ORIGINAL SUBMISSION

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College Park, MD 20740

RECEIVED
SEP 4 2013
OFFICE OF
FOOD ADDITIVE SAFETY

August 29th, 2013

GRAS Notice for Buglossoides oil

Dear Dr. Mattia:

Technology Crops International ("TCI") is hereby submitting the enclosed GRAS exemption claim for refined Buglossoides oil ("Buglossoides oil") for use as a food ingredient. Buglossoides oil has been determined by TCI to be Generally Recognized as Safe (GRAS) for the specified food uses, consistent with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This determination is based on scientific procedures and has been evaluated by experts qualified by scientific training and experience to assess the safety of Buglossoides oil under the conditions of its intended use in food. On the basis of this GRAS determination, and in accordance with proposed 21 CFR §170.36, the use of Buglossoides oil in food as detailed in the enclosed notice is exempt from the requirement of pre-market approval.

There is no data or information in the enclosed documents which TCI considers to be confidential.

If there are any questions which arise during the agency's review of this notification, or if there is any further information required, please contact me at the address above.

Yours sincerely

(b) (6)

Andrew Hebard
Chief Executive Officer

TECHNOLOGYCROPS
INTERNATIONAL

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Chief Executive Officer

GRAS NOTICE

FOR

REFINED BUGLOSSOIDES OIL

Prepared for: Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Pkwy
College Park
MD 20740

Prepared by: Technology Crops International LLC
7996 North Point Blvd
Winston Salem
NC 27106

August 30, 2013

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SEP 4 2013
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FOOD ADDITIVE SAFETY

GRAS NOTICE

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Prepared for:

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I. GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval

As defined herein, refined Buglossoides oil (Buglossoides oil), produced from the seeds of *Buglossoides arvensis* (L.) I.M.Johnst., has been determined by Technology Crops International LLC (TCI) to be Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This determination is based on scientific procedures as described in the following sections, and on the consensus opinion of an independent panel of experts qualified by scientific training and expertise to evaluate the safety of Buglossoides oil under the conditions of Its intended use in food. Therefore, the use of TCI's Buglossoides oil in food as described below is exempt from the requirement of premarket approval.

Signed:

(b) (6)

29th Aug 2013

Andrew Hebard
on behalf of
Technology Crops International LLC

Date

B. Name and Address of Notifier

Andrew Hebard
Technology Crops International LLC
7996 North Point Blvd
Winston Salem
NC 27106

C. Common Name of the Notified Substance

Refined Buglossoides oil

D. Conditions of Intended Use in Food

It is intended that Buglossoides oil will be used in the United States as a food ingredient which provides a source of fatty acids, including stearidonic acid (SDA), in a range of food products consumed by the general population, including baked goods and baking mixes, breakfast cereals and grains, cheeses, dairy product analogs, fats and oils, fish products, frozen dairy desserts and mixes, grain products and pastas, gravies and sauces, meat products, milk products, nuts and nut products, poultry products, processed fruit juices, processed vegetable products, puddings and fillings, snack foods, soft candy, and soups and soup mixes. Buglossoides oil will be added to foods at levels that provide up to 375 mg of SDA per serving.

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, Buglossoides oil has been determined by TCI to be GRAS for use in foods as defined herein on the basis of scientific procedures. See Appendix 2 entitled

"Expert Panel Report on the Generally Recognized As Safe Status of the Intended Use of Buglossoides Oil as a Nutritional Ingredient in Various Food Products".

F. Availability of Information

The data and information that are the basis for TCI's GRAS determination will be sent to the Food and Drug Administration (FDA) upon request, or are available for the FDA's review and copying at reasonable times at the following address:

Technology Crops International LLC
7996 North Point Blvd
Winston Salem
NC 27106
USA

(Contact: Andrew Hebard, email: ahebard@techcrops.com)

II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

A. Identity

A.1 Botanical identity

Buglossoides oil is a refined edible oil obtained from the seeds of *Buglossoides arvensis* (L.) I.M.Johnst. (previously *Lithospermum arvense* L.), an herbaceous annual plant (NRCS 2011) which has not been modified using modern methods of biotechnology such as recombinant DNA techniques. Common names include Corn Gromwell and Bastard Alkanet (Clapham *et al.* 1962).

Buglossoides is a member of the Boraginaceae family. As a result of the upheaval which has occurred in taxonomy following the discovery of molecular methods for assessing the relationships between clades, the position of this family within the overall taxonomic scheme is in a state of flux. However, a traditional view would place the family as shown below.

Plantae – Plants
└Tracheobionta – Vascular plants
  └Spermatophyta – Seed plants
    └Magnoliophyta – Flowering plants
      └Magnoliopsida – Dicotyledons
        └Asteridae
          └Lamiales
            └Boraginaceae – Borage family

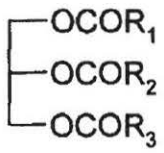
(NRCS 2012)

A.2 Chemical Name

Buglossoides oil is a natural plant oil containing a mixture of constituents. There is therefore no specific chemical name and it has no Chemical Abstracts Services (CAS) number.

Buglossoides oil consists primarily of triglycerides with small amounts of di- and mono-glycerides, free fatty acids, and an unsaponifiable fraction, comprised mostly of sterols and tocopherols. The triglycerides are of the form shown in Figure 1 where R1, R_2 and R_3 may be the same or different and represent various fatty acid moieties.

Figure 1 – Triglyceride structure



The fatty acids normally present in Buglossoides oil at levels greater than 1% (by fatty acid methyl ester gas chromatography (FAME GC) peak area) are shown in Table 1.

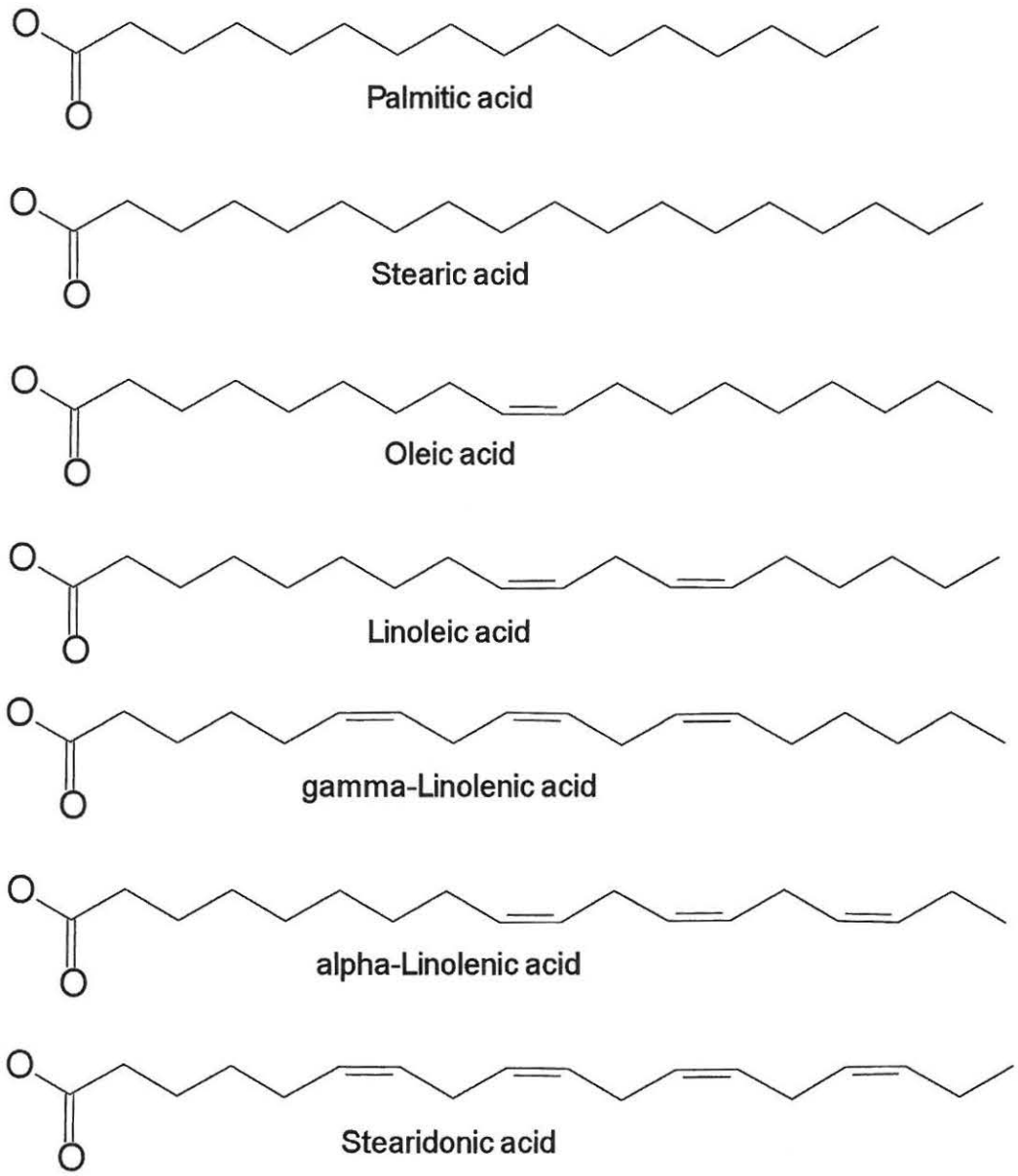
Table 1 – Identity of principal fatty acids

Common Name	Empirical formula	Scientific Name	CAS Number	Molecular weight
Palmitic acid	$C_{16}H_{32}O_2$	Hexadecanoic acid	57-10-3	256.42
Stearic acid	$C_{18}H_{36}O_2$	Octadecanoic acid	57-11-4	284.48
Oleic acid	$C_{18}H_{34}O_2$	9c-Octadecenoic acid	112-80-1	282.46
Linoleic acid	$C_{18}H_{32}O_2$	9c,12c - Octadecadienoic acid	60-33-3	280.45
gamma-Linolenic acid (GLA)	$C_{18}H_{30}O_2$	6c,9c,12c - Octadecatrienoic acid	506-26-3	278.43
alpha-Linolenic acid (ALA)	$C_{18}H_{30}O_2$	9c,12c,15c- Octadecatrienoic acid	463-40-1	278.433
Stearidonic acid (SDA), Moroctic acid	$C_{18}H_{28}O_2$	6c,9c,12c,15c- Octadecatetraenoic acid	20290-75-9	276.417

A.3 Chemical Structure

The structure of the principal fatty acids present in Buglossoides oil is shown in Figure 2.

Figure 2 – Fatty acid structures



A.4 Chemical and Physical Properties

The parameters in Table 2 were measured on three non-consecutive batches of Buglossoides oil.

Table 2 – Chemical and physical properties of Buglossoides oil

	NZ00053	NZ00056	NZ00058	Analytical Method
Flash point (°C)	187	176	185	AOCS Cc 9b-55 (Closed cup)
Iodine Value	233	227	222	AOCS Cd 1b-87
Specific Gravity	0.942	0.935	0.935	AOCS To 1a-64
Appearance	Pale yellow liquid	Pale yellow liquid	Pale yellow liquid	Visual AP-041 (TCI 2012a)
Refractive Index	1.4867	1.4640	1.4861	AOCS Cc 7-25
Viscosity (cps)	42.2	46.4	49.0	Brookfield Instrument Method

B. Method of Manufacture

B.1 Manufacturing Process

Buglossoides oil is manufactured in accordance with cGMP using food-grade raw materials and processing agents. All processing aids and additives employed in the manufacture of Buglossoides oil are used in compliance with appropriate federal regulations and current good manufacturing practice (cGMP). Traditional food oil manufacturing processes are used to produce Buglossoides oil which do not alter the fatty acid profile of the oil. Schematic diagrams of the manufacturing process are provided in Figure 3 and Figure 4 below.

Figure 3 – Extraction process for Buglossoides oil

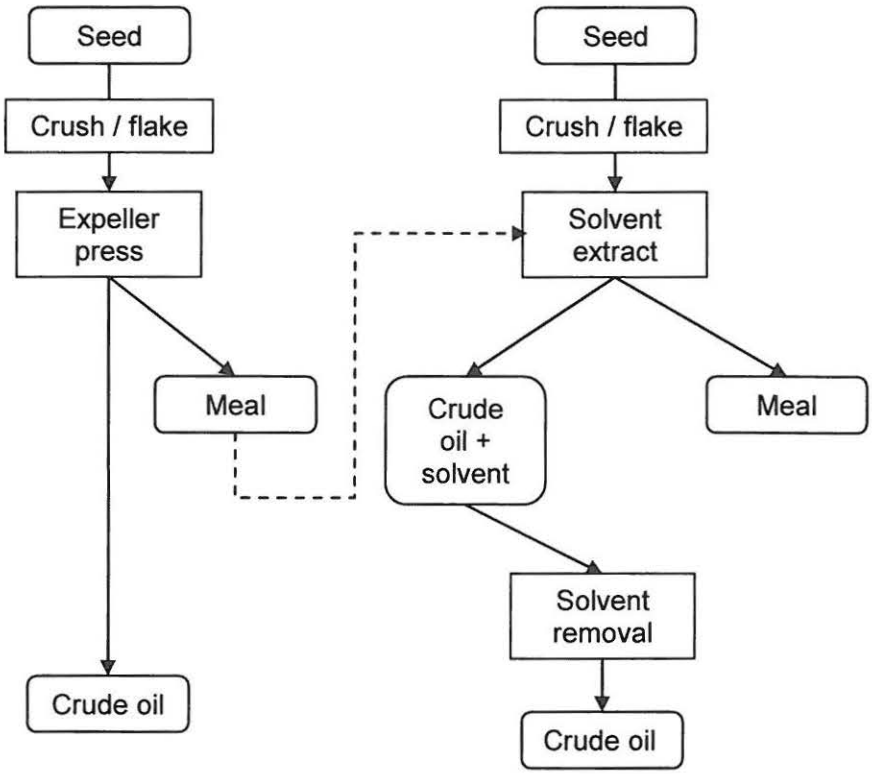
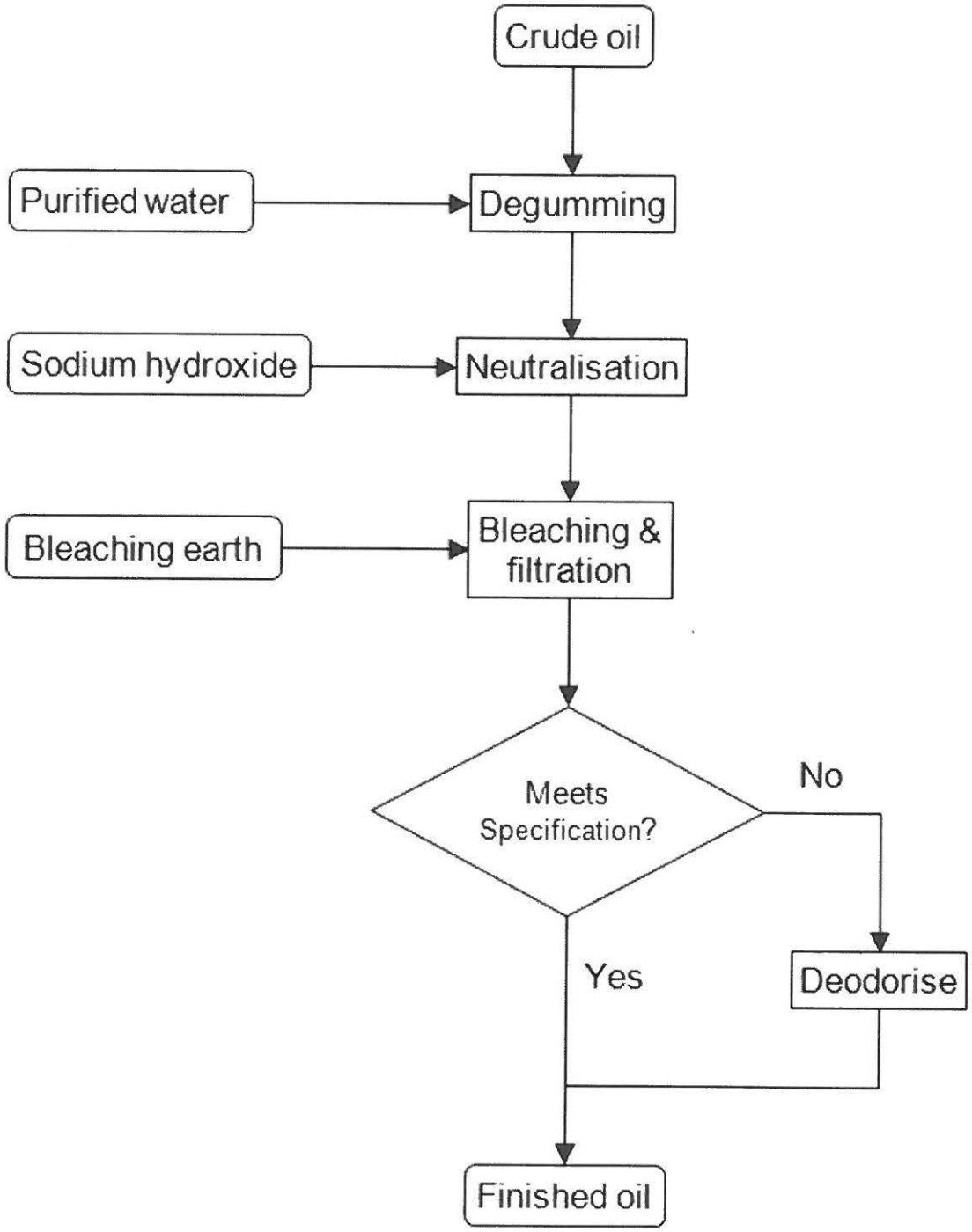


Figure 4 – Refining process for Buglossoides oil



B.2 Monitoring

The seed used for producing Buglossoides oil is produced under contract to TCI or to seed production companies acting on its behalf. The terms of the contract allow for all crops to be visited by TCI's representatives at least once during the growing season. All harvested seed is tested for purity and moisture and crop identity prior to delivery. Manufacture is in conformance with cGMP and with ISO 22000:2005 (which incorporates the Hazard Analysis and Critical Control Points (HACCP) system). These processes ensure that the product is monitored throughout the production process. Once processing is complete the oil is tested against the product specification. In addition, a sampling system for random batches of oil will be employed to monitor a much wider range of parameters, including the levels of undesirable substances such as PCBs, dioxins and pesticides. Records are kept in order to ensure full traceability of each finished batch of oil back to the individual farms on which the seed was grown.

C. Specifications for Food-Grade Material

The specifications for Buglossoides oil are given in Table 3, together with the results of analyses on three non-consecutive batches which were produced by the applicant in their own premises in November 2012 (batch numbers NZ00053, NZ00056 and NZ00058). Further analytical data on the three representative non-consecutive batches are given in Appendix 1. These data demonstrate that the manufacturing process results in a product with consistent properties which meets the specification.

Table 3 – Specifications for Buglossoides oil

	Specification	NZ00053	NZ00056	NZ00058	Analytical Method
Appearance	Pale yellow to clear liquid	Pale yellow liquid	Pale yellow liquid	Pale yellow liquid	Visual inspection AP-041 (TCI 2012a)
Color	<2.0R; <20.0Y	0.4R; 4.2Y	0.8R; 8.7Y	0.6R; 7.1Y	AOCS Cc 13j-97
Cold test*	Passes test	Pass	Pass	Pass	AOCS Cc 11-53
Fatty acid composition (Area %)					AOCS Ce 1j-07
Palmitic acid	10 – 50% in total	27.3	27.5	27.2	
Stearic acid					
Oleic acid					
Linoleic acid					
gamma-Linolenic acid	3.5 – 8.5%	6.4	6.2	6.3	
alpha-Linolenic acid	35 - 55%	44.0	44.4	43.5	
Stearidonic acid	15 - 30%	20.5	19.7	20.8	
Erucic acid	NMT 2.0%	0.2	0.2	0.2	
Other fatty acids	NMT 5%	0.2	<0.1	0.1	
Peroxide value	NMT 10 meq/kg	2.03	1.55	1.22	AOCS Cd 8-53
Free fatty acids	NMT 0.3%	0.011	0.06	0.17	AOCS Ca 5a-40
Unsaponifiable matter	NMT 1.5 %	0.28	0.43	0.73	AOCS Ca 6a-40
Moisture	NMT 0.2%	0.09	0.02	0.07	AOCS Ca 2e-84
Residual solvent**	NMT 1ppm	N/A	N/A	< 1.0 ppm	AOCS Ca 3b-87

*Required for oil to be marketed as a retail product
**Only applicable to oil extracted using solvent (N/A = Not applicable)

D. Stability

Immediately after refining, Buglossoides oil is packed in airtight containers. The containers are normally filled to capacity and the headspace is flushed with nitrogen gas. Buglossoides oil is normally stored at cool ambient temperature in light-proof containers. Stability tests conducted on samples of Buglossoides oil show that the oil remains within specification for peroxide value for a period of 8 weeks at all temperatures. This confirms that the oil is stable, even when stored under extreme conditions. The results are shown in Table 4.

Table 4 – Oxidation levels in Buglossoides oil during storage

Test	Temp	Anti-oxidant	Buglossoides oil samples					
			NZ00070		NZ00073		NZ00074	
			Start	8 weeks	Start	8 weeks	Start	8 weeks
Peroxide value	4°C	-	0.36	0.75	0.41	0.88	0.37	1.01
		+	0.36	0.81	0.41	0.78	0.37	0.93
	22°C	-	0.34	1.41	0.31	1.20	0.41	1.36
		+	0.34	1.14	0.31	0.95	0.41	1.31
	60°C	-	0.26	0.78	0.30	0.59	0.34	0.54
		+	0.26	0.59	0.30	0.90	0.34	0.62

+ Includes 1000 ppm mixed tocopherols
- No tocopherols

E. Potential Human Toxicants

Erucic acid

Erucic acid is present in Buglossoides oil and is also present in the diet. Diets rich in erucic acid have been reported to cause a transient accumulation of triacylglycerol (lipidosis) in the heart and other tissues of rats (Bremer & Norum 1982). However, this has not been reported in humans. Mustard seed oil (containing 40-60% erucic acid) is the main edible oil in parts of India. In a study comparing post-mortem hearts from one of these areas with those from areas where the main edible oils were peanut, sesame and coconut, fatty acid differences were observed in the heart tissue but it could not be associated with any observed heart damage. In France, where (high-erucic) rapeseed oil is consumed, an epidemiological study in 1974 of 254,788 cases of death due to heart failure found 269 cases (0.11%) with a somewhat similar histology to that seen in rats. The researchers found, in these cases, that there was a significant association with alcohol consumption but not with dietary fat and vegetable oil consumption. These studies and others, reviewed by Food Standards Australia New Zealand (FSANZ 2003) led the authors to conclude that a tolerable level of exposure for humans would be 7.5mg erucic acid/kg bodyweight/day, equivalent to about 500 mg erucic acid/day for the average adult. This would be unlikely to be exceeded if the levels of erucic acid in canola oil (the fully refined, bleached, and deodorized edible oil obtained from certain varieties of *Brassica napus* or *B. campestris*) did not exceed 2%. Similarly, canola oil

is considered GRAS in the USA provided that it contains less than 2% erucic acid (21CFR 184.1555) and the same limit has been adopted by TCI for Buglossoides oil.

Unsaponifiable fraction

The unsaponifiable fraction of Buglossoides oil has been characterized, as detailed in Table 18. This fraction comprises mainly of phytosterols, principally ß-sitosterol, campesterol and stigmasterol (Table 20), which are all found in major commercial food oils such as corn oil and soybean oil (Gunstone *et al.* 2007). Phytosterols are considered to be beneficial to human health in that they inhibit the uptake of cholesterol from the diet (Moreau *et al.* 2002). Buglossoides oil also contains some vitamin E, mostly in the form of γ-tocopherol (Table 21) which is also found at similar levels in a range of common food oils, such as palm oil (Codex 2005).

Pyrrolizidine alkaloids

A number of species from the Boraginaceae family, including *Buglossoides arvensis* contain naturally-produced pyrrolizidine alkaloids (PAs) some of which are liver toxins at high doses. They are carcinogenic in rats although, up to 2001, there was no reported evidence of carcinogenicity in humans (ANZFA 2001). A tentative no-effect level of 10 µg PAs/kg bodyweight/day in the diet has been suggested, based upon limited human data on the incidence of veno-occlusive disease. Applying an uncertainty factor of 10 to this figure allowed the Australia New Zealand Food Authority to propose a provisional tolerable daily dietary intake of 1 µg PAs/kg bodyweight/day (ANZFA 2001).

The European Food Safety Agency (EFSA) has produced a scientific opinion on pyrrolizidine alkaloids in food and feed (EFSA 2011) in which they found that there were no available substantial long-term follow-up data or epidemiological studies to assess whether exposure to 1,2-unsaturated PAs results in cancer in humans. They concluded however that, on the basis of current knowledge of metabolism and other areas, PAs may act as carcinogens and that therefore the data from experimental animals were relevant to humans. On this basis, they calculated the $BMDL_{10}$ (the lower confidence limit on the benchmark dose associated with a 10% response) for the induction of liver hemangiosarcomas by lasiocarpine in male rats at 70 µg/kg body weight/day. This compound is one of the most toxic of the PAs and so represents a conservative choice. With a PA content of 4 ppb in the oil and a daily intake at the 95th percentile of 19.6g/day, a 70 kg consumer would be exposed to 1.12×10^{-3} µg/kg body weight/day, giving a margin of exposure (MOE) of 62,500. The EFSA Scientific Committee has concluded that a MOE of 10,000 or higher, based on a $BMDL_{10}$ from an animal study, is of low concern from a public health point of view.

PAs are present in several foods. Honey made from the nectar of *Echium plantagineum* is believed to make up 10-25% of Australian production (Culvenor 1985 cited in Croda 2006a) and such honey has been shown to contain between 270 and 950 ppb (µg/kg) of PAs. Given usual patterns of honey consumption, it was concluded that this posed little if any human health risk (Coulomb 2003). Refined seed oil from *Echium plantagineum* has been approved as a novel food in the European Union (EU Commission 2008) and has been notified as a New Dietary Ingredient in the United States without questions being raised by the FDA (Croda 2006b) with a demonstrated upper limit on the concentration of PAs of 4 ppb.

PAs are water-soluble and therefore the bulk of any PAs present in *Buglossoides arvensis* seed would be expected to be left in the seed meal on extraction, and the level in the oil to be further reduced during refining. A sample of unrefined Buglossoides oil has been analyzed and found to contain 44 ppb of PAs, but refining reduces this to below 1 ppb, as indicated in Table 18. At this

level, an individual weighing 70 kg would have to consume 70 kg/day of Buglossoides oil to reach the tolerable daily intake of PAs set by ANZFA (above).

Allergenic proteins

No references in the literature or in the Allergome database have been found to allergic reactions to *Buglossoides arvensis*. Furthermore, the database does not contain any reference to dietary allergens in any members of the Boraginaceae, apart from an unreferenced entry for *Symphytum officinale* (comfrey) which indicates that the leaf contains an unknown allergen (Allergome 2013b). No references to this example have been found in the literature, so it is possible that it is referring to the well known hepatotoxicity of comfrey leaves which is caused by pyrrolizidine alkaloids (EFSA 2011). (The Allergome database does contain two entries for Ehretia species, but these are no longer considered to be members of the Boraginaceae family (Valdés 2004).)

However concerns were expressed by the Advisory Committee for Novel Food Products (the UK government's novel food advisory body) regarding *Echium plantagineum* which is closely related to *Buglossoides arvensis* (ACNFP 2007).

Cytochrome c has been reported as a respiratory allergen from echium pollen (Matthews *et al.* 1988), and Sharma *et al.* (2010) consider it to be an important respiratory allergen in the fungus *Curvularia lunata*. It has also been reported as an allergen in grass pollen, but a comprehensive review of grass pollen allergens concluded that "Taken together, the available evidence indicates that cytochrome c is not a relevant grass pollen allergen and certainly not an important one." (Andersson & Lidholm 2003). The Allergome database, which contains the allergen data extracted from nearly 6000 scientific papers, does not record any instance of cytochrome c causing an allergic reaction through oral administration (Allergome 2013a). Cytochrome c is an extremely common protein. It is a key enzyme in the mitochondrial respiratory chain and, as such is found in almost all eukaryotic cells, that is to say in all tissues of all multicellular organisms (including mammals, birds, fish, mollusks, insects, plants and algae) and is therefore a component of virtually all foodstuffs which contain protein (Lehninger 1975). Given that no reports of dietary allergy to cytochrome c have been recorded, it is reasonable to conclude that the protein is non-allergenic when ingested.

Respiratory exposure to Buglossoides oil is extremely unlikely as, in common with all vegetable oils, it has an extremely low vapor pressure at room temperature and would decompose or combust in air before reaching its boiling point. The cost and composition of the oil make it unsuitable for high temperature cooking, such as deep fat frying which, in any case, would tend to denature any protein present.

The oil could be atomized to form a mist, and this might occur to some degree if the product was presented in the form of spray container (either pump-action or pressurized) such as the type sold to consumers for applying small amounts of oil for frying or on salads. However, these devices are designed to produce relatively large droplets which do not remain suspended in the air. The protein content of Buglossoides oil is in any case sufficiently low that it would be physically impossible to breathe in sufficient oil to accumulate a meaningful quantity of protein.

It is therefore reasonable to conclude that there is no *a priori* reason to suppose that refined Buglossoides oil poses any significant allergenic risk.

Notwithstanding this, it is prudent to ensure that protein levels are reduced as far as practicable, and this is done during processing. Protein is polar in nature and will naturally partition into an

aqueous phase from a non-polar phase, such as oil. This will happen during extraction (especially when a solvent is used) and during refining, when the oil is washed with water and aqueous solutions. The oil is also filtered on at least one occasion, normally down to 1 micron, which will remove any pollen or particulate plant material. *Buglossoides arvensis* pollen is reported as being 8.8 – 11 µm across on its smallest dimension. (Perveen *et al.* 1995). Treatment with absorbent clay or bleaching earth would also act to reduce protein levels.

Concerns were expressed by the ACNFP committee, when they were considering the application for the approval of Refined Echium Oil as a novel food, about the allergenic potential of residual protein in the oil. In their statement of opinion, they accepted the applicant's proposal for a maximum limit of 20 µg protein/ml oil which was adopted by the European Commission in its final approval (ACNFP 2007).

The Buglossoides oil samples were initially analysed for protein content using two methods (Bradford method and chemiluminescence method) as shown in Table 18.

These results, although supportive, are not of themselves sufficient to demonstrate conclusively that Buglossoides oil meets the requirements of the Echium oil regulatory specification. The AFNCP has expressed concerns about the Bradford method in their guidance in these terms: ". . . *the Bradford Assay utilizes the dye reagent Coomassie Blue to bind to protein and reacts with larger peptides and intact proteins. This means the assay performance can be affected by treatments that affect protein integrity. As a consequence, the Bradford method is not itself sufficient to confirm the absence of protein from novel foods.*" (ACNFP 2011). The chemiluminescence method avoids these problems but the sensitivity of the machine used was only sufficient to demonstrate an absence of protein down to a level of 10 ppm total N, equivalent to 65 ppm of protein, which is above the limit specified for Echium oil.

Rigby *et al.* (2011) compared the efficiency of four different extraction methods (low-temperature acetone, phosphate-buffered saline, Olszewski bicarbonate, and sodium tetraborate) and three quantification methods (bicinchoninic acid (BCA), Bradford dye-binding, and 3-(4-carboxybenzoyl) quinolone-2-carboxaldehyde (CBQCA) fluorescence assays). They found that the bicarbonate and borate extraction methods gave the best extraction profiles, with the borate method being preferred as it was simpler, used a smaller volume of oil and allowed direct analysis of protein by the CBQCA method. The researchers then compared the variance of the results from the three analytical methods and were able to rank them with the CBQCA method giving the lowest variance (greatest precision) and the Bradford method the highest, with the differences between the methods being statistically significant at the 0.05 level. They ascribed the poorer performance of the Bradford method principally to the fact that some of the samples measured were below the lower limit of quantification for the method, but noted that the results could also be confounded because the method relies on the presence of binding sites on largely intact proteins, shows variability in responsiveness between proteins and is more susceptible to interference from other substances. The authors expressed a preference for the CBQCA method over the BCA method on the basis of its greater precision, which reflected its greater sensitivity (down to 0.075µg/ml).

Further analyses were therefore performed on Buglossoides oil samples using the borate extraction method and CBQCA (ATTO-TAG™) analytical procedure using a commercial analytical kit (Molecular Probes 2001) as described by Rigby *et al.* (2011). The results are given in Table 18 and show that the protein content of the Buglossoides oil is substantially below the limit set for Echium oil and slightly lower than that found in a commercial sample of Echium oil.

Given the absence of reports of allergenic activity in *Buglossoides arvensis* together with the fact that Buglossoides oil, when prepared using the methods described, does not contain sufficient protein to pose a significant allergenic risk, it is reasonable to conclude that a specification for protein content is unnecessary.

Other components

A literature survey has been carried out in order to identify possible additional undesirable substances which might be present in the product.

Buglossoides arvensis (and its synonym *Lithospermum arvense*) has not featured widely in the literature, either for reports of its use as food or medicine, or for undesired side effects.

Sandroni (2001), in an historical review of aphrodisiacs found that the leaf and seeds of *L. arvense* had been reported to increase the libido through their androgenic, gonadotropic, and estrogenic properties, but that no toxicity was known. By contrast, Findley & Jacobs (1980) reported that certain Indian tribes in Nevada used a related species (*L. ruderale*) as a contraceptive. They identified antigonadotropic activity in aqueous extracts from the roots.

B. arvensis seeds and leaves were found to give a positive response when treated with appropriate antisera which indicated the presence of phytoecdysteroids (plant-produced analogues of steroidal insect hormones) (Dinan *et al.* 2001). The authors noted that these compounds were apparently non-toxic to mammals and suggested that the ability to synthesize them could be usefully elevated in crop species for the control of insect predators. Phytoectysteroids are found in a wide range of plant species, including food plants. They are highly polar molecules and, if present in cultivated *Buglossoides arvensis* seed, they would be expected to partition into the aqueous fractions during extraction and refining (Soriano *et al.* 2004).

The roots of *Lithospermum erythrorhizon* Siebold & Zucc. have been commonly used in traditional Chinese medicine since at least the 16th century. The active component has been identified as shikonin, a naphthoquinone which has demonstrated wound healing, antitumor and antimicrobial effects in trials. No toxic effects were observed in oral feeding studies in mice or rats, but some toxicity was observed with intraperitoneal administration in mice, giving an LD_{50} of 20 ± 5 mg/kg. Shikonin has not been reported from *Buglossoides arvensis* but has been found in *Echium vulgare* L. Related compounds have been reported from the roots of *Buglossoides arvensis* and various Echium species as shown in Table 5. (Papageorgiou *et al.* 1999)

Table 5 – Shikonin analogues in *B. arvensis* and *Echium spp.*

	Buglossoides arvensis	Presence in Echium spp.
shikonin	NR	*E. vulgare*
acetylshikonin	Yes	*E. vulgare*
isobutyrylshikonin	Yes	*E. vulgare*
isovalerylshikonin	Yes	*E. vulgare*
isobutyrylshikonin	Yes	*E. vulgare*
a-methylbutyrylshikonin	NR	*E. vulgare*
b,b-dimethylacrylshikonin	NR	*E. vulgare*
b-hydroxyisovalerylshikonin	Yes	*E. lycopsis*
deoxyshikonin,	NR	*E. vulgare*
alkannan	NR	*E. vulgare*

NR = Not reported

Weston *et al.* (2012) have reported finding a range of naphthoquinones, including shikonin, acetylshikonin, and 1,3 dihydroxy-3-methylanthraquinone, in roots of *Echium plantagineum*, which they found to provide strong inhibition of plant, insect, fungal, and bacterial growth. Echium oil has been approved for use as a food ingredient in the EU and no concerns have been expressed about the presence of these compounds.

In summary, no reports of significant adverse effects or toxicity in *Buglossoides arvensis* have been discovered in the literature, apart from those which are also associated with Echium oil, which have been discussed above.

III. SELF-LIMITING LEVELS OF USE

The use of Buglossoides oil is limited by the level of fat that can be added to the proposed foods.

IV. BASIS FOR GRAS DETERMINATION

A. Documentation to Support the Safety of Buglossoides oil

The determination that Buglossoides oil is GRAS for the specified food uses is on the basis of scientific procedures and the information supporting this determination includes:

- Information relating to the manufacturing of Buglossoides oil using well-known, standard methods for edible oil production, in accordance with cGMP, resulting in a product which meets all appropriate food specifications.
- Detailed analyses of representative batches of Buglossoides oil which determined its properties in terms of physical and chemical characteristics, gross composition, fatty acid composition, composition of the unsaponifiable fraction, and which indicated the absence of inherent or external toxicants or contaminants.
- A long history or safe consumption of all of the fatty acids present in Buglossoides oil as components of other plant- or marine-derived oils, and the fact that SDA is a normal component of human metabolism, being an intermediate in the synthesis of EPA from ALA consumed in the diet.
- Published toxicological and clinical studies on SDA and GLA from other oils.
- The compositional similarity between Buglossoides oil and SDA Soybean Oil and published scientific data demonstrating the safety of SDA Soybean Oil.
- The regulatory status of SDA Soybean Oil which has been notified to the FDA as GRAS without objection.
- The estimated consumption of SDA from Buglossoides oil which is identical to that estimated for SDA Soybean Oil.
- The taxonomic similarity between *Buglossoides arvensis* and *Echium plantagineum*, the compositional similarity between Buglossoides oil and Refined Echium Oil and published scientific data demonstrating the safety of Refined Echium Oil.
- The regulatory status of Refined Echium Oil which has been notified as a New Dietary Ingredient without objection from the FDA and which has been approved for sale as a food ingredient in the European Union.

A summary of these data is presented herein.

This information was reviewed by a panel of experts, qualified by scientific training and experience to evaluate the safety of food and food ingredients who concluded that the proposed uses of Buglossoides oil are safe and GRAS based on scientific procedures. Their report appears as Appendix 2 to this document.

B. Estimated Intake

B.1 Natural presence in diet

Buglossoides oil has no history of use in food and, therefore, is not normally present in the diet. However, the component fatty acids are commonly found in a wide range of foods. (All percentage values quoted below represent the given fatty acid as a proportion of the total fatty acids present in the source oil.)

The fatty acids normally present in Buglossoides oil at levels greater than 1% (peak area) are shown in Table 6, in comparison to the principal fatty acids found in other food oils (Gunstone 2005). The data for Buglossoides oil was measured on samples taken from a range of genotypes, a range of seasons and a range of environments. The observed ranges were then widened to allow for anticipated improvements in the crop arising from research into agronomy and conventional plant breeding.

Table 6 – Fatty acid profiles

Fatty acid	**Buglossoides oil**	**Cocoa butter**	**Corn oil**	**Flaxseed oil**	**SDA soybean oil[1]**
Palmitic 16:0	4.5 – 7.5	26	9 – 17		9 – 13
Stearic 18:0	1.5 – 4.0	34			2.0 – 5.5
Oleic 18:1	4.0 – 10.0	35	20 – 42	16	10 – 20
Linoleic 18:2	7.0 – 15.0		39 – 63	24	15 – 30
GLA 18:3 n-6	3.5 – 8.5				5 – 8
ALA 18:3 n-3	35 – 55			50 – 60	9 – 12
SDA 18:4 n-3	15 – 30				15 – 30

All figures are expressed as percentage of total fatty acids.
[1] Monsanto 2009a

Gunstone (2005) and Haas (2005) give further examples of the contents of these fatty acids in a range of common food oils as follows:

> Palmitic acid is found in beef tallow (20.9–28.9 %), chicken fat (23.2%), palm oil (44%) and soybean oil (11%)
>
> Stearic acid is found in beef tallow (7.0–26.5%), and chicken fat (6.4%),

Oleic acid is found in beef tallow (30.4–48.0%), chicken fat (41.6%), olive oil (78%), rapeseed (canola) oil (56%), soybean oil (22%) and sunflower oil (20%)

Linoleic acid is found in groundnut oil (41%), rapeseed (canola) oil (26%), soybean oil (53%) and sunflower oil (60%)

Alpha-linolenic acid is found in rapeseed (canola) oil (10%) and soybean oil (10%).

Alpha-Linolenic acid (ALA) is an omega-3 essential fatty acid and is the parent compound for the omega-3 series of fatty acids. It is converted in the human body to SDA and thence to EPA and, to a lesser extent, DHA. As such it is an important component of the diet (de Lorgeril & Salen 2004, Gebaur 2006). The American Heart Association recommends consumption of foods containing ALA such as tofu and other forms of soybeans, canola, walnut and flaxseed, and their oils with the aim of consuming 1.3 – 2.7 g per day of ALA (Kris-Etherton *et al.* 2002, AHA 2010). Hiomega™ flaxseed oil, which has been notified to the FDA as GRAS, provides up to 25g of ALA per day and the FDA raised no questions (Polar Foods 2008, OFAS 2009a). Most American diets provide more than 10 times more omega-6 than omega-3 fatty acids. There is general agreement that individuals should consume more omega-3 and less omega-6 fatty acids to promote good health (ODS 2005).

Gamma-linolenic acid (GLA) is an omega-6 fatty acid and is an intermediate in human metabolism in the synthesis of dihomo-gamma-linolenic acid (DGLA) and arachidonic acid from the omega-6 parent compound, linoleic acid (LA) (Kapoor & Huang 2006). It is estimated that adult humans synthesize between 100 and 1,000 mg/day of GLA from dietary LA (Horrobin 1992). GLA (together with its metabolite DGLA) is present in human breast milk at a level of 100 to 400 mg/ℓ and a fully breast-fed infant may be consuming 20 to 80 mg/kg/day of GLA+DGLA. GLA is present in small quantities (less than 0.25%) in the lipids from beef, chicken and egg yolk (Horrobin 1992).

GLA is found in a number of oils and foods which are used as dietary supplements. It was estimated in 1990 that one million kilograms of evening primrose oil (*Oenothera biennis L.*) had been sold since the mid 1970s, with a GLA content of 7-10%, equivalent to approximately one million person-years of consumption. No major adverse events had been reported (Horrobin 1990). Borage oil, also known as Starflower oil, (*Borago officinalis* L.) contains 23 – 26% GLA and blackcurrant seed oil (*Ribes nigrum* L.) contains 15 – 20% (Horrobin 1990, Sarubin-Fragakis & Thomson 2007). Hempseed oil (*Cannabis sativa* L.) is reported to contain 4% GLA (Callaway 2004); the cyanobacterium *Spirulena platensis* contains 18-21% and the fungus *Mucor javanicus* contains 15 - 18% (Lindemann & Merolli 2006). Echium seed oil (*Echium plantagineum* L.) contains 10 -11%. GLA in echium oil has been approved in the European Union as a novel food ingredient for use in milk-based products and drinkable yogurt products delivered in a single dose, cheese preparations, spreadable fat and dressings, breakfast cereals, food supplements, dietary foods for special medical purposes, and foods intended for use in energy-restricted diets for weight reduction (EU Commission 2008). Echium oil has also been notified as a New Dietary ingredient (NDI) in the USA without raising any questions from the FDA (Croda 2006b, Anonymous 2008). Sonova 400® is a modified safflower variety (*Carthamus tinctorius* L.) which contains 45% GLA in its seed oil. It was notified as a new dietary ingredient (NDI) to the FDA in 2009 and has been marketed since June 2010 (Watkins 2010).

Stearidonic acid (SDA) is an omega-3 fatty acid and is an intermediate in human metabolism in the synthesis of eicosapentaenoic acid (EPA) from the omega-3 parent compound ALA. EPA and docosahexaenoic acid (DHA) are important components in the diet. The American Heart Association recommends eating two servings of fatty fish per week (to achieve an intake of 0.3 –

0.5 g per day of EPA plus DHA). Dietary SDA has been shown to be 17% to 30% as effective as dietary EPA in raising EPA levels in red blood cells and plasma phospholipids (Whelan 2009).

Stearidonic acid (SDA) is also found in a variety of foods and ingredients. Fish and other seafood is a major source, normally containing 0.5 – 2.0% of the total fatty acids, although the content can be as high as 7% (Ackman 2005). SDA is uncommon amongst terrestrial plants but is found in blackcurrant seed oil (*Ribes nigrum* L.) at 2 – 6% (Whelan 2009). Hemp seed oil also contains SDA, at levels between 0.4% and 1.9% (Callaway *et al.* 1996). The richest non-GM dietary source is echium oil which, as has been noted above, has been approved in the European Union for use as an ingredient in a range of foods and has been notified in the USA as an NDI. It contains at least 10% SDA.

The Monsanto company has developed a genetically modified soybean variety which produces a seed oil containing 9-12% ALA, 5-8% GLA and 15-30% SDA. In 2009, Monsanto provided notification to the FDA that it considered this oil to be GRAS and the FDA raised no questions (Monsanto 2009a, OFAS 2009b).

In summary, the main fatty acids present in Buglossoides oil are already present in the food supply. The safety of other constituents of Buglossoides oil is addressed in section II.E

B.2 Current regulatory status

There are no FDA regulations that specifically address the use of Buglossoides oil, SDA, GLA or ALA as ingredients in food. Certain uses of palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid and stearidonic acid (or materials containing substantial concentrations of them) as food ingredients have been affirmed or notified as GRAS in the USA as shown in Table 7.

SDA soybean oil has been notified as GRAS for use in a range of foods without objection from the FDA (OFAS 2009b). Echium oil has been notified as a new dietary ingredient without objection from the FDA and so may legally be sold for use as a dietary supplement in liquid or encapsulated form (Croda 2006b, Anonymous 2008). Both oils contain the same fatty acids as those found in Buglossoides oil.

In Europe palmitic acid, stearic acid and oleic acid are approved for use as a food additives in their own right (as E570):. All of the fatty acids present in Buglossoides oil may be used as food additives in the form of mono- and diglycerides (designated as E471) as they are all present in food oils and fats, as described below (EU Commission 2012). These additives are permitted in a very wide range of foodstuffs following the '*quantum satis*' principle, which is to say that:

> ". . .no maximum level is specified. However, additives shall be used in accordance with good manufacturing practice, at a level not higher than is necessary to achieve the intended purpose and provided that they do not mislead the consumer."(EU Commission 1996)

Echium oil has been approved as a food in the EU (EU Commission 2008).

Table 7 – Components of Buglossoides oil affirmed or notified as GRAS

Component	CFR/GRN code	Use
Palmitic acid	21 CFR 184.1065	In food with no limitation other than cGMP
	21 CFR 184.1329	In food with no limitation other than cGMP (as Glyceryl palmitostearate)
	GRN 248 (Compass Foods 2008, OFAS 2008)	Sucrose fatty acid esters (i.e., sucrose monoesters of lauric acid, palmitic acid, and stearic acid) manufactured by reaction of sucrose with vinyl esters of lauric, palmitic, and stearic acids
Stearic acid	21 CFR 172.860	In food as a lubricant, binder or defoaming agent in accordance with cGMP
	21 CFR 184.1505	In food with no limitation other than cGMP (as mono- and diglycerides)
	GRN 248 (Compass Foods 2008, OFAS 2008)	Sucrose fatty acid esters (i.e., sucrose monoesters of lauric acid, palmitic acid, and stearic acid) manufactured by reaction of sucrose with vinyl esters of lauric, palmitic, and stearic acids
Oleic acid	21 CFR 172.862	In food as a lubricant, binder or defoaming agent in accordance with cGMP
	21 CFR 184.1505	In food with no limitation other than cGMP (as mono- and diglycerides)
	GRN 306 (Monsanto 2009b, OFAS 2010)	Soybean oil with reduced palmitic and linolenic acids and increased oleic acid
Linoleic acid	21 CFR 184.1065	In food with no limitation other than cGMP
	21 CFR 184.1505	In food with no limitation other than cGMP (as mono- and diglycerides)
alpha-Linolenic acid	GRN 256 (Polar Foods 2008, OFAS 2009a)	High linolenic acid flaxseed oil
gamma-Linolenic acid	GRN 283 (Monsanto 2009a, OFAS 2009b)	Stearidonic acid soybean oil
Stearidonic acid	GRN 283 (Monsanto 2009a, OFAS 2009b)	Stearidonic acid soybean oil

GRN = Generally Recognized as Safe Notification

B.3 Intended use in human food

Technology Crops International (TCI) intends to market Buglossoides oil for use as a nutritional ingredient which provides a source of fatty acids, including SDA, in various food products in the United States, as listed in Table 8. Buglossoides oil will be added to foods at levels that provide up to 375 mg SDA per serving. For foods governed by a standard of identity, Buglossoides oil will be added consistent with the governing standard.

Table 8 – Intended food uses

FDA Food Classifications (21 CFR §170.3(n))	**Relevant Examples**
Baked goods and baking mixes	Biscuits, bagels, tortillas, English muffins breads, cookies, cakes, crackers, bars
Breakfast Cereals & Grains	Breakfast Cereals
Cheeses	Cottage Cheese Cheese
Dairy Product Analogs	Cream substitutes Soy milk
Fats & Oils	Margarine/Spreads Mayonnaise Dressings for Salads
Fish Products	Entrees with Sauce
Frozen Dairy Desserts and Mixes	Milk desserts and frozen yogurt Novelties
Grain Products and Pastas	Pasta
Gravies and Sauces	Main entrée sauces (spaghetti sauces)
Meat Products	Entrées with sauce, hot dogs, luncheon meat
Milk Products	Milk Based Drinks Milk Shakes Yogurt
Nuts and Nut Products	Peanut Butter
Poultry Products	Entrées with sauce, luncheon meat
Processed Fruit Juices	Fruit Drinks, Fruit Smoothies

Processed Vegetable Products	Vegetable Juices
Puddings and fillings	Pudding
Snack Foods	All varieties
Soft Candy	Candy bars
Soups and soup mixes	Processed soups (not home made)

B.4 Estimated daily intake

The Monsanto company notified the FDA in 2009 that its 'Stearidonic (SDA) Omega-3 Soybean Oil' was GRAS (Monsanto 2009a) and the FDA raised no questions (OFAS 2009b). As can be seen from the comparative fatty acid profiles in Table 9, Buglossoides oil is very similar to Monsanto's product, with the exception of the notably higher level of ALA in Buglossoides oil.

Buglossoides oil is intended to be used in the same food products as those identified in the Monsanto notification and in the same amounts which will also provide up to 375 mg of SDA per serving of food. James et al. (2003) reported an increase in erythrocyte EPA levels, using a dose of 1.5 g SDA per day, which in combination with DHA has been reported to correlate with reduced risk of cardiovascular disease (Harris & von Schacky, 2004). On the basis of this work, it was considered by Monsanto that the minimum consumption should be 1.5 g SDA per day and Technology Crops International (TCI) have reached the same conclusion.

Table 9 – Comparison of fatty acid contents

Fatty Acid	SDA Soybean Oil (20%)[1]	SDA Soybean Oil (29%)[2]	Buglossoides oil (20%)[3]
Palmitic 16:0	11.6	(Not reported)	5.2
Stearic 18:0	4.3	4.4	1.8
Oleic 18:1	16.0	16.1	7.6
Linoleic 18:2	22.2	18.7	12.7
ALA 18:3 n-3	10.3	10.9	43.8
GLA 18:3 n-6	6.1	7.4	6.3
SDA 18:4	20.2	28.8	20.3

[1] Batch 070118018S (Monsanto 2009a)
[2] 29% SDA Soybean Oil, Table B-2-1 (Monsanto 2009a)
[3] Mean values for three non-consecutive samples (NZ00053, NZ00056, NZ00058)

Monsanto estimated the consumption of SDA Soybean Oil from all proposed food uses and use levels in conjunction with food consumption data included in the National Health and Nutrition Examination Surveys (NHANES 1999-2002) (CDC 2007). They state:

> "The current intake of fat, *trans* fat, and fatty acids in the diet as well as intake following the addition of 20 or 30% SDA soybean oil to the proposed food uses was calculated. SDA soybean oil was either added to foods or replaced an unhydrogenated oil (including soybean oil, oil 'not further specified', corn oil, cottonseed oil, peanut oil, sunflower oil, coconut oil, olive oil, canola oil, or palm oil). In order to achieve 375 mg SDA per serving of food, every target food needs to have 1.8 g of 20% SDA soybean oil or 1.3 g of 30% SDA soybean oil per serving of food. Recipes were used to determine the amount and type of oil in each food, and either added in or replaced soybean oil or non-soybean liquid oil with SDA soybean oil to ensure this measured oil quantity. When the main oil variety for a food was a hydrogenated oil, it was assumed that this hydrogenated oil contained a blend of 60% solid fat and 40% liquid oil [Proprietary formulation, Stuart Clegg (Leatherhead Food International) to Richard Wilkes (Monsanto), Aug. 9, 2006]. Only the liquid 40% portion of the hydrogenated oil blend was then made available for substitution of SDA soybean oil in order to maintain functionality of the oil blend."

Their results for per capita intakes of 20% SDA Soybean Oil are summarized in Table 10 below (reproduced from Monsanto 2009a)

Table 10 – *Per capita* intake of SDA Soybean Oil (Monsanto 2009a)

Table 7 Per capita Intake of SDA Soybean Oil Post 20% SDA Soybean Oil Introduction[1,2]

Population Group	20% SDA Soybean Oil Intake (g/day)		20% SDA Soybean Oil Intake (g/kg/day)	
	Per capita Mean	Per capita 90th Percentile	Per capita Mean	Per capita 90th Percentile
U.S. population	10.1	19.6	0.18	0.38
Males 1-8 years	8.8	14.8	0.45	0.80
Females 1-8 years	7.8	13.5	0.41	0.73
Males 9-19 years	11.0	20.8	0.21	0.42
Females 9-19 years	9.1	16.7	0.18	0.35
Males 20-49 years	11.9	23.1	0.14	0.28
Females 20-49 years	9.9	19.0	0.14	0.28
Males 50+ years	11.1	20.6	0.13	0.25
Females 50+ years	9.6	17.9	0.14	0.26

[1] The amount of SDA soybean oil consumed was calculated for each NHANES participant by subtracting the amount of SDA consumed at baseline from the POST SDA inclusion intake of SDA and dividing by 20.7% (SDA content of 20% SDA) (g/day) and divided by each individual's bodyweight (g/kg/day).

[2] Soybean oil was either added to foods or replaced an unhydrogenated oil (including soybean oil, oil 'not further specified', corn oil, cottonseed oil, peanut oil, sunflower oil, coconut oil, olive oil, canola oil, or palm oil) so that the amount of SDA soybean oil in each food was equivalent

Table 11 – *Per capita* intake of fatty acids (Monsanto 2009a)

Table 8	U.S. Population Per capita Intake of Fat and Fatty Acids from the Total Diet - NHANES 1999-2002 (g/day)			
Fatty Acid	**Current Intake of Fatty Acids in Total Diet**		**Post 20%[1] SDA Soybean Oil Introduction in Total Diet**	
	Mean	**90th Percentile**	**Mean**	**90th Percentile**
U.S. Population				
Total Fat	78.8	136.5	84.0	143.6
Palmitic 16:0	13.9	24.7	14.6	25.7
Stearic 18:0	7	12.6	7.1	12.7
Oleic 18:1	23.4	41.8	23.7	41.9
Linoleic 18:2	10.1	19.5	11.8	21.9
ALA 18:3	0.9	1.8	1.9	3.6
GLA 18:3n-6[2]	0.03	0.1	0.7	1.3
SDA 18:4	0.004	0.004	2.1	4.1
Trans fat	5.9	11.6	4.9	9.8
EPA (20:5)	0.02	0.06	0.02	0.06
DHA (22:6)	0.02	0.06	0.02	0.06

[1] SDA soybean oil was either added to foods or replaced an unhydrogenated oil (including soybean oil, oil 'not further specified', corn oil, cottonseed oil, peanut oil, sunflower oil, coconut oil, olive oil, canola oil, or palm oil) so that the amount of SDA soybean oil in each food was equivalent to 1.8 grams of oil per serving. Serving sizes based upon FDA's reference amounts customarily consumed located in 21CFR §101.12 (U.S. FDA, 2008).
[2] Gamma-linolenic acid (18:3n-6) is only quantified in the Post 20% SDA soybean oil analyses. The other analyses present 18:3 not n-3 that includes gamma-linolenic acid.

The consumption figures for Buglossoides oil will be identical to the figures given in Table 10 for 20% SDA Soybean Oil, given that the SDA content, the inclusion rates and the food types are all the same.

Monsanto then examined the per capita intake of fat and fatty acids from the current diet and from the diet modified by the addition of 20% SDA Soybean Oil and summarized the results in the table reproduced below as Table 11.

The changes in the total fat, *trans* fat, SDA, EPA and DHA will be identical for Buglossoides oil. The figures for the other fatty acids will be different, because the fatty acid contents of the two oils are different. However it is possible to calculate the equivalent figures as follows.

For a given fatty acid:

$$W_B = W_S - (D_S \times S) + (D_B \times B)$$

Where:
W_B = Total weight of the fatty acid in the Buglossoides oil diet
W_S = Total weight of the fatty acid in the 20% SDA Soybean Oil diet
D_S = Weight of 20% SDA Soybean Oil in the diet
S = Proportion of the fatty acid in 20% SDA Soybean Oil
D_B = Weight of Buglossoides oil in the diet
B = Proportion of the fatty acid in SDA Soybean 20%

Using the above formula, it is possible to calculate an equivalent table of *per capita* intakes for Buglossoides oil as shown in Table 12 below. It is intended that Buglossoides oil should be used as a replacement for the SDA Soybean oil in products, such that the combined total of SDA present in a product should not exceed that which has been notified as GRAS for SDA Soybean oil.

In Table 12 only the shaded cells contain data which are different between Buglossoides oil and 20% SDA Soybean Oil. The principal difference is that ALA consumption is considerably increased when Buglossoides oil is introduced, compared to both the original diet and that containing 20% SDA Soybean Oil, as shown in Figure 5 and Figure 6.

The omega-6 to omega-3 ratios of the different diets are shown in Table 13. Diets with Buglossoides oil or 20% SDA soybean oil yield a lower ratio.

Table 12 – *Per capita* intake of fatty acids with Buglossoides oil

	U.S. Population *Per Capita* Intake of Fat and Fatty Acids from the Total Diet – NHANES 1999-2002 (g/day)			
Fatty Acid	**Current Intake of Fatty Acids in Total Diet**		**Post 20%[1] Buglossoides oil Introduction in Total Diet**	
	Mean	**90th Percentile**	**Mean**	**90th Percentile**
U.S. Population				
Total Fat	78.8	136.5	84.0	143.6
Palmitic 16:0	13.9	24.7	13.9	24.3
Stearic 18:0	7	12.6	6.8	12.2
Oleic 18:1	23.4	41.8	22.9	40.4
Linoleic 18:2	10.1	19.5	10.8	20.0
ALA 18:3	0.9	1.8	5.1	9.7
GLA 18:3n-6[2]	0.03	0.1	0.7	1.3
SDA 18:4	0.004	0.004	2.1	4.1
Trans fat	5.9	11.6	4.9	9.8
EPA (20:5)	0.02	0.06	0.02	0.06
DHA (22:6)	0.02	0.06	0.02	0.06

Figure 5 – *Per capita* mean intake of fatty acids

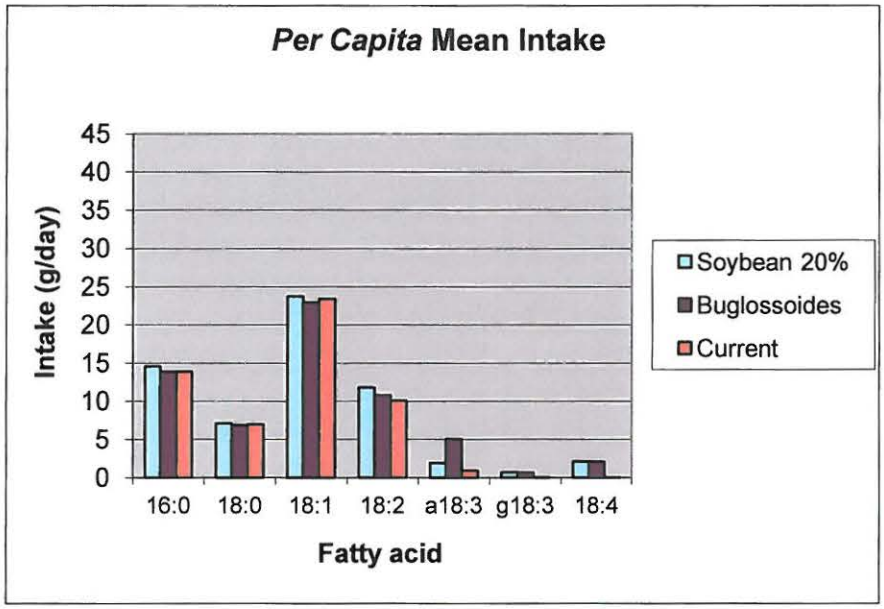


Figure 6 – *Per capita* 90th percentile intake of fatty acids

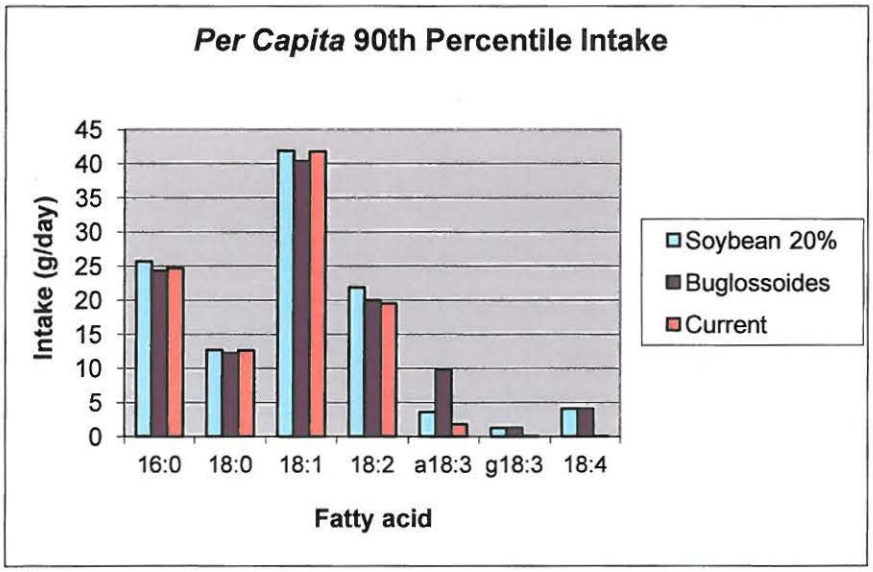


Table 13 – Omega-6 to omega-3 ratios for various diets

Diet	Mean	90th percentile
Original diet	11.2	10.9
20% SDA Soybean Oil diet	3.1	3.0
Buglossoides oil diet	1.6	1.5

C. Absorption, Distribution, Metabolism, and Excretion (ADME)

Like all dietary vegetable oils, the primary fate of the fatty acids in Buglossoides oil is beta-oxidation for use as energy. The essential fatty acids can also be metabolized to longer chain or more unsaturated fatty acids. Both the ALA and SDA in Buglossoides oil can be elongated and desaturated to EPA, the omega-3 fatty acid typically found in fish oils. Pre-clinical and clinical studies all show that dietary supplementation with either ALA or SDA results in an enrichment of tissue phospholipids with EPA, although the efficiency of this conversion is greater with SDA than with ALA (James *et al.* 2003).

Despite an enrichment of tissues with EPA following the consumption of oils containing SDA, the efficiency of dietary SDA is inferior to that of dietary EPA itself as shown in both animal and human studies (Hansen-Petrik *et al.* 2000, Ishihara *et al.* 2002, James *et al.* 2003, Harris *et al.* 2007, Harris *et al.* 2008, Lemke *et al.* 2010). In humans, 3 to 5 times more dietary SDA than dietary EPA must be consumed to attain an equivalent enrichment of tissues with EPA (James *et al.* 2003, Harris *et al.* 2008). As such, the consumption of 4.1g of SDA per day, representing the 90th percentile of the proposed food use, would fall well below the maximum level of 3g/day for the combined intake of EPA and DHA as set by the FDA for menhaden oil (21 CFR 184.1472(a)(3)). Similarly, approximately 10 times more dietary ALA than dietary EPA must be consumed to attain an equivalent enrichment of tissues with EPA (James *et al.* 2003). Thus, the consumption of 9.7g of ALA per day, representing the 90th percentile of the proposed food use, would also fall well below the maximum level of 3g/day for the combined intake of EPA and DHA as set by the FDA for menhaden oil (21 CFR 184.1472(a)(3)). The combined contribution from ALA and SDA, using the lowest conversion ratio, also falls well below the FDA maximum limit.

As indicated above, the fate of dietary SDA is mainly mitochondrial beta-oxidation or conversion to EPA. Human and animal studies show that dietary SDA does not accumulate in tissues. The consumption by humans of 0.75g SDA ethyl esters/day for 3 weeks followed by 1.5g/day for an additional 3 weeks resulted in no measurable impact on erythrocyte, platelet or mononuclear leukocyte SDA content, nor on plasma cholesterol ester and triglyceride SDA content (James *et al.* 2003). Similarly, the consumption by hypertriglyceridemic subjects of 15g echium oil per day containing 1.9g SDA and 4.3g ALA for 28 days resulted in no change in peripheral blood neutrophil SDA levels and only a slight increase in total plasma SDA to 0.2% of total plasma fatty acids (Surette *et al.* 2004). Similar results were obtained using SDA-enriched soybean oil where Harris *et al.* (2008) and Lemke *et al.* (2010)) measured no increase or small increases in erythrocyte SDA

levels not exceeding 0.05% of total cellular fatty acids after consuming 4.2g SDA/day for 12 weeks and 3.66g SDA/day for 16 weeks, respectively.

D. Preclinical Studies

D.1 Studies on SDA and GLA

The published studies involving SDA and a selection of studies on GLA are summarized in Table 14.

Table 14 – Summary of preclinical toxicity studies

Citation	Objective	Subjects[1]	Test article	Dose	Duration	Results
Hammond *et al.* 2008	Subchronic oral toxicity	30M + 30F Sprague–Dawley Crl:CD(SD) rats	20% SDA soybean oil	Up to 3g oil/kg/day by gavage	28 days	NOAEL = c.0.6 g/kg/day SDA[2] (highest level tested)
Hammond *et al.* 2008	Subchronic, reproductive & developmental oral toxicity	25M + 45F Sprague–Dawley Crl:CD(SD) rats	26% SDA soybean oil	Up to 4g oil/kg/day in diet (variable percentage)	90 days	NOAEL = c.1.0 g/kg/day SDA[3] (highest level tested)
Kawabata *et al.* 2012	Subchronic oral toxicity	14 M Wistar rats	23.5% SDA soybean oil	2.5% and 5% of oil in diet (approx 2.5 and 5.0g oil/kg bodyweight/day)	4 weeks	No adverse effects reported
Engler 1993	Subchronic oral toxicity	16-19 M spontaneously hypertensive rats	Blackcurrant oil	11% of oil in the diet (approx. 330 mg SDA/kg/day & 880 - 2860 mg GLA/kg/day)	49 days	No compound-related adverse effects reported
Barzanti *et al.* 1995	Subchronic oral toxicity	16M Wistar rats	Blackcurrant oil	Up to 10% of oil in diet (approx. 132 mg SDA/kg/day)	3 months	No adverse effects reported
Yamazaki *et al.* 1992	Subchronic oral toxicity	12M Wistar rats	SDA ethyl ester	1% of ethyl ester in diet (approx. 1.0 g ester/kg/day)	One or three weeks	No difference in body weight or serum lipid concentrations
Ishihara *et al.* 2002	Subchronic oral toxicity	7M Balb/c mice	SDA triglyceride	10% of TG in diet (approx 2.1 g SDA/kg/day[2])	3 weeks	No differences observed compared to EPA or ALA diets
Crozier *et al.* 1989	Subchronic oral toxicity	7M Dunkin-Hartley guinea pigs	Blackcurrant oil	10% of oil in diet (approx. 104 mg SDA/kg/day)	40 days	No differences observed compared to walnut oil or lard diets

Harris *et al.* 2007	Subchronic oral toxicity	45M Beagle dogs	SDA ethyl esters	Up to 193 mg ester/kg/day in diet (variable percentage)	Up to 12 weeks	No compound-related effects on body weight, food consumption or histopathalogical findings.
Everett *et al.* 1988	Subchronic oral toxicity	75M + 75F Sprague-Dawley rats	Evening primrose oil	Up to 2.5 ml oil/kg/day (approx. 230 mg GLA/kg/day) by gavage	53 weeks	No adverse effects following evaluation of multiple endpoints
Everett *et al.* 1988	Subchronic oral toxicity	15M + 15F beagle dogs	Evening primrose oil	Up to 5.0 ml oil/kg/day (approx. 460 mg GLA/kg/day) by gavage	52 weeks	No adverse effects following evaluation of multiple endpoints
Wainwright *et al.* 2003	Subchronic & reproductive oral toxicity	60F B6D2F$_1$ mice	Borage oil and GM rapeseed oil	10% of oil in the diet (up to approx. 5.4 g GLA/kg/day)	6 months	No adverse effects attributable to GLA consumption

[1] Numbers treated, excluding controls and animals receiving other treatments. M = Male, F = Female
[2] The authors state the NOAEL > 0.6 g/kg/day SDA but 0.6 g/kg/day was the highest dose tested
[3] The authors state the NOAEL > 1.0 g/kg/day SDA but 1.0 g/kg/day was the highest dose tested

The safety of SDA Soybean Oil was evaluated in a 28-day gavage study and a 90-day one-generation reproduction feeding study in rats (Hammond *et al.* 2008).

In a GLP study that was compliant with FDA, EPA and OECD guidelines, male and female Sprague–Dawley Crl:CD(SD) rats received 20% SDA Soybean Oil at doses of 0, 0.3, 1.0 and 3.0 ml/kg bodyweight by gavage for 28 days. The no-observed-adverse-effect-level (NOAEL) was approximately 3 g/kg/day SDA Soybean Oil, the highest dosage administered. Since the SDA Soybean Oil tested contained 20% SDA, the NOAEL for SDA was approximately 600 mg/kg/day. (The authors state the NOAEL as 'greater than' 600 mg/kg/day SDA but 600 mg/kg/day was the highest dose tested.)

The reproductive and subchronic toxicity of 26% SDA Soybean Oil were evaluated in a GLP-compliant feeding study that was consistent with FDA, EPA and OECD guidelines. Male and female Sprague–Dawley Crl:CD(SD) rats received 26% SDA Soybean Oil as a dietary admixture during a 90-day one generation reproduction feeding period at levels of 0, 1.5 and 4.0 g/kg/day (up to 1000mg SDA/kg body weight/day and 273 mg GLA/kg body weight/day). There were no consistent, dose-dependent, statistically significant adverse effects on any of the parameters tested including clinical observations, hematology, clinical chemistry and urinalysis, organ weights, macroscopic and microscopic pathology and reproductive and developmental endpoints. The NOAEL for reproductive and developmental toxicity and for subchronic toxicity was determined to be 4 g/kg/day, the highest dosage of SDA Soybean Oil tested. Since this SDA Soybean Oil contained 26% SDA, the NOAEL for SDA was 1000 mg/kg/day. (The authors state the NOAEL as 'greater than' 1,000 mg/kg/day SDA but 1,000 mg/kg/day was the highest dose tested.)

Kawabata *et al.* (2012) studied the safety of SDA Soybean Oil containing approximately 23.5% SDA in male Wistar rats. 42 animals were divided into 6 groups of 7 animals and given an *ad libitum* standard diet containing 10% of test oil, the composition of which varied between groups. The FFC (control) group received 50% refined lard and 50% mixed vegetable oil; the C group received 46% lard, 50% vegetable oil and 4% fish oil; the SDA25 group received 46% lard, 25% vegetable oil. 25% SDA Soybean Oil and 4% fish oil (approximately equivalent to 590 mg SDA/kg body weight/day and 140 mg GLA/kg body weight/day (FDA 1993)); the SDA50 group received 46% lard, 50% SDA Soybean Oil and 4% fish oil (approximately equivalent to 1180 mg SDA/kg body weight/day and 280 mg GLA/kg body weight/day (FDA 1993)); the ALA group received 46% lard, 16% vegetable oil, 34% flaxseed oil and 4% fish oil; and the EPA+DHA group received 12% lard, 50% vegetable oil, and 38% fish oil. Initially, the animals were all fed for one week on the FFC diet and then on the diet corresponding to their group for a further four weeks, whereupon the animals were sacrificed. There were no significant differences in initial and final body weights, body weight gain, body weight gain ratio, food intake, serum total cholesterol, LDL-cholesterol, and HDL-cholesterol levels between the six groups. Rats fed diets containing SDA Soybean Oil showed no significant differences in blood biochemistry data with respect to liver and kidney function relative to rats on other diets, and no other adverse effects from SDA Soybean Oil were reported.

A number of other preclinical (safety) studies have been conducted in rats using oils containing SDA. Between 16 and 19 spontaneously hypertensive male rats fed diets containing 11% blackcurrant oil providing approximately 330 mg SDA per kg body weight per day (FDA 1993) for 49 days were not reported to exhibit any compound related adverse effects compared to control animals (Engler 1993). In another study, male Wistar rats received, as dietary admixtures, up to 10% black currant oil (132 mg SDA/kg/day (FDA 1993)) or 10% olive oil (control). The authors reported that the animals appeared healthy and no adverse effects on body, liver, brain or heart weights were observed (Barzanti *et al.* 1995). Yamazaki *et al.* (1992) fed male Wistar rats diets containing 9% lard supplemented with 1% ALA or SDA ethyl esters (approximately 1.0[1] g ester/kg body weight

/day (FDA 1993)) for one or three weeks and reported no differences in body weight or serum lipid concentrations between groups.

Ishihara *et al.* (2002) provided 3-week-old male Balb/c mice with diets containing 1% ALA, SDA or EPA as a triglyceride oil for 3 weeks. There were no reported differences in body weight gain, food consumption or relative liver weight in the animals. The animals ingested approximately 2,108 mg of each of the supplemented fatty acids/kg body weight/day[1].

In male Dunkin-Hartley guinea pigs fed diets containing 10% black currant oil (BCO), walnut oil or lard for 40 days no differences in body weight or liver weights were reported. The BCO diets provided approximately 104 and 684 mg/kg body weight/day of SDA and GLA, respectively (Crozier *et al.* 1989, FDA 1993).

In 15 male Beagle dogs provided diets that contained up to 193 mg of SDA ethyl esters/kg body weight/day for up to 12 weeks, no compound-related effects on body weight or food consumption were reported and liver, heart and kidney appeared normal upon histological examination (Harris *et al.* 2007).

In addition to the above studies with blackcurrant oil, other repeated-dose studies have been conducted with oils containing GLA. For example, in a 53-week study in male and female Sprague-Dawley rats administered evening primrose oil by gavage providing up to 230 mg GLA/kg body weight /day, no adverse effects were reported on any of the multiple endpoints related to safety. A similar study in beagle dogs over 52 weeks providing up to 460 mg GLA/kg/day also reported no adverse effects (Everett *et al.* 1988).

Engler (1993) assessed the effects of dietary GLA from different sources on blood pressure in spontaneously hypertensive rats. 16-19 male rats aged 6-7 weeks were fed diets containing 11% fat which was comprised of either sesame oil (control), evening primrose oil, borage oil, blackcurrant oil or fungal oil for 7 weeks. The diets provided approximately 0, 880, 2090, 1870, 2860 mg GLA/kg body weight/day respectively (FDA 1993). No significant differences in body weight were reported and all GLA treatments were considered to have reduced blood pressure relative to the control. A significant increase in serum cholesterol in the borage oil group was noted after treatment, but no other adverse effects were reported.

Wainwright *et al.* (2003) assessed the effects of dietary GLA from different sources and at different concentrations on reproduction, growth, brain and behavioral development in $B6D2F_1$ mice. Twenty, 8-week old female mice per group were fed diets containing 10% fat which was comprised of either a mixture of 92.7% corn oil and 7.3% soybean oil (control), or borage oil (containing 23% GLA), or genetically modified canola oil (HGCO) containing 23% GLA or HGCO containing 36% GLA for 6 months. The diets provided approximately 0, 3.4, 3.4, and 5.4 g GLA/kg body weight/day respectively (FDA 1993). No significant differences were reported in the number of successful pregnancies, gestation length, or maternal weight gain during gestation and lactation between the groups. Both HGCO groups lost slightly more pups than the control group in the first 2 days after birth. This finding was not considered to be toxicologically significant because of variation in litter sizes and the lack of any other significant reproductive findings.

There were significant differences in the growth of the pups in different groups. The authors stated:

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[1] Calculated as (Food intake over 3 weeks) / 21 x 1% / (Initial body weight)
(79.7 / 21) x 1% / 0.018 = 2.108 mg/kg/day

'The present findings indicated that some of the effects of GLA provided as HGCO differed from those of equivalent amounts of GLA provided as BO. Specifically, 23% GLA from HGCO reduced pup body weight and was associated with a slight increase in neonatal pup attrition. There were no significant effects on behavioral development or on performance in the plus maze. An increase in dietary GLA resulted in an increase in brain n-6 FA and a corresponding decrease in brain n-3. Moreover, despite their similar levels of GLA, the effects on brain FA composition were greater in the mice provided 23% GLA as HGCO than with those receiving the same amount from BO. Comparison of the group receiving 23% GLA from HGCO with that receiving 36% indicated that at the higher level the effects on growth were greater, as were those on brain FA composition, particularly in the PE fraction. These findings support the use of HGCO as an alternative source of GLA.'

D.2 Studies on Buglossoides oil

Buglossoides oil has been the subject of two unpublished studies in mice (Surette 2013 (in part), Surette & Matar 2012) and one in salmon fry (Plante & Surette 2012), using oil supplied by TCI.

In the first study, two groups of ten female BALB/c mice (18g) were fed modified Monsanto US17 Rodent Diets supplemented with 0.1g arachidonic ethyl ester/kg of diet. In the treatment group the diet contained Buglossoides oil (26 g/kg diet) so as to provide 1% of the energy as SDA (equivalent to approximately 3.9 g/kg body weight/day of Buglossoides oil and 0.78 g/kg body weight/day of SDA (FDA 1993)). Mice were inspected daily by the animal facility staff for general health status: respiration, color of paws, muscle tone and signs of distress and dehydration. After three weeks on the diets, 5 animals from each group were given 100 µg/day of biopeptides derived from microbial/enzymatic hydrolysis of dietary proteins by gavage for 7 days, the other 5 animals per group receiving its diluent (saline). The animals were then sacrificed. There were no significant differences in body weight between the dietary groups. Additionally, inspection of the general health status of the animals did not reveal health concerns in any dietary groups.

In the second study, two groups (1 and 2) of 15 female BALB/c mice (18-20g) were fed the control diet and two groups (3 and 4) the diet containing Buglossoides oil (as in the first experiment) for 3 weeks and then groups 1 and 3 were given 100 µg/day of biopeptides by gavage for 7 days, the other two groups receiving its diluent (saline). This was followed by 5 days without treatment and the cycle repeated for the duration of the experiment. After the first 7 days of treatment with biopeptide, mice were then injected with 0.5ml containing 1.4 x 104 4T1 mammary carcinoma cells per ml into the right mammary gland. Tumor volume was measured on days 10, 14, 18, 22 and 27 post-injection. Tumor mass was measured on days of sacrifice; five mice per group were sacrificed on days 12, 20 and 27 post-injection. Mice were inspected daily by the animal facility staff for general health status: respiration, color of paws, muscle tone and signs of distress and dehydration. There were no significant differences between dietary groups for body weight at day 28 (day of tumor cell injections) and inspection of the general health status of the animals did not reveal health concerns in any dietary group. No significant differences in animal weights between dietary subgroups were observed during the cancer stage of the experiment. The SDA-oil diet showed a trend for decreased tumor growth, with tumor mass being significantly less than the control ($p<0.05$) on days 20 and 27. No safety concerns associated with the dietary regimens were noted.

Plante & Surette studied the effects of Buglossoides oil in Atlantic salmon (*Salmo salar* L.) fry (Plante & Surette 2012). Two isoproteinaceous diets were prepared based on a standard salmonid diet: diet 1 contained 11.5% herring oil and diet 2 was identical except that the herring oil was replaced by Buglossoides oil containing 18.56% SDA. 120 fry weighing on average 1.86g were

divided equally amongst six aquaria and fed for four weeks on diet 1, whereupon thirty fish were sampled. The fish in three tanks were then fed for a further 8 weeks on diet 1 whilst the remaining fish were fed for 8 weeks on diet 2. At the end of this period, a further 10 fish per tank (30 per diet) were sampled and fish condition, specific growth rate, mortality, percent lipid deposited (plus fatty acids analysis), and gross energy content were measured. No mortality occurred during the trial in either treatment. After 56 days of feeding, no significant difference was found in terms of growth, fish condition, energy content and specific growth rate between fish fed herring and Buglossoides oil. Small differences in body fatty acid composition between fish fed the two diets were reported and were ascribed by the authors to the higher content of 18-carbon polyunsaturated fatty acids in Buglossoides oil, and the more elevated content of 20- and 22-carbon monounsaturated fatty acids in herring oil. The content of long chain n-3 fatty acids in the fish bodies was not affected by the type of dietary oil.

E. Studies in Humans

Published toxicity studies in humans involving SDA and GLA and ALA are summarized in Table 15.

Table 15 – Summary of toxicity studies in humans

Citation	Objective	Subjects[1]	Test article	Dose	Duration	Results
Harris *et al.* 2008	Oral toxicity	5M + 6F healthy aged 38.4 ± 9.8 years	SDA Enriched Soybean Oil as liquid oil consumed with food	3.66 g/day SDA plus 1.2 g/day GLA	16 weeks	No clinically significant differences reported relative to EPA group or soybean oil control
Lemke *et al.* 2010	Oral toxicity	24M + 30F overweight (BMI 25 to 35 kg/m^2) aged 49.1 ± 12.3 years	SDA Enriched Soybean Oil as liquid oil consumed twice daily with food	4.2 g/day SDA plus 1.13 g/day GLA	12 weeks	No clinically significant differences reported relative to EPA group or soybean oil control
James *et al.* 2003	Oral toxicity	9M + 6 postmenopausal F healthy aged 18 - 65	SDA ethyl ester in capsules	1.5 g/day	6 weeks	No significant change in inflammatory mediators or blood lipids relative to EPA or ALA ethyl ester controls
Surette *et al.* 2004	Oral toxicity	6M + 5F hyper-triglyceridemic aged 56 ± 11 years	Echium oil in capsules consumed thrice daily	1.9 g/day SDA plus 1.7 g/day GLA	28 days	No change in vital signs or clinical laboratory analyses were reported compared to baseline values and no adverse events were attributed to the supplementation
Miles *et al.* 2004a,b and 2006	Oral toxicity	8 M + F healthy aged 33.7 ± 2.6 years	Echium oil in capsules	1 g/day SDA plus 0.9 g/day GLA	12 weeks	No adverse effects on immune response or serum lipids
		12 M + F healthy aged 31.9 ± 2.1 years	Borage oil in capsules	2 g/day GLA		
Kelley *et al.* 1993	Oral toxicity	10 M healthy aged 27.3 ± 1.9 years	Flaxseed oil as liquid with food	20 g/day ALA	56 days	No changes in serum lipids or in coagulation parameters or adverse events

Gracious *et al.* 2010	Oral toxicity	8 M + 7 F bipolar aged 13.4 ± 2.8 years	Flaxseed oil in capsules twice daily	Up to 6.6 g/day ALA	16 weeks	No difference was reported in adverse events or continuation rate in comparison with placebo (olive oil)
Takeuchi *et al.* 2007	Oral toxicity	11 M + 8 F normal or mild hypertensive aged 39.5 ± 13.3 or 36.7 ± 10.9 years	Blended oil as liquid on food	9.1 g/day ALA	4 weeks	No adverse effects were reported relative to control (rapeseed soybean blend)

[1] Numbers treated, excluding controls and subjects receiving other treatments. M = Male, F = Female

Two clinical studies were conducted using Monsanto's SDA-enriched soybean oil. In the first study (Harris *et al.* 2008), 33 healthy subjects (21 to 70 years old, 14 male, 19 female) were divided into three equal-sized groups and provided with oils in packets and capsules to be consumed with food: either SDA-enriched soybean oil (which provided 3.66g SDA/day and 1.2g GLA/day) in packets with regular soybean oil in capsules; or regular soybean oil in packets and EPA ethyl esters in capsules; or regular soybean oil in both packets and capsules, for 16 weeks. No clinically-significant adverse events were reported from SDA consumption and there were no clinically-significant differences in clinical chemistry parameters compared to subjects consuming 0.98g of EPA ethyl ester/day or regular soybean oil placebo. In the second study (Lemke *et al.* 2010), 252 overweight subjects (aged 49.1 ± 12.3 years) with a Body Mass Index (BMI) of 25 to 35 kg/m^2, consumed SDA-enriched soybean oil providing 4.2g SDA/day and 1.13g GLA/day (24 males plus 30 females), 1g EPA/day (27 males plus 35 females) or regular soybean oil placebo (28 males plus 37 females) for 12 weeks. No significant adverse effects of treatment were reported, including no significant effects on standard clinical chemistry and complete blood count panels, except for two analyses. Total bilirubin was lower in the EPA and SDA groups but remained within the normal clinical range and gamma-glutamyl transpeptidase was lower in the EPA group. The authors stated: "*For both of these differences, no known pathology was associated with the lower values, and the differences were not considered to be important.*"

The consumption of 1.5g/day of SDA ethyl ester in capsules by 9 male and 6 postmenopausal female healthy subjects, aged between 18 and 65, for 6 weeks, resulted in no significant change in inflammatory mediators or blood lipids compared to subjects consuming EPA ethyl esters or ALA ethyl esters (James *et al.* 2003).

Six male and five female hypertriglyceridemic subjects, aged 56 ± 11 years, consumed 15g of echium oil per day containing 1.9g SDA, 1.7g GLA and 4.3g ALA, in capsules three times per day, for 28 days. No change in vital signs or clinical laboratory analyses were reported compared to baseline values and no adverse events were attributed to the supplementation (Surette *et al.* 2004). Serum TG concentrations decreased by 21% during the study, an effect that is well documented following the consumption of dietary omega-3 PUFA from fish oils (Phillipson *et al.* 1985) and which is considered beneficial to health (Tanne *et al.* 2001).

In another study, eight healthy male and female subjects aged 33.7 ± 2.6 years consumed echium oil in capsules which provided 1g SDA/day and 0.9 g GLA /day, and twelve healthy male and female subjects aged 31.9 ± 2.1 years consumed borage oil in capsules which provided 2.0 g/day GLA, both for 12 weeks. No adverse effects on immune response or serum lipids were reported, and no difference in adverse events were reported compared to controls (Miles *et al.* 2004a,b and 2006).

It was reported in 1992 that around 4000 patients had been involved in trials involving the consumption of GLA for more than 3 months, mostly in the form of evening primrose oil. In most of the studies the oil dose was 3-6 g/day providing about 240 – 480 mg GLA/day. In the placebo controlled studies, not a single adverse event occurred more frequently in the GLA group as compared to the control. Evening primrose oil was licensed for use as a pharmaceutical in the UK and, by 1992, about half a million prescriptions for one month's supply had been dispensed. The incidence of reported adverse events was far lower than for most drugs and no pattern suggesting a causal relationship to GLA consumption was detected (Horrobin 1992).

Some concern has occasionally been expressed that evening primrose oil (containing 7 – 10% GLA) might cause, or lower the threshold for, epileptic seizures. This was based entirely on two studies reported in the early 1980s. In one study, three patients diagnosed with schizophrenia, who were

taking evening primrose oil, developed electroencephalogical features of temporal lobe epilepsy. None of them suffered from a seizure whilst taking evening primrose oil and all three were re-diagnosed during the study as suffering from temporal lobe epilepsy. In the second study, three out of 23 patients with schizophrenia developed seizures, one taking placebo and two taking evening primrose oil. In all cases the patients were also taking phenothiazine-based treatments. There is a strong link between schizophrenia and epilepsy, and it has long been established that phenothiazines lower the threshold for seizures. Puri (2007) therefore concluded that concern about evening primrose oil in relation to epileptic seizures is not warranted.

ALA is one of the two essential fatty acids in humans and is found in many dietary oils including soybean, canola, walnut and flax oils. ALA is the main dietary omega-3 fatty acid in the US diet and its consumption is generally considered to be beneficial to human health. One crossover study of 10 subjects that provided 31g/day of flaxseed oil (20g ALA/day) in the diet for 56 days showed no changes in serum lipids or in coagulation parameters compared to control diets and there were no adverse events reported (Kelley *et al.* 1993). In another study, eight male and seven female bipolar subjects with a mean age of 13.4 ± 2.8 years were provided with flaxseed oil in capsules, twice daily, supplying up to 6.6 g ALA/day. No difference was reported in adverse events or continuation rate in comparison with a placebo group (olive oil) (Gracious *et al.* 2010). In a third study, two groups of subjects (three male and 5 female normotensive aged 39.5 ± 13.3 years and eight male and three female high normal or mild hypertensive aged 36.7 ± 10.9 years) were provided with 7.8 g/day ALA in a blended oil added to food which, in combination with the remainder of their diet provided 9.1 g/day ALA, for four weeks. No adverse effects were observed relative to control groups receiving rapeseed/soybean blended oil (Takeuchi *et al.* 2007)

F. Other Data Pertaining to the Safety of Buglossoides oil

Croda in their application for approval for Echium oil as a novel food in Europe (Croda 2006a) identified two groups which they considered might be at risk from consuming SDA from Echium oil: those receiving anticoagulant therapy; and those with normal or suboptimal blood triglyceride levels. They concluded that there was no significant risk to either group and did not raise these concerns in their NDI notification to the FDA later the same year (Croda 2006b).

Croda's concern for those receiving anticoagulant therapy was unreferenced, but was based on the conversion of SDA to EPA (although not DHA) as described in section 4.III.3 above. They stated that:

> *". . . the combined intake of EPA and DHA in excess of 3 grams per day has been associated with reductions in platelet aggregation and increases in bleeding time, subjects receiving anticoagulant therapy (e.g., acetylsalicylic acid, warfarin, heparin, etc.) should avoid consuming oils or foods rich in EPA and DHA."*

In the United States, the Food and Drug Administration, has affirmed that menhaden oil is Generally Recognized as Safe (GRAS) provided that the combined intake of EPA and DHA are limited to no more than 3.0 g/person/day (FDA 1997). This limit was imposed because, after a review of the then available evidence, they found that there were no reports of increased bleeding times outside of the normal range at or below that intake level. At intake levels above 3.0 g/person/day they found evidence from a number of trials of increased bleeding time, but found it to be inconclusive for one or more of the following reasons: the extended bleeding times were within the normal range; in the case that final bleeding times were beyond the normal range, so also were

the initial bleeding times; the small number of subjects made the data difficult to interpret; in the case of coronary heart disease patients, the changes were not clinically significant; the differences were not statistically significant. Because of the lack of data and because of the potential risk of excessive bleeding in sensitive individuals with intakes at higher levels, the FDA concluded that the safety of menhaden oil is generally recognized only at levels that limit intake of EPA and DHA to 3 g/person/day.

More recently, Bays (2007), in a review of the literature, concluded that clinical trials have shown high-dose fish oil omega-3 fatty acid consumption to be safe, even when concurrently administered with other agents that may increase bleeding, such as aspirin and warfarin. Salisbury *et al.* (2012) reported on a study of 1523 patients across 24 centers who had the levels of red blood cell EPA and DHA (omega-3 index) assessed at the time of their presentation with acute myocardial infarction. The authors had found previously that the omega-3 index was strongly influenced by dietary intake of EPA and DHA in non-fried fish and fish oil supplements. They found no relationship between omega-3 index and bleeding events during hospitalization, even though the patients were at high risk of bleeding through the use of potent antithrombotic medications and invasive management.

In 2012, the European Food Safety Authority concluded in an extensive safety review that

> *". . .the available data are not sufficient to establish a tolerable upper intake level for n-3 LCPUFA (DHA, EPA, and DPA, individually or combined) for any population group."*

And that

> *"At observed intake levels, consumption of n-3 LCPUFA has not been associated with adverse effects in healthy children or adults."*

On the basis of the observed intake levels they suggested that supplemental intakes of EPA and DHA combined at doses up to 5 g/day, and supplemental intakes of EPA alone up to 1.8 g/day, do not raise safety concerns for the adult population (EFSA 2012).

Although there is an enrichment of tissues with EPA following the consumption of oils containing SDA, the efficiency of dietary SDA is inferior to that of dietary EPA itself as shown in both animal and human studies (Hansen-Petrik *et al.* 2000, Ishihara *et al.* 2002, James *et al.* 2003, Harris *et al.* 2007, Harris *et al.* 2008, Lemke 2010). In humans, 3 to 5 times more dietary SDA than dietary EPA must be consumed to attain an equivalent enrichment of tissues with EPA (James *et al.* 2003, Harris *et al.* 2008). As such, the consumption of 4.1g of SDA per day in Buglossoides oil (representing the 90th percentile of the proposed food use in the population group with the highest consumption per person) would fall well below the maximum level of 5g/day for the combined intake of EPA and DHA. Similarly, approximately 10 times more dietary ALA than dietary EPA must be consumed to attain an equivalent enrichment of tissues with EPA (James *et al.* 2003).

Using the mean fatty acid content of Buglossoides oil as shown in Table 19, it may be calculated that the consumption of 4.1g of SDA per day in Buglossoides oil (representing the 90th percentile of the proposed food use in the population group with the highest consumption per person) with the oil containing 20.3% SDA would require

4.1 x 100/20.3 x 1/0.89 = 22.7g of Buglossoides oil.

This amount of oil, with an ALA content of 43.8% would supply

22.7 x 43.8/100 x 0.89 = 8.8 g ALA

Thus the combined production of EPA resulting from this maximal consumption of Buglossoides oil may be calculated as:

4.1 / 3.0 + 8.8/10 = 2.2g EPA

This falls well below the level which is considered to be GRAS and which EFSA considered to be safe when consumed as EPA + DHA. It is slightly above the tentative upper limit set by EFSA, but this limit was chosen because of the lack of evidence from trials at higher intake levels, not because of evidence of toxicity.

The second group identified by Croda who might be at risk were those with normal or sub-normal triglyceride levels. This concern was based on the results of a single study which assessed the effects of Echium oil on 11 subjects with mild to moderate hypertriglyceridemia (Surette *et al.* 2004). The authors found that administration of approximately 1.9 g/person/day of SDA in the form of 15g Echium oil resulted in a significant reduction in serum triacylglycerol from baseline levels. Croda extrapolated from this that there may be a theoretical risk that individuals with normal or low plasma triglyceride levels may be similarly affected, but concluded that this was not an effect which needed to be taken into account. It seems likely that the triglyceride lowering effect of echium oil is due to the conversion of SDA to EPA in the body, which is confirmed by the fact that fish oil is well known to have a similar effect. TCI has not been able to discover any evidence in the literature that either fish oils or SDA-containing oils lower triglyceride levels in normal and sub-normal individuals. It therefore seems likely that the observed reduction represents a normalization and would only therefore affect those with high initial triglyceride levels, where it would be a desirable effect. The EFSA safety review (EFSA 2012) did not consider triglyceride lowering to be a safety issue.

G. Summary and Basis for GRAS Conclusion

Buglossoides oil is a refined edible oil obtained from the seeds of *Buglossoides arvensis*, an herbaceous annual plant which has not been genetically modified. Buglossoides oil has no history of use in food. However, the component fatty acids are commonly found in a wide range of foods. Buglossoides oil consists primarily of triglycerides with small amounts of di- and mono-glycerides, free fatty acids, and an unsaponifiable fraction, comprised mostly of sterols and tocopherols. The triglycerides contain a range of fatty acid moieties. The principal fatty acids (with a minimum content of 1%) include palmitic stearic, oleic, linoleic, gamma-Linoleic, alpha-Linolenic and stearidonic acid (SDA).

Buglossoides oil is manufactured using food-grade raw materials and processing agents. Oil is extracted from the seeds of *Buglossoides arvensis* by expeller pressing or organic solvent (usually hexane or isohexane) extraction and then refined. Traditional food oil manufacturing processes are used which do not alter the fatty acid profile of the oil.

Buglossoides oil is intended to be used as a nutritional ingredient in various food products at levels that will provide up to 375 mg SDA per serving. The estimated consumption of Buglossoides oil will be similar to that of SDA Soybean Oil since it is intended to be used in the same food products and in the same amounts. Consistent with Monsanto's recommendation, TCI recommends minimum daily consumption of 1.5 g SDA/day. TCI estimated that the intake from the proposed uses will result in per capita mean consumption of the oil of 10.1 g/day (0.18 g/kg bw/day) and the 90th percentile of 19.6 g/day (0.38 g/kg bw/day).

The safety of Buglossoides oil is based on the safety of its components and on the published safety studies of SDA soybean oil. Under the conditions of intended use, Buglossoides oil will provide a comparable level of SDA to that provided by SDA Soybean Oil. The safety of other fatty acids at the levels present in Buglossoides oil is well recognized. A critical evaluation of the available information on other constituents of Buglossoides oil including erucic acid, phytosterols, pyrrolizidine alkaloids failed to identify significant adverse health effects at the levels found in Buglossoides oil. It is highly unlikely that Buglossoides oil is allergenic since the level of protein in the oil is below the level which is considered safe for susceptible individuals in other edible oils.

On the basis of the preclinical, clinical, and other information presented above, TCI has concluded that the intended use of Buglossoides oil is safe. A panel of experts qualified by their scientific training and experience to evaluate the safety of food and food ingredients, undertook an independent and collective critical evaluation of the available published and unpublished data and other information pertinent to safety of the intended use Buglossoides oil as a nutritional ingredient in various food products. The panel concluded that Buglossoides oil, manufactured consistent with cGMP and meeting appropriate food grade specifications, is safe and generally recognized as safe based on scientific procedures.

ABBREVIATIONS AND TERMS

ACNFP	Advisory Committee on Novel Foods and Processes
ADME	Absorption, Distribution, Metabolism and Excretion
ALA	Alpha-Linolenic Acid
AOAC	AOAC International (formerly Association of Analytical Communities)
AOCS	American Oil Chemists Society
AOCS xx xx-xx	Standardized analytical method as published by the American Oil Chemists Society, where xx xxxx is the method reference code.
BCA	Bicinchoninic acid
$BMDL_{10}$	The lower confidence limit on the benchmark dose associated with a 10% response
Buglossoides	*Buglossoides arvensis* (L.) I.M.Johnst.
Buglossoides oil	Refined edible oil obtained from the seeds of *Buglossoides arvensis* (L.) I.M.Johnst.
CAS	Chemical Abstracts Service
CBQCA	3-(4-Carboxybenzoyl) quinolone-2-carboxaldehyde
CFR	Code of Federal Regulations
CFU	Colony Forming Units
cGMP	Current Good Manufacturing Practice
DGF	German Society for Fat Science (Deutsche Gesellschaft für Fettwissenschaft)
DGLA	Dihomo-Gamma-Linolenic Acid
DHA	Docosahexaenoic Acid
DL	Dioxin-like polychlorinated biphenyl
DNA	Deoxyribonucleic Acid
Echium	*Echium plantagineum* L.
Echium oil	Refined edible oil obtained from the seeds of *Echium plantagineum* L.
Ed	Editor
Eds	Editors
Edn	edition
EFA	Essential Fatty Acid
EFSA	European Food Safety Agency
EPA	Eicosapentaenoic Acid
EPA	Environmental Protection Agency
et al.	*et alia* (and others)
EU	European Union
F	Female
FA	Fatty acid
FAME	Fatty Acid Methyl Ester

FCC	Food Chemicals Codex
FDA	Food and Drug Administration
FDA LIB	FDA Laboratory Information Bulletin
FSANZ	Food Standards Australia New Zealand
GC	Gas Chromatography
GC/MS	Gas chromatography followed by mass spectrometry
GLA	Gamma-Linolenic Acid
GLP	Good Laboratory Practice
GM	Genetically modified
GMP	Good Manufacturing Practice
GRAS	Generally Recognized as Safe
GRN	Generally Recognized as Safe Notification
HACCP	Hazard analysis and critical control points
Hg	Mercury
HGCO	Genetically modified canola oil
hr	hour
ICP-MS	Inductively coupled plasma mass spectrometry
ISO	International Organisation for Standardisation
LD_{50}	Median lethal dose
M	Male
meq	milliequivalent: the mass in grams of a substance which will react with 0.001 mol of electrons
MFHPB	Microbiology Food Health Protection Branch (Canada)
MFLP	Microbiology Food Laboratory Procedure (Canada)
µg	microgram (10^{-6} gram)
MOE	Margin of exposure
n-3	Omega-3 (fatty acid)
n-6	Omega-6 (fatty acid)
n/a	Not applicable
ND	Not Detected
NDI	New Dietary Ingredient
NF	National Formulary
NGT	Not Greater Than
NIH	National Institutes of Health
NLT	Not Less Than
NMT	Not More Than
NR	Not reported
NS	Not Specified
OECD	Organisation for Economic Co-operation and Development

PAs	Pyrrolizidine alkaloids
PAFA	Priority-based assessment of food additives
PAH	Polycyclic aromatic hydrocarbon
PAM	Pesticide Analytical Manual
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzodioxin
PCDF	Polychlorinated dibenzofuran
PE	Ethanolamine phosphoglycerides
pp	Pages
ppb	Parts per billion (1,000 million)
ppm	parts per million
PUFA	Polyunsaturated fatty acid
SDA	Stearidonic Acid
spp	Species
TCI	Technology Crops International LLC
TEQ	Toxic equivalent
TG	Triglyceride
w/w	Mass fraction (weight/weight)

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APPENDIX 1 SUMMARY OF ANALYTICAL DATA

Three non-consecutive batches of manufactured Buglossoides oil were analyzed and the results are shown in Table 16 to Table 23. These indicate that the chemical composition of the finished oil is consistent and suitable for use as a foodstuff.

Table 16 – Physical analyses

Parameter	Specification	NZ00053	NZ00056	NZ00058	Analytical Method
Appearance	Pale yellow to clear liquid	Pale yellow liquid	Pale yellow liquid	Pale yellow liquid	Visual inspection AP-041 (TCI 2012a)
Odor	NS	Slight, characteristic	Slight, characteristic	Slight, characteristic	Organoleptic AP-005 (TCI 2012b)
Color	<2.0R; <20.0Y	0.4R; 4.2Y	0.8R; 8.7Y	0.6R; 7.1Y	AOCS Cc 13j-97
Refractive Index @ 25°C	NS	1.4867	1.4640	1.4861	AOCS Cc 7-25
Viscosity (cps) @ 25°C	NS	42.2	46.4	49.0	Brookfield Instrument Method
Specific Gravity @25°C	NS	0.942	0.935	0.935	AOCS To 1a-64
Cold test	Pass	Pass	Pass	Pass	AOCS Cc 11-53

NS – Not specified

Table 17 – Chemical analyses

Parameter	Specification	NZ00053	NZ00056	NZ00058	Analytical Method
Iodine Value	NS	233	227	222	AOCS Cd 1b-87
Flash point (°C)	NS	187	176	185	AOCS Cc 9b-55 (Closed cup)
Peroxide value	NMT 10 meq/kg	2.03	1.55	1.22	AOCS Cd 8-53
p-Anisidine value	NS	12.42	13.13	6.07	AOCS Cd 18-90
Oxidative Stability Index (hours @ 100°C)	NS	0.30	0.33	0.31	AOCS Cd 12b-92

NS – Not specified

Table 18 – Primary constituents of Buglossoides oil

Analyte (%)	Specification	NZ00053	NZ00056	NZ00058	Method
Triglycerides	NS	92.58	86.72	89.49	AOCS Cd 11c-93
Diglycerides	NS	6.11	2.00	2.48	AOCS Cd 11c-93
Monoglycerides	NS	2.33	3.72	3.93	AOCS Cd 11c-93
Glycerol		1.08	<1.0	<1.0	AOCS Cd 11c-93
Free fatty acids (%)	NMT 0.3%	0.011	0.06	0.17	AOCS Ca 5a-40
Unsaponifiable matter	NS	0.28%	0.43%	0.73%	AOCS Ca 6a-40
Sterols (mg/kg)	NS	2,560	2,160	2,430	ISO 12228
Tocopherols (mg/kg)	NS	546	258	390	DGF-F-II 4a
Tocotrienols (mg/kg)	NS	0	0	0	DGF-F-II 4a
Pyrrolizidine alkaloids (ppb)	NMT 4	<1	<1	<1	GC-MSD (Wiedenfeld 2012)
Protein (µg/ml)	NS	< 10	< 10	< 10	Bradford protein assay
		<62.5	<62.5	<62.5	Antek 9000NS Analyzer (combustion/ chemiluminescence)
		1.3	1.0	1.3	Borate extraction/ CBQCA assay
Moisture (%)	NMT 0.2%	0.09	0.02	0.07	AOCS Ca 2e-84
Residual solvent (ppm)	NMT 1ppm	N/A*	N/A*	< 1.0	AOCS Ca 3b-87

NS = Not specified N/A – Not applicable (cold-pressed batch)

Table 19 – Fatty acid composition of Buglossoides Oil

Fatty acid (area%)	Specification	NZ00053	NZ00056	NZ00058
16:0	NS	5.2	5.3	5.2
16:1	NS	0.1	0.1	0.1
18:0	NS	1.8	1.9	1.8
18:1 n-9	NS	7.6	7.6	7.5
18:2	NS	12.7	12.7	12.7
18:3 n-6 (GLA)	3.5 – 8.5%	6.4	6.2	6.3
18:3 n-3 (ALA)	35 - 55%	44.0	44.0	43.5
18:4 (SDA)	15 - 30%	20.5	19.7	20.8
20:1	NS	0.7	0.8	0.8
22:1 (erucic)	NMT 2.0	0.2	0.2	0.2
24:1	NS	0.1	0.2	0.0
Total epoxy fatty acids	NS	<0.1	<0.1	<0.1
Total *trans* fatty acids	NS	< 1.0	< 1.0	< 1.0

Method: AOCS Ce 1j-07 NS = Not specified
All fatty acids with content at or greater than 0.1% have been identified and included.

Table 20 – Phytosterol content of Buglossoides oil

Analyte (%)	NZ00053	NZ00056	NZ00058	Canola Oil (CODEX 2005)	Vegetable Oil Phytosterols* (Cargill 2000)	Arabica Coffee (Valdenebro et al. 1999)
Cholesterol	0.7	0.6	0.7	ND – 1.3	0.36	1.2
Campesterol	37.1	39.4	38.7	24.7 – 38.6	23.58	15.5
Campestanol	<0.1	2.3	1.8	Not reported	0.89	0.73
Stigmasterol	0.4	0.4	0.5	0.2 – 1.0	23.24	18.9
Δ-7-Campesterol	0.8	0.6	0.5	Not reported	0.71	0.6
Chlerosterol	0.4	0.3	0.4	Not reported	Not reported	0.87
β-Sitosterol	47.2	43.1	42.8	45.1 – 57.9	42.27	52.7
Sitostanol	0.5	0.7	0.7	Not reported	Not reported	2.41
Δ-5-avenasterol	7.3	5.5	7.8	2.5 – 6.6	0.82	2.84
Δ-5,24-Stigmasterol	Not reported	Not reported	Not reported	Not reported	Not reported	0.6
Δ-7-Stigmastenol	0.3	0.2	0.2	ND – 1.3	0.72	2.04
Δ-7-Avenasterol	1.4	1.2	1.2	ND – 0.8	0.26	1.74
24-methylene-cholesterol	2.2	1.7	2.6	Not Reported	Not Reported	Not Reported
Brassicasterol	<0.1	<0.1	<0.1	5.0 – 13.0	0.45	Not Reported
** Δ 5,23 stigmastadienol	0.2	2.7	1.1	Not reported	Not reported	Not reported
** Δ 5,24 stigmastadienol	1.5	1.3	1.0	Not reported	Not reported	Not reported
Others	< 0.1	< 0.1	< 0.1	ND – 4.2	4.78	Not reported

Method: ISO 12228
ND = Not Detected. Results are expressed as % of total sterols
*Mean results from five samples. This material has been notified as GRAS without objection (OFAS 2000).
** Δ 5,23 and Δ 5,24 stigmastadienols are present in olive oil and are two of the six phytosterols which are required to form at least 93% of the sterol content under the International Olive Oil Council trade standard. (Vossen 2007).

Table 21 – Tocopherol and tocotrienol content of Buglossoides oil

Analyte (mg/kg)	Codex soybean oil specification*	NZ00053	NZ00056	NZ00058
α-tocopherol	9 – 352	< 10	< 10	< 10
β-tocopherol	ND – 36	< 10	< 10	< 10
γ-tocopherol	89 – 2307	535	258	390
δ-tocopherol	154 – 932	11	< 10	< 10
α-tocotrienol	ND – 69	< 10	< 10	< 10
β-tocotrienol	Not specified	< 10	< 10	< 10
γ- tocotrienol	ND – 103	< 10	< 10	< 10
δ- tocotrienol	ND	< 10	< 10	< 10
Total	600 – 3370	546	258	390

* CODEX 2005 NS = Not specified
Method: DGF F-II 4a

Table 22 – Potential external contaminants of Buglossoides Oil

Analyte	NZ00053	NZ00056	NZ00058	Method
Arsenic (ppm)	<0.007	<0.007	<0.007	ICP-MS/AOAC 993.14
Cadmium (ppm)	<0.002	<0.002	<0.002	
Copper (ppm)	<0.013	<0.013	<0.014	
Iron (ppm)	<0.95	<0.94	1.16	
Lead (ppm)	<0.007	<0.007	<0.007	
Mercury (ppm)	<0.004	0.010	0.004	
Nickel (ppm)	0.018	0.013	0.020	
Silver (ppm)	<0.07	<0.07	<0.07	
Tin (ppm)	<0.03	<0.03	<0.03	
Total heavy metals as lead (ppm)	<10	<10	<10	USP/NF 231
Pesticides	None detected	None detected	None detected	Eurofins FDA PAM 304 E3C5
Polychlorinated biphenyls (inc. dioxin)	0.531 pg/g (upperbound) <0.002 pg/g (lower bound)	0.31 pg/g (upperbound) ND pg/g (lower bound)	0.309 pg/g (upperbound) ND pg/g (lower bound)	Eurofins internal methods A7158, GF004, A7347
Polyaromatic Hydrocarbons	None Detected (LoD 1.0 – 4.0 µg/kg)	None Detected (LoD 1.0 – 4.0 µg/kg)	2.3 ppb Phenanthrene	Eurofins internal method QA 049
Melamine	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg	FDA method LIB 4422
Cyanuric acid	<0.25 mg/kg	<0.25 mg/kg	<0.25 mg/kg	

Table 23 – Microbiological test results for Buglossoides oil

Test (cfu/g)	NZ00053	NZ00056	NZ00058
Total aerobic plate count	<5	<5	<5
Osmophilic yeasts	<5	<5	<5
Yeasts & mold	<5	<5	<5
Enterobacteria	ND	ND	ND
Staphylococcus aureus	ND	ND	ND

Methods used: USP <61>,MFLP ,43 MFHPB-22
ND – Not detected

The refining and filtering process used in processing Buglossoides oil ensures that no microbial contamination is present in the final product. Furthermore, Buglossoides oil contains virtually no water and therefore does not support microbial growth.

APPENDIX 2 EXPERT PANEL REPORT

Expert Panel Report on the Generally Recognized As Safe Status of the Intended Use of Buglossoides Oil as a Nutritional Ingredient in Various Food Products

Technology Crops International ("TCI") convened a panel of experts ("Expert Panel"), qualified by their scientific training and experience to evaluate the safety of food and food ingredients, to determine whether the intended use of Buglossoides Oil obtained from the seeds of *Buglossoides arvensis* as a nutritional ingredient in various food products is Generally Recognized As Safe ("GRAS") based on scientific procedures. The Expert Panel Members included Joseph F. Borzelleca, Ph.D. (Professor Emeritus, Virginia Commonwealth University School of Medicine; Robert J. Nicolosi, Ph.D. (Professor, University of Massachusetts-Lowell and University of Connecticut); and Michael W. Pariza Ph.D. (Professor Emeritus, University of Wisconsin-Madison).

The Expert Panel, independently and collectively, critically evaluated a dossier, "GRAS Dossier, Refined Buglossoides Oil - May 9, 2013" ("GRAS Dossier") prepared by TCI and other materials deemed appropriate or necessary by the Expert Panel. The GRAS Dossier includes information derived from a comprehensive search of the scientific literature conducted by TCI through April, 2013. The GRAS Dossier also includes the product (chemical) identity of Buglossoides Oil, method of manufacture including specifications and stability data, conditions of intended use including use levels and functionality, estimated dietary exposures, and biological studies pertinent to the safety of Buglossoides Oil under the conditions of intended use.

Following its independent and collective critical evaluation of the available published and unpublished data and other information pertinent to safety of the intended use, the Expert Panel convened in a teleconference on 10 June 2013. The Expert Panel independently, jointly, and unanimously concluded that the intended use of Buglossoides Oil as a nutritional ingredient in various food products is safe, and GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion follows.

Description of Buglossoides Oil, Manufacturing Process, Product Specifications

Buglossoides oil is a refined edible oil obtained from the seeds of *Buglossoides arvensis* (L.) I.M.Johnst. (previously *Lithospermum arvense* L.), an herbaceous annual plant which has not been genetically modified. Common names include Corn Gromwell and Bastard Alkanet.

Buglossoides oil consists primarily of triglycerides with small amounts of di- and mono-glycerides, free fatty acids, and an unsaponifiable fraction, comprised mostly of sterols and tocopherols. The triglycerides contain a range of fatty acid moieties. The principal fatty acids (with a minimum content of 1%) include palmitic stearic, oleic, linoleic, gamma-Linoleic, alpha-Linolenic and stearidonic acid ("SDA"). Buglossoides Oil may contain up to 30% SDA.

Buglossoides oil is manufactured using food-grade raw materials and processing agents. Oil is extracted from the seeds of Buglossoides arvensis by expeller pressing or organic solvent (usually hexane or isohexane) extraction and then refined. Traditional food oil manufacturing processes are used which do not alter the fatty acid profile of the oil. All processing aids and additives are used in compliance with applicable regulations of the U.S. Food and Drug Administration ("FDA") and current Good Manufacturing Practice ("cGMP").

Food grade specifications for Buglossoides oil and the analytical method employed were presented in the dossier. TCI provided analytical data for three non-consecutive batches of Buglossoides Oil demonstrating that the product can be consistently produced; i.e., a product that consistently meets appropriate food grade specifications.

TCI provided stability data on Buglossoides Oil that demonstrated that the oil remains within specifications for peroxide value for a period of 8 weeks at temperatures of 4°C, 22°C or 60°C in the dark.

Proposed Uses, Use Levels and Estimated Daily Intakes

TCI intends to market Buglossoides Oil for use as a nutritional ingredient in various food products marketed in the U.S., namely baked goods and baking mixes, breakfast cereals and grains, cheeses, dairy product analogues, fats and oils, fish products, frozen dairy desserts and mixes, puddings and fillings, grain products and pastas, gravies and sauces, meat products, milk products, nuts and nut products, poultry products, processed fruit juices, processed vegetable products, puddings and fillings, snack foods, soft candy and soups and soup mixes. Buglossoides Oil will be added to foods at levels that provide up to 375 mg SDA per serving. For foods governed by a standard of identity, Buglossoides Oil will be added consistent with the governing standard.

The use of Buglossoides Oil is limited by the level of fat that can be added to the proposed foods. As noted above, under the conditions of intended use, Buglossoides Oil will be added to foods at levels that provide up to 375 mg SDA per serving.

Buglossoides Oil has no history of use in food and, therefore, is not normally present in the diet. However, the component fatty acids are commonly found in a wide range of foods.

The estimated consumption of Buglossoides Oil tracks the estimates provided by the Monsanto company for its SDA Soybean Oil (Monsanto 2009) which was notified as GRAS in 2009, in response to which the FDA raised no questions (OFAS 2009). Buglossoides Oil is very similar to SDA Soybean Oil, with the exception of the higher level of ALA in Buglossoides Oil.

Buglossoides Oil is intended to be used in the same food products as those identified in the Monsanto notification and in the same amounts which will also provide up to 375 mg of SDA per serving of food. Buglossoides Oil may be used as a replacement for, or in

combination with, SDA Soybean oil, such that the combined total of SDA present in a product does not exceed that which has been notified as GRAS for SDA Soybean oil.

Monsanto estimated the consumption of SDA Soybean Oil from all proposed food uses and use levels in conjunction with food consumption data included in the National Health and Nutrition Examination Surveys (NHANES 1999-2002) (CDC 2007). Consistent with Monsanto's calculations, TCI estimated that the introduction of Buglossoides Oil with 20% SDA into the same foods will result in the per capita mean and mean 90th percentile intakes of the Buglossoides Oil of 10.1 g/day (0.18 g/kg body weight/day) and 19.6 g/day (0.38 g/kg body weight/day), respectively. Per capita mean and mean 90th percentile intakes of fat are estimated to increase from 78.8 g/day to 84.0 g/day at the mean and from 136.5 g/day to 143.6 g/day at the 90th percentile with the addition of Buglossoides Oil. This does not take into account the possibility that food manufacturers may adjust their formulations to negate some of the increased energy content. Intake of ALA from all dietary sources would increase from 0.9 to 5.1 g/day at the mean and 1.8 to 9.7 g/day at the 90th percentile. Intake of GLA would increase from 0.03 to 0.7 g/day at the mean and 0.1 to 1.3 g/day at the 90th percentile. Intake of SDA would increase from 0.004 to 2.1 g/day at the mean and 0.004 to 4.1 g/day at the 90th percentile. Changes in intake of the other fatty acids present in Buglossoides Oil would be less than 1 g/day.

Under the conditions of intended use, Buglossoides Oil would be added in an amount needed to deliver up to 375 mg of SDA per serving. This would require use of smaller amounts of any Buglossoides Oil that contains higher levels of SDA (e.g., 30%), which would reduce the contribution to the diet of the other fatty acids present in the oil.

The principal difference between the consumption of Buglossoides Oil and SDA Soybean Oil is that ALA consumption is increased when Buglossoides Oil is used. The consumption of other fatty acids would be comparable to or less than that which would occur with SDA Soybean Oil.

Safety Assessment

Absorption, Distribution, Metabolism and Excretion

Like all dietary vegetable oils, the primary fate of the fatty acids in Buglossoides oil is beta-oxidation for use as energy. The essential fatty acids can also be metabolized to longer chain or more unsaturated fatty acids. Both the ALA and SDA in Buglossoides oil can be elongated and desaturated to EPA, the omega-3 fatty acid typically found in fish oils. Pre-clinical and clinical studies all show that dietary supplementation with either ALA or SDA results in an enrichment of tissue phospholipids with EPA, although the efficiency of this conversion is greater with SDA than with ALA (James *et al.* 2003).

Preclinical Toxicity Studies

The safety of Buglossoides Oil is supported by published studies on SDA Soybean Oil, and on SDA and other components of Buglossoides Oil.

The safety of SDA Soybean Oil was evaluated in a 28-day gavage study and a 90-day one-generation reproduction feeding study in rats (Hammond *et al.* 2008). In the 28-day GLP study that was compliant with FDA, EPA and OECD guidelines, male and female Sprague–Dawley Crl:CD(SD) rats received 20% SDA Soybean Oil at doses of 0, 0.3, 1.0 and 3.0 ml (g)/kg bodyweight by gavage for 28 days. The no-observed-adverse-effect-level (NOAEL) was 3 g/kg/day SDA Soybean Oil, the highest dosage administered. Since the SDA Soybean Oil tested contained 20% SDA, the NOAEL for SDA was approximately 600 mg/kg/day.

The reproductive and subchronic toxicity of 26% SDA Soybean Oil were evaluated in a GLP- compliant feeding study that was consistent with FDA, EPA and OECD guidelines. Male and female Sprague–Dawley Crl:CD(SD) rats received 26% SDA Soybean Oil as a dietary admixture during a 90-day one generation reproduction feeding period at targeted doses of 0, 1.5 and 4.0 g /kg/day (equivalent to 1000mg SDA/kg body weight/day and 273 mg GLA/kg body weight/day at the highest dose; cf Table 1). There were no consistent, dose-dependent, statistically significant adverse effects on any of the parameters tested including clinical observations, hematology, clinical chemistry and urinalysis, organ weights, macroscopic and microscopic pathology and reproductive and developmental endpoints. The NOAEL for reproductive and developmental toxicity and for subchronic toxicity was determined to be approximately 4 g/kg/day, the highest dosage of SDA Soybean Oil tested. Since this SDA Soybean Oil contained 26% SDA, the NOAEL for SDA was approximately1000 mg/kg/day.

Table 1 – Actual mean doses per animal during 90-day feeding trial (Hammond et al. 2008)

		GLA		SDA	
		1.5 g/kg/day	4.0 g/kg/day	1.5 g/kg/day	4.0 g/kg/day
Start of trial	Male	0.027	0.072	0.098	0.262
	Female	0.019	0.051	0.071	0.189
End of trial	Male	0.056	0.149	0.204	0.545
	Female	0.031	0.083	0.114	0.304

A number of preclinical (safety) studies with oils containing SDA or GLA including blackcurrant, walnut, evening primrose, borage, and fungal, were conducted in rats, mice guinea pigs and dogs and the only adverse effect reported was a significant increase in serum cholesterol in spontaneously hypertensive rats receiving borage oil. There are no acute, chronic, reproductive or genetic toxicity studies conducted on Buglossoides Oil.

Clinical Studies

Monsanto conducted two clinical studies with SDA Soybean Oil, one with 33 healthy subjects and one with 252 overweight subjects. In the first study, 11 subjects received the SDA-enriched soybean oil which provided 3.66 g SDA/day and 1.2 g GLA/day for 16 weeks. No adverse effects were reported in any of the parameters evaluated including clinical chemistry. The overweight subjects (BMI of 25 to 35 kg/m^2) consumed SDA-enriched soybean oil that provided 4.2 g SDA/day and 1.13 g GLA/day for 12 weeks. No

adverse effects were reported in any of the parameters evaluated including clinical chemistry and hematology except lower bilirubin which was not considered clinically significant. Clinical studies conducted with borage oil, echium oil, evening primrose oil and flaxseed oil did not identify significant oil-related adverse effects under the test conditions.

SDA is "efficiently converted to EPA" (Croda 2006) but not to DHA. The FDA affirmed in 1997 that menhaden oil is GRAS for use as a nutritional ingredient in certain foods, provided that the combined intake of EPA and DHA is limited to no more than 3.0 g/person/day. Although the FDA found reports of increased bleeding at intakes above 3 g/person/day, it could not definitively conclude that the increases reported posed a significant threat to health based on its critical review of the available information. Nevertheless, the Agency took a very conservative position based on the potential risk of excessive bleeding in sensitive individual at intakes above 3 g/person/day. The consumption of 4.1g of SDA per day, representing the 90th percentile intake under the conditions of intended use of Buglossoides Oil, would fall well below the maximum level of 3g/day for the combined intake of EPA and DHA set by the FDA for menhaden oil. The combined contribution from SDA and ALA (some of which is converted to EPA and DHA) would also fall well below the FDA's maximum level for combined intake of EPA and DHA.

There have been no reports of allergies related to *Buglossoides arvensis*. The level of protein in Buglossoides Oil is below the level which is considered safe for susceptible individuals in other edible oils.

A critical evaluation of the available information on other constituents of Buglossoides Oil, including erucic acid, phytosterols, and pyrrolizidine alkaloids, failed to identify significant adverse health effects at the levels found in Buglossoides Oil.

Basis for Determination of the GRAS Status of Buglossoides Oil Under the Conditions of Intended Use

Buglossoides Oil is a refined edible oil obtained from the seeds of *Buglossoides arvensis*, an herbaceous annual plant which has not been genetically modified. Buglossoides Oil has no history of use in food. However, the component fatty acids are commonly found in a wide range of foods. Buglossoides Oil consists primarily of triglycerides with small amounts of di- and mono-glycerides, free fatty acids, and an unsaponifiable fraction, comprised mostly of sterols and tocopherols. The triglycerides contain a range of fatty acid moieties. The principal fatty acids (with a minimum content of 1%) include palmitic stearic, oleic, linoleic, gamma-Linoleic, alpha-Linolenic and stearidonic acid (SDA).

Buglossoides Oil is manufactured using food-grade raw materials and processing agents. Oil is extracted from the seeds of *Buglossoides arvensis* by expeller pressing or organic solvent (usually hexane or isohexane) extraction and then refined. Traditional food oil manufacturing processes are used which do not alter the fatty acid profile of the oil.

Buglossoides Oil is intended to be used as a nutritional ingredient in various food products at levels that will provide 375 mg SDA per serving. The estimated consumption of Buglossoides Oil will be similar to that of SDA Soybean Oil since it is intended to be used in the same food products and in the same amounts which will provide 375 mg SDA/serving. Consistent with Monsanto's recommendation, TCI recommends minimum daily consumption of 1.5 g SDA/day. TCI estimated that the intake from the proposed uses will result in per capita mean consumption of the oil of 10.1 g/day (0.18 g/kg bw/day) and the 90th percentile of 19.6 g/day (0.38 g/kg bw/day).

The safety of Buglossoides Oil is based on the safety of its components and on the published safety studies of SDA soybean oil. Under the conditions of intended use, Buglossoides Oil will provide a comparable level of SDA to that provided by SDA Soybean Oil. The safety of other fatty acids at the levels present in Buglossoides Oil is well recognized. A critical evaluation of the available information on other constituents of Buglossoides Oil including erucic acid, phytosterols, pyrrolizidine alkaloids failed to identify significant adverse health effects at the levels found in Buglossoides Oil. It is highly unlikely that Buglossoides Oil is allergenic since the level of protein in the oil is below the level which is considered safe for susceptible individuals in other edible oils.

Conclusion

We, the members of the Expert Panel, have independently and collectively, critically evaluated the published and unpublished data and information summarized above, as well as any other data and information that we deemed pertinent to the safety of the intended use of TCI's Buglossoides Oil in various food products. We unanimously conclude that the intended use as a nutritional ingredient in various food products of Buglossoides Oil, manufactured consistent with cGMP and meeting appropriate food grade specifications, is safe.

We further unanimously conclude that the intended use as a nutritional ingredient in various food products of Buglossoides Oil, manufactured consistent with cGMP and meeting appropriate food grade specifications, is GRAS based on scientific procedures.

It is our opinion that other experts, qualified by scientific training and experience, and evaluating the same data and information, would concur with these conclusions.

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