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June 14, 2023

Susan J. Carlson, Ph.D., Director
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835

RE: GRAS Notification for Edible Pongamia Oil (EPO)

Dear Dr. Carlson:

We respectively submit the attached GRAS Notification on behalf of our client, Terviva, Inc., for the addition of edible pongamia oil (EPO) to foods as a substitute for existing fats and oils, consistent with the uses provided in 21CFR§170.3(n)(12) "fats and oils, including margarine, dressings for salads, breads, crackers, butter, salad oils, shortenings and cooking oils" with the exception of infant formula, meat-containing products, or those foods with a standard of identity. EPO is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because Terviva, Inc. has determined that the intended use is Generally Recognized As Safe (GRAS) based on scientific procedures in accordance with 21CFR§170.30(b) and in conformance with the guidance issued by the Food and Drug Administration (FDA) under 21CFR§170.36, 81 Fed. Reg. 54960 (Aug. 17, 2016). More detailed information regarding product identification, the manufacturing process, intended use levels, and safety of the ingredient is provided in the attached GRAS Notification.

A CD is enclosed containing Form 3667 and the notification of GRAS that contains the signatures of the members of the GRAS panel.

The information that is the basis for this GRAS Notification is available for FDA review and copying at reasonable times at Burdock Group, 859 Outer Road, Orlando, FL, 32814, or will be sent to FDA upon request. If you have any questions regarding this notification, please feel free to contact me at 407-802-1400 or rmatulka@burdockgroup.com.

Sincerely,

Ray A. Matulka, Ph.D.
Director of Toxicology
Executive Vice President
Burdock Group

BURDOCK GROUP
CONSULTANTS

NOTICE TO US FOOD AND DRUG ADMINISTRATION OF THE CONCLUSION THAT THE INTENDED USE OF EDIBLE PONGAMIA OIL (EPO) AS A FOOD INGREDIENT IS GENERALLY RECOGNIZED AS SAFE (GRAS)

Submitted by the Notifier:
Terviva, Inc.
980 Atlantic Ave. #105
Alameda, CA 94501

Prepared by the Agent of the Notifier:
Burdock Group
859 Outer Road
Orlando, FL 32814

June 14, 2023

NOTICE TO US FOOD AND DRUG ADMINISTRATION OF THE CONCLUSION THAT THE INTENDED USE OF EDIBLE PONGAMIA OIL (EPO) AS A FOOD INGREDIENT IS GENERALLY RECOGNIZED AS SAFE (GRAS)

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Part 1: Signed Statements and Certification

1.1 Basis of Conclusion

Terviva, Inc. (hereinafter referred to as Terviva), on the advice of a Panel of qualified experts, has concluded that Edible Pongamia Oil (EPO) is Generally Recognized As Safe (GRAS) in accordance with 21 CFR Part 170, Subpart E, and, therefore, exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act, under the conditions of its intended use as described below. The basis for this finding and supporting information is provided below.

1.2 Name and Address of the Notifier and Agent of the Notifier

Notifier

James D. Astwood, Ph.D.
Chief Technology Officer
Terviva, Inc.
980 Atlantic Ave. #105
Alameda, CA 94501

Agent of the Notifier

Ray A. Matulka, Ph.D.
Burdock Group
859 Outer Road
Orlando, FL 32814

Additional Agent Representative

Sharon Mayl
DLA Piper Law
33 Arch Street #26
Boston, MA 02110

1.3 Name of the Substance

The name of the substance of this GRAS conclusion is Edible Pongamia Oil (EPO). EPO (MW: 892.7 g/mol; CASN 247588-54-1) which is produced from the oilseeds of the tree whose genus and species is *Millettia pinnata*, a legume, also known as *Pongamia pinnata* (L.) Pierre. EPO will be referred to as Ponova™ Oil in commerce to distinguish this oil from otherwise non-food pongamia oils.

1.4 Intended Conditions of Use

EPO is a nutritive, plant-sourced macro ingredient oil, intended to be used as a substitute for existing fats and oils, consistent with the uses provided in 21CFR§170.3(n)(12) "Fats and oils, including margarine, dressings for salads, breads, crackers, butter, salad oils, shortenings and

cooking oils[1]" with the exception of infant formula, meat-containing products, or those foods with a standard of identity. Terviva does not intend to add EPO to any meat and/or poultry products that come under USDA jurisdiction. Therefore, 21 CFR §170.270 does not apply.

The proposed use is substitutive for existing fats and oils, rather than additive in use. EPO is to be added to foods identified herein (Table 9), such that the 90th percentile consumption from all categories may be up to and including 7,703.32 mg/day, approximately equivalent to 127.33 mg/kg bw/day.

1.5 Statutory Basis for GRAS conclusion

This GRAS conclusion is based on scientific procedures in accordance with 21 CFR §170.30(a) and (b).

1.6 Not Subject to Premarket Approval

EPO is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on the conclusion that the notified substance is GRAS under the conditions of its intended use.

1.7 Data and Information Availability Statement

The data and information that serve as the basis for this GRAS conclusion will be available for review and copying during customary business hours at the office of Burdock Group, 859 Outer Road, Orlando, FL 32814, or will be sent to FDA upon request.

1.8 Exemption from Disclosure under the Freedom of Information Act

None of the data and information in Parts 2 through 7 of this GRAS notice are considered exempt from disclosure under the Freedom of Information Act (FOIA). They do not contain any privileged or confidential information such as trade secrets and/or commercial or financial information and can be made publicly available.

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[1] Not intended for use in meat-containing products.

1.9 Certification

The undersigned authors of this document— a notice of the GRAS conclusion for the use of Edible Pongamia Oil (EPO)—hereby certify that, to the best of their knowledge, this GRAS notice is based on a complete, representative, and balanced submission that includes both favorable and unfavorable information, that are pertinent to the evaluation of the safety and GRAS status of EPO under the condition of its intended use.

June 14, 2023

Ray A. Matulka, Ph.D. – Agent to the Notifier
Fellow, American College of Nutrition
Director of Toxicology,
Executive Vice President, Burdock Group
859 Outer Road
Orlando, FL 32814

Date

Part 2: Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

Edible Pongamia Oil (EPO)[2] (MW: 892.7 g/mol; CASN 247588-54-1) is manufactured from the beans of the Pongamia tree, *Millettia pinnata* (L.) Panigrahi. The tree produces the beans in pods; the beans may contain oil at a concentration of approximately 30 – 40% of the weight of air-dried beans. The beans are pressed to separate oil from solids, and the oil is subsequently solvent extracted to remove bitter tasting and potentially toxic compounds, collectively known as furanoflavonoids, including karanjin, pongamol, and multiple pongapinnols (Yadav *et al.*, 2004; Al Muqarrabun *et al.*, 2013).

The resulting processed oil is clear/slightly yellow with a neutral odor and buttery taste. The compositional analysis of Edible Pongamia Oil product demonstrates the oil is comprised substantially of triglycerides (>95%), of which, greater than 50% are mono-unsaturated fatty acids, principally oleic acid (Omega-9). Other fatty acids include linoleic (Omega-6 polyunsaturated fatty acid), stearic (saturated fatty acid), and palmitic fatty acids (saturated fatty acid) (Burdock, 2022). The different fatty acid classes are provided in Table 1.

Table 1. Fatty acid classes of EPO*

Class	Relative abundance (% of total FAs)
Total Identified Fatty Acids	98.90
Total Monounsaturated Fatty Acids	52.09
Total Polyunsaturated Fatty Acids	18.44
Total Saturated Fatty Acids	21.93
Total *Trans* Fatty Acids	0.24
Total Omega 3 Fatty Acids	1.99
Total Omega 5 Fatty Acids	<0.05
Total Omega 6 Fatty Acids	16.37
Total Omega 7 Fatty Acids	0.65
Total Omega 9 Fatty Acids	51.12

*Data represents one processing batch (TV_OIL_0028).
FA=Fatty acid

[2] While the common and usual name for Edible Pongamia Oil (EPO) is outside the scope of this document, it is anticipated that Edible Pongamia Oil will be referred to as Ponova™ Oil in commerce to distinguish this oil from otherwise non-food pongamia oils.

2.2 Botanical identification

EPO is obtained from the beans[3] (*i.e.*, seeds) of the Pongamia tree. The Pongamia tree belongs to the family Fabaceae (Leguminosae), the third largest family of flowering plants with over 730 genera and greater than 19,400 species. The Pongamia tree (also known as, *Millettia pinnata* (L.) Panigrahi; *Pongamia pinnata* (L) Pierre) (Table 2), is cosmopolitan in distribution and mostly propagated through its beans, but vegetatively as well. Although the exact origin of the tree is not known, it is thought to be native to the area encompassed by present day India, Indonesia, Malaysia, and Myanmar where it evolved to its present form of growing and propagating in tropical climates throughout the world (Yadav *et al.*, 2011; Usharani *et al.*, 2019). Pongamia is nitrogen-fixing (it is a legume and a member of the pea family) and enriches the soil in which it has been planted and its complex root system lends itself to erosion control; it provides shade, fragrant flowers and has favorable growth characteristics (*e.g.*, tolerance to temperature extremes (high and low), drought, waterlogging, saltwater exposure, and insects) (Usharani *et al.*, 2019). Sometime during the late Holocene period up to modernity, various parts of Pongamia were found to be useful to humans as a traditional medicine (*e.g.*, as an antioxidant, antimicrobial, antiprotozoal, antischistosomal, anti-inflammatory, anticonvulsant, antidiabetic and spermicidal; Al Muqarrabun *et al.*, 2013). Other uses are discussed below (Sections 3.2). The bark can be used to make twine and yields a black gum historically used to treat wounds caused by poisonous fish (Bhalerao and Sharma, 2014). The oil from the beans is used as a fuel for lamps, soap making and as a lubricant (Usharani *et al.*, 2019). More recently, the bean oil has been used for biodiesel production (Halder *et al.*, 2014; Morgan *et al.*, 2019). The beans have a high lipid content (30 – 40%), nearly half of which is oleic acid (Yadav *et al.*, 2011). Once the oil is extracted from the beans, the press cake (*i.e.*, seed meal) remains, which is rich in protein (28 – 34%). The press cake has been evaluated for use in animal feed for ruminants (Raj *et al.*, 2016) and poultry (Husna *et al.*, 2017) (although it is not approved as such in the US). As a result of many of these useful applications, Pongamia have been transplanted to parts of Australia, New Zealand, Africa, and the United States (Yadav *et al.*, 2011).

Due to its cosmopolitan growth, its nomenclature is varied and may include local names. It has been classified into several genera including, but not limited to, *Cytisus*, *Dalbergia*, *Derris*, *Millettia*, *Pongamia*, and *Robinia* (Table 2). The basis of this distinction as one genus or another may stem from the presence of one or more distinguishing characteristics such as being the only member of the species that has pinnate shaped leaves, and one of the few nitrogen-fixing trees to produce beans with substantial oil content.[4]

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[3] The Pongamia tree is of the legume family and so technically the seeds are termed "beans".

[4] https://jatropha.pro/pongamia-pinnata/; site last visited December 19, 2022.

Table 2. *Millettia pinnata* bean oil (s.o.) synonyms; CASN 247588-54-1

Cytisus pinnatus s.o.	Honge oil	*Millettia pinnata* s.o.	*Pongamia pinnata* (L.)[c] oil
Dalbergia arborea s.o.	Karanja oil	Oils, pongamia glabra	*Pongamia pinnata* s.o.
Derris indica (Lam.) Benn.[b] s.o.	Karum s.o.[a]	*Panigrahi pongame* *Paraderris elliptica*-Adema Tubaroot Pongam s.o.	*Pongamia pinnata* (L.) Pierre[c]
Galedupa indica s.o.	Karumtree s.o.	*Pongamia glabra* Vent oil	*Pongamia xerocarpa* s.o.
Galedupa pinnata s.o.	Millet seed	*Pongamia glabra* s.o.	Poonga s.o.
Galedupa pungum s.o.	*Millettia novo-guineensis* s.o.	*Pongamia mitis* s.o.	*Pterocarpus flavus* s.o.
Hongay oil	*Millettia pinnata*	Pongamia oil	*Robinia mitis* s.o.

[a]ChemIDplus favored name; [b]USDA synonyms; [c]Name used in scientific literature

The USDA Plants Database (USDA, 2020a) describes the taxonomy of *Millettia pinnata* as follows (Table 3):

Table 3. *Millettia pinnata* (L.): Taxonomic hierarchy (USDA Plants Database, 2020a)*

Rank	Scientific Name and Common Name	Scientific Name and Common Name
Kingdom	Plantae – Plants	
Subkingdom	Tracheobionta – Vascular plants	
Superdivision	Spermatophyta – Seed plants	
Division	Magnoliophya – Flowering plants	
Class	Magnoliopsida – Dicotyledons	
Subclass	Rosidae	
Order	Fabales	
Family	Fabaceae/Leguminosae – Pea family	
Genus	*Millettia* Wight & Arn. – oiltree	*Paraderris* (Miq.) R. Geesink – paraderris
Species	*Millettia pinnata* (L.) Panigrahi – pongame oiltree	*Paraderris elliptica* (Wall.) Adema – tubaroot

*https://plants.usda.gov/core/profile?symbol=MIPI9

EPO can be used as a dietary source of oleic and linoleic fatty acids in the diet. Linoleic acid, an omega-6 fatty acid, is an essential fatty acid. Oleic acid has been reported to have health benefits and nutritional value (Teres *et al.*, 2008).

2.3 Description and specifications

EPO (MW: 892.7 g/mol; CASN 247588-54-1) is an edible oil produced from the seeds of the tree *Millettia pinnata*. The oil appears as clear/slightly yellow with neutral odor. The oil is mainly (>95%) comprised of triglycerides, of which greater than 50% are mono-unsaturated fatty acids, principally oleic acid (> 50%), as a percentage of the total fatty acid content. Other fatty acids include linoleic (> 15%), stearic (>7%), and palmitic (> 8%) fatty acids, on a total fatty acid basis. The identifying properties of Edible Pongamia Oil are provided in Table 4. A comparison of the fatty acid content of EPO to other commonly consumed vegetable oils is provided in Table 5.

Table 4. Compositional analysis of EPO*

Component (unit of measure)	Value
Free Fatty Acids (FFA)(% g/100g)	0.13
Peroxide Value (meq O2/kg)	4.55
p-Anisidine Value (AnV)	4.7
Neutral Oil (%)	99.91
Iodine value (calculated from FA profile)	84.6
Insoluble impurities (%)	<0.01
OSI (Oxidative Stability Index; hours)	9.6
Phosphorus (oil specific) (ppm)	22.5
Chlorophyll (ppm)	<0.1
Residual solvents (%)	<0.05
Moisture (%)	0.03
Lovibond Color - AOCS Scale	0.5R, 18.0Y
Smoke point (°F)	347
Glycerol %	<1
Monoglycerides %	<1
Diglycerides %	1.0
Triglycerides %	95.9

*Data represents the average of 2 samples from two processing batches: TV_OIL_0028 and TV_OIL_0030; AOCS = American Oil Chemists Society; FA = fatty acid; FFA = free fatty acid; meq = milli-equivalents; ppm = parts *per* million

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Table 5. Fatty acid composition (Percent total fatty acids) of EPO compared to other commonly consumed vegetable oils

Fatty acid	Common name	EPO (g) (N = 5 lots)	Olive Oil (g)#	Canola Oil (g)*	Soybean Oil (g)**
14:0	Myristic acid	0.03	ND	ND	ND
16:0	Palmitic Acid	9.35	11.3	4.30	10.5
16:1c9	Palmitoleic Acid	0.05	1.26	0.21	ND
17:0	Margaric Acid (Heptadecanoic)	ND	0.02	ND	0.03
17:1c9	Heptadecenoic Acid	ND	0.13	ND	ND
18:0	Stearic Acid	8.35	1.95	2.09	4.44
18:1c9	Oleic Acid	52.89	71.3	61.7	22.6
18:2n6	Linoleic Acid	17.02	9.76	19.0	51
18:3n3	*alpha;*-Linolenic Acid	2.60	0.76	9.14	6.79
20:0	Arachidic Acid	1.76	ND	0.65	0.36
20:1c11	Gondoic Acid	1.03	0.31	1.32	0.23
22:0	Behenic Acid	4.29	ND	0.33	0.37
22:1c13	Erucic Acid	0.05	ND	ND	ND
24:0	Lignoceric Acid	1.48	ND	ND	ND
Total Fatty Acids Identified (% Total Fatty Acids)		98.90	96.8	98.74	96.32

https://fdc.nal.usda.gov/fdc-app.html#/food-details/1103861/nutrients; site last visited December 19, 2022.
* https://fdc.nal.usda.gov/fdc-app.html#/food-details/172336/nutrients; site last visited December 19, 2022.
** https://fdc.nal.usda.gov/fdc-app.html#/food-details/171411/nutrients; site last visited December 19, 2022.
ND = Analyzed, but not detected; NR = Not reported.
Total Fatty Acids *per* Certificate of Analysis may differ from additive amounts in table in parenthesis.

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Specifications for EPO from the average of 5 different batches are provided in Table 6. Analysis of each of these five lots of EPO are provided in GRAS dossier (Burdock, 2022) and is in Appendix I. Total sterol content is approximately 1000 ppm, with the major sterol being *beta*-sitosterol (~70%), campesterol (~7%), stigmasterol (~13%) and *delta*-5-avenasterol (~9%).[5]

Table 6. Specifications of EPO

			Batch Analysis Results ($N = 5$)	
Analysis	**Method**	**Specification**	**Range**	**Average**
Color	Sensory - Visual	Clear, light/golden yellow	N/A	Complies
Odor	Sensory - Smell	Pleasant; faint nuttiness	N/A	Complies
Moisture (max.)	AOCS Ca 2b-38	<0.25% g/100g m/m	0.02 - 0.07	0.04%
Oxid. stability index	AOCS Cd 12b-92	>1 hrs	1.07 - 4.96	2.22
Unsaponifiable matter	AOCS Ca 6a-40	<2.0% g/100g	0.25 - 0.38	0.31
Peroxide value	AOCS Cd 8-53	<20.0 meqO_2/kg	1.5 - 3.8	2.8
p-Anisidine value	AOCS Cd 18-90	<20 AnV	5.7 - 12.9	7.9
Triglycerides	AOAC 966.06	>95%	94.1 - 97.2	96.2
Total Fatty acids	AOAC 996.06 mod.	>90% g/100g	90.1 - 93.1	92.1
Oleic acid (18:1)	AOAC 996.06 mod.	>50.0 g/100g FA	52.1 - 53.2	52.9
Linoleic acid (18:2n-6)	AOAC 996.06 mod.	>15.0 g/100g FA	16.5 - 18.0	17.0
Palmitic acid (16:0)	AOAC 996.06 mod.	>8.0 g/100g FA	9.1 - 9.9	9.4
Stearic acid (18:0)	AOAC 996.06 mod.	>7.0 g/100g FA	7.9 - 8.6	8.4
Behenic acid (22:0)	AOAC 996.06 mod.	>3.0 g/100g FA	3.4 - 5.0	4.3
Erucic acid (22:1c13)	AOAC 996.06 mod.	<1.0 g/100g FA	<0.1	<0.1
Free Fatty acids	AOCS Ca 5a-40	<1.0% g/100g	<0.1 - 0.2	0.1
Microbials				
Total coliforms	AOAC 991.14	<10 cfu/g	<10	<10
Escherichia coli	AOAC 991.14	<10 cfu/g	<10	<10
Salmonella spp.	AOAC-RI 121501	Negative cfu/25g	Negative	Negative
Mold	BAM Chap. 18	<10 cfu/g	<10	<10
Yeast	BAM Chap. 18	<10 cfu/g	<10	<10
Heavy metals	ICP-MS			
Lead (Pb)	AOAC Ca 17-01	<10 ppm	<0.01	<0.010
Cadmium (Cd)	AOAC Ca 17-01	<0.1 ppm	<0.01	<0.010
Mercury (Hg)	AOAC Ca 17-01	<1 ppm	<0.01	<0.010
Arsenic (As)	AOAC Ca 17-01	<0.3 ppm	<0.01	<0.010
Furanoflavonoids				
Karanjin	TV-STM_001.01	<150 ppm	15 - 135	75.4
Pongamol	TV-STM_001.01	<150 ppm	93 - 156	120.4

[5] Terviva internal data, 2021.

Table 6. Specifications of EPO

Analysis	Method	Specification	Batch Analysis Results ($N = 5$) Range	Average
Mycotoxins				
Aflatoxin B1, B2,G1,G2	AOAC 999.07	0.5 - 15** µg/kg	<4	<4
Fumonisin (total)	J AOAC 92(2),496	2000 - 4000 µg/kg	<30	<30
Ochratoxin A	AOAC 999.07	1 - 5 µg/kg	<1.0	<1.0
T-2/HT-2 Toxin	Food Add Cont 2013:30(3),541	5 - 10 µg/kg	<1/10	<1/10
Zearalenone	Food Add Cont 2013:30(3),541	75 - 200 µg/kg	<5.0	<5.0

AOAC = Association of Official Analytical Collaboration; AOCS = American Oil Chemists Society; BAM = Bacteriological Analytical Manual; FA = Fatty acids; cfu = colony forming units; ICP-MS = Inductively coupled Plasma-Mass Spectrometry; max = maximum; meq = milliequivalents; mod = modified; N/A = Not Applicable; ND = Not Detected; TV-STM = Terviva-Standard Test Method. *Measured at 110°C; 1 hr = approximately 1 month; Food Add Cont = Food Additives & Contaminants.

2.4 Manufacturing Process

Edible Pongamia Oil (>99.5% pure) is manufactured from the oilseeds of the leguminous tree species *Pongamia pinnata* (L.) Pierre, also known as *Millettia pinnata*. The beans are pressed to separate oil from solids (presscake). *M. pinnata* beans are procured from Florida, Hawaii, and India, shipped packaged in food grade approved sealed Eurotainers with a nitrogen blanket. The beans are analyzed for pesticides, mycotoxins, heavy metals, microbials, foreign material, and moisture before pressing. The resulting crude oil is allowed to settle, then filtered with diatomaceous earth and degummed with a food grade citric acid solution, removing phospholipids and other water-soluble substances such as proteins. The resulting oil is then dewaxed by chilling (~16°C) and then refined with pure, food-grade ethanol at < 80°C to remove bitter tasting compounds, free fatty acids and unsaponifiable substances (Figure 1). Greater than 99% of the ethanol used in the manufacturing process is recycled back into the process, with the remainder evaporated from the product. The bitter compounds are removed in the process; they are comprised mainly of furanoflavonoids, the most abundant of which are karanjin and pongamol (Thakur *et al.*, 2021). The resulting edible oil is clear/slightly yellow with a slight nutty odor and agreeable, neutral taste, containing < 0.01% of total volatiles and < 0.05% ethanol. The manufacturing process to produce EPO is similar to conventional vegetable oil production in that the manufacturing process includes seed preparation and crushing, degumming, dewaxing/winterization, and solvent removal. The EPO process utilizes food-grade ethanol which extracts the unsaponifiables, proteins, sterols, free fatty acids and other foreign substances from the pongamia oil. The end result of either process is isolation and purification the final oil product (Appendix II).

The final oil product is packaged in food grade bulk stainless steel containers, sealed with a nitrogen blanket and stored at air-conditioned room temperature (<25 °C). Potential consumer product would be packaged in glass, PET, or HDPE opaque food grade bottles that are safe and suitable for their intended use (Piscopo and Poiana, 2012). Karanjin and pongamol were quantified

in the edible oil (to analyze and evaluate if the stated limit of <150 ppm of each had been met) by high pressure liquid chromatography using purified karanjin and pongamol as reference standards.

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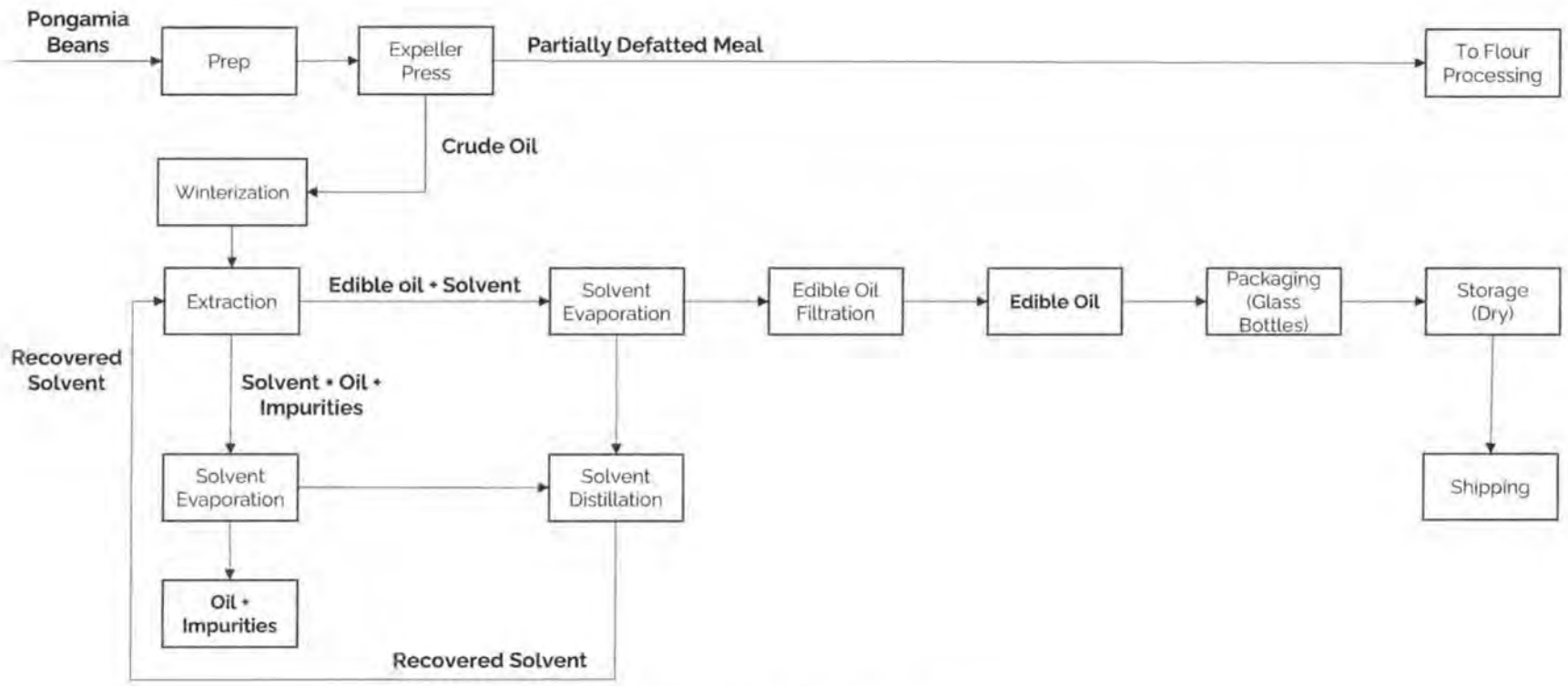

Figure 1. EPO production scheme

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The beans and the resulting oil have been analyzed for pesticide residues. The results indicate that EPO does not contain residual pesticides at levels of toxicological or regulatory concern (pesticide concentrations are <10 ppb overall). Future testing will be performed on the *M. pinnata* beans from every lot (currently established at 50 metric tons/lot); from each lot, testing will be completed on two composite samples prepared from approximately 100 primary samples collected from 100 different bags (~200 g collected from each bag) at the time of procurement. Terviva tests one batch of the finished product for residual pesticides once a year.

The manufacturing process for conventionally produced refined, bleached, deodorized (RBD) oils can form unintentional contaminants by chemical reactions during processing with elevated temperatures or extreme pH conditions. Some processed foods and vegetable oils have been found to contain 2- and 3-monochloropropane-1,2-diols (2-MCPD and 3-MCPD, respectively) through the use of chlorinated ingredients (*e.g.*, hydrochloric acid) in the presence of acylglycerols during deodorization at high temperature (>200 °C), although a direct correlation with temperature has not been found (JECFA, 2017). Palm oil has been reported to contain the highest concentrations of 3-MCPD esters, compared to other edible oils. The EPO manufacturing process does not utilize chlorinated chemicals, and so the production of these types of chemicals are not expected to be present in the final product. Glycidyl esters may also be produced in the manufacture of RBD oils when the vegetable oil is heated to > 200°C, the main driver in the production of glycidyl esters in oils (JECFA, 2017). The EPO manufacturing process does not heat the oil to > 200°C and therefore glycidyl esters would not be expected to be present in the final refined EPO. Polycyclic aromatic hydrocarbons (PAH) are formed during the incomplete combustion of carbon-containing organic matter and may end up in RBD oil.[6] High-temperature cooking, especially during open-flame cooking and smoking/drying, will form PAHs in meat and other foods (Sampaio *et al.*, 2021). As the manufacture of EPO does not include the use of temperatures above 100°C and does not include open-flame processes, it is highly unlikely that PAHs would be formed during the production of EPO. A detailed discussion of the manufacturing process utilized to produce EPO is provided in Appendix II.

2.5 Stability

Current data demonstrate that with the addition of food grade antioxidants, EPO is stable for at least six months. Food grade antioxidant preservatives (*e.g.*, mixed tocopherols and/or green tea extract) are added by Terviva to EPO to maintain stability of the oil. Ongoing evaluations will determine the length of time EPO containing antioxidants is stable under the stated conditions. Additional stability analysis (Table 7) showed that EPO containing the antioxidants: (1) green tea extract (at a total of 5,000 ppm); or (2) mixed tocopherols + green tea extract (at a total 5,000 ppm) is stable for six months.

[6] https://www.cdc.gov/biomonitoring/PAHs_FactSheet.html#:~:text=Related%20Pages,small%20particles%20in%20the%20air.; site last visited July 21, 2022.

Table 7. Stability of EPO with antioxidants* at 20°C over a period of 24 weeks

Lot	OSI (110°C; hours) (>1 hr)[a]	Peroxide value (meq/kg) (<20)[a]	*p*-Anisidine value (<20)[a]
Neat			
0 weeks	2.6	5.0	8.0
8 weeks	1.2	21.0	9.5
16 weeks	0.05	59.0	11.7
24 weeks	0.05	147.0	22.0
Green tea extract*			
0 weeks	18.8	5.0	8.3
8 weeks	18.1	5.4	6.6
16 weeks	17.2	7.1	6.2
24 weeks	15.8	7.9	7.6
Mixed tocopherols + Green tea extract**			
0 weeks	19.7	5.2	6.1
8 weeks	18.8	6.4	7.6
16 weeks	18.0	9.1	7.7
24 weeks	17.3	13.0	8.0

[a] Specification limit; OSI: Oxidative Stability Index

*Total antioxidant (green tea extract) concentration at 5g/kg (5,000 ppm) added to the oil.

* Total antioxidant (mixed tocopherols + green tea extract) concentration at 5 g/kg (5,000 ppm) added to the oil. The total mixed tocopherols added is a minimum of 2.0 g/kg oil (2000 ppm) mixed tocopherols (minimum 1.1 g/kg non-*alpha* tocopherol and 3 g/kg (3,000 ppm) green tea extract.

Approximate inherent tocopherol content is provided in Table 8, which adds to the ability to maintain the stability of the oil.

Table 8. Tocopherols in EPO*

Tocopherol	Abundance (ppm)
alpha- Tocopherol (ppm)	<55.2
beta-Tocopherol (ppm)	<55.2
delta-Tocopherol (ppm)	<55.2
gamma-Tocopherol (ppm)	<55.2
Total Tocopherols (ppm)	<55.2

*Data represents one processing batch (TV_OIL_0314)

Part 3: Dietary Exposure

3.1 Estimated Daily Intake

The intake profile (amount and frequency) by individuals in USDA's What We Eat in America (WWEIA) Continuing Survey of Food Intakes by Individuals 2017-2018 (USDA, 2021) was used to calculate the estimated daily intake (EDI) of EPO for individuals consuming the food groups selected for the addition of EPO *per* this GRAS evaluation. The food groups as defined by the WWEIA database are provided in Table 9.

Table 9. Food groups selected for EPO addition*

Food Category	Maximum intended use level (ppm)
Dairy drinks and substitutes	21,000
Cheeses (included imitation & spreads)	58,000
Meats (imitations)	34,000
Poultry (imitations)	32,000
Seafood (imitations)	30,000
Eggs (imitation products)	30,000
Plant-based protein foods: nuts and seeds; processed soy products	55,000
Breads and bread products, rolls and tortillas; cooked cereals	27,000
Crackers, snack/meal bars/sweet bakery products	30,000
Chips, popcorn, pretzels	351,000
Cooked vegetables	50,000
Sweetened beverages; nutritional and smoothie-type beverages	25,000
Protein and nutritional powders	85,000
Fats and oils	100,000
Condiments and sauces	222,000

*The food categories correspond to those listed in the WWEIA 2017-2018 database https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/1718/Key%20Points%20Using%20WWEIA%20NHANES%202017-2018.pdf; site last visited Dec 19, 2022.
ppm=parts *per* million

The mean and 90th percentile EDIs were calculated for EPO intake following addition of the EPO to the selected food groups. The mean and 90th percentile EDIs were not calculated for current EPO intake from natural sources as no information regarding current intake of oil from the Pongamia (*M. pinnata*) tree was found following a thorough search of the published literature. The addition of EPO to the selected foods at the levels specified in Appendix III would provide a mean and 90th percentile EPO intake of 3,228.45 and 7,703.32 mg/day, respectively, as indicated in Table 10. This mean and 90th percentile daily intake on a body weight basis is estimated at 55.19 and 127.32 mg/kg bw[7]/day of EPO, respectively.

The USDA has also estimated total *per capita* intake of oils for the 2017-2018 data cycle, reporting consumption of oils at 29.12 ± 0.707 g/day for males and females two years of age and older,[8] approximately equivalent to 485 mg/kg bw/day for a 60 kg person. Using this value as the mean for current consumption, the 90th percentile current EDI of oils is estimated by assuming two times greater consumption than the reported mean current EDI.[9] Thus, the estimated 90th percentile current EDI of oil intake for people two years and older is approximately 58.24 g/day (970.67 mg/kg bw/day), based on the USDA database. The overall oil intake in the U.S. indicates that

[7] bw=bodyweight
[8] https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/FPED/Tables_1-4_FPED_1718.pdf; site last visited December 20, 2022.
[9] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-estimating-dietary-intake-substances-food#:~:text=Examination%20of%20food%20frequency%20and,approximately%204%20times%20the%20mean.; site last visited December 20, 2022.

replacement of oil in the selected categories would result in only a fraction of the total amount of oil intake being replaced by EPO. In addition, the above calculations reflect 100% market replacement of the oil content in the stated foods in Table 10 by EPO, which cannot be obtained by current agricultural production of EPO, as there are not enough *M. pinnata* trees worldwide to produce enough oil for 100% market replacement. Therefore, the estimated consumption is a gross overestimation of potential EPO intake.

All food categories designated by Terviva have been utilized in the calculations as appropriate. The high-oleic EPO will be added only to foods for which a standard of identity does not exist and, would be a substitute for other edible oils used in the production of stated food products; therefore, oil intake will not be increased.

Table 10. Predicted intake of EPO following supplementation of selected foods at the indicated levels and total intake for individuals consuming selected supplemented foods

	Per User (mg/day)	
EPO intake from:	**Mean**	**90th Percentile**
Possible maximum consumption with EPO as an added ingredient to food	3,228.45	7,703.32

EPO does contain flavonoids. Based on the concentrations identified from EPO analysis, the major flavonoids in EPO (<150 ppm) include karanjin and pongamol each. Assuming an approximate 300 ppm flavonoid content in the oil and 90th percentile intake of the oil at 7.703 g/day would result in an estimated flavonoid intake of 2.31 mg/day. Kent *et al.* (2018) reported that total dietary flavonoid intake in older adults (N=79, mean age of 70.1 years) was estimated at 678.69 mg/day. Other assessments place flavonoid intake (depending on analysis of aglycone or glycoside content) in different countries ranging from approximately 200 – 696 mg/day (Escobar-Cevoli *et al.*, 2017). Therefore, the consumption of the flavonoids contained in EPO is insignificant in comparison to overall flavonoid intake from other sources in the human diet.

3.2 History of use of Pongamia oil

Pongamia oil has historically been considered an inedible oil because of the furanoflavonoid content (*e.g.*, karanjin, pongamol) which impart a bitter taste and disagreeable odor (Fu *et al.*, 2021). Heretofore the methods for extraction of the furanoflavonoids had been inefficient and laborious, were not standardized and not altogether suitable for producing a commercially viable consumable oil. As a result, crude Pongamia oil has been used for commercial purposes including as a water-paint binder, a mechanical lubricant and as fuel for cooking and lamps in rural India (Meher *et al.*, 2004). Pongamia oil has been evaluated for biofuel. As noted earlier, no commercial production for human consumption was identified in the public literature (Fu *et al*, 2021).

Pongamia oil is used as a raw material in the production of cosmetics, including hair products, and soap in rural areas; as a component in the leather tanning process, and as a lamp and

cooking fuel oil, but the dark color and off-odor limit its commercial use (Meher *et al.*, 2004; Halder *et al.*, 2014; Good Scents Company, 2021).[10] In southern India, a mixture of the oil with the leaf of *Eupatorium odorata* is applied topically to wounds (Arote and Yeole, 2010; Al Muqarrabun *et al.*, 2013) One vendor indicated that Karanja capsules (*M. pinnata*)[11] should be taken orally (470 mg/capsule, 2 – 4 times/day);[12] although for most products, vendors stipulated external use only, while others did not supply directions for use.[13] Claims for relieving various maladies *via* external application are prominent.

3.3 Summary of EPO Exposures

The food groups selected for the addition of EPO *per* this GRAS evaluation, as defined by the WWEIA database (USDA, 2021), are provided in Table 9. In summary, exposure estimates to the EPO based on their intended use may be up to and including 7,703.32 mg/day, approximately equivalent to 127.33 mg/kg bw/day (90th percentile).

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[10] https://hort.purdue.edu/newcrop/duke_energy/Pongamia_pinnata.html; site last visited February 14, 2022.

[11] Vendor states the capsules contain "…100 % pure ingredients specially formulated and include green leaves, flowers, seeds, roots & barks.

[12] https://www.amazon.com/DR-WAKDES%C2%AE-Ayurvedic-Supplement-multiples/dp/6040338555; site last visited Apr 26, 2020.

[13] https://www.amazon.com/Premium-Karanja-Organic-Unrefined-Undiluted/dp/B00Y128NO8/ref=sr_1_3?gclid=CjwKCAiA9tyQBhAIEiwA6tdCrNOuhf9U5HrsTCoTIyQfpyiQe5M0grQypjkOQHA4xq36iF-HFwb18BoCx6AQAvD_BwE&hvadid=190516135384&hvdev=c&hvlocphy=9011784&hvnetw=g&hvqmt=b&hvrand=10828006598535040 97&hvtargid=kwd-488002879432&hydadcr=29135_10164395&keywords=karanja+seed+oil&qid=1645739016&sr=8-3; site last visited February 24, 2022.

Part 4: Self-limiting Levels of Use

The use of EPO in foods is considered to be self-limiting for technological reasons, such as product texture and/or flavor profile, either of which could affect consumer acceptance.

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Part 5: Experience Based on Common Use in Food Prior to 1958

The statutory basis for the conclusion of the GRAS status of EPO in this document is not based on common use in food before 1958. The GRAS assessment is based on scientific procedures.

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Part 6: Narrative

6.1. Absorption, distribution, metabolism, and elimination (ADME)

The major fatty acids of the triacylglycerols (TAG) in EPO (on a fatty acid percentage of total) include, but are not limited to, oleic acid (approximately 50%), linoleic acid (approximately 17%), palmitic acid (approximately 8%), stearic acid (approximately 8%), and behenic acid (approximately 4%), making up at least 90% of the total fatty acid content. Other fatty acids in EPO are approximately 2% or less.

The TAG that comprises EPO are expected to be digested, absorbed, and metabolized and the metabolites excreted in a manner identical to other commonly consumed TAG from plant and animal sources. Oleic acid is found in many different edible oils, including olive, sunflower, and canola oils. Linoleic acid is found in many vegetable oils, nuts, and seeds, while palmitic acid is common in butter, cheese, and milk. Stearic acid is commonly found in animal fats, including small amounts in milk. Stearic acid may not be completely absorbed, with estimates at 68 – 98% absorbed (Jones *et al.*, 1999; Baer *et al.*, 2003).

Fatty acids are released from triacylglycerol molecules in the small intestine, largely producing free fatty acids (FFA) and monoacyl glycerols. The monoacyl glycerols and free fatty acids are absorbed by enterocytes, then re-assembled as TAG in the endoplasmic reticulum by acyltransferases; monoacylglycerols are esterified with FFAs by monoacylglycerol acyltransferases to form diacylglycerols, which are converted to triacylglycerols by diacylglycerol acyltransferases, "packaged" into chylomicrons and transported to adipocytes and stored in cytosolic lipid droplets (Hussain, 2014). When needed, the triacylglycerols are released from the cellular lipid stores by diacylglycerol lipase and monoacylglycerol lipase that work together to provide complete hydrolysis of the triacylglycerol molecule to release the fatty acids (Tardelli, 2020). Once in the peripheral tissues and absorbed into the cells, fatty acids are degraded by β-oxidation in the cellular mitochondria, resulting in successive release of two-carbon acetyl coenzyme A (acetyl-CoA) molecules. The citric acid cycle uses the acetyl-CoA molecules to produce reducing equivalents. The reducing equivalents produce adenosine triphosphate (ATP) for energy *via* the electron transport chain (Gotoh *et al.*, 2008). This indicates that the triglycerides and fatty acids that make up EPO would be absorbed, distributed, metabolized, and excreted in a manner identical to other commonly consumed TAG from plant and animal sources.

6.2 Acute studies

A non-GLP limit test of the acute oral toxicity of EPO was conducted in female Sprague-Dawley rats ($N = 5$). The EPO was administered by gavage (Marone *et al.*, 2022). The study was conducted according to EPA guidelines and consisted of dosing a single, fasted rat with 5,000 mg/kg by gavage. The dosed rat was observed for 48 hours and when no abnormalities were noted, an additional four animals were dosed in a similar manner. According to the authors "[A]cute administration of Debittered Pongamia Oil followed by observation for 14 days and euthanization [sic] with thoracotomy[sic] resulted[sic] in the animals tolerating the exposure well with no toxic

signs or abnormalities observed in any of the tested animals."[14] The authors concluded "[T]he acute oral LD_{50}[15] of Debittered Pongamia Oil was deemed greater than 5,000 mg/kg in Sprague-Dawley female rats."

Gandhi and Cherian (2000) evaluated the acute toxicity of raw karanja oil (not extracted), and the same oil extracted with aqueous methanol. This extraction process was not the same manufacturing process as for EPO. Oral doses of raw karanja oil[16] at 10 and 20 ml/kg bw (approximately equivalent to 10,000 and 20,000 mg/kg bw) to rats (Haffkine-Wistar, N = 2/sex/dose) resulted in 100% mortality within 12 – 48 hours following dosing; the same doses of the extracted oil elicited no adverse effect. The authors stated that "the fatty acid composition of the raw and extracted karanja oils showed no difference" (Gandhi and Cherian, 2000). A material data safety sheet (Neem Pro Inc., 2015) reported an oral LD_{50} of >4,000 mg/kg in the rat, and a dermal LC_{50}[17] in the rat at >2,000 mg/kg. No additional information was provided in the publication.

An acute oral toxicity study of a crude bean extract (*via* Soxhlet apparatus) of *M. pinnata* was conducted in five female Wistar rats at a single dose of 2,000 mg/kg/bw, then observed for 14 days. No adverse effects were reported. All animals gained weight throughout the study. Lipid peroxidation was significantly ($P < 0.05$) increased in the liver, brain, kidney, heart, and spleen of the *M. pinnata* extract-treated animals compared to controls. Glutathione levels in the spleen and oxidized glutathione levels in the liver were significantly increased in treated over control animals, but not in the lung, brain, kidney or heart (Aneela *et al.*, 2011). Bose *et al.* (2013) analyzed the effects of the hexane extract of *M. pinnata* beans in an adjuvant-induced arthritic rat model, which included the evaluation of some safety-related parameters. Male Wistar rats were administered a single gavage dose of the seed extract in graded doses (10 – 30 g/kg) to determine the minimum lethal dose (MLD). After determining the MLD of 28,000 mg/kg bw hexane seed extract, additional Wistar rats (N = six/group) were treated with a single dose (by gavage) with the extract at 300 or 500 mg/kg bw/day, which the authors stated were 1/50th and 1/75th of the MLD dose and observed for 14 days. At the end of the 14-day observation period, there were no statistically significant ($P < 0.05$) changes in urinary creatinine values, or serum AST, ALT or LDH concentrations, relative to the control group (Bose *et al.*, 2013).

In streptozotocin-induced diabetic rats, single oral doses of pongamol (50 mg/kg) and karanjin (100 mg/kg) lowered the blood glucose by 12.8% ($P < 0.05$) and 11.7% ($P < 0.05$) at 50 mg/kg dose and 22.0% ($P < 0.01$) and 20.7% ($P < 0.01$) at 100 mg/kg dose, respectively after 6 hours post administration. The results showed that pongamol and karanjin isolated from the fruits of *M. pinnata* possesses significant antihyperglycemic activity in streptozotocin-induced diabetic rats (Tamrakar *et al.*, 2008).

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[14] "Debittered Pongamia Oil" is synonymous with EPO.

[15] LD_{50} = (the) Lethal Dose at which 50% of the animals die when a substance is administered internally.

[16] Karanjin, pongamol or other flavonoid contents not analyzed.

[17] LC_{50}=(the) Lethal concentration at which 50% of the animals die when a substance is applied topically.

The acute study of EPO did not induce toxicity in female Wistar rats at 5,000 mg/kg (*i.e.*, LD_{50} > 5,000 mg/kg in rats). A methanolic extract of pongamia oil of 10,000 and 20,000 mg/kg did not elicit signs of toxicity. Crude pongamia oil was lethal at 10,000 mg/kg and 20,000 mg/kg bw. These acute oral toxicity studies show that crude oil from the pongamia plant is different from Terviva's EPO in crucial safety-related parameters and therefore studies conducted with raw, unrefined pongamia oil have little relevance to the safety of EPO.

6.3 Subacute and subchronic studies

A 14-day range-finding palatability and general toxicity study was conducted on EPO, conducted consistent with current good laboratory practice (GLP) standards, meeting the requirements of FDA GLP (21 CFR Part 58), which is compatible with OECD GLP principles (Marone *et al.*, 2022). The 14-day study utilized individually housed male and female Sprague Dawley rats (CRL SD CD® IGS; 5/sex/group) that were fed a standard rodent chow diet (Group 1), an isocaloric diet that was utilized as a control (Group 2) that contained cocoa butter, high oleic sunflower and soybean oils, or the basal diet that had decreasing levels of the above fatty acid sources to which was added 50,000 ppm (Group 3), 100,000 ppm (Group 4), and 150,000 ppm (Group 5) EPO. The diets were formulated weekly. Test substance stability and homogeneity in the diet were conducted. The rats were fed the stated diets with target doses of 4,167, 8,333 and 12,500 mg/kg bw/day for Groups 3 – 5, respectively. The rats were evaluated daily for clinical signs, gross toxicity, and behavioral changes, and at least weekly for body weight and food consumption. Just prior to scheduled necropsy, blood was obtained to evaluate clinical chemistry, hematology, and coagulation parameters. Gross necropsies were completed, absolute and relative organ weights obtained, and gross and histological evaluation of liver tissues were conducted, as the liver was believed to be a sensitive organ for potential toxicity, as the liver is the primary source for general metabolism and therefore a likely indicator organ for toxicity of an ingredient with unknown toxicity (Chandni and Ashwani, 2017a).

No test-substance-related mortalities were reported during the 14-day study, and any clinical observations were incidental and of no toxicological significance (*i.e.*, slight alopecia and some fur/skin oily coat soiling). No changes were reported in body weight, body weight gain, food consumption or food efficiency were attributable to EPO administration (Marone *et al.*, 2022). The authors stated, "there were no adverse test substance-related changes attributed to the dietary administration of EPO for 14-days". No absolute or relative organ weight changes or macroscopic or microscopic liver changes were attributable to EPO (Marone *et al.*, 2022). The mean daily doses were 4,328.0, 8,036.5 and 12,023.0 mg/kg/day for males and 4,560.1, 9,694.3, and 11,898.5 mg/kg/day for females for Groups 3 – 5, respectively. The rats tolerated 15% (150,000 ppm) of EPO, the highest dose tested.

Beans from the *M. pinnata* tree were collected, dried, powdered, and subject to a petroleum ether extraction process (Mandal *et al.*, 1984). The resulting oil (termed karanja oil) was refined, and the authors stated that "toxic flavonoids including karanjin and pongamol were removed" (Mandal *et al.*, 1984). Male Wistar rats (N = 12/group) were individually housed and fed a stock

standard diet that contained either 10% peanut oil (control group), 10% karanja oil, or 10% akashmoni[18] oil for twelve weeks. At the end of the twelve weeks, the rats were euthanized, blood and liver samples obtained, and total lipids, phospholipids, free fatty acid, cholesterol, and triglycerides were determined. The liver, kidney, heart, spleen, pancreas, intestine (large and small), and reproductive organs) were subjected to histopathology.

There was a decreased growth rate in the rats consuming the pongamia oil (Table 11). Serum and liver total lipid levels of the rats fed karanja oil were significantly greater than the control group, and the liver triglyceride content was significantly increased in the karanja oil group ($P < 0.01$). Liver weight was also increased in the pongamia oil group, compared to the control group, but the authors did not state if this was a significant effect. Mild to moderate fatty infiltration was reported in the liver of the rats consuming 10% karanja oil. The authors concluded that "The present study therefore indicates that the refined karanja oil is not suitable as a dietary source of fat, unless a suitable method is found for its complete detoxification" (Mandal *et al.*, 1984).

Table 11. Growth rate, serum and liver lipid content of rats fed peanut or Pongamia oil (Mandal *et al.*, 1984)

Parameters Studied	Peanut Oil	Pongamia (karanja) Oil
Growth Rate		
Gain in body weight (average, g) at Day 84	126.4 ± 10.2[a]	84.2 ± 11.4*
Feed Efficiency Ratio	22.5 ± 2.1	18.1 ± 1.8
Fat digestibility (%)	94	91
Liver weight (% of body weight)	3.5 ± 2.1	4.1 ± 2.2
Serum		
Total lipids (mg/100 ml)	278.3 ± 8.5	312.2 ± 7.4*
Total phospholipids (mg/100 ml)	84.3 ± 3.9	80.1 ± 4.3
Total cholesterol (mg/100 ml)	68.2 ± 4.1	70.4 ± 3.8
Triglycerides (mg/100 ml)	20.3 ± 2.1	25.2 ± 2.6
Free Fatty acids (mmol/L)	0.32 ± 0.04	0.34 ± 0.05
Liver		
Total lipids (mg/100 ml)	132.1 ± 4.2	288.7 ± 5.8**
Total phospholipids (mg/100 ml)	78.2 ± 3.4	75.1 ± 3.8
Total cholesterol (mg/100 ml)	6.2 ± 0.4	6.8 ± 0.6
Triglycerides (mg/100 ml)	24.1 ± 1.6	48.4 ± 2.1**
Free Fatty acids (mmol/L)	3.4 ± 0.7	3.8 ± 0.5

[a]Values are mean ± SEM ($N = 12$ animals/group)
*$P < 0.01$; **$P < 0.001$

In a follow-up study, Mandal *et al.* (1985) conducted a 30-day toxicity study in which adult male albino rats (in-house laboratory strain) were fed an extracted pongamia oil at 15% in the diet, and plasma and liver lipids and plasma and depot fat fatty acid composition were evaluated. The pongamia oil used was extracted from the ground pongamia bean by Soxhlet solvent (petroleum

[18] A similar seed-bearing tree, *Acacia auriculiformis*.

ether), refined by removal of "the toxic materials", color odor and bitterness, and then hydrogenated. The male albino rats (N = 12/group) were provided either the pongamia oil, corn oil, coconut oil, or a mixture of fatty acids[19] (control group): lauric acid (2.4g/kg diet), myristic acid (2.1 g/kg diet), palmitic acid (15.5 g/kg diet), stearic acid (2.4 g/kg diet), oleic acid (12.4 g/kg diet), linoleic acid (20.2 g/kg diet), and arachidonic acid (2.5 g/kg diet). Body weights and food intake were recorded daily. At study conclusion, the rats were euthanized and blood, liver, and fat depots (defined by the authors as a site in the body in which large quantities of fat are stored, as in adipose tissue) fat analysis were conducted. Blood was analyzed for serum lipids, phospholipids, and fatty acids, blood hemoglobin, glucose, and urea. Samples of liver were analyzed for total lipid, phospholipid, cholesterol, and fatty acid content.

There was no difference between the control and pongamia oil groups for food intake, body weight gain, blood hemoglobin, glucose, or urea levels. Compared to the control group, the group that consumed the refined pongamia oil had increased plasma concentrations of total lipid, cholesterol, and free fatty acids. The plasma level of oleic acid was increased in the pongamia oil-fed group, compared to the control group. The fatty acid content of the fat depot of the pongamia oil-fed group showed an increase in oleic acid, compared to the control group. The authors did not indicate that these plasma or fat depot changes were adverse in nature, concluding that "[I]n light of the present results it appears therefore that [refined pongamia oil] causes no growth retardation, health hazards or toxic effects on the biochemical parameters of rats. Thus [refined pongamia oil] could be incorporated in foods as a fat ingredient" (Mandal *et al.*, 1985).

Baki *et al.* (2007) reported a 14-day toxicologic study of pongamol-based bean extract isolated from *M. pinnata* and administered to Long Evans male rats at a dose of 300 µg/day (*i.p.*)[20] for 14 days. There were no changes in body weight, hematology, clinical pathology parameters and no histopathologic changes in liver, kidney, heart, or lung of the experimental group compared with control (Baki *et al.*, 2007), indicating that low levels of this component did not induce toxicity, even when administered *i.p.* in this study.

Vismaya *et al.* (2011) reported a 14-day oral toxicity study in male Wistar albino rats (N = six/group) fed karanjin (95% pure) at 10 and 20 mg/kg bw/day as part of a study to determine karanjin's effects on stomach ulcer formation. Oral administration (no additional information provided) of karanjin for 14 days at 20 mg/kg bw/day did not change plasma protein levels, alkaline phosphatase, or ALT levels, compared to the control group. AST levels were significantly ($P < 0.05$) decreased in the karanjin group, compared to the control group. Stated values were within the author's reference range, to which the authors stated that the results were "...indicating no adverse effect on major organs at the ingested concentrations" (Vismaya *et al.*, 2011). During the same study, karanjin did not alter gastric mucin or H+K+-ATPase levels. Administration of karanjin for 14 days (20 mg/kg bw/day) prior to ethanol-induced ulcer formation resulted in the limiting of ethanol-induced decreases in gastric mucin content, and inhibited ethanol-induced

[19] The manuscript only states that a mixture of fatty acids was provided, not a specific oil (Mandal *et al*, 1985).

[20] *i.p.*=Intraperitoneal

increases in gastric H+K+-ATPase levels, compared to ethanol treatment alone. There was no adverse effect of karanjin on food and water intake, body weight gain, gross organ structure or clinical parameters[21] measured (Vismaya *et al.*, 2011), indicating that low levels of this component did not induce toxicity in this study, when administered orally to rats.

A 13-week toxicity study, which included a 28-day recovery period, was conducted in male and female Sprague Dawley (CRL SD CD® IGS) rats (N = 10/sex/group, 7 – 8 weeks of age at start) analyzing the potential toxicity of EPO (Marone *et al.*, 2022). The study was conducted consistent with OECD guidelines[22] and adhered to FDA GLP standards to evaluate the potential toxicity of EPO when administered as a dietary admixture for at least 90 days (Marone *et al.*, 2022). Isocaloric dietary concentrations of the basal control[23] diet (Group 1) and increasing concentrations of 25,000 ppm (Group 2), 50,000 ppm (Group 3), 100,000 ppm (Group 4) EPO and a comparator control[24] (Group 6), which provided ratios of fatty acids from commonly consumed fats (cocoa butter, high oleic sunflower oil, and soybean oil) at levels comparable to the high-dose (100,000 ppm) EPO dose group. Additional control and high-dose animals (N = 10/sex/group, 7 – 8 weeks of age at start) that were a part of the 90-day main test were fed control diet for another 28 days (recovery phase).

All animals (main study and recovery animals) were observed at least once daily for gross toxicity, behavioral changes and other adverse effects, as *per* FDA Redbook guidelines.[25] A functional observational battery (FOB) was conducted on Day 89 (males) and Day 90 (females) to evaluate potential adverse effects on excitability, autonomic function, gait, reactivity, sensitivity, and other clinical parameters (Mattsson *et al.*, 1996). Grip strength, foot splay, and motor activity parameters were also analyzed. Individual body weights were recorded prior to study start, on Day 0, and then weekly thereafter until the end of the main study (Day 91) or the end of the recovery phase (Day 119). Individual food consumption was measured weekly, and food efficiency calculated.

At the end of each test phase (main test and recovery), all surviving animals were fasted overnight, blood samples obtained for clinical chemistry, hematology, and coagulation parameters, then euthanized and necropsied. Samples for thyroid hormone parameters were not collected from the recovery animals since treatment-related effects were not identified in the dosing phase animals. Urine was collected utilizing metabolism cages prior to obtaining blood samples, and

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[21] Clinical parameters as defined within the Contract Laboratory's Standard Operating Procedures (PSL, 2018) and Mattsson *et al.* (1996).

[22] https://www.oecd-ilibrary.org/docserver/9789264070707-en.pdf?expires=1641851256&id=id&accname=guest&checksum=CA325FB0AEEB25515DC622EB3DC20531; site last visited January 10, 2022.

[23] Diet for Group 1 was the Standard 2016 Certified Envigo Teklad Global Rodent Diet® (Marone *et al.*, 2022).

[24] The Comparator Control (also referred to as Reference Control) provided fatty acid ratios similar to those found in EPO.

[25] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/redbook-2000-ivc10-neurotoxicity-studies#vc3ai; site last visited August 18, 2022.

vaginal smears for estrus staging were obtained on the day of terminal sacrifice. All animals were weighed immediately prior to necropsy which included examinations of the external body surface, orifices, musculoskeletal system, and the cranial, thoracic, abdominal and pelvic cavities and associated organs and tissues. Select organs were weighed and fixed in appropriate buffer.[26] Tissues from all animals in both control and high dose groups (Groups 1 and 5, respectively) from the main study were prepared for histopathological evaluation which was by a board-certified veterinary pathologist. Spermatogenesis and interstitial testicular cell structure[27] of all Groups 1 and 5 main study male rats was conducted as suggested by the optional parameters in the OECD 408 guideline protocol.

No statistically significant differences in daily food consumption or food efficiency between the control and EPO groups were reported (Marone et al, 2022). The mean daily intakes (main test) of EPO fed as a dietary admixture were 0, 1,285.9, 2,667.7, 3,784.1 and 5,162.7 mg/kg bw/day for males and 0, 1,564, 3,159, 4,480.9 and 6,468.6 mg/kg bw/day for females over the course of the study (Days 0-91) for Groups 1 – 5, respectively (Marone et al, 2022). One Group 5 female rat (in the recovery group) was sacrificed on Day 45 for tumor development that was discovered on study Day 9 and was not related to test substance administration). There were no statistically significant, treatment-related, dose-dependent adverse effects on survivability, body weights, body weight gain, food consumption, functional observation battery and motor activity, ophthalmological findings, absolute or relative organ weights or gross and histopathological findings (Marone, et al, 2022). Slight-to-moderate alopecia, superficial eschar, and slight desquamation were reported, particularly in Groups 1 and 5, but were not test substance-specific and the authors attributed it to "food jar gathering/scraping against the skin" (Marone *et al.*, 2022).

There were no statistically significant, treatment-related, dose-dependent adverse effects on hematology (Marone *et al.*, 2022), clinical chemistry (Table 12 and Table 13), coagulation, urinalysis (data not shown), or thyroid hormone (Table 14) findings associated with administration of EPO. Some reported effects included alanine aminotransferase (ALT) decreased in Group 5 males ($P < 0.001$), and aspartate aminotransferase (AST) in decreased in Groups 2 ($P < 0.05$), 3 ($P < 0.01$), 5 ($P < 0.01$) and 6 ($P < 0.05$) males; increased ($P < 0.05$) alkaline phosphatase (ALKP) in Group 5 females; decreased globulin (GLOB) ($P < 0.05$) in Group 5 males. Urine glucose (GLUC) was decreased ($P < 0.05$) in Groups 2 – 6 females, a response to two notable individual animals in the basal control group with significantly large, discrepant, levels.

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[26] Adrenals (combined), brain, epididymides (combined, males), heart, kidneys, liver, ovaries with oviducts (combined, females), spleen, testes (combined, males), thymus, and uterus. The thyroid/parathyroid, pituitary gland, and the prostate and seminal vesicles with coagulating gland (combined, males only) were weighed at least 24 hours after preservation in 10% neutral buffered formalin. The epididymides, testes, eyes, and optic nerve from all animals were preserved in modified Davidson's fixative, and then transferred to ethanol.

[27] Parameters included a qualitative evaluation of Leydig cells and other parameters of testicular damage, and an approximation of stage frequency, comparisons of tubular diameters of selected stages between the groups, and cell counts of round spermatids, leptotene and pachytene spermatocytes, and Sertoli cells.

Table 12. Clinical chemistry results (mean ± SD) from male rats (N = 9-10/group) administered EPO for 13 weeks (Marone *et al.*, 2022)

Group	1	2	3	4	5	6
Dietary Level (ppm)	0	25,000	50,000	75,000	100,000	0
Animals In Group	9*	10	10	10	10	10
ALT (U/L)	71.0 ± 58.6	31.2 ± 11.6	37.6 ± 43.5	31.2 ± 15.4	20.7 ± 4.1***	46.1 ± 46.2
ALB (g/dL)	4.08 ± 0.22	4.08 ± 0.26	4.11 ± 0.19	4.09 ± 0.29	3.98 ± 0.27	4.18 ± 0.29
ALKP (U/L	73.6 ± 11.0	73.4 ± 14.7	71.9 ± 15.7	80.0 ± 12.0	78.9 ± 17.4	72.2 ± 8.5
AST (U/L	149.7 ± 67.6	95.3 ± 24.3*	95.2± 42.3**	103.3 ± 19.0	84.6 ± 10.7**	100.3 ± 39.3*
CALC (mg/dL)	11.74 ± 0.49	11.61 ± 0.67	11.61 ± 0.37	11.21 ± 0.78	11.24 ± 0.52	11.66 ± 0.72
CL (mmol/L)	101.70 ± 0.89	100.93 ± 1.18	101.37 ± 1.62	101.67 ± 1.31	101.02 ± 2.91	101.17 ± 2.26
CHOL (mg/dL)	62.7 ± 18.3	60.3 ± 15.4	55.3 ± 16.1	67.7 ± 22.1	56.8 ± 13.9	70.2 ± 12.4
CREA (mg/dL)	0.249 ± 0.030	0.263 ± 0.060	0.246 ± 0.040	0.250 ± 0.047	0.264 ± 0.028	0.263 ± 0.043
CK (U/L)	228.778 ± 173.139	255.600 ± 124.254	199.200 ± 93.963	268.100 ± 123.606	212.300 ± 80.869	278.900 ± 197.078
GLOB (g/dL)	2.38 ± 0.16	2.30 ± 0.24	2.16 ± 0.24	2.16 ± 0.28	2.06 ± 0.25*	2.28 ± 0.17
GLUC (mg/dL)	194.3 ± 33.2	195.9 ± 34.2	185.2 ± 28.0	188.4 ± 30.5	189.5 ± 48.0	203.0 ± 40.0
HDL (mmol/L)	1.000 ± 0.308	1.070 ± 0.298	0.980 ± 0.270	1.230 ± 0.395	1.030 ± 0.283	1.180 ± 0.210
IPHS (mg/dL)	8.92 ± 1.20	9.39 ± 1.15	9.73 ± 1.43	8.88 ± 1.50	9.26 ± 1.06	8.88 ± 1.17
LDL (mmol/L)	0.356 ± 0.101	0.310 ± 0.099	0.250 ± 0.097	0.320 ± 0.155	0.240 ± 0.070	0.330 ± 0.116
K (mmol/L)	7.009 ± 1.428	7.884 ± 1.556	7.631 ± 1.070	6.899 ± 1.113	6.853 ± 1.332	7.446 ± 1.160
NA (mmol/L)	143.33 ± 2.00	142.10 ± 2.33	142.70 ± 1.89	142.60 ± 2.80	142.30 ± 3.30	142.70 ± 2.54
SDH (U/L)	29.96 ± 26.78	17.68 ± 7.32	17.55 ± 14.52	17.52 ± 11.14	11.79 ± 3.95	21.31 ± 16.95
BILI (mg/dL)	0.092 ± 0.050	0.073 ± 0.014	0.069 ± 0.013	0.068 ± 0.029	0.063 ± 0.018	0.054 ± 0.022
TP (g/dL)	6.46 ± 0.29	6.38 ± 0.42	6.27 ± 0.24	6.25 ± 0.46	6.04 ± 0.38	6.46 ± 0.37
TRIG (mg/dL)	62.6 ± 28.1	56.7 ± 19.8	63.1 ± 48.3	60.5 ± 16.9	72.2 ± 24.2	82.9 ± 25.6
BUN (mg/dL)	14.6 ± 1.4	14.2 ± 2.4	14.0 ± 1.2	14.9 ± 2.8	13.9 ± 2.3	13.9 ± 2.0

ALB, albumin; ALKP, alkaline phosphatase; ALT, alanine aminotransferase; AST, serum aspartate aminotransferase; BILI, total bilirubin; BUN, urea nitrogen; CALC, calcium; CHOL, total cholesterol; CL, chloride; CREA, blood creatinine; GLOB, globulin; GLUC, fasting glucose; HDL, high density lipoprotein; ; IPHS, inorganic phosphorus; K, potassium; LDL, low density lipoprotein; NA, sodium; SD, standard deviation; SDH, sorbitol dehydrogenase; TP, total serum protein; TRIG, triglycerides; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

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Table 13. Clinical chemistry results (mean ± SD) from female rats (N = 10/group) administered EPO for 13 weeks (Marone *et al.*, 2022)

Group	1	2	3	4	5	6
Dietary Level (ppm)	**0**	**25,000**	**50,000**	**75,000**	**100,000**	**0**
ALT (U/L)	17.8 ± 4.7	15.3 ± 2.9	21.2 ± 12.3	15.7 ± 3.3	17.5 ± 4.2	23.8 ± 23.1
ALB (g/dL)	5.38 ± 0.38	5.40 ± 0.30	5.33 ± 0.51	5.29 ± 0.72	5.16 ± 0.51	5.45 ± 0.55
ALKP (U/L)	31.5 ± 5.0	31.3 ± 7.6	37.7 ± 7.1	39.0 ± 10.6	43.8 ± 12.7*	30.8 ± 7.4
AST (U/L)	70.8 ± 19.9	67.9 ± 13.5	85.7 ± 32.7	77.0 ± 10.2	76.7 ± 12.7	97.8 ± 80.8
CALC (mg/dL)	11.81 ± 0.50	11.66 ± 0.56	11.32 ± 0.41	11.50 ± 0.98	11.56 ± 0.93	11.89 ± 0.87
CL (mmol/L)	100.49 ± 1.95	101.49 ± 2.12	101.89 ± 1.44	100.75 ± 1.92	101.66 ± 2.17	101.37 ± 2.23
CHOL (mg/dL)	64.5 ± 18.0	62.4 ± 18.2	57.6 ± 8.4	73.2 ± 13.0	71.9 ± 16.2	65.6 ± 18.0
CREA (mg/dL)	0.291 ± 0.049	0.294 ± 0.046	0.295 ± 0.052	0.281 ± 0.087	0.299 ± 0.044	0.287 ± 0.056
CK (U/L)	134.200 ± 70.438	263.900 ± 437.158	177.000 ± 93.791	233.600 ± 174.973	188.100 ± 174.973	203.500 ± 120.335
GLOB (g/dL)	1.45 ± 0.39	1.50 ± 0.22	1.49 ± 0.31	1.52 ± 0.41	1.63 ± 0.32	1.49 ± 0.30
GLUC (mg/dL)	194.6 ± 40.4	185.5 ± 27.6	168.2 ± 7.2	184.0 ± 38.9	186.3 ± 58.5	185.7 ± 45.6
HDL (mmol/L)	1.475 ± 0.388	1.435 ± 0.373	1.348 ± 0.206	1.682 ± 0.303	1.648 ± 0.315	1.529 ± 0.407
IPHS (mg/dL)	8.44 ± 1.09	7.95 ± 1.22	7.68 ± 0.82	8.59 ± 1.17	7.99 ± 1.65	8.21 ± 0.99
LDL (mmol/L)	0.136 ± 0.046	0.133 ± 0.037	0.117 ± 0.026	0.148 ± 0.059	0.162 ± 0.065	0.124 ± 0.065
K (mmol/L)	7.250 ± 2.117	6.666 ± 1.297	6.294 ± 1.134	6.397 ± 0.838	6.684 ± 1.964	6.798 ± 1.402
NA (mmol/L)	140.10 ± 2.64	141.10 ± 2.13	141.00 ± 2.11	141.60 ± 3.60	141.10 ± 2.23	141.50 ± 2.27
SDH (U/L)	12.96 ± 6.26	8.93 ± 2.14	13.22 ± 8.63	10.44 ± 3.28	12.00 ± 5.60	13.47 ± 14.82
BILI (mg/dL)	0.067 ± 0.036	0.079 ± 0.031	0.059 ± 0.021	0.072 ± 0.021	0.067 ± 0.022	0.070 ± 0.029
TP (g/dL)	6.83 ± 0.43	6.90 ± 0.44	6.82 ± 0.44	6.81 ± 0.44	6.79 ± 0.52	6.94 ± 0.66
TRIG (mg/dL)	58.6 ± 24.9	53.2 ± 14.6	54.0 ± 21.0	44.0 ± 14.2	52.4 ± 25.2	61.2 ± 19.9
BUN (mg/dL)	15.0 ± 1.6	15.6 ± 2.1	16.3 ± 2.1	14.6 ± 3.0	16.4 ± 3.0	15.7 ± 2.4

ALB, albumin; ALKP, alkaline phosphatase; ALT, alanine aminotransferase; AST, serum aspartate aminotransferase; BILI, total bilirubin; BUN, urea nitrogen; CALC, calcium; CHOL, total cholesterol; CL, chloride; CREA, blood creatinine; GLOB, globulin; GLUC, fasting glucose; HDL, high density lipoprotein; ; IPHS, inorganic phosphorus; K, potassium; LDL, low density lipoprotein; NA, sodium; SDH, sorbitol dehydrogenase; TP, total serum protein; TRIG, triglycerides; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Thyroid stimulating hormone (TSH) was significantly increased ($P< 0.001$) in males and females of Groups 3 – 6. Thyroxine (T4) was significantly decreased in males (Groups 3 and 4, $P < 0.001$; and 6, $P < 0.01$), and females (Groups 3 and 6 $P < 0.05$; Group 4, $P < 0.001$); and triiodothyronine (T3) was significantly increased in males (Groups 2, $P < 0.001$; and 4 $P < 0.01$), decreased in males (Groups 5 and 6, $P < 0.001$); and decreased in females (Groups 5 and 6, $P < 0.001$) compared with basal diet control (Table 14).

Table 14. Thyroid parameters (mean ± SD) of animals consuming EPO for 13 weeks (Marone *et al.*, 2022)

Group	1	2	3	4	5	6
Dietary Level (ppm)	**0**	**25,000**	**50,000**	**75,000**	**100,000**	**0**
Males						
TSH (ng/mL)	3.1459 ± 0.0972	3.2088 ± 0.0704	3.7614 ± 0.1695***	3.7264 ± 0.2394***	4.1364 ± 0.3510***	3.8281 ± 0.1582***
T4 (ng/mL)	40.1268 ± 3.0014	41.7971 ± 3.9557	34.8165 ± 1.8474***	35.2786 ± 2.7729***	37.8873 ± 1.4493	36.0073 ± 2.3337**
T3 (ng/mL)	1.5080 ± 0.4776	2.4990 ± 0.5439***	1.7785 ± 0.3522	2.1859** ± 0.5514	0.8681 ± 0.0376***	0.9688 ± 0.0621***
Females						
TSH (ng/mL)	3.2550 ± 0.1846	3.1560 ± 0.0669	3.6400 ± 0.1274***	3.8175 ± 0.2494***	3.7807 ± 0.1961***	3.9818 ± 0.3131***
T4 (ng/mL)	37.2025 ± 3.4360	34.6738 ± 2.5840	33.5537 ± 2.6854*	31.5715 ± 2.2678***	34.7726 ± 2.9215	32.9335 ± 4.4448*
T3 (ng/mL)	2.3345 ± 0.7510	2.3702 ± 0.7899	2.6418 ± 1.0045	2.2147 ± 0.4403	1.0359 ± 0.1101***	1.0333 ± 0.0973***

ppm = parts *per* million; SD = Standard deviation; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone.

*9 animals/group T3 males; 9 animals/group T3 females; ** = $P < 0.01$; *** = $P < 0.001$

Non-dose dependent findings include the following: There were statistically significant decreases in the mean absolute heart weight in the Group 4 females ($P < 0.05$) and kidney weights in Groups 3 and 4 ($P < 0.05$) females. Spleen-to-body weight ratio was reduced ($P < 0.05$) in comparator control males, and kidney-to-brain weight ratio was increased ($P < 0.05$) in females of Group 5. All organ weight values were within known historical control values and without histologic correlate (Marone et al, 2022). There were no changes in thyroid-parathyroid absolute or relative weights for males and females in any test group during the treatment period (data not shown).

After the 28-day recovery period, there were no body weight, body weight gain, food consumption and food efficiency or clinical observation changes in Group 5 compared with basal diet control group (Marone et al, 2022). Organ weight changes after 28-day recovery included significantly ($P < 0.001$) increased the absolute pituitary gland organ weight, and absolute thyroid-parathyroid weights ($P < 0.05$); and pituitary- ($P < 0.001$), uterus- ($P < 0.05$), and thyroid-parathyroid- ($P < 0.01$, $P < 0.05$) to-body weight and body-to-brain weight ratios, respectively, in females of recovery Group 5. Kidney-to-body weight ratio was significantly increased in males ($P < 0.01$) and females ($P < 0.05$) of recovery Group 5. However, the authors stated

> Without any adverse toxicological findings in the main study animals, no further assessment of blood or urine samples collected from the recovery animals for potential further analysis was conducted. Except for that of the Group 5 decedent female and those organs having necropsy findings mentioned previously, no other histological evaluation was indicated or performed for any of the other procured organs of the recovery animals (Marone *et al.*, 2022).

The authors stated that although there were significant increases in TSH levels in the EPO treatment groups and the comparator control group, T3 increases in Groups 2 and 4 and a decrease in Group 5 of the EPO male groups and decreased T3 in the female Group 5, as well as significant ($P < 0.05$) decreases in T4 levels in the male and female Groups 3 and 4 and the control Group 1, these changes were uncorrelated with any disturbances in body weight or clinical appearance/behavior, microscopic findings, or thyroid-parathyroid organ weights. In addition, the authors referred to publications indicating that plant polyphenols, alkaloids, and other plant-based substances can interfere with thyroid function (action and metabolism); an increase in thyroid hormonogenesis will cause a decreased production of T3 and T4 hormones, subsequently increasing TSH levels (Moudgal *et al.*, 1958; Gaitan, 1990).

The comparator control diet utilized in the Marone *et al.* (2022) study contained a blend of oils to provide a similar fatty acid composition to the diet containing EPO. Consumption by the rats of this comparator control diet resulted in similar thyroid hormone changes as seen from consumption of the EPO-containing diet. These results indicate that a specific fatty acid formulation such as an increase in saturated fatty acids (without an overall change in fat content), may result in changes in thyroid hormone levels that are statistically significant but not considered biologically significant. There are numerous publications that document high fat diet consumption by rodents results in thyroid gland and thyroid hormone changes. Araujo *et al.* (2010) reported that a mixed high-fat lard/soybean oil diet consumed by rats for eight weeks resulted in elevated serum TSH with normal serum T3 and T4 levels, while Han *et al.* (2012) reported time-dependent decreases in serum total triiodothyronine (TT3) and total thyroxine (TT4) in BALB/c mice that consumed a high fat diet for three- and six-month durations. Lard-based diets (containing 15% fat) fed to male Sprague Dawley rats for 24 weeks resulted in significantly increased triglyceride levels, increased serum TSH levels and decreased serum T4 and free thyroxine levels (Shao *et al.*, 2014). El-Sayed and Ibrahim (2020) reported that administration of high-fat diets for 12 weeks that induce obesity in female albino rats (25% lard in the diet) resulted in a significant ($P<0.05$) increase in TSH and corresponding decreases in serum T3 and T4, similar to the results seen by the administration of EPO for 12 weeks. These studies and the Marone *et al.* (2022) study provided high fatty acid intake levels that would be considered excessive when compared to the much lower expected average daily human intake of the EPO ingredient at approximately 450 mg/kg bw/day, based on 27 g oil consumed by a 60 kg person (USDA, 2020b). It is highly unlikely that the much lower intake by humans could induce thyroid hormone changes that would be considered biologically or toxicologically adverse.

Marone *et al.* (2022) referred to a recent review (Di Dalmazi and Giuliani, 2021) that concluded that thyroid effects of phytochemical constituents (*e.g.*, flavanones such as naringenin, flavonols such as rutin, and flavones such as apigenin), depending on the plant and quantity of component, can modulate thyroid hormone concentrations, but do not appear to be adverse except in conditions of iodine deficiency or excessive intake. The authors concluded that

In the present toxicologic review of a novel edible pongamia oil food ingredient, purified from a validated analytic method, the GLP, OECD-compliant 90-day dietary study established a NOAEL of 100,000 ppm, the highest dose tested. This intake, equaling exposures of 5,162.7 and 6,468.6 pongamia oil mg/kg bw/day for male and female rats, respectively (Marone *et al.*, 2022).

Sasmal *et al.* (1997) administered purified *M. pinnata* oil to Wistar rats ($N = 10$/sex/group) at 4 ml/kg bw/day (approximately equivalent to 4,000 mg/kg bw/day) for 22 weeks. Parameters evaluated at study termination included blood levels of cholesterol, bilirubin, alkaline phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, total proteins, glucose, free fatty acids, urea, uric acid, and creatinine. Food and water intake, gain in body weight, and hematological parameters were also compared. Results showed no significant difference between *M. pinnata* oil compared to groundnut oil. It was concluded by the authors that the purified *M. pinnata* oil used had no hepatotoxic or nephrotoxic effect.

In summary, the subchronic toxicity studies conducted in rats that evaluated the potential toxicity of oil from the beans of *M. pinnata* show that adherence to a critical and standardized oil refining process results in a refined oil (EPO) that does not induce toxicity under the conditions of a standardized, internationally accepted protocol.

6.4 Other studies

Historical reports on the toxicity of *M. pinnata* are limited, with results often dependent upon the variable isolation and purification techniques of the bean and oil extracts. While early studies reporting behavioral changes in mice and rats (Mahli *et al.*, 1989) and body weight changes in broiler chickens (Natanam *et al.*, 1989) exist, more recent reports validating the bioavailability of the test product using standardized oil isolation techniques (Shejawal *et al.*, 2014) and its detoxification of furanoflavonoid fractions (Gandhi and Cherian, 2000) indicate pongamia's potential as an edible vegetable oil with no demonstrable toxicity to rats (Singh *et al.*, 2021).

Broiler chicks fed a crude *M. pinnata* oil at 1% and 2% in the diet had body weight gains lower than those fed the control diet; feed efficiency was significantly different between 1% and 2% oil. The liver and pancreas of the chicks receiving the 1% oil were significantly heavier, whereas the hemoglobin and packed cell volume values were lower than those of the basal diet group (Natanam *et al.*, 1989), indicating that refinement process of the *M. pinnata*-sourced oil contributes to the safety of such oil.

Further studies testing the immunomodulatory effect of Karanja *M. pinnata*[28] bean churna (powder)[29] in aqueous solution administered by gavage to Charles Foster albino rats[30] ($N = 6$/test

[28] Stated as *Pongamia pinnata* Pierre in the manuscript.

[29] It is assumed that this powder was made from the whole *M. pinnata* bean, and therefore contained substantial quantities of the oil.

[30] The manuscript cites both Swiss albino mice and Charles Foster albino rats utilized in the study, but only results from administration to rats was provided in the publication (Chandni and Ashwani, 2017a; 2017b).

group)[31] at 400 and 800 mg/kg bw, respectively for 10 days reported significant cell mediated immunity at 800 mg/kg without significance to spleen and thymus antibody formation or organ weight (Chandni and Ashwani, 2017b). A second study by the same authors evaluating karanja bean churna, (powder) *M. pinnata* in Charles Foster albino rats at 1,600 mg/kg bw daily for 20 days reported no toxic effects to the heart, liver, spleen, kidney, testes, or jejunum, but a significant ($P < 0.02$) decrease in body weight in the karanja bean churna-treated group. There was a significant ($P < 0.001$) decrease in absolute liver weight (no effect on liver-to-body weight parameter) and an increase in the heart-to-body weight parameter ($P < 0.01$). All hematological parameters were non-significant except increased ($P < 0.001$) hemoglobin percentage in comparison to the control group. Microscopic analysis of the liver showed moderate fatty degenerative changes and increased proportions of white pulp reported in the spleen. Microscopic analyses of the jejunum, heart, kidney, testis, and uterus did not find any histopathological changes significantly different from the control group. The authors concluded that "it is proved that the test drug is safe for human use at this dose but should be administered cautiously in impaired liver functions" (Chandni and Ashwani, 2017a).

Hyperglycemic, hyperlipidemic, and hyperinsulinemic db/db mice treated with pongamol (50 and 100 mg/kg/day) and karanjin (50 and 100 mg/kg/day) (assumed by gavage as an "orally administered suspension" was stated by the authors) for ten consecutive days significantly lowered the blood glucose level in streptozotocin-induced diabetic rats in a dose-dependent fashion. Treatment of type 2 db/db mice with either pongamol or karanjin (100 mg/kg bw/day) for ten days decreased (in a time-dependent fashion) the blood glucose profile, with a lowering activity of 35.7% ($P < 0.01$) and 30.6% ($P < 0.01$), respectively (Tamrakar *et al.*, 2008). The results showed that pongamol and karanjin isolated from the fruits of *M. pinnata* possesses significant antihyperglycemic activity in type 2 diabetic db/db mice and protein tyrosine phosphatase-1β may be the possible target for their activity.

6.5 Genotoxicity

The ability of EPO to induce mutagenic activity was evaluated in a bacterial reverse mutation assay conducted consistent with OECD guidelines[32] at up to 5,000 µg/plate (Marone *et al.*, 2022). The assay utilized *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* tester strain WP2uvrA; the EPO was evaluated in a plate incorporation test and subsequent confirmatory pre-incubation test, both with/without (±) metabolic activation (rat liver S9 microsomal fraction) conducted in DMSO at oil levels of 1.58, 5.0, 15.8, 50, 158, 500, 1580 and 5,000 µg/plate (precipitation was reported at 5,000 µg/plate EPO).

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[31] The manuscript states that "the animals for this trial were rats of either sex and blood that was used was sheep's blood." No other information was provided concerning how many animals/sex/group were used (Chandni and Ashwani, 2017a; 2017b).

[32] OECD guideline No. 471 "Bacterial Reverse Mutation Test", https://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en; site last visited May 10, 2022.

The results of this assay showed that EPO did not cause gene mutation activity in the tester strains by either base pair changes or frameshifts in either the plate incorporation (Table 15) or pre-incubation (Table 16) tests. There were no concentration-related or substantial test substance-related increases in the number of revertant colonies reported in any of the tester strains and revertant colony counts were within the expected colony strain range. The positive control substances for the different strains and conditions (sodium azide, ICR 191 acridine, daunomycin, methyl methanesulfonate, and 2-aminoanthracene) substantially increased the mutation factor, confirming assay functionality. The authors concluded that "Debittered Pongamia Oil did not elicit bacterial mutagenicity in the bacterial reverse mutation assay" (Marone *et al.*, 2022).[33]

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[33] "Debittered Pongamia Oil" was the term used in the study for "Edible Pongamia Oil" produced by Terviva.

Table 15. Plate-incorporation bacterial test (Experiment I) of EPO mutagenicity potential (Marone *et al.*, 2022)

Test Substance	Dose Level (µg/plate)	S9 mix	Revertant Colony Counts (mean)[a]									
			TA 98	Mut. Factor	TA 100	Mut. Factor	TA 1535	Mut. Factor	TA 1537	Mut. Factor	WP2 *uvrA*	Mut. Factor
	1.58		24	0.89	100	1.01	12	1.50	11	0.92	32	0.91
	5		21	0.78	91	0.92	9	1.13	14	1.17	33	0.94
	15.8		27	1.00	89	0.90	8	1.00	13	1.08	37	1.06
Pongamia Oil	50		25	0.93	94	0.95	13	1.63	7	0.58	35	1.00
	158		27	1.00	90	0.91	12	1.50	10	0.83	38	1.09
	500		23	0.85	98	0.99	11	1.38	16	1.33	37	1.06
	1580	-	27	1.00	92	0.93	10	1.25	10	0.83	36	1.03
	5000		26*	0.96	91*	0.92	12*	1.50	10*	0.83	25*	0.71
Daunomycin	6		1049	38.85								
2-AA	10		-	-	-	-	-	-	-	-	-	-
NaN3	1.5				669	6.76	620	77.50				
MMS	2.5										599	17.11
ICR 191	1								789	65.75		
DMSO	N/A		27	1.00	99	1.00	8	1.00	12	1.00	35	1.00
	1.58		23	0.92	100	1.14	10	1.00	11	0.92	42	1.00
	5		27	1.08	106	1.20	9	0.90	10	0.83	45	1.07
	15.8		23	0.92	92	1.05	9	0.90	9	0.75	40	0.95
Pongamia Oil	50	+	25	1.00	94	1.07	9	0.90	11	0.92	45	1.07
	158		23	0.92	104	1.18	11	1.10	13	1.08	42	1.00
	500		25	1.00	95	1.08	8	0.80	15	1.25	42	1.00
	1580		25	1.00	112	1.27	8	0.80	15	1.25	37	0.88
	5000		22*	0.88	105*	1.19	9*	0.90	14*	1.17	43*	1.02
Daunomycin	6		-	-								
2-AA[c]	10		2911	116.44	2050	23.30	226	22.60	240	20.00	120	2.86
NaN3	1.5				-	-	-	-				
MMS	2.5										-	-
ICR 191	1								-	-		
DMSO[d]	N/A		25	1.00	88	1.00	10	1.00	12	1.00	42	1.00

[a]Mean of triplicate (3) plates; *Precipitate obscured assessment of background lawn; 2-AA=2-aminoanthracene; DMSO=dimethyl sulfoxide; ICR 191=ICR 191 acridine; MMS=methyl methane sulfonate; NaN3=sodium azide

Table 16. Pre-incubation bacterial test (Experiment II) of EPO mutagenicity potential (Marone *et al.*, 2022)

Test Substance	Dose Level (µg/plate)	S9 mix	Revertant Colony Counts (mean)[a]									
			TA 98	Mut. Factor	TA 100	Mut. Factor	TA 1535	Mut. Factor	TA 1537	Mut. Factor	WP2 *uvrA*	Mut. Factor
	1.58		21	0.89	88	0.93	11	1.22	9	0.69	39	1.00
	5		22	0.78	86	0.91	10	1.11	8	0.62	43	1.10
	15.8		22	1.00	94	0.99	10	1.11	7	0.54	36	0.92
Pongamia Oil	50		25	0.93	84	0.88	8	0.89	6	0.46	32	0.82
	158		27	1.00	91	0.96	11	1.22	6	0.46	33	0.85
	500		25	0.85	91	0.96	9	1.00	9	0.69	32	0.82
	1580	-	21	1.00	91	0.96	7	0.78	7	0.54	33	0.85
	5000		20*	0.96	90*	0.95	10*	1.11	13*	1.00	34*	0.87
Daunomycin	6		1088	41.85								
2-AA	10		-	-	-	-	-	-	-	-	-	-
NaN3	1.5				731	7.69	740	82.22				
MMS	2.5										532	13.64
ICR 191	1								3640	280.00		
DMSO	N/A		26	1.00	95	1.00	9	1.00	13	1.00	39	1.00
	1.58		22	0.92	84	0.82	8	0.80	8	0.80	42	0.88
	5		24	1.08	93	0.91	8	0.80	6	0.60	46	0.96
	15.8		22	0.92	94	0.92	10	1.00	7	0.70	38	0.79
Pongamia Oil	50	+	21	1.00	85	0.83	9	0.90	7	0.70	40	0.83
	158		21	0.92	90	0.88	7	0.70	8	0.80	41	0.85
	500		25	1.00	95	0.93	8	0.80	7	0.70	38	0.79
	1580		24	1.00	104	1.02	7	0.70	12	1.20	33	0.69
	5000		21*	0.88	94*	0.92	10*	1.00	16*	1.60	33*	0.69
Daunomycin	6		-	-								
2-AA	10		2269	94.54	1904	18.67	238	23.80	265	26.50	108	2.25
NaN3	1.5				-	-	-	-				
MMS	2.5										-	-
ICR 191	1								-	-		
DMSO	N/A		24	1.00	102	1.00	10	1.00	10	1.00	48	1.00

[a]Mean of triplicate (3) plates; *Precipitate obscured assessment of background lawn; 2-AA=2-aminoanthracene; DMSO=dimethyl sulfoxide; ICR 191=ICR 191 acridine; MMS=methyl methane sulfonate; NaN3=sodium azide

A mammalian bone marrow chromosome aberration test was also conducted to evaluate the potential of EPO to induce genotoxicity, consistent with OECD guideline study #475.[34] EPO was dissolved in corn oil and administered *via* gavage to male Crl:WI(Han) rats ($N = 5$/group) at 2,000 mg/kg bw, selected as the Maximum Tolerated Dose (MTD). The animals were euthanized and necropsied at 24- and 48- hours post-dosing, at which time the femur bones were removed, and the bone marrow cells isolated. The cells were evaluated at metaphase for structural chromosome aberrations and the mitotic index determined for each animal to assess potential toxicity. Cyclophosphamide (40 mg/kg bw/day, *i.p.*) was administered to a separate group of animals as the positive control. Toxicity was assessed by comparing the relative mitotic index of the control and test groups. The results of the study showed that the mean number of chromosomal aberrations for the control and EPO-treated group at 2,000 mg/kg bw/day were within the historical control data range and no biologically relevant increase in chromosomal aberrations occurred after EPO treatment (Table 17). Treatment with the positive control resulted in the expected significant increase in chromosomal aberrations, confirming the sensitivity of the study. The authors concluded that EPO was "non-mutagenic with respect to clastogenicity in this Mammalian Bone Marrow Chromosome Aberration Test" (Marone *et al.*, 2022).

Table 17. Results of a Mammalian Bone Marrow Chromosome Aberration Test in Crl: WI(Han) Male Rats (Marone *et al.*, 2022)

Test Substance	Dose Level (mg/kg bw)	Prep (hr)	Statistics		
			Mitotic Index in mean % (p value)	Aberrant Cells/1000 (p value)	
				Total	% ± SD
EPO	2000	24	10.18 ($p = 0.3016$)	1	0.01 ± 0.04 ($p = 0.3968$)
Corn Oil Control	0		8.5	1	0.01±0.04
Cyclophosphamide	40		1.44 ($p = 0.0079$)*	78	55.7±12.3 ($p = 0.0079$)*
EPO	2000	48	10.28 ($p = 0.5238$)	5	0.5 ± 1.2 ($p = 0.4286$)
Corn Oil Control	0		8.64	2	0.2 ± 1.6

Mean of 5 male rats/group, single application; bw range: 257.0 - 295.0 g. body weight (bw) mean [g] ± S.D.: 273.7 ± 9.1. bw variation [%]: ± 6.9. The bw variation of the animals did not exceed ± 20% of the mean bw of each sex, as recommended in OECD guideline 475; SD = Standard deviation.
*Indicates statistically significant increase at $P < 0.05$.

[34] https://www.oecd-ilibrary.org/docserver/9789264264786-en.pdf?expires=1641505259&id=id&accname=guest&checksum=A2959E4E71E65C41B3AC28393948CAB1; site last visited January 6, 2022.

No other studies were identified in the literature survey for the potential genotoxicity of a crude or refined pongamia oil.

6.6 *In vitro* studies

In vitro studies have been published on the beneficial effects of *M. pinnata* components (Al Muqarrabun *et al.*, 2013). However, very few *in vitro* studies were identified that evaluated the toxicity of pongamia oil, or some of the components. Gandhi and Cherian (2000) evaluated the toxicity of raw pongamia oil,[35] the same oil that was subject to an aqueous methanol-extract process, and the aqueous methanol extract, utilizing a red cell hemolysis test (rabbit cells) and estimating the subsequent lactate dehydrogenase (LDH) levels. Emulsions (5%) of the raw pongamia oil, the extracted pongamia oil, and ground nut oil (control) were evaluated. The raw and extracted pongamia oils were added to saline at 5, 10, 50, 100, and 200 mg oil/tube. The ground nut oil was similarly added at 100 and 200 mg/tube. Washed rabbit red blood cells were added to each tube and maintained at 15°C overnight, then analyzed for hemolysis and lactate dehydrogenase (LDH) release. The ground nut oil and the extracted pongamia oil did not induce hemolysis, but the raw pongamia oil induced concentration dependent hemolysis starting at 10 mg/tube. The authors reported that the raw oil and the methanolic extract released substantial LDH, correlating with hemolytic activity. The extracted oil did not induce LDH release. The authors concluded that "in the present studies, it is shown that the non-lipid constituents, which include furanoflavone compounds, can haemolyse the red cells and the toxicity may be due to the direct action on the cells." The authors stated, "rabbit red cell haemolysis test thus can be used as a predictive test for evaluating the method of detoxification" (Gandhi and Cherian, 2000).

Isolated karanjin was applied to cultured human cervical cancer cells (HeLa) and mouse fibroblast cells (L929) to determine the potential for cytotoxicity by karanjin on mammalian cells (Raghav *et al.*, 2019). The mammalian cells were seeded into 96-well culture plates ($0.5x10^5$ cells/ml), then treated with karanjin (0-200 mM) after 24 hours. Sulforhodamine B (SRB) was utilized to evaluate inhibition of cell proliferation. The authors stated that "[T]reatment with 20, 40, 60, 80, 100 and 180 µM karanjin inhibited the HeLa cell proliferation by 7.4, 3, 17, 23, 27 and 48%, respectively" (Raghav *et al.*, 2019). Karanjin at 200 µM inhibited HeLa cell proliferation by 55%, and a half-maximal inhibitory concentration (IC_{50}) after 24 hours was reported to be 186 µM. Karanjin concentrations of 10, 20, 50, 100, 150, and 200 µM inhibited the proliferation of L929 cells by 27, 31, 43, 53, 61, and 70%, respectively. The IC_{50} value was calculated at 86 µM.

These studies show that while crude pongamia oil can induce toxicity when applied to mammalian cells *in vitro*, refined (extracted) pongamia oil did not induce a toxicological response. Karanjin, one of the *M. pinnata* bean constituents (karanjin) that is minimized during EPO production, was found to inhibit cell proliferation when administered to mammalian cells *in vitro*, while the refined (extracted) pongamia oil did not inhibit cellular proliferation when evaluated under the study conditions.

[35] Stated as Karanja oil in the publication.

6.7 Allergenicity potential

6.7.1 Background

Foods which cause allergies are categorized on the basis of prevalence, potency and severity as either major allergenic sources or minor sources (FAO and WHO, 2022). Major allergens are the most prevalent, potent and severe and receive specialized attention in food safety regulations. Because avoidance of offending allergens remains the most effective approach to protect allergic patients from unwanted reactions, many jurisdictions, including the United States, have introduced labeling regulations for the major food allergens when used as ingredients in finished foods.[36] The major allergenic foods in the U.S. include milk, fish, crustacea, eggs, tree nuts, wheat, soy, peanuts and sesame, yet over 170 other foods have been documented to cause allergic reactions (Hefle *et al.*, 1996; Boyce *et al.*, 2010).

In all cases, the allergenic substances within major allergenic foods are proteins (Sathe *et al.*, 2016). Highly processed vegetable oils from major allergenic sources such as soybeans and peanuts have been exempt from source labeling regulations because these oils contain no or very low levels of protein as a result of processing.[34] This concept has been corroborated by clinical studies (Crevel *et al.*, 2000) and indeed, guidance on labeling has considered the impact of processing in the context of clinical factors such as: (1) the level of a protein unlikely to cause a reaction, (2) the type of protein unlikely to cause a reaction, and (3) the type and degree of processing conditions in the manufacturing of the final food (FAO and WHO, 2022).

As major allergens are of the highest clinical and public health importance – especially peanut and shrimp for adults and cow's milk for children (Taylor, 2022), the thresholds at which allergic individuals react to these allergens have been extensively evaluated over the last decade. The dose distribution of adverse effect levels for the major allergenic foods has been determined and is referred to as the "ED" or "eliciting dose" (Houben *et al.*, 2020; Remington *et al.*, 2020). For example, the ED_{50} for a food allergen refers to "the eliciting dose predicted to provoke a reaction in 50% of the allergic population" (FAO and WHO, 2022).

EPO is produced from *M. pinnata*, a member of the Fabaceae (legume) family, which includes many edible legumes such as soybeans, peas, beans, chickpeas, lentils, and peanuts (Smits *et al.*, 2021). Within the edible legume clade, *M. pinnata* is more closely related phylogenetically to peas and beans and more distantly related to peanuts, having diverged from peanut *via* a common ancestor approximately 59 million years ago (Wang *et al.*, 2017; Koenen *et al.*, 2020).

With the above facts in mind, Terviva has evaluated whether protein residues remain in EPO after processing and if so, at what level (Burdock, 2022). Further, on the basis of the observed protein levels in EPO, a safety assessment utilizing calculated serving doses of residual protein in oil *versus* the known threshold eliciting doses (EDs) of a hypothetical worst-case reference

[36] https://www.fda.gov/food/food-allergensgluten-free-guidance-documents-regulatory-information/food-allergen-labeling-and-consumer-protection-act-2004-falcpa ; site last visited December 13, 2022

allergen (peanuts) was conducted. FAO/WHO Expert Consultation of Risk Assessment of Food Allergens recommends the health-based guidance value for peanut at 2 mg of total peanut protein (ED_{05}) (Baumert, 2022; Burdock, 2022).[37] Despite that a very conservative ED_{01} (0.2 mg of peanut protein) was selected as the benchmark threshold, a level at which the most sensitive 1% of peanut-allergic individuals would react, albeit with very minor symptoms (Remington *et al.*, 2020).

6.7.2 Studies of potential allergenic risk

In the first study, Terviva evaluated the protein content of EPO manufactured (Burdock, 2022). The total protein extraction and quantitation was performed on two non-consecutive lots of EPO according to the published method of Rigby *et al.* (2011) which had been developed for vegetable oils. The total extracted protein was quantified using the 3-(4-carboxybenzoyl) quinolone-2-carboxyaldehyde (CBQCA) assay kit[38] using bovine serum albumin as an external protein standard. The suggested lower limit of detection was 100 ng according to standard assay variability (Burdock, 2022).

Rigby *et al.* (2011) had measured total protein concentrations of different soybean oil products, including crude degummed soybean oil (300 – 20,330 ng/g), neutralized soybean oil (64 – 1700 ng/g), neutralized bleached soybean oil (26 – 320 ng/g) and neutralized bleached deodorized soybean oil (82 – 698 ng/g total protein). Terviva reported total protein from two lots of EPO ranging from 1470.3 – 1965.2 ng/g (average at 1717.8 ng/g), similar in magnitude to results on neutralized soybean oil reported by Rigby *et al.* (2011).

In the second study, the risk potential for allergenic reactions to residual pongamia protein in EPO was evaluated by Dr. Joseph Baumert (Appendix IV). Because no previous evaluations or history of allergenicity to Pongamia and EPO have been reported, the safety assessment study was based on a worst-case scenario in which any protein found in EPO oil was considered to be equivalent to, and as allergenic as, peanut protein. As such, EPO was evaluated in an acute dietary intake assessment using: (1) population dietary intake data from the 2003-2020 NHANES consumption data,[39] (2) the maximum intended use level of EPO in food products as indicated in this document, and (3) the calculated absolute level of pongamia protein in those foods (Baumert, 2022; Burdock, 2022; Appendix IV). The use of peanut allergen data to define a hypothetical ED (Eliciting Dosage) for Pongamia was considered by Dr. Baumert to be a conservative approach that "overestimates the true risk associated with the EPO and its application in finished food products as consumed" (Baumert, 2022; Burdock, 2022).

Based on a population dose distribution model for peanut allergenicity, an international scientific panel (VITAL Scientific Expert Panel, Australia and New Zealand, Taylor *et al.*, 2019)

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[37] The summary of Part 2 of the FAO/WHO expert consultation can be reviewed at the following website: https://cdn.who.int/media/docs/default-source/food-safety/jemra/2nd-allergen-summary-report-20aug2021.pdf?sfvrsn=915a8417_8; site last visited Dec 14, 2022.

[38] Invitrogen, Thermo-Fisher Cat. #165305, (Waltham, MA)

[39] https://wwwn.cdc.gov/Nchs/Nhanes/search/datapage.aspx?Component=Dietary;site last visited December 13, 2022.

recommended a reference dose of 0.2 mg total peanut protein, which is the estimated dose that the most sensitive 1% of peanut protein-sensitive people (ED_{01}). Even this threshold is considered conservative as allergic reactions between the threshold of ED_{01} and ED_{05} are mild (FAO and WHO, 2022). Consumption quantities of EPO containing products for adults, teens and children were calculated based on the intended EPO use categories described in Table 9 (Part 3), from which the highest levels from calculations of Pongamia protein levels in EPO-containing food products, popcorn, chips, and pretzels, were identified as the food products with the highest estimated exposure when consumed during a single eating occasion. Based on the assessment as stated above, Dr. Baumert concluded:

> When considering the totality of the conservative estimates for the calculated concentration of total Pongamia protein that may be present in finished food products which would contain EPO and threshold data from peanut-allergic individuals (used as a conservative surrogate to represent the allergenicity of Pongamia protein) that underpin the safety assessment, the risk of an allergic reaction occurring, assuming Pongamia protein would elicit an IgE-mediated allergic response, would be very low and would not pose a reasonable likelihood of an adverse response in individuals with IgE-mediated food in my expert opinion.

In addition, Dr. Baumert (Baumert, 2022; Appendix IV) assessed that "For all of the food products evaluated, the quantity of food that would need to be consumed during a single eating occasion to reach the ED_{01} reference dose of 200 µg of protein from the allergenic source was a minimum of 3.4 times higher than the 90th percentile consumption quantity." Indeed, most of the products stated in Table 9 would require the consumption of more than 1 kg of that food in a single occasion to reach the peanut-equivalent ED_{01}. Importantly, to reach the ED_{05} of 2 mg of protein, a threshold that is widely considered appropriate for allergen labeling purposes (FAO and WHO, 2022), 10 times that amount would be required (*i.e.*, providing a margin of safety of 34 times). Therefore, when EPO is consumed according to the intended use described in this document (including at the 90th percentile), the consumer is safe, to the degree of a reasonable certainty of no harm from an allergic response.

6.7.3 Summary and conclusions

The manufacturing process, the very low protein content of EPO, and the significant margin of safety relative to a conservatively assigned allergen ED_{01} when modeling on the basis of equivalent allergenic potency to peanuts, in total confirm that there is minimal likelihood of allergic reactions when consuming EPO in the intended food categories of use.

6.8 Observations in humans

No clinical trials with raw pongamia or with EPO were identified. Ancheril *et al.* (2015) reported a clinical study to determine the efficacy of oil from *M. pinnata* beans (no statement on oil refinement)[40] on the symptoms of psoriasis. This open-label study utilized male and female

[40] Authors stated the test substance as "seed oil of *Pongamia pinnata*" (Ancheril *et al.*, 2015).

subjects ($N = 30$) that had been diagnosed with psoriasis who applied the *M. pinnata* oil topically to the skin areas that had psoriatic appearance (*e.g.*, erythema, scaling, thickening of the skin) for 14 days, along with oral pills taken internally with consumption of a tincture of twelve different herbs. No adverse events were reported, and the combined regimen of topical *M. pinnata* oil and consumption of twelve different herbs was effective in reducing the main symptoms of psoriasis. This study is of limited value in the assessment of the safety of ingested EPO.

6.9 Regulatory status

Oil from the Pongamia tree beans has not obtained regulatory compliance for use in food in the United States.

FDA has several databases that include a variety of foods, food ingredients, food contact substances, *etc.* with which FDA has had past experience. These databases are listed in Table 18. There were no "hits" in response to "*Pongamia*", "*Millettia*" or "*pinnata*"; therefore, pongamia products are not known to meet regulatory compliance for use in the United States in food products, nor was any publicly available information identified that indicated that FDA has previously provided regulatory compliance for an oil from *M. pinnata* beans.

Table 18. Food and Feed Ingredient Regulatory Database Search

Dataset Name	Last Updated	Last search for Millettia, pongamia, pinnata or karanja
Consultations on Food from New Plant Varieties	Mar 3, 2020	July, 2022
Food Additive and Color Additive Petitions Under Review or Held in Abeyance	Dec 2, 2021	July, 2022
GRAS Notices	March 1, 2022	July, 2022
Indirect Additives used in Food Contact Substances	Feb 3, 2022	July, 2022
Inventory of Effective Food Contact Substance (FCS) Notifications	Jan 31, 2022	July, 2022
Inventory of Environmental Impact Decisions for Food Contact Substance Notifications	Jan 31, 2022	July, 2022
New Protein Consultations (Early Food Safety Evaluation)	Mar 28, 2018	July, 2022
SCOGS (Select Committee on GRAS Substances)	July 31, 2020	July, 2022
Submissions on Post-Consumer Recycled (PCR) Plastics for Food-Contact Articles	Dec 12, 2021	July, 2022
Substances Added to Food (formerly EAFUS)	Feb 18, 2022	July, 2022
Threshold of Regulation (TOR) Exemptions	Nov 3, 2021	July, 2022
American Herbal Products Association*	NA**	July, 2022
Association of American Feed Control Officials (AAFCO)***	2021	July, 2022

*Because the FDA's Office of Dietary Supplements has been slow to update its database, the American Herbal Products Association tracks submissions of NDINs in its proprietary database.
**NA = Date of last update not available
***AAFCO is a private organization not associated with FDA but is instrumental in approval of animal feed ingredients at the State level.

There is no record in the American Herbal Products Association (AHPA) database[41] indicating pongamia oil was ever submitted to FDA as a New Dietary Ingredient Notification (searched March 4, 2022). The Association of American Feed Control Officials (AAFCO) is a private organization that has the most complete list of substances approved for addition to animal feed in its *Official Publication*. The is no record of approval of *M. pinnata* (or as *P. glabra*) oil or seed meal for animal feed use stated in the 2021 AAFCO *Official Publication*.

The European Food Safety Authority (EFSA) cites in the European Compendium of Botanicals several references to components within the *M. pinnata* seed to include: pongamol, karanjin, phytates, and erucic acid.[42] In addition, the European Chemicals Agency (ECHA) indicates that "karanjin oil" is undergoing a re-registration process in the EU as part of the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) program.[43] No additional information was provided. The Food Safety and Standards Authority of India (FSSAI, 2021) made reference to a method to determine the presence of pongamia oil [referenced as Karanja (*Pongamia glabra*) oil], while previously publishing draft regulations stating that the oil from karanja may be consumed at 1-2 ml/day as an article of food for adults (FSSAI, 2020).

6.10 Safety evaluation summary

Edible Pongamia Oil (EPO) is a clear vegetable-based oil with a mildly nutty taste that is obtained from the beans of the Pongamia tree, a member of the Leguminosae family. The genus/species name has recently been reclassified in some publications from *Pongamia pinnata* (L) Pierre] to *Millettia pinnata* (L) Panigrahi. There are many common names, including Karanja and Pongamia. The Pongamia tree is a nitrogen-fixing plant that produces beans with a lipid content ranging from 30 – 40%, and at least half is oleic acid. The oil from the beans has been used as a heating oil and for soap production. The furanoflavonoid content of the crude oil causes a bitter taste and may result in adverse biological events (*e.g.*, cell apoptosis, decreased blood glucose) that, without costly or reliable extraction processing, previously unavailable, makes the oil unfit for consumption. Terviva has developed an efficient extraction and refinement process to produce EPO that contains limited furanoflavonoids, karanjin and pongamol. The flavonoid content in the EPO oil is significantly less than total flavonoid intake from other dietary sources in an average diet.

The major fatty acids in EPO include (as a percentage of the total fatty acid content) approximate levels of oleic acid at 50%, linoleic acid at 17%, stearic acid at 8%, palmitic acid at 9%, behenic acid at 4%, and *alpha*-linolenic and arachidic acids at 2% each. Other fatty acids are included at approximately 1% or less. Similar fatty acid compositions are readily found in commonly consumed vegetable oils (olive, Canola and soybean oils have oleic acid contents at approximately 71.3%, 61.7%, and 22.6%, respectively, linoleic acid contents at approximately 9.76%, 19.0%, and 51%, respectively, and palmitic acid contents at 11.3%, 4.3%, and 10.5%,

[41] Database searched in 2021.

[42] https://combodb.ecomole.com/report/2065?botanic_page=20; site last visited February 2, 2022.

[43] https://echa.europa.eu/substance-information/-/substanceinfo/100.118.034; site last visited February 2, 2022.

respectively). The manufacturing process removes the majority of furanoflavonoids, as shown by the marker substances karanjin and pongamol. EPO is stable at ambient temperature for at least six months. Longer term stability is currently being evaluated.

The EPO ingredient was evaluated for potential toxicity in a 14-day range-finding dietary toxicity study and in a 13-week dietary toxicity study in Sprague Dawley male and female rats, conducted consistent with OECD guidelines. In the 14-day range-finding study, EPO was provided as a dietary admixture at levels of 0, 5%, 10% and 15%. There were no changes in body weight or body weight gain, food consumption or food efficiency in male and female rats attributable to the dietary administration of EPO. The authors of the study stated that there were no adverse test substance-related changes attributed to EPO in the 14-day study, concluding that rats are expected to tolerate dietary concentrations of up to 150,000 ppm, which was the highest level fed, and equivalent to 12,023 mg/kg bw/day for males and 11,899 mg/kg bw/day for females.

In a 30-day study in male albino rats (in-house laboratory strain) utilizing a refined pongamia oil (specifications not stated), consumption of the oil did not result in adverse effects at 15% in the diet when plasma and liver lipids and plasma and depot fat fatty acid composition were evaluated; and that extracted pongamia oil causes no growth retardation, health hazards or toxic effects on the biochemical parameters of rats (Mandal, 1985). The NOAEL for this study was 15% in the diet, the highest level fed, approximately equivalent to 12,150 mg/kg bw/day.

In a 13-week toxicity OECD-compliant toxicity study with 28-day recovery period, male and female Sprague Dawley rats were randomly allocated into groups and provided 50,000 ppm, 75,000 ppm, and 100,000 ppm EPO in the diet. There were no changes of toxicological significance in body weight, body weight gain, food consumption, food efficiency, FOB/MA, organ weight, clinical-pathology or histopathological parameters, during either the dosing or recovery phases. No adverse effects were attributed to the consumption of EPO in either study. The NOAEL for this study was the highest level fed, 100,000 ppm equivalent to 5,162.7 mg/kg bw/day for male rats and 6,468.6 mg/kg bw/day for the female rats.

A bacterial reverse mutation assay in *S. typhimurium* and *E. coli* strains in the presence and absence of metabolic activation and consistent with OECD guidelines showed that EPO was not mutagenic at concentrations up to 5,000 µg/plate, the highest concentration tested, and at which point it precipitated. An *in vivo* chromosomal aberration assay conducted in Sprague Dawley rats according to OECD guidelines did not show formation of chromosomal aberrations in the bone marrow of the rats when administered EPO by gavage at up to 2,000 mg/kg bw/day, the highest dose tested.

The fatty acids and triglyceride found in EPO are commonly found in vegetable oils that have previously been concluded safe for general consumption in the U.S. food supply as general-purpose food oils. The NOAEL for EPO in rats is at 5,162.7 mg/kg/day.

The estimated daily intake of EPO is based on the 90th percentile consumption level of the EPO from foods supplemented with the oil, and is approximately 7,703.32 mg/day, or 127.33

mg/kg bw/day. This theoretical intake represents a conservative estimate because it is highly unlikely that an individual would consume the EPO from all stated conventional foods at the 90th percentile level and all selected food products would contain EPO. The protein content of EPO was reported at an average of 1717.8 ng/g.

The risk of allergic reactions to EPO was considered to be insignificant based on a safety assessment in which actual amounts of protein in EPO relative to the known amounts required to elicit reactions (the ED_{01}) in a highly sensitive reference allergic populations was calculated. Specifically, the margin of safety for peanut allergic consumers for a 90th percentile acute serving of EPO was 3.4 times when modeling an ED_{01}, and 34 times when modeling an ED_{05}.

6.11 GRAS Panel statement

At the request of Terviva, Inc. (Terviva), a Panel of Experts (the "GRAS Panel") of independent scientists, qualified by their scientific training and relevant national and international experience in the safety evaluation of food ingredients, conducted a critical and comprehensive assessment of data and information pertinent to the safety of Edible Pongamia Oil (EPO) produced from the *M. pinnata* plant when used as a vegetable oil, as described in the GRAS dossier (Burdock, 2022), to evaluate the EPO status as Generally Recognized As Safe (GRAS) based on scientific procedures. The GRAS Panel consisted of the below-signed scientific experts: Professor Emeritus Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine); Professor Emeritus Michael W. Pariza and Professor Emeritus Stephen L. Taylor (University of Nebraska).

The GRAS Panel critically evaluated a comprehensive package of publicly available scientific data and information compiled from a search of the scientific literature through July 2022 by the Burdock Group and from Terviva documents, and summarized by the Burdock Group in a dossier entitled "Dossier in support of the generally recognized as safe (GRAS) status of edible pongamia oil (EPO) as a food ingredient according to the conditions of use cited herein" (Burdock, 2022) that included an evaluation of the available scientific data and information (favorable and unfavorable) relevant to the safety of intended use of EPO manufactured from the *Millettia pinnata* (L). plant. The dossier included information on the characterization, identity and purity of the EPO ingredient, manufacturing information, ingredient specifications, analytical data, stability information, conditions of use, consumption information and safety data. Terviva assures Burdock Group that all relevant, unpublished information in its possession related to the safety of EPO has been supplied to Burdock Group and has been summarized in this dossier. This dossier and supporting documentation were made available to the GRAS Panel. The GRAS Panel also independently evaluated other materials deemed appropriate and necessary and unanimously concluded EPO GRAS under the stated intended conditions of use. The following page contains the conclusion statement and signatures of the GRAS Expert Panel.

GRAS PANEL CONCLUSION

Following its independent and collective critical evaluation of the information available, the GRAS Panel, knowledgeable about the safety of substances directly or indirectly added to food, concluded that, based on common knowledge in the scientific community, there is reasonable certainty that Edible Pongamia Oil (EPO), produced in accordance with current Good Manufacturing Practice (cGMP), and meeting the food grade specifications presented in the dossier, is safe for its intended conditions of use when the estimated consumption is up to and includes 7,703.32 mg EPO/day (equivalent to an estimated 127.33 mg/kg bw/day) when added to the foods specified in the dossier.

The GRAS Panel further concluded that the proposed use as a food ingredient of EPO, produced consistent with current Good Manufacturing Practice (cGMP) and meeting food grade specifications presented in the dossier, is Generally Recognized As Safe (GRAS) based on scientific procedures.

It is our opinion that other experts qualified by scientific training and experience to evaluate the safety of food and food ingredients would agree with these conclusions.

SIGNATURES

[redacted]

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8 Nov 2022
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Part 7: Supporting Data and Information

A comprehensive search[44] of the scientific literature was conducted through July, 2022 for safety and toxicity information on *Millettia pinnata* (L.)-derived oil and related substances and was described in Part 6 of this GRAS notice.

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[44] Relevant literature cited in the electronic database search was reviewed. Literature not cited in the search or literature published subsequent to the search, may not have been included in the review.

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7.2 Appendices

7.2.1. Appendix I. EPO Specification lot analysis

Parameter	Method	Specification Range	TVOil0188	TVOil0312	TVOil0349	TVOil0370	TVOil0393	Average
Color	Sensory - Visual	Clear, light/golden yellow	Complies	Complies	Complies	Complies	Complies	Complies
Odor	Sensory - Smell	Pleasant; faint nuttiness	Complies	Complies	Complies	Complies	Complies	Complies
Moisture (max.)	AOCS Ca 2b-38	<0.25% g/100g m/m	0.02	0.07	0.02	0.03	0.04	0.04
Oxid. stability index	AOCS Cd 12b-92	>1 hrs	2.1	1.07	2.82	4.96	2.24	2.22
Unsaponifiable matter	AOCS Ca 6a-40	<2.0% g/100g	0.25	0.33	0.29	0.38	0.29	0.31
Peroxide value	AOCS Cd 8-53	<20.0 meqO_2/kg	1.5	3.1	3.8	3.1	2.4	2.8
p-Anisidine value	AOCS Cd 18-90	<20 AnV	6	6.9	7.8	12.9	5.7	7.9
Triglycerides	AOAC 966.06	>95%	96.2	97.2	97.2	94.1	96.2	96.2
Total Fatty acids	AOAC 996.06 mod.	>90% g/100g	92.1	93.1	93.1	90.1	92.0	92.1
Oleic acid (18:1)	AOAC 996.06 mod.	>50.0 g/100g FA	52.1	53.0	53.0	53.2	53.1	52.9
Linoleic acid (18:2n-6)	AOAC 996.06 mod.	>15.0 g/100g FA	16.7	16.5	16.5	18.0	17.5	17.0
Palmitic acid (16:0)	AOAC 996.06 mod.	>8.0 g/100g FA	9.6	9.1	9.1	9.9	9.1	9.4
Stearic acid (18:0)	AOAC 996.06 mod.	>7.0 g/100g FA	8.4	8.6	8.6	7.9	8.3	8.4
Behenic acid (22:0)	AOAC 996.06 mod.	>3.0 g/100g FA	5.0	4.5	4.5	3.4	4.0	4.3
Erucic acid (22:1c13)	AOAC 996.06 mod.	<1.0 g/100g FA	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Free Fatty acids	AOCS Ca 5a-40	<1.0% g/100g	<0.1	<0.1	0.1	0.2	0.2	0.1
Microbials								
Total coliforms	AOAC 991.14	<10 cfu/g	<10	<10	<10	<10	<10	<10
E. coli	AOAC 991.14	<10 cfu/g	<10	<10	<10	<10	<10	<10
Salmonella spp.	AOAC-RI 121501	Negative cfu/25g	Neg.	Neg.	Neg.	Neg.	Neg.	Neg
Mold	BAM Chap. 18	<10 cfu/g	ND	ND	<10	<10	<10	<10
Yeast	BAM Chap. 18	<10 cfu/g	ND	ND	<10	<10	<10	<10
Heavy metals	**ICP-MS**							
Lead (Pb)	AOAC Ca 17-01	<10 ppm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Cadmium (Cd)	AOAC Ca 17-01	0.1 ppm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Mercury (Hg)	AOAC Ca 17-01	<1 ppm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Arsenic (As)	AOAC Ca 17-01	<3 ppm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Furanoflavonoids								
Karanjin	TV-STM_001.01	<150 ppm	135	84	78	ND	80	75.4
Pongamol	TV-STM_001.01	<150 ppm	93	126	126	156	101	120.4
Mycotoxins								
Aflatoxin B1,B2,G1,G2	AOAC 999.07	0.5-15** µg/kg	<4	<4	<4	<4	<4	<4
Fumonisin (total)	J AOAC 92(2),496	2000-4000 µg/kg	<30	<30	<30	<30	<30	<30
Ochratoxin A	AOAC 999.07	1-5 µg/kg	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

T-2/HT-2 Toxin	Food Add Cont 30(3),541	5-10 µg/kg	<1/10	<1/10	<1/10	<1/10	<1/10	<1/10
Zearalenone	Food Add Cont 30(3),541	75-200 µg/kg	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0

ND = not detected; All data derived from Terviva Certificate of Analyses for the given lot.; *Measured at 110 ^{0}C. 1 hr = approx. 1 month

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7.2.2. Appendix II. Manufacturing process

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Title: Description of the Pongamia Edible Oil (EPO) Manufacturing Process

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A. Introduction

Millettia pinnata (Pongamia) is a tree species belonging to the pea family, *Fabaceae*, which is native to eastern and tropical Asia, Australia, and the Pacific Islands. It is often referred to by its synonym, *Pongamia pinnata*. Common names also include *Indian beech* and *Pongame oil tree*.

The pongamia bean is a rich source of mid-oleic oil, containing as much as 30% to 40% of the fresh weight of the bean.

Terviva's patented Edible Pongamia Oil (EPO) (Terviva, Inc., 2022) process is a hybrid between the edible oil's industry-standard physical extract refining process and solvent extraction refining process. A defining characteristic of this process is the removal of furanoflavonoids (principally karanjin and pongamol), which have unpleasant sensory attributes.

Terviva's proprietary EPO manufacturing process is described below. The resulting refined EPO has unique properties, including richness of oleic fatty acid (C18:1) and behenic fatty acid (C22:0) content, which confer desirable nutritional and functional properties relevant to the food industry. The Pongamia bean is not only rich in oil, but also contains a significant amount of other useful compounds (e.g., carbohydrates and proteins) which can also be refined into plant-derived edible protein and flour ingredients.

The following is a description of the step-by-step process spanning the preparation of Pongamia beans to the finished, refined EPO with a view to illuminating the chemical constituents that are removed at each step and comprising the resulting composition of each intermediate fraction.

B. Terviva's Edible Pongamia Oil (EPO) Manufacturing Process

1. **Bean Preparation – Bean Pod Drying and Shelling:**
   Harvested Pongamia pods are dried to remove excess moisture during which the shells become brittle in preparation for shelling. Shells may be removed manually or mechanically. The shelled beans are then mechanically cleaned to remove foreign matter such as sticks, rocks, or other foreign debris. Pongamia beans typically contain approximately 30% oil, 10% moisture, 20% protein, 38% carbohydrates, and 2% ash by weight (Terviva, Inc. Pongamia Processing Master Dataset v3.xlsx[1])

   Step Summary: Pongamia shells and harvest-related foreign matter are removed at this stage.

2. **Crushing:**
   Shelled and cleaned Pongamia beans are then crushed in an expeller press. Crushing is a mechanical process during which the beans are fed into a rotating screw press that extracts two products: crude oil and press cake. The crude oil is then fed through either a centrifuge or a simple cloth filter that removes residual solids, such as bean particulate. Crude Pongamia oil is comprised of major lipids including triacylglycerol (90%), diacylglycerol (1-2%), and monoacylglycerol (1-2%), < 5% free fatty acids, 1-3% of furanoflavonoids, and other impurities that will be removed in subsequent processing steps (Terviva, Inc. Pongamia Processing Master Dataset v3.xlsx[1]).

   Step Summary: Bean meal particulate has been substantially removed from crude oil at this stage.

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[1]Terviva, Inc. (2022). Reference dataset, including beans, press cake, crude oil, and refined oil. - Pongamia Processing Master Dataset v3.xlsx Terviva Shared Drive\ Product and Processing

3. **Crude Oil Pre-treatment Process:**
This is the first refinement step of Terviva's Pongamia crude oil resulting from the prior crushing step.

   a. **Settling/Decanting:**
   Physical solid/liquid separation process is an industry-standard process that allows non-lipid compounds, such as starches, protein, cell debris and sediment that settles to the bottom of the processing vessel to be physically removed by decanting the liquid oil upper portion.

   b. **Pre-filtration with Diatomaceous Earth (DE):**
   To further remove non-lipid compounds via solid/liquid separation, diatomaceous earth (DE) filter aid is applied to the oil. DE is a high-surface area, siliceous filter aid that can easily adsorb impurities, such as excess moisture, volatile matter and insoluble impurities in the oil while increasing the flow rate through the filter press.

Step Summary: Insoluble impurities (e.g., starch, cell debris, protein) are substantially removed during this stage.

Table 1: Composition of crude oil produced by settling/decanting and DE treatment

Fractions	TAG (%)	FFA (%)	Protein (%)	Unsaponifiable matter (%)	Insoluble impurities (%)	Moisture & Volatiles (%)	Furano-flavonoids (ppm)	Phosphorous (ppm)
Clarified Crude oil	89.22	7.50	1.06	0.62	0.03	N/A	20,000	20
Decanted Solids Sludge	N/D	N/D	N/D	0.64	30.89	N/A	N/D	1050

FFA = free fatty acids, TAG = triacylglycerols, Unsaponifiable matter are tocopherols, sterols, phytosterols and hydrocarbons. N/D = not determined. N/A = not applicable

4. **Crude Oil Degumming:**
Acid degumming is a process whereby pre-treated crude oil is mixed with a water and food-grade citric acid solution. This process removes hydratable gums, such as phospholipids, and other similar water-soluble compounds such as proteins. After this mixing step, the solution is centrifuged to separate the aqueous fraction from the degummed crude Pongamia oil.

Step Summary: Polar lipids, water- and acid-soluble proteins/compounds are removed at this stage.

Table 2: Composition of crude oil after degumming

Fraction	FFA (%)	Unsaponifiable matter (%)	Moisture & Volatiles (%)	Protein (%)	Insoluble impurities (%)	Furano-flavonoids (ppm)	Phosphorous (ppm)	Total Gums
Degummed Crude oil	7.28	1.01	0.12	0.00015	<0.01	20,000	20	N/D

N/D = non-detectable
[1]Protein content determined by the method of Rigby et al., 2011.

5. **Crude Oil Dewaxing:**
After degumming, the crude oil is chilled to a set temperature (~16°C), sometimes with slow agitation, to promote the crystallization of waxes and long-chain saturates. This chilled oil is then mixed with food-grade DE and fed into a filter press. As in the previous step, the high surface area DE will easily adsorb impurities in the oil while increasing the flow rate through the filter press. The filtered oil has reduced level of waxes and saturated fatty acids such as stearic acid (C18:0), behenic acid (C22:0) and lignoceric acid (C24:0).

Step Summary: Waxes and related long-chain saturates are removed at this stage

Table 3: Composition of crude oil after dewaxing

Fraction	FFA (%)	Unsaponifiable Matter (%)	Moisture & Volatiles (%)	Furano-flavonoids (ppm)	Phosphorous (ppm)	Waxes (ppm)	Tocopherols (ppm)	Sterols (ppm)	Carotenoids (ppm)
Degummed dewaxed Crude oil	6.81	1.01	0.12	20,000	20	56	545	1910	1.62

6. **Liquid-Liquid Extraction:**

   a. Extraction:
   Degummed and dewaxed crude oil is then exposed to a liquid-liquid extraction process. In this process, crude oil is mixed with food-grade ethanol at elevated temperatures (<80°C) to extract the furanoflavonoids that are responsible for unpleasant sensory attributes of crude Pongamia oil. During this ethanol extraction process, other compounds are removed from the oil such as free fatty acids and unsaponifiable (i.e., tocopherols, sterols, carotenoids) compounds. The resulting oil has a lighter color, milder flavor, and no detectable bitterness. Total furanoflavonoids, principally karanjin and pongamol, are reduced to < 250 ppm.

   b. De-solventization:
   Following liquid-liquid extraction, the refined Pongamia oil contains residual ethanol. Ethanol is removed using vacuum filtration to lower the boiling point and modest heat (< 60°C) to assure complete evaporation. The resulting edible oil contains < 0.01% of total volatiles and < 0.05% ethanol.

Step Summary: Furanoflavonoids, free fatty acids, tocopherols, sterols, carotenoids are removed at this process step

Table 4: Composition of refined oil after liquid: liquid extraction

Fraction	FFA (%)	Unsaponifiable matter (%)	Moisture & Volatiles (%)	Volatiles (%)	Furano-flavonoids (ppm)	Tocopherols (ppm)	Sterols (ppm)	Carotenoids (ppm)
Refined Oil	<0.09	0.27	0.04	<0.01	217	62.2	1530	0.52

7. **Finished Refined Oil Filtration – Cleaning/polishing step:**
   A final filtration step with DE is incorporated to ensure that incidental particles that may have been introduced during processing, including remaining trace amounts of minor compounds, such as moisture, unsaponifiable matter and other minor impurities, are removed.

8. **Antioxidant Addition:**
   DE filtration and liquid-liquid extraction remove >90% of the native tocopherols (natural antioxidants) from the oil. Therefore, the addition of antioxidants is intended as a protective measure to assure stability over time. Food-grade mixed tocopherols (α, β, γ) and green tea extract are added to the refined Edible Pongamia Oil to improve the oxidative stability and shelf life. This is achieved by reducing the

formation of pro-oxidants, such as peroxides (free radicals), aldehydes (beta-oxidant) and free fatty acids (Terviva, Inc. Pongamia Processing Master Dataset v3.xlsx').

C. Commentary on Potential Process Contaminants

Unintentional contaminants of refined, bleached, and deodorized (RBD) oils can be formed by unwanted chemical reactions during processing, especially under extreme conditions such as elevated temperatures or extreme pH; and/or through their use in food processes (i.e., cooking). For example, in palm oils these unwanted contaminants can include 2-monochloropropane-1,2-diol (2-MCPD), 3-monochloropropane-1,2-diol (3-MCPD) and its related fat-soluble forms (3-MCPD esters), glycidyl esters (GE), and polycyclic aromatic hydrocarbons (PAHs) (WHO, 2006).

- ***2- and 3-MCPD esters:*** Found in some processed foods and vegetable oils (mainly palm oil), 2- and 3-MCPDs are formed by the reaction between a source of chlorine (e.g., chlorinated water or salt) and a lipid source such as a vegetable oil under elevated temperatures. These esters are food processing contaminants. 2- and 3-MCPD and its esters are formed unintentionally in these foods during oil refining processes. The manufacturing process described above does not include chlorine or chlorinated chemicals (e.g., hydrochloric acid) and would therefore not be expected to introduce such compounds during the manufacturing process. (EFSA, 2008).

- ***Glycidyl Ester (GE):*** Glycidyl esters are formed from a group of substances that are naturally present in all vegetable oils (diglycerides or monoglycerides) when they are heated to temperatures greater than 200°C (during the deodorization stages of traditional RBD refining process). Deodorization and concomitant high temperatures (> 200°C) are not part of the Pongamia oil manufacturing process. As such, glycidyl esters would not be expected to be present in the final refined edible pongamia oil product. (EU, 2020).

- ***Polycyclic Aromatic Hydrocarbons (PAHs):*** Polycyclic aromatic hydrocarbons (PAHs) are a group of over 100 different chemicals that are formed during the incomplete combustion of carbon-containing organic matter, such as coal, petroleum-based oil and gas, food, and other organic substances. PAHs are usually found as a mixture containing two or more of these compounds, such as soot. According to the Centers of Disease Control (CDC), PAHs generated from these sources can bind and form small particles in the air. High-temperature cooking, particularly open-flame grilling and smoking, will form PAHs in meat and in other foods where burning occurs. Therefore, the presence or formation of PAHs as described by the CDC would not be expected to be produced as part of this process. (EFSA, 2008; CDC, 2013).

D. Conclusion

The manufacturing process for Edible Pongamia Oil (EPO) produces a substantially refined oil comprising greater than 95% triacylglycerol from which numerous impurities and undesirable substances have been removed. The process described is similar in effect to more traditional approaches, such as standard industry refining, bleaching, and deodorizing (RBD) processing. A defining characteristic of this process is the removal of *furanoflavonoids, principally karanjin and pongamol,* which are associated with unpleasant sensory attributes including bitter taste. The processing methods described herein for the manufacturing of EPO produce an oil which is substantially equivalent to the purity and safety of refined oils produced by traditional refining, bleaching, and deodorizing.

Table 5 below highlights similarities between the EPO refining process and standard RBD oil processing. (Erickson, 1995; O'Brien *et al.*, 2000; Ruiz-Méndez, *et al.*, 2021). Table 6 represents a summary of the Terviva process.

Table 5: Comparison of the Edible Pongamia Oil (EPO) refining process vs. industry-standard soybean oil refined, bleached, and deodorized (RBD) process.

Processing Step	Edible Pongamia oil processing (Compounds removed)	Soybean oil RBD processing (Compounds removed)
Bean Preparation	Shells, harvest-related foreign matter	Shells, harvest-related foreign matter
Bean Crushing	Bean meal	Bean meal
Degumming	Polar lipids, water- or acid-soluble proteins and compounds	Polar lipids, water- or acid-soluble proteins and compounds
Neutralization	N/A	Free fatty acids, soap stock
Bleaching	N/A	Oxidation products, pigments
Dewaxing Winterization	Waxes, saturated fatty acids, solid particles	Waxes, saturated fatty acids, solid particles
Liquid-Liquid Extraction	Furanoflavonoids, free fatty acids, tocopherols, sterols, carotenoids, proteins*	N/A
De-solventization	Volatiles*	N/A
Deodorization	N/A	Free fatty acids, tocopherols, sterols, carotenoids, oxidation products, volatiles

*Terviva Inc., 2022
N/A = not applicable

Table 6: Data Summary of Edible Pongamia Oil (EPO) Processing Steps (in step sequence)

Processing Step	Fraction	TAG (%)	FFA (%)	Protein (%)[1]	Un-saponifiable matter (%)	Insoluble impurities (%)	Moisture & Volatiles (%)	Furano-flavonoids (ppm)
Crude Oil Pre-treatment	Clarified Crude oil	89.2	7.50	1.06	0.62	0.03	N/A	20,000
Crude Oil Degumming	Degummed Crude oil	N/A	7.28	0.00015	1.01	<0.01	0.12	20,000
Crude Oil Dewaxing	Degummed dewaxed Crude oil	N/A	6.81	0.00015	1.01	<0.01	0.12	20,000
Liquid. liquid refining	Refined Oil	>95.0	<0.09	0.00015	0.27	<0.01	0.04	<250

[1] Protein content determined by the method of Rigby *et al.*, 2011. Clarified crude oil protein determined by nitrogen combustion.

E. References:

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7.2.3. Appendix III. Consumption analysis of EPO in selected foods at maximum intended use level

Table 1. Consumption analysis of EPO in selected foods at maximum intended use level

FOODCODE	DESCRIPTION	Amount (ppm)
11300100	Non-dairy milk, NFS	11700
11320000	Soy milk	14700
11320100	Soy milk, light	7700
11321000	Soy milk, chocolate	15300
11321100	Soy milk, light, chocolate	6400
11350000	Almond milk, sweetened	9300
11350010	Almond milk, sweetened, chocolate	9500
11350020	Almond milk, unsweetened	9600
11350030	Almond milk, unsweetened, chocolate	10000
11360000	Rice milk	9700
11370000	Coconut milk	20800
11512030	Hot chocolate / Cocoa, ready to drink, made with non-dairy milk	9800
11512120	Hot chocolate / Cocoa, ready to drink, made with non-dairy milk and whipped cream	20000
11513310	Chocolate milk, made from dry mix with non-dairy milk	12700
11513375	Chocolate milk, made from reduced sugar mix with non-dairy milk	13400
11513385	Chocolate milk, made from dry mix with non-dairy milk (Nesquik)	12700
11513395	Chocolate milk, made from no sugar added dry mix with non-dairy milk (Nesquik)	13400
11513750	Chocolate milk, made from syrup with non-dairy milk	10400
11513805	Chocolate milk, made from light syrup with non-dairy milk	11700
11513855	Chocolate milk, made from sugar free syrup with non-dairy milk	13100
11514150	Hot chocolate / Cocoa, made with dry mix and non-dairy milk	15700
11514360	Hot chocolate / Cocoa, made with no sugar added dry mix and non-dairy milk	13500
11519215	Strawberry milk, non-dairy	11300
14410500	Cheese, processed cheese food	57650
14420300	Cheese spread, pressurized can	53075
14502000	Imitation cheese	80000
21003000	Beef, NS as to cut, fried, NS to fat eaten	30000
21102110	Beef steak, fried, NS as to fat eaten	30000
21102120	Beef steak, fried, lean and fat eaten	30000
21102130	Beef steak, fried, lean only eaten	30000
22000200	Pork, NS as to cut, fried, NS as to fat eaten	30000
22000210	Pork, NS as to cut, fried, lean and fat eaten	30000
22000220	Pork, NS as to cut, fried, lean only eaten	30000
22101200	Pork chop, fried, NS as to fat eaten	30000
22101210	Pork chop, fried, lean and fat eaten	30000
22101220	Pork chop, fried, lean only eaten	30000
22201200	Pork steak or cutlet, fried, NS as to fat eaten	30000
22201210	Pork steak or cutlet, fried, lean and fat eaten	30000
22201220	Pork steak or cutlet, fried, lean only eaten	30000
22210310	Pork, tenderloin, breaded, fried	30000
22210450	Pork, tenderloin, battered, fried	30000
24127200	Chicken breast, fried, coated, skin / coating eaten, from raw	30000
24127202	Chicken breast, fried, coated, prepared skinless, coating eaten, from raw	30000
24127210	Chicken breast, fried, coated, skin / coating eaten, from pre-cooked	30000
24127220	Chicken breast, fried, coated, skin / coating eaten, from fast food / restaurant	30000
24137300	Chicken leg, drumstick and thigh, fried, coated, skin / coating eaten	30000

Table 1. Consumption analysis of EPO in selected foods at maximum intended use level

FOODCODE	DESCRIPTION	Amount (ppm)
24147300	Chicken drumstick, fried, coated, skin / coating eaten, from raw	30000
24147302	Chicken drumstick, fried, coated, prepared skinless, coating eaten, from raw	30000
24147310	Chicken drumstick, fried, coated, skin / coating eaten, from pre-cooked	30000
24147320	Chicken drumstick, fried, coated, skin / coating eaten, from fast food / restaurant	30000
24157300	Chicken thigh, fried, coated, skin / coating eaten, from raw	30000
24157302	Chicken thigh, fried, coated, prepared skinless, coating eaten, from raw	30000
24157310	Chicken thigh, fried, coated, skin / coating eaten, from pre-cooked	30000
24157320	Chicken thigh, fried, coated, skin / coating eaten, from fast food	30000
24157330	Chicken thigh, fried, coated, skin / coating eaten, from restaurant	30000
24167200	Chicken wing, fried, coated, from raw	30000
24167210	Chicken wing, fried, coated, from pre-cooked	30000
24167220	Chicken wing, fried, coated, from fast food	30000
24167230	Chicken wing, fried, coated, from restaurant	30000
24201060	Turkey, light meat, breaded, baked or fried, skin not eaten	30000
24201070	Turkey, light meat, breaded, baked or fried, skin eaten	30000
24201360	Turkey, light or dark meat, fried, coated, skin not eaten	30000
24201370	Turkey, light or dark meat, fried, coated, skin eaten	30000
26100120	Fish, NS as to type, baked or broiled, made with oil	30000
26100130	Fish, NS as to type, coated, baked or broiled, made with oil	30000
26100140	Fish, NS as to type, coated, fried, made with oil	30000
26107120	Catfish, baked or broiled, made with oil	30000
26107130	Catfish, coated, baked or broiled, made with oil	30000
26107140	Catfish, coated, fried, made with oil	30000
26109120	Cod, baked or broiled, made with oil	30000
26109130	Cod, coated, baked or broiled, made with oil	30000
26109140	Cod, coated, fried, made with oil	30000
26118020	Halibut, baked or broiled, made with oil	30000
26118030	Halibut, coated, baked or broiled, made with oil	30000
26118040	Halibut, coated, fried, made with oil	30000
26137120	Salmon, baked or broiled, made with oil	30000
26137130	Salmon, coated, baked or broiled, made with oil	30000
26137140	Salmon, coated, fried, made with oil	30000
26151120	Trout, baked or broiled, made with oil	30000
26151130	Trout, coated, baked or broiled, made with oil	30000
26151140	Trout, coated, fried, made with oil	30000
26158010	Tilapia, baked or broiled, made with oil	30000
26158020	Tilapia, coated, baked or broiled, made with oil	30000
26158030	Tilapia, coated, fried, made with oil	30000
26319120	Shrimp, baked or broiled, made with oil	30000
26319140	Shrimp, coated, fried, made with oil	30000
26319145	Shrimp, coated, fried, from fast food / restaurant	30000
26319160	Shrimp, coated, baked or broiled, made with oil	30000
31105005	Egg, whole, fried, NS as to fat	30000
31105030	Egg, whole, fried with oil	30000
31105090	Egg, whole, fried, from fast food / restaurant	30000
32103020	Egg salad, made with mayonnaise-type salad dressing	25660
32103025	Egg salad, made with light mayonnaise-type salad dressing	26000
32130010	Egg omelet or scrambled egg, made with oil	30000
32130080	Egg omelet or scrambled egg, from fast food / restaurant	30000
32130110	Egg omelet or scrambled egg, with cheese, made with oil	30000
33001010	Egg substitute, omelet, scrambled, or fried, fat added	30000

Table 1. Consumption analysis of EPO in selected foods at maximum intended use level

FOODCODE	DESCRIPTION	Amount (ppm)
33401000	Egg substitute, omelet, scrambled, or fried, with cheese	30000
33401100	Egg substitute, omelet, scrambled, or fried, with meat	30000
33401200	Egg substitute, omelet, scrambled, or fried, with vegetables	30000
33401400	Egg substitute, omelet, scrambled, or fried, with cheese and vegetables	30000
33401500	Egg substitute, omelet, scrambled, or fried, with meat and vegetables	30000
33401600	Egg substitute, omelet, scrambled, or fried, with cheese, meat, and vegetables	30000
22300120	Ham, fried, NS as to fat eaten	30000
22300130	Ham, fried, lean and fat eaten	30000
22300140	Ham, fried, lean only eaten	30000
22300150	Ham, breaded or floured, fried, NS as to fat eaten	30000
22300160	Ham, breaded or floured, fried, lean and fat eaten	30000
22300170	Ham, breaded or floured, fried, lean only eaten	30000
42200500	Almond butter	27750
42200510	Almond butter, lower sodium	27750
42200600	Almond paste	13870
42201000	Cashew butter	26515
42202000	Peanut butter	25550
42202010	Peanut butter, lower sodium	25680
42202100	Peanut butter, lower sodium and lower sugar	26560
42202130	Peanut butter, lower sugar	27445
42202150	Peanut butter, reduced fat	17000
42202200	Peanut butter, vitamin and mineral fortified	25405
42203000	Peanut butter and jelly	12780
42203100	Peanut butter and chocolate spread	17175
41420380	Yogurt, soy	1800
41480020	Frozen dessert, non-dairy	22350
41810200	Bacon strip, meatless	73800
41810400	Breakfast link, pattie, or slice, meatless	45400
41810600	Chicken, meatless, NFS	31825
41810610	Chicken, meatless, breaded, fried	31925
41811400	Frankfurter or hot dog, meatless	34325
41811600	Luncheon slice, meatless-beef, chicken, salami or turkey	27775
41811800	Meatball, meatless	22500
41811890	Vegetarian burger or patty, meatless, no bun	15750
41812000	Sandwich spread, meat substitute type	22500
41812600	Vegetarian, fillet	30000
41901020	Soyburger, meatless, with cheese on bun	16500
42203200	Soy nut butter	27750
59003000	Meat substitute, cereal- and vegetable protein-based, fried	30000
56205002	Rice, white, cooked, made with oil	31200
56205012	Rice, brown, cooked, fat added, made with oil	32700
51000100	Bread, NS as to major flour	3590
51000110	Bread, NS as to major flour, toasted	3940
51000180	Bread, made from home recipe or purchased at a bakery, NS as to major flour	3410
51000190	Bread, made from home recipe or purchased at a bakery, toasted, NS as to major flour	3750
51123010	Bread, high protein	2200
51123020	Bread, high protein, toasted	2420
51127010	Bread, potato	3130
51127020	Bread, potato, toasted	3440
51134000	Bread, sweet potato	2840

Table 1. Consumption analysis of EPO in selected foods at maximum intended use level

FOODCODE	DESCRIPTION	Amount (ppm)
51134010	Bread, sweet potato, toasted	3120
51135000	Bread, vegetable	3230
51135010	Bread, vegetable, toasted	3550
51140100	Bread, dough, fried	3590
51300050	Bread, whole grain white	2150
51300060	Bread, whole grain white, toasted	2360
51300110	Bread, whole wheat	3550
51300120	Bread, whole wheat, toasted	3900
51300140	Bread, whole wheat, made from home recipe or purchased at bakery	4070
51300150	Bread, whole wheat, made from home recipe or purchased at bakery, toasted	4480
51300175	Bread, chappatti or roti, wheat	9200
51301010	Bread, wheat or cracked wheat	4530
51301020	Bread, wheat or cracked wheat, toasted	4980
51301040	Bread, wheat or cracked wheat, made from home recipe or purchased at bakery	3640
51301050	Bread, wheat or cracked wheat, made from home recipe or purchased at bakery, toasted	4000
51301510	Bread, wheat or cracked wheat, reduced calorie and/or high fiber	2550
51301520	Bread, wheat or cracked wheat, reduced calorie and/or high fiber, toasted	2800
51301600	Bread, pita, whole wheat	1710
51301610	Bread, pita, whole wheat, toasted	1880
51301620	Bread, pita, wheat or cracked wheat	1710
51301630	Bread, pita, wheat or cracked wheat, toasted	1880
51401010	Bread, rye	3300
51401020	Bread, rye, toasted	3630
51601010	Bread, multigrain, toasted	4650
51601020	Bread, multigrain	4230
51602010	Bread, multigrain, reduced calorie and/or high fiber	2550
51602020	Bread, multigrain, reduced calorie and/or high fiber, toasted	2800
51801010	Bread, barley	4530
51801020	Bread, barley, toasted	4980
51804010	Bread, soy	3440
51804020	Bread, soy, toasted	3780
51805010	Bread, sunflower meal	8200
51805020	Bread, sunflower meal, toasted	9010
51806010	Bread, rice	5240
51806020	Bread, rice, toasted	5760
51808000	Bread, gluten free	5240
51808010	Bread, gluten free, toasted	5760
51000200	Roll, NS as to major flour	3910
51000300	Roll, hard, NS as to major flour	4300
51000400	Roll, bran, NS as to type of bran	6000
51150000	Roll, white, soft	3910
51153000	Roll, white, hard	4300
51154010	Roll, white, hot dog bun	3910
51154100	Roll, white, hamburger bun	3910
51183990	Breadsticks, NFS	12850
51184200	Breadsticks, soft, NFS	12850
51184210	Breadsticks, soft, from fast food / restaurant	12850
51184220	Breadsticks, soft, from frozen	9160
51320010	Roll, wheat or cracked wheat	6300
51320060	Roll, wheat or cracked wheat, hot dog bun	3610

Table 1. Consumption analysis of EPO in selected foods at maximum intended use level

FOODCODE	DESCRIPTION	Amount (ppm)
51320070	Roll, wheat or cracked wheat, hamburger bun	3610
51320500	Roll, whole wheat	6300
51320550	Roll, whole wheat, hot dog bun	4380
51320560	Roll, whole wheat, hamburger bun	4380
51320700	Roll, whole grain white	3490
51320710	Roll, whole grain white, hot dog bun	3490
51320720	Roll, whole grain white, hamburger bun	3490
51620000	Roll, multigrain	6000
51620020	Roll, multigrain, hot dog bun	6000
51620030	Roll, multigrain, hamburger bun	6000
51808100	Roll, gluten free	2650
51180010	Bagel	1320
51186010	Muffin, English	1690
51300100	Bagel, whole grain white	1530
51301700	Bagel, wheat	1530
51301750	Bagel, whole wheat	1530
51301900	Bagel, wheat bran	1530
51302500	Muffin, English, wheat bran	2000
51303010	Muffin, English, wheat or cracked wheat	2000
51303030	Muffin, English, whole wheat	2000
51303100	Muffin, English, whole grain white	2000
51630000	Bagel, multigrain	1530
51630200	Muffin, English, multigrain	2000
52215000	Tortilla, NFS	5420
52215100	Tortilla, corn	2850
52215200	Tortilla, flour	7990
52215260	Tortilla, whole wheat	9760
52101000	Biscuit, NFS	18920
52101040	Crumpet	1690
52102040	Biscuit, from refrigerated dough	11220
52103000	Biscuit, from fast food / restaurant	18920
52104010	Biscuit, home recipe	15460
52104040	Biscuit, wheat	18190
52105100	Scone	17840
52201000	Cornbread, prepared from mix	9580
52202060	Cornbread, made from home recipe	9600
52206010	Cornbread muffin, stick, round	8400
52206060	Cornbread muffin, stick, round, made from home recipe	10280
52301000	Muffin, NFS	16070
52303500	Muffin, wheat	17780
52304000	Muffin, whole grain	17780
52304010	Muffin, wheat bran	5820
52304100	Muffin, oatmeal	18690
52304150	Muffin, oat bran	7400
52306010	Muffin, plain	15950
55100005	Pancakes, NFS	11920
55100010	Pancakes, plain, from frozen	6830
55100030	Pancakes, whole grain, from frozen	6750
55100040	Pancakes, gluten free, from frozen	4550
55100050	Pancakes, plain, from fast food / restaurant	15330
55100065	Pancakes, whole grain, from fast food / restaurant	15330
55101000	Pancakes, plain	11920
55105200	Pancakes, whole grain	12130

Table 1. Consumption analysis of EPO in selected foods at maximum intended use level

FOODCODE	DESCRIPTION	Amount (ppm)
55106000	Pancakes, gluten free	4550
55200010	Waffle, NFS	9490
55200020	Waffle, plain, from frozen	9490
55200060	Waffle, whole grain, from frozen	8390
55200090	Waffle, gluten free, from frozen	8840
55200100	Waffle, plain, from fast food / restaurant	26190
55200130	Waffle, whole grain, from fast food / restaurant	26770
55201000	Waffle, plain	18640
55205000	Waffle, whole grain	19020
55208000	Waffle, gluten free	8840
55300020	French toast, plain, from frozen	3710
55300030	French toast, whole grain, from frozen	3890
55300040	French toast, gluten free, from frozen	4490
55300050	French toast, plain, from fast food / restaurant	14090
55300055	French toast, whole grain, from fast food / restaurant	14310
55301000	French toast, plain	11150
55301015	French toast, whole grain	11350
55301025	French toast, gluten free	11980
55301030	French toast sticks, NFS	9120
55301031	French toast sticks, plain, from frozen	9120
55301040	French toast sticks, plain, from fast food / restaurant	20870
55301050	French toast sticks, plain	17740
55301055	French toast sticks, whole grain	11350
55400010	Crepe, NFS	10930
55401000	Crepe, plain	10930
55702100	Dosa (Indian), plain	4050
57100100	Cereal, ready-to-eat, NFS	20000
57124200	Cereal, chocolate flavored, frosted, puffed corn	20000
57206715	Cereal (General Mills Fiber One Raisin Bran Clusters)	20000
57216000	Cereal, frosted rice	20000
57227000	Cereal, granola	20000
57301511	Cereal (Kashi GOLEAN Crunch)	20000
57308190	Cereal, muesli	20000
57309100	Cereal (Nature Valley Granola)	20000
57329000	Cereal, raisin bran	20000
57348000	Cereal, frosted corn flakes	20000
57416010	Cereal, puffed wheat, sweetened	20000
57000100	Cereal, oat, NFS	20000
57134000	Cereal, corn flakes	20000
57137000	Cereal, corn puffs	20000
57151000	Cereal, crispy rice	20000
57206700	Cereal (General Mills Fiber One)	20000
57207000	Cereal, bran flakes	20000
57208000	Cereal (Kellogg's All-Bran Complete Wheat Flakes)	20000
57228000	Granola, homemade	20000
57301500	Cereal (Kashi 7 Whole Grain Puffs)	20000
57301505	Cereal (Kashi Autumn Wheat)	20000
57301510	Cereal (Kashi GOLEAN)	20000
57337000	Cereal, rice flakes	20000
57340000	Cereal, puffed rice	20000
57401100	Cereal, toasted oat	20000
57416000	Cereal, puffed wheat, plain	20000
56202900	Oatmeal, from fast food, plain	10000

Table 1. Consumption analysis of EPO in selected foods at maximum intended use level

FOODCODE	DESCRIPTION	Amount (ppm)
56203075	Oatmeal, regular or quick, made with non-dairy milk, NS as to fat	15000
56203076	Oatmeal, regular or quick, made with non-dairy milk, no added fat	10000
56203077	Oatmeal, regular or quick, made with non-dairy milk, fat added	15000
56203105	Oatmeal, instant, plain, made with non-dairy milk, NS as to fat	10000
56203106	Oatmeal, instant, plain, made with non-dairy milk, no added fat	10000
56203107	Oatmeal, instant, plain, made with non-dairy milk, fat added	15000
56201065	Grits, regular or quick, made with non-dairy milk, NS as to fat	10000
56201066	Grits, regular or quick, made with non-dairy milk, no added fat	10000
56201067	Grits, regular or quick, made with non-dairy milk, fat added	15000
56201350	Grits, instant, made with non-dairy milk, NS as to fat	10000
56201355	Grits, instant, made with non-dairy milk, no added fat	10000
56201360	Grits, instant, made with non-dairy milk, fat added	15000
56205090	Rice, cream of, cooked, fat added	5000
56205092	Rice, cream of, cooked, NS as to fat	5000
56207025	Cream of wheat, regular or quick, made with non-dairy milk, NS as to fat	15000
56207026	Cream of wheat, regular or quick, made with non-dairy milk, no added fat	10000
56207027	Cream of wheat, regular or quick, made with non-dairy milk, fat added	15000
56207101	Cream of wheat, instant, made with non-dairy milk, NS as to fat	15000
56207102	Cream of wheat, instant, made with non-dairy milk, no added fat	10000
56207103	Cream of wheat, instant, made with non-dairy milk, fat added	15000
71200400	Potato chips, baked, plain	30000
71200410	Potato chips, baked, flavored	30000
41310900	Bean chips	30000
41410015	Soy chips	30000
54318000	Chips, rice	30000
54401075	Tortilla chips, plain	50000
54401085	Tortilla chips, flavored	50000
54401095	Tortilla chips, popped	50000
54420210	Multigrain chips (Sun Chips)	50000
54440020	Cracker chips	50000
71220000	Vegetable chips	50000
71905410	Plantain chips	50000
73410210	Sweet potato chips	50000
54403045	Popcorn, popped in oil, unbuttered	50000
54403046	Popcorn, popped in oil, with added butter or margarine	75000
54403056	Popcorn, microwave, butter flavored	10000
54403057	Popcorn, microwave, butter flavored, light	10000
54403085	Popcorn, ready-to-eat packaged, butter flavored	10000
54403086	Popcorn, ready-to-eat packaged, butter flavored, light	10000
54408000	Pretzels, NFS	7325
54408015	Pretzels, hard, NFS	7325
54408016	Pretzels, hard, plain, salted	7325
54408035	Pretzels, hard, flavored	9700
54408070	Pretzels, hard, multigrain	7400
54408081	Pretzels, hard, plain, gluten free	16675
54408082	Pretzels, hard, flavored, gluten free	18950
54408190	Pretzels, hard, coated, NFS	44100
54408260	Pretzels, hard, coated, gluten free	38925
54408400	Pretzels, soft, NFS	4000
54408405	Pretzels, soft, ready-to-eat, NFS	4000
54408450	Pretzels, soft, from frozen, NFS	4000
54408455	Pretzels, soft, from frozen, salted	3060

Table 1. Consumption analysis of EPO in selected foods at maximum intended use level

FOODCODE	DESCRIPTION	Amount (ppm)
54408456	Pretzels, soft, from frozen, unsalted	3100
54408480	Pretzels, soft, multigrain	3130
54408485	Pretzels, soft, gluten free	5740
51184000	Breadsticks, hard, NFS	10000
51184100	Breadsticks, hard, reduced sodium	10000
51185000	Croutons	10000
51306000	Breadsticks, hard, whole wheat	10000
51808050	Breadsticks, hard, gluten free	10000
54001000	Crackers, NFS	20000
54102050	Crackers, oatmeal	20000
54103000	Crackers, breakfast biscuit	50000
54200100	Crackers, butter, reduced sodium	20000
54201010	Crackers, matzo, reduced sodium	20000
54204020	Crackers, wheat, reduced sodium	20000
54204030	Crackers, woven wheat, reduced sodium	20000
54301010	Crackers, butter, plain	20000
54301030	Crackers, butter (Ritz)	20000
54305010	Crackers, crispbread	20000
54305020	Crackers, flatbread	20000
54307000	Crackers, matzo	20000
54308000	Crackers, milk	20000
54318500	Rice cake	20000
54319000	Crackers, rice	20000
54319005	Crackers, rice and nuts	20000
54319020	Popcorn cake	20000
54326000	Crackers, multigrain	20000
54328000	Crackers, sandwich	20000
54337010	Crackers, woven wheat	20000
54337020	Crackers, woven wheat, plain (Triscuit)	20000
54337030	Crackers, woven wheat, flavored (Triscuit)	20000
54337060	Crackers, woven wheat, reduced fat	20000
54338000	Crackers, wheat	20000
54338010	Crackers, wheat, plain (Wheat Thins)	20000
54338020	Crackers, wheat, flavored (Wheat Thins)	20000
54338100	Crackers, wheat, reduced fat	20000
54339000	Crackers, corn	20000
54340100	Crackers, gluten free, plain	20000
54340110	Crackers, gluten free, flavored	20000
54202020	Crackers, saltine, reduced sodium	20000
54313000	Crackers, oyster	20000
54325000	Crackers, saltine	20000
54325010	Crackers, saltine, reduced fat	10000
54325060	Crackers, saltine, multigrain	20000
53710400	Cereal or granola bar (General Mills Fiber One Chewy Bar)	50000
53710500	Cereal or granola bar (Kellogg's Nutri-Grain Cereal Bar)	50000
53710502	Cereal or granola bar (Kellogg's Nutri-Grain Yogurt Bar)	50000
53710504	Cereal or granola bar (Kellogg's Nutri-Grain Fruit and Nut Bar)	50000
53710600	Milk 'n Cereal bar	50000
53710700	Cereal or granola bar (Kellogg's Special K bar)	50000
53710810	Cereal or granola bar (KIND Fruit and Nut Bar)	50000
53710900	Cereal or granola bar (General Mills Nature Valley Chewy Trail Mix)	50000
53710902	Cereal or granola bar, with yogurt coating (General Mills Nature Valley Chewy Granola Bar)	50000

Table 1. Consumption analysis of EPO in selected foods at maximum intended use level

FOODCODE	DESCRIPTION	Amount (ppm)
53710904	Cereal or granola bar (General Mills Nature Valley Sweet and Salty Granola Bar)	50000
53710906	Cereal or granola bar (General Mills Nature Valley Crunchy Granola Bar)	50000
53711000	Cereal or granola bar (Quaker Chewy Granola Bar)	50000
53711002	Cereal or granola bar (Quaker Chewy 90 Calorie Granola Bar)	50000
53711004	Cereal or granola bar (Quaker Chewy 25% Less Sugar Granola Bar)	50000
53711006	Cereal or granola bar (Quaker Chewy Dipps Granola Bar)	50000
53711100	Cereal or granola bar (Quaker Granola Bites)	50000
53712000	Snack bar, oatmeal	50000
53712100	Cereal or Granola bar, NFS	50000
53712200	Cereal or granola bar, lowfat, NFS	50000
53713000	Cereal or granola bar, reduced sugar, NFS	50000
53713010	Cereal or granola bar, fruit and nut	50000
53713100	Cereal or granola bar, peanuts, oats, sugar, wheat germ	50000
53714200	Cereal or granola bar, chocolate coated, NFS	50000
53714210	Cereal or granola bar, with coconut, chocolate coated	50000
53714220	Cereal or granola bar with nuts, chocolate coated	50000
53714230	Cereal or granola bar, oats, nuts, coated with non-chocolate coating	50000
53714250	Cereal or granola bar, coated with non-chocolate coating	50000
53714300	Cereal or granola bar, high fiber, coated with non-chocolate yogurt coating	50000
53714400	Cereal or granola bar, with rice cereal	50000
53714500	Breakfast bar, NFS	50000
53714510	Breakfast bar, date, with yogurt coating	50000
53714520	Breakfast bar, cereal crust with fruit filling, lowfat	50000
53710800	Cereal or granola bar (Kashi Chewy)	50000
53710802	Cereal or granola bar (Kashi Crunchy)	50000
53720100	Nutrition bar (Balance Original Bar)	50000
53720200	Nutrition bar (Clif Bar)	50000
53720210	Nutrition bar (Clif Kids Organic Zbar)	50000
53720300	Nutrition bar (PowerBar)	50000
53720400	Nutrition bar (Slim Fast Original Meal Bar)	50000
53720500	Nutrition bar (Snickers Marathon Protein Bar)	50000
53720600	Nutrition bar (South Beach Living Meal Bar)	50000
53720610	Nutrition bar (South Beach Living High Protein Bar)	50000
53720700	Nutrition bar (Tiger's Milk)	50000
53720800	Nutrition bar (Zone Perfect Classic Crunch)	50000
53729000	Nutrition bar or meal replacement bar, NFS	50000
53100070	Cake batter, raw, not chocolate	50000
53100100	Cake or cupcake, NS as to type	50000
53101100	Cake, angel food, without icing or filling	3000
53108200	Snack cake, chocolate, with icing or filling	50000
53109200	Snack cake, not chocolate, with icing or filling	50000
53115310	Cake or cupcake, nut, without icing or filling	50000
53115320	Cake or cupcake, nut, with icing or filling	50000
53115410	Cake or cupcake, oatmeal	50000
53115450	Cake or cupcake, peanut butter	50000
53116000	Cake, pound, without icing or filling	50000
53116020	Cake, pound, with icing or filling	50000
53116270	Cake, pound, chocolate	50000
53116390	Cake, pound, reduced fat, cholesterol free	2000
53118100	Cake, sponge, without icing or filling	50000

Table 1. Consumption analysis of EPO in selected foods at maximum intended use level

FOODCODE	DESCRIPTION	Amount (ppm)
53118200	Cake, sponge, with icing or filling	50000
53118300	Cake, sponge, chocolate	50000
53122080	Cake, shortcake, biscuit type, with fruit	10000
53123080	Cake, shortcake, sponge type, with fruit	10000
53391000	Pie shell	50000
53391100	Pie shell, graham cracker	50000
53391150	Pie shell, chocolate wafer	50000
53391200	Vanilla wafer dessert base	50000
53410100	Cobbler, apple	10000
53415100	Crisp, apple, apple dessert	10000
53440000	Strudel, apple	10000
53450000	Turnover or dumpling, apple	50000
53200100	Cookie, batter or dough, raw	50000
53201000	Cookie, NFS	50000
53203500	Cookie, biscotti	50000
53204000	Cookie, brownie, NS as to icing	50000
53204010	Cookie, brownie, without icing	50000
53204840	Cookie, brownie, reduced fat, NS as to icing	50000
53205260	Cookie, bar, with chocolate	50000
53206000	Cookie, chocolate chip	50000
53234000	Cookie, peanut butter	50000
53240000	Cookie, animal	50000
53261000	Cookie, gluten free	50000
54102010	Graham crackers	50000
54102015	Graham crackers (Teddy Grahams)	50000
51166000	Croissant	50000
51167000	Brioche	50000
53520000	Doughnut, NFS	100000
53520100	Doughnut, cake type, plain	100000
53520510	Beignet	100000
91703040	Caramel candy, chocolate covered	30000
91760100	Toffee, chocolate covered	30000
91703010	Caramel, chocolate-flavored roll	30000
91703020	Caramel, flavor other than chocolate	30000
91703080	Caramel, all flavors, sugar free	30000
91760000	Toffee, plain	30000
63403030	Fruit salad, including citrus fruits, with nondairy whipped topping	8040
73102217	Carrots, fresh, cooked with oil	30000
73102224	Carrots, frozen, cooked with oil	30000
73102227	Carrots, canned, cooked with oil	30000
73103021	Carrots, canned, reduced sodium, cooked with oil	30000
73402021	Sweet potato, baked, peel eaten, made with oil	30000
73403021	Sweet potato, baked, peel not eaten, made with oil	30000
73405021	Sweet potato, boiled, made with oil	30000
72201223	Broccoli, fresh, cooked with oil	30000
72201226	Broccoli, frozen, cooked with oil	30000
72125217	Spinach, fresh, cooked with oil	30000
72125224	Spinach, frozen, cooked with oil	30000
72125227	Spinach, canned, cooked with oil	30000
72107227	Collards, fresh, cooked with oil	30000
72107230	Collards, frozen, cooked with oil	30000
72107233	Collards, canned, cooked with oil	30000
75205044	Green beans, fresh, cooked with oil	30000

Table 1. Consumption analysis of EPO in selected foods at maximum intended use level

FOODCODE	DESCRIPTION	Amount (ppm)
75205047	Green beans, frozen, cooked with oil	30000
75205050	Green beans, canned, cooked with oil	30000
75205131	Green beans, canned, reduced sodium, cooked with oil	30000
75211031	Cabbage, green, cooked with oil	30000
75216134	Corn, fresh, cooked with oil	30000
75216137	Corn, frozen, cooked with oil	30000
75216141	Corn, canned, cooked with oil	30000
75216321	Corn, canned, reduced sodium, cooked with oil	30000
71900200	Plantain, cooked with oil	30000
75224043	Green peas, fresh, cooked with oil	30000
75224046	Green peas, frozen, cooked with oil	30000
75224049	Green peas, canned, cooked with oil	30000
75224131	Green peas, canned, reduced sodium, cooked with oil	30000
75202027	Asparagus, fresh, cooked with oil	30000
75202031	Asparagus, frozen, cooked with oil	30000
75202034	Asparagus, canned, cooked with oil	30000
75214027	Cauliflower, fresh, cooked with oil	30000
75214030	Cauliflower, frozen, cooked with oil	30000
75219033	Mushrooms, fresh, cooked with oil	30000
75233027	Summer squash, yellow or green, fresh, cooked with oil	30000
75233030	Summer squash, yellow or green, frozen, cooked with oil	30000
75233033	Summer squash, yellow or green, canned, cooked with oil	30000
75311027	Classic mixed vegetables, frozen, cooked with oil	30000
75311030	Classic mixed vegetables, canned, cooked with oil	30000
72202030	Fried broccoli	30000
74205010	Fried green tomatoes	30000
75205200	Fried green beans	30000
75409020	Fried cauliflower	30000
75411020	Corn fritter	30000
75412010	Fried eggplant	30000
75414030	Fried mushrooms	30000
75414500	Fried okra	30000
75415022	Fried onion rings	30000
75418010	Fried summer squash, yellow or green	30000
75511300	Pickles, fried	30000
14670000	Mozzarella cheese, tomato, and basil, with oil and vinegar dressing	50000
74506000	Tomato and cucumber salad made with tomato, cucumber, oil, and vinegar	50000
71103030	Potato, boiled, from fresh, peel not eaten, made with oil	30000
71103135	Potato, boiled, from fresh, peel eaten, made with oil	30000
71104070	Potato, roasted, from fresh, peel eaten, made with oil	30000
71104130	Potato, roasted, from fresh, peel not eaten, made with oil	30000
71401015	Potato, french fries, from fresh, baked	30000
71401020	Potato, french fries, from frozen, baked	30000
71404000	Potato, hash brown, NFS	60000
71601020	Potato salad with egg, made with mayonnaise-type salad dressing	20000
71601030	Potato salad with egg, made with creamy dressing	20000
71603020	Potato salad, made with mayonnaise-type salad dressing	20000
71603030	Potato salad, made with creamy dressing	20000
95101000	Nutritional drink or shake, ready-to-drink (Boost)	4225
95101010	Nutritional drink or shake, ready-to-drink (Boost Plus)	13450
95102000	Nutritional drink or shake, ready-to-drink (Carnation Instant Breakfast)	4800
95103000	Nutritional drink or shake, ready-to-drink (Ensure)	6325

Table 1. Consumption analysis of EPO in selected foods at maximum intended use level

FOODCODE	DESCRIPTION	Amount (ppm)
95103010	Nutritional drink or shake, ready-to-drink (Ensure Plus)	11300
95104000	Nutritional drink or shake, ready-to-drink, sugar free (Glucerna)	7700
95105000	Nutritional drink or shake, ready-to-drink (Kellogg's Special K Protein)	4000
95106000	Nutritional drink or shake, ready-to-drink (Muscle Milk)	5425
95106010	Nutritional drink or shake, ready-to-drink, light (Muscle Milk)	2875
95110000	Nutritional drink or shake, ready-to-drink (Slim Fast)	4825
95110010	Nutritional drink or shake, ready-to-drink, sugar free (Slim Fast)	4825
95110020	Nutritional drink or shake, high protein, ready-to-drink (Slim Fast)	8450
95120000	Nutritional drink or shake, ready-to-drink, NFS	6325
95120010	Nutritional drink or shake, high protein, ready-to-drink, NFS	8450
95120020	Nutritional drink or shake, high protein, light, ready-to-drink, NFS	13450
95120050	Nutritional drink or shake, liquid, soy-based	13450
64134025	Fruit smoothie, with whole fruit, non-dairy	9500
78101115	Fruit and vegetable smoothie, non-dairy	4500
78101118	Fruit and vegetable smoothie, non-dairy, added protein	5300
92101903	Coffee, Latte, with non-dairy milk	10300
92101906	Coffee, Latte, with non-dairy milk, flavored	10100
92101913	Coffee, Latte, decaffeinated, with non-dairy milk	10300
92101919	Coffee, Latte, decaffeinated, with non-dairy milk, flavored	10100
92101923	Frozen coffee drink, with non-dairy milk	6200
92101928	Frozen coffee drink, with non-dairy milk and whipped cream	20000
92101933	Frozen coffee drink, decaffeinated, with non-dairy milk	7400
92101938	Frozen coffee drink, decaffeinated, with non-dairy milk and whipped cream	20000
92101960	Coffee, Cafe Mocha, with non-dairy milk	9400
92101975	Coffee, Cafe Mocha, decaffeinated, with non-dairy milk	9400
92102020	Frozen mocha coffee drink, with non-dairy milk	5800
92102050	Frozen mocha coffee drink, with non-dairy milk and whipped cream	20000
92102080	Frozen mocha coffee drink, decaffeinated, with non-dairy milk	5800
92102110	Frozen mocha coffee drink, decaffeinated, with non-dairy milk and whipped cream	24100
92102502	Coffee, Iced Latte, with non-dairy milk	6500
92102505	Coffee, Iced Latte, with non-dairy milk, flavored	6400
92102512	Coffee, Iced Latte, decaffeinated, with non-dairy milk	6500
92102515	Coffee, Iced Latte, decaffeinated, with non-dairy milk, flavored	6400
92102602	Coffee, Iced Cafe Mocha, with non-dairy milk	5900
92102612	Coffee, Iced Cafe Mocha, decaffeinated, with non-dairy milk	5900
92161002	Coffee, Cappuccino, with non-dairy milk	6400
92162002	Coffee, Cappuccino, decaffeinated, with non-dairy milk	6400
81104490	Butter-oil blend, NFS	391500
81104500	Butter-oil blend, stick	405550
81104510	Butter-oil blend, tub	391500
81104550	Butter-oil blend, light	275500
81103035	Margarine-oil blend, NFS	299050
81103040	Margarine-oil blend, stick	403550
81103080	Margarine-oil blend, tub	299050
81103090	Butter replacement, liquid	299050
81104010	Margarine-oil blend, tub, light	189550
81104020	Margarine-oil blend, stick, light	189550
81200100	Oil or table fat, NFS	873400
81203000	Shortening, NS as to vegetable or animal	99970
12140000	Cream, whipped	67880
12220200	Whipped topping	50620

Table 1. Consumption analysis of EPO in selected foods at maximum intended use level

FOODCODE	DESCRIPTION	Amount (ppm)
12220280	Whipped topping, sugar free	26200
12320100	Sour cream, imitation	39040
14301010	Cream cheese, regular, plain	68880
14420200	Cheese spread, cream cheese, regular	57200
12210520	Coffee creamer, soy, liquid	99700
83108000	Vegan mayonnaise	96000
82108500	Sunflower oil	1000000
83100100	Salad dressing, NFS, for salads	222700
83112000	Avocado dressing	216650
83200100	Salad dressing, light, NFS	70000
11440070	Vegetable dip, yogurt based	40075
12350230	Ranch dip, regular	114600
12350235	Ranch dip, light	38125
12350250	Vegetable dip, regular	114450
12350255	Vegetable dip, light	37975
95201000	Nutritional powder mix (Carnation Instant Breakfast)	7000
95201010	Nutritional powder mix, sugar free (Carnation Instant Breakfast)	25500
95201200	Nutritional powder mix (EAS Whey Protein Powder)	25650
95201300	Nutritional powder mix (EAS Soy Protein Powder)	17850
95201500	Nutritional powder mix, high protein (Herbalife)	53550
95201600	Nutritional powder mix (Isopure)	5800
95202000	Nutritional powder mix (Muscle Milk)	85700
95202010	Nutritional powder mix, light (Muscle Milk)	60000
95210000	Nutritional powder mix (Slim Fast)	66700
95210010	Nutritional powder mix, sugar free (Slim Fast)	66700
95210020	Nutritional powder mix, high protein (Slim Fast)	67300
95220000	Nutritional powder mix, NFS	7000
95220010	Nutritional powder mix, high protein, NFS	53550
95230000	Nutritional powder mix, whey based, NFS	7800
95230010	Nutritional powder mix, protein, soy based, NFS	27800
95230020	Nutritional powder mix, protein, light, NFS	60000
95230030	Nutritional powder mix, protein, NFS	7800

7.2.4 Appendix IV. Safety Assessment Report (Baumert, 2022)

Safety Assessment Report

Prepared on behalf of the Burdock Group Consultants and Terviva

Allergenicity Safety Assessment of the Potential Exposure to Residual Pongamia Protein in Edible Pongamia Oil (EPO)

Prepared By:
Joseph L. Baumert, Ph.D.
Baumert Consulting Group, LLC.

October 5, 2022

Edible Pongamia Oil (EPO) is derived from the beans of the Pongamia (*Millettia pinnata)* tree which belongs to the diverse Fabaceae (legume) family. Clinical cross-reactivity has been documented to occur between certain species of legumes in the Fabaceae family, although this clinical cross-reactivity is relatively rare. Within the legume family, peanut is the most prominent allergenic food due to its relatively high prevalence of allergenicity in industrialized countries and the reported severity of allergic reactions that can occur when exposed to peanut protein. Pongamia oil has not been traditionally used for human consumption and therefore no clinical evaluations or history of the potential allergenicity or cross-reactivity are available. EPO undergoes a refining process which does not include bleaching and deodorizing steps that would be considered key steps in the process to derive highly refined oils which meet the source allergen labeling exemption set forth by Congress in the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA). Protein is removed to a degree through the oil refining process outlined in Figure 1 of the dossier; however, some residual Pongamia bean proteins remain in refined EPO product. Terviva tested two lots of EPO using the method of Rigby et al. (2011) and found total protein levels of 1470 and 1965 ng protein/gram of EPO (1.47 and 1.965 ppm; µg/g).

The focus of this safety assessment was to evaluate the potential allergenic exposure risk associated with the residual protein found in the EPO. Since no reports of allergenicity to EPO or the proteins found within the Pongamia bean are available, and thus no exposure/threshold data exist, peanut threshold data and reported population reference doses were used as conservative surrogates for the exposure assessment of the residual Pongamia protein found in the EPO. As mentioned, peanut is considered the most potent allergenic food source within the legume family and therefore serves as the worst-case legume source for this safety assessment. No data are available which suggest that Pongamia protein is allergenic (or equivalent in allergenicity to peanut). It is important to note that the approach used in this assessment serves as the worse-case scenario and overestimate the true risk associated with the EBO and its application in finished food products as consumed.

Exposure Dose Analysis:

IgE-mediated food allergies involve an acute immune response upon the exposure of the offending protein(s) from the allergenic source which can manifest into allergic signs and symptoms within minutes to hours after exposure. Given the acute nature of IgE-mediated food allergies, this exposure assessment evaluated consumption patterns on an individual eating occasion basis for the U.S. population using data from the 2003-2020 National Health and Nutrition Examination Surveys (NHANES). Representative food products within the food groups identified in Table 10 of the dossier were analyzed to derive the U.S. population consumption estimates, including the gram quantities of consumption per eating occasion associated with the average, 75th percentile (p75) and 90th percentile (p90) of the U.S. population. Consumption data for children, teens and adults were combined to derive the population estimates for each food product. These consumption quantities were then used to calculate the exposure dose of residual Pongamia protein based upon the maximum intended use level of EPO in the various food products (see Table 10 of the dossier) and the maximum concentration of Pongamia protein that was detected in the EPO using the method of Rigby et al. (2011). The final concentration of Pongamia protein in each finished food product and the calculated exposure dose (µg Pongamia protein) are provided in Table 1 of this report.

Clinical Threshold/Reference Dose Analysis:

The Food Allergy Research and Resource Program (FARRP) and TNO (Zeist, the Netherlands) have reviewed the published clinical literature and unpublished clinical records containing low-dose dose food challenge data of allergic children and adults to obtain individual NOAEL and LOAEL values as described by Remington et al. (2020). This clinical data was used these data to generate population threshold distribution curves for various priority allergenic sources where data exist. For the purposes of this safety assessment, the peanut threshold distribution which was derived from 1306 peanut-allergic individuals (children and adults) was utilized as a surrogate allergenic source as mentioned previously. Based upon the population dose distribution model, the VITAL Scientific Expert Panel recommended a reference dose of 0.2 mg total peanut protein based upon the estimated dose that the most sensitive 1% (ED_{01}) of the peanut-allergic population may react to upon consumption. This reference dose is currently used in the Allergen Bureau of Australia and New Zealand's VITAL® 3.0 calculator. Recently, the Ad hoc Joint FAO/WHO Expert Consultation of Risk Assessment of Food Allergens also reviewed available clinical threshold data for priority allergenic food sources as well as the severity of response associated with exposure doses associated with the ED_{01} (0.2 mg of peanut protein) and ED_{05} (2 mg of peanut protein) estimates. Based upon the clinical data available for priority allergens, the Committee noted that all objective reactions that occurred between the ED_{01} and ED_{05} doses were mild or moderate and the likelihood of severe anaphylaxis was considered to be very low. The Committee recommended that reference doses based upon the ED_{05} would meet the safety objective of minimizing the probability of any clinically relevant objective allergic response occurring at the defined reference doses for priority allergenic foods. The recommended health-based guidance value for peanut was set at 2 mg of total peanut protein. The recommendations of the Committee are currently under consideration by the Codex Committee on Food Labeling (CCFL). The summary documents of Part 2 of the FAO/WHO expert consultation can be reviewed at the following website:

- https://cdn.who.int/media/docs/default-source/food-safety/jemra/2nd-allergen-summary-report-20aug2021.pdf?sfvrsn=915a8417_8

- https://www.fao.org/3/cb9312en/cb9312en.pdf

For the purposes of this safety assessment, the ED_{01} reference dose of 0.2 mg of total peanut protein was conservatively used as the benchmark dose for comparison to the calculated exposure dose of residual Pongamia protein for each finished food product that would contain EPO.

Conclusions of the Safety Assessment of the Potential Allergenic Risk to Consumers Exposed to Residual Pongamia Protein in Food Products Containing EPO:

This safety assessment is predicated upon the use of several conservative assumptions as noted above. When considering the totality of the conservative estimates for the calculated concentration of total Pongamia protein that may be present in finished food products which would contain EPO and threshold data from peanut-allergic individuals (used as a conservative surrogate to represent the allergenicity of Pongamia protein) that underpin the safety assessment, the risk of an allergic reaction occurring, assuming Pongamia protein would elicit an IgE-mediated allergic response, would be very low and would not pose a reasonable likelihood of an adverse response in individuals with IgE-mediated food in my expert opinion.

For all of the food products evaluated, the quantity of food that would need to be consumed during a single eating occasion to reach the ED_{01} reference dose of 200 µg of protein from the allergenic source was a minimum of 3.4 times higher than the 90th percentile consumption quantity. In this instance, pretzels served as the food product with the highest estimated exposure dose due to the combination of the highest inclusion of EPO of the selected food products and the quantity of pretzels consumed during a single eating occasion in the population. For the majority of the food products, over 1 kg of the food would need to be consumed during a single eating occasion to reach the ED_{01} reference dose which would be unlikely to occur based upon the consumption data available from the NHANES database. Therefore, the amount of residual Pongamia protein present in the EPO that would be used in various food product applications would not pose an allergenic risk in my expert opinion.

References:

Remington BC, Westerhout J, Meima MY, Blom WM, Kruizinga AG, Wheeler MW, Taylor SL, Houben GF, Baumert JL. (2020). Updated population minimal eliciting dose distributions for use in risk assessment of 14 priority food allergens. *Food and Chemical Toxicology*. 139:111259. *https://doi.org/10.1016/j.fct.2020.111259*

Rigby, N.M., Sancho, A.I., Salt, L.J., Foxall, R., Taylor, S., Raczynski, A., Cochrane, S.A., Crevel, R.W.R., and Mills, E.N.C. (2011) Quantification and partial characterization of the residual protein in fully and partially refined commercial soybean oils. *Journal of Agricultural and Food Chemistry*. 59:1752-1759.

Table 1. Exposure Dose Assessment of Pongamia Protein in Food Products that Would Contain Edible Pongamia Oil.

Food Group (Population Percentile Consumption)*	Grams Consumed Per Eating Occasion	Maximum Intended Use Level of Pongamia Oil in Food Product (ppm; µg/g)	Maximum Protein Concentration in Pongamia Oil (ppm; µg/g)	Concentration of Pongamia Oil Protein in Food Product as Consumed (ppm; µg/g)	Estimated Exposure Dose of Pongamia Oil Protein per Eating Occasion (µg protein)	ED_{01} Dose for Peanut (µg protein)**	Gram Quantity of Food Needed to Reach the ED_{01}
Dairy Drinks and Substitutes n=8247							
Average	235	21,000	1.965	0.041	9.697	200	4847
p75	250	21,000	1.965		10.316		
p90	395	21,000	1.965		16.300		
Cheeses (including imitation and spreads) n=28,191							
Average	26	58,000	1.965	0.114	2.963	200	1755
p75	28	58,000	1.965		3.191		
p90	50	58,000	1.965		5.699		
Alternative/Imitation Meats n=516							
Average	70	34,000	1.965	0.067	4.677	200	2994
p75	71	34,000	1.965		4.744		
p90	140	34,000	1.965		9.353		
Egg Substitute (Omelet) n=120							
Average	108	30,000	1.965	0.059	6.367	200	3393
p75	135	30,000	1.965		7.958		
p90	176	30,000	1.965		10.375		
Nuts/Seeds n=4410							
Average	40	55,000	1.965	0.108	4.323	200	1851
p75	55	55,000	1.965		5.944		
p90	73	55,000	1.965		7.889		
Bread n=34,199							
Average	48	27,000	1.965	0.053	2.547	200	3770
p75	55	27,000	1.965		2.913		
p90	73	27,000	1.965		3.873		

Table 1. Exposure Dose Assessment of Pongamia Protein in Food Products that Would Contain Edible Pongamia Oil (continued).

Food Group (Population Percentile Consumption)*		Grams Consumed Per Eating Occasion	Maximum Intended Use Level of Pongamia Oil in Food Product (ppm; µg/g)	Maximum Protein Concentration in Pongamia Oil (ppm; µg/g)	Concentration of Pongamia Oil Protein in Food Product as Consumed (ppm; µg/g)	Estimated Exposure Dose of Pongamia Oil Protein per Eating Occasion (µg protein)	ED_{05} Dose for Peanut (µg protein)**	Gram Quantity of Food Needed to Reach the ED_{05}
Rolls n=16,107								
	Average	55	27,000	1.965	0.053	2.918	200	3770
	p75	60	27,000	1.965		3.183		
	p90	94	27,000	1.965		4.987		
Tortilla n=8972								
	Average	64	27,000	1.965	0.053	3.396	200	3770
	p75	84	27,000	1.965		4.457		
	p90	120	27,000	1.965		6.367		
Cooked Cereal n=125								
	Average	315	27,000	1.965	0.053	16.712	200	3770
	p75	318	27,000	1.965		16.871		
	p90	720	27,000	1.965		38.200		
Snack/Meal Bars n=4731								
	Average	38	30,000	1.965	0.059	2.240	200	3392
	p75	43	30,000	1.965		2.535		
	p90	62	30,000	1.965		3.655		
Crackers n=11,553								
	Average	25	30,000	1.965	0.059	1.474	200	3392
	p75	35	30,000	1.965		2.063		
	p90	45	30,000	1.965		2.653		
Popcorn n=6039								
	Average	36	351,000	1.965	0.690	24.830	200	290
	p75	46	351,000	1.965		31.727		
	p90	85	351,000	1.965		58.626		

Table 1. Exposure Dose Assessment of Pongamia Protein in Food Products that Would Contain Edible Pongamia Oil (continued).

Food Group (Population Percentile Consumption)*	Grams Consumed Per Eating Occasion	Maximum Intended Use Level of Pongamia Oil in Food Product (ppm; µg/g)	Maximum Protein Concentration in Pongamia Oil (ppm; µg/g)	Concentration of Pongamia Oil Protein in Food Product as Consumed (ppm; µg/g)	Estimated Exposure Dose of Pongamia Oil Protein per Eating Occasion (µg protein)	ED_{01} Dose for Peanut (µg protein)**	Gram Quantity of Food Needed to Reach the ED_{01}
Chips n=21,395							
Average	32	351,000	1.965	0.690	22.071	200	290
p75	36	351,000	1.965		24.830		
p90	58	351,000	1.965		40.003		
Pretzels n=3448							
Average	39	351,000	1.965	0.690	26.899	200	290
p75	42	351,000	1.965		28.968		
p90	85	351,000	1.965		58.626		
Cooked Vegetables n=544							
Average	118	50,000	1.965	0.098	11.594	200	2036
p75	165	50,000	1.965		16.211		
p90	240	50,000	1.965		23.580		
Sweetened Beverages n=41,397							
Average	315	25,000	1.965	0.049	15.474	200	4071
p75	370	25,000	1.965		18.176		
p90	521	25,000	1.965		25.594		
Nutritional and Smoothie-Type Beverages n=5879							
Average	417	25,000	1.965	0.049	20.485	200	4071
p75	534	25,000	1.965		26.233		
p90	783	25,000	1.965		38.465		

Table 1. Exposure Dose Assessment of Pongamia Protein in Food Products that Would Contain Edible Pongamia Oil (continued).

Food Group (Population Percentile Consumption)*	Grams Consumed Per Eating Occasion	Maximum Intended Use Level of Pongamia Oil in Food Product (ppm; µg/g)	Maximum Protein Concentration in Pongamia Oil (ppm; µg/g)	Concentration of Pongamia Oil Protein in Food Product as Consumed (ppm; µg/g)	Estimated Exposure Dose of Pongamia Oil Protein per Eating Occasion (µg protein)	ED_{01} Dose for Peanut (µg protein)**	Gram Quantity of Food Needed to Reach the ED_{01}
Protein and Nutritional Powders n=1081							
Average	38	85,000	1.965	0.167	6.347	200	1197
p75	44	85,000	1.965		7.349		
p90	70	85,000	1.965		11.692		
Fats and Oils n=5504							
Average	9	100000	1.965	0.197	1.7685	200	1015
p75	14	100000	1.965		2.751		
p90	14	100000	1.965		2.751		
Condiments and Sauces n=10,926							
Average	29	222,000	1.965	0.436	12.651	200	459
p75	32	222,000	1.965		13.959		
p90	64	222,000	1.965		27.919		

*n= the number of individuals in the NHANES dietary surveys that indicated consuming food products within the respective category during a single eating occasion.

**Clinical threshold data and population reference doses for Pongamia protein allergencity are not available. Threshold data and the associated ED_{01} reference dose for peanut were used as a surrogate legume source to represent the potential allergenicity of Pongamia protein. This is a very conservative assumption as there is no data available that would indicate that Pongamia protein is allergenic or that Pongamia protein allergenicity would be equivalent in potency to peanut.