard to intake from food sources, and taking into account the aforementioned expense, market share and organoleptic property overall factors, it is reasonable and conservative to estimate that approximately 10% of fats and oils in a high consumers diet might come from Provinal™ daily over a lifetime. This is equivalent to approximately 10 g of Provinal™, or 5 g of palmitoleic acid per day for an average user, and 20 g of Provinal™, or 10 g of palmitoleic acid per day for a 90th percentile user.

While the above estimation of exposure is considered to be reasonable, it should be noted that there are not expected to be safety issues even if 100% of fats and oils currently consumed were to be replaced by Provinal™. The fats found in this ingredient, including palmitoleic acid, undergo normal beta-oxidation catabolism in the body along with other commonly consumed dietary fats.

It should also be reiterated that exposure to Provinal™ is not expected to significantly increase the total consumption of edible fatty acids in the diet, but as previously stated, may modify the ratio of fatty acids toward that of a more beneficial fatty acid profile that is higher in monounsaturated fats.

Basis for the GRAS Determination

Provinal™ consists of a wide range of edible fatty acids derived from crude anchovy or menhaden oil, standardized to a high percentage of palmitoleic acid (C16:1, approximately 50% of the ingredient), and not more than 1% palmitic acid (C16:0). It also contains approximately 34% oleic acid (C18:1), based on batch assessments. The C16:1 and C18:1 monounsaturated fatty acids are endogenously produced, ubiquitous in the diet, and undergo catabolism in the body via normal beta-oxidation reactions. A large body of research suggests that

replacing saturated fats in the diet with monounsaturated fats may result in health benefits.[45 20, 46]

That Provinal™contains fatty acids found in many commonly consumed foods (including high levels of palmitoleic acid found in macadamia nuts, which as stated previously is equivalent to approximately 3.7 g/serving of 10–12 nuts), that FDA considers anchovies and menhaden oil (the raw materials for the production of Provinal™) GRAS as is recorded in the Code of Federal Regulations (menhaden oil is approximately 10.5% palmitoleic acid); that fats and oils derived from edible sources are considered GRAS by FDA pursuant to *21 CFR 184.1505* for various intended uses; that FDA has filed without question numerous fish oil ingredient GRAS Notifications in recent years; and that research and USDA recommendations suggest that replacing consumption of saturated fats with monounsaturated fatty acids leads to health benefits and no health concerns; are pivotal in the GRAS determination of Provinal™. Corroborative evidence includes human studies using macadamia nut-based diets and Sea buckthorn oil supplementation have demonstrated no significant adverse effects. No evidence to date has surfaced that would suggest safety concerns with regard to consumption of this ingredient under its conditions of intended use.

General Recognition

The data and information used to evaluate the safety of Provinal™ products for this GRAS determination are widely published and available in the public domain. This includes FDA regulatory information, as well as information from scientific peer-reviewed articles and texts. The reference section of this notification cites all cited published studies. The information and published data provide ample evidence of consensus among qualified experts that there is reasonable certainty that consumption of mixtures of fatty acids including Provinal™ (containing fatty acids that are endogenously produced and ubiquitously found in the diet) are safe for their intended use. The general availability of this information satisfies the common knowledge component of this GRAS determination.

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