APPLICATION FOR AUTHORISATION OF REFINED BUGLOSSOIDES OIL AS A NOVEL FOOD

SIMPLIFIED PROCEDURE

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A Administrative Information

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Name of Novel Food:	Refined Buglossoides Oil
Date of application:	22 January 2013

A.1 Basis of application

The applicant is of the view that Refined Buglossoides Oil is substantially equivalent, in terms of composition, level of undesirable substances, nutritional value, metabolism and intended use, to Refined Echium Oil, which has been admitted to the market in the European Union (Anonymous 2008). On this basis, since Buglossoides oil falls within the category of "foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals (except for foods and food ingredients obtained by traditional propagating or breeding practices and having a history of safe food use)" (ACNFP 2005), the applicant believes that the simplified procedure for approval is appropriate.

Buglossoides oil is a commercially viable alternative to Refined Echium Oil because the crop yields are higher, leading to cheaper oil, and less oil is required to provide an equivalent nutritional benefit because Buglossoides oil has a higher content of the desirable component stearidonic acid (SDA).

B Composition

B.1 Identity of the source

Buglossoides oil is a refined edible oil obtained from the seeds of *Buglossoides arvensis* (L.) I.M.Johnst. (previously *Lithospermum arvense* L.), (NRCS 2012a) an herbaceous plant which has not been genetically modified using modern methods of biotechnology such as recombinant DNA techniques. Common names include Corn Gromwell and Bastard Alkanet (Clapham *et al*. 1962). The botanical identity of the seed used for extraction of the representative oil samples listed in Appendix 1 was confirmed by a seed testing laboratory (Appendix 2).

Refined Echium Oil is a refined edible oil obtained from the seeds of *Echium plantagineum* (L.), an herbaceous plant which has not been genetically modified using modern methods of biotechnology such as recombinant DNA techniques (Croda 2006).

Lapinskas (2012) has reported that *Buglossoides arvensis* and *Echium plantagineum* are both members of the Boraginoideae sub-family within the Boraginaceae family and are thus closely related (See also IBIS 2012, Kelley *et al*. 2012, NRCS 2011, Valdés 2004).

Echium crops are grown in the United Kingdom (Croda 2006). Buglossoides crops will primarily be cultivated in the United Kingdom and North America where it is common as a wild plant (Clapham *et al*. 1962, NRCS 2012a). Echium is considered to be a noxious weed in New South Wales, Australia (Naughton *et al* 2006) and Oregon state, USA (NRCS 2012b). *Buglossoides arvensis* has been classified as “can be weedy or invasive” in the USA but is not considered to be noxious (NRCS 2012a).

B.2 Product specification

The proposed regulatory specification for Buglossoides oil is the same as that which has been adopted for Refined Echium oil, as shown in Table 1. The Table also shows that this specification has been met for three representative non-consecutive batches of Buglossoides oil (NZ00053 (Batch 4), NZ00056 (Batch 5) and NZ00058 (Batch 6)) which were manufactured during November 2012.

Table 1 - Regulatory specifications

Test	Echium Oil Specification (Anonymous 2008)	Proposed Buglossoides Oil Specification	Buglossoides oil samples		
			NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6
Stearidonic acid content (% w/w of total fatty acids)	NLT 10	NLT 10	20.5	19.7	20.8
Trans fatty acids (% w/w of total fatty acids)	NMT 2	NMT 2	<1.0	<1.0	<1.0
Acid value (mg KOH/g)*	NMT 0.6	NMT 0.6	0.22	0.12	0.34
Peroxide value (meq O_2/kg)	NMT 5	NMT 5	2.03	1.55	1.22
Unsaponifiable Content (%)	NMT 2	NMT 2	0.28	0.43	0.73
Protein Content (total nitrogen µg/ml)	NMT 20	NMT 20	<10	<10	<10
Pyrrolizidine alkaloids (µg/kg)	Not detectable with a detection limit of 4	NMT 4	<1	<1	<1

NLT = Not less than; NMT = Not more than
* Acid Value = 1.99 x Free Fatty Acids %

B.3 Preparation method

Both Echium oil and Buglossoides oil are extracted from seed grown by farmers as conventional field crops. The farmers are contracted to the applicant or to a production company acting on his behalf and the resultant seed is cleaned, dried and transported to the extraction facility.

Echium oil may be extracted using hexane solvent (Croda 2006) or by mechanical pressing (NFU 2009), or by first mechanically pressing the seed, solvent extracting the resultant meal and combining the two oil fractions.(NFU 2010). Buglossoides oil may be extracted by any of these, as shown in Figure 1, but mostly by the third route.

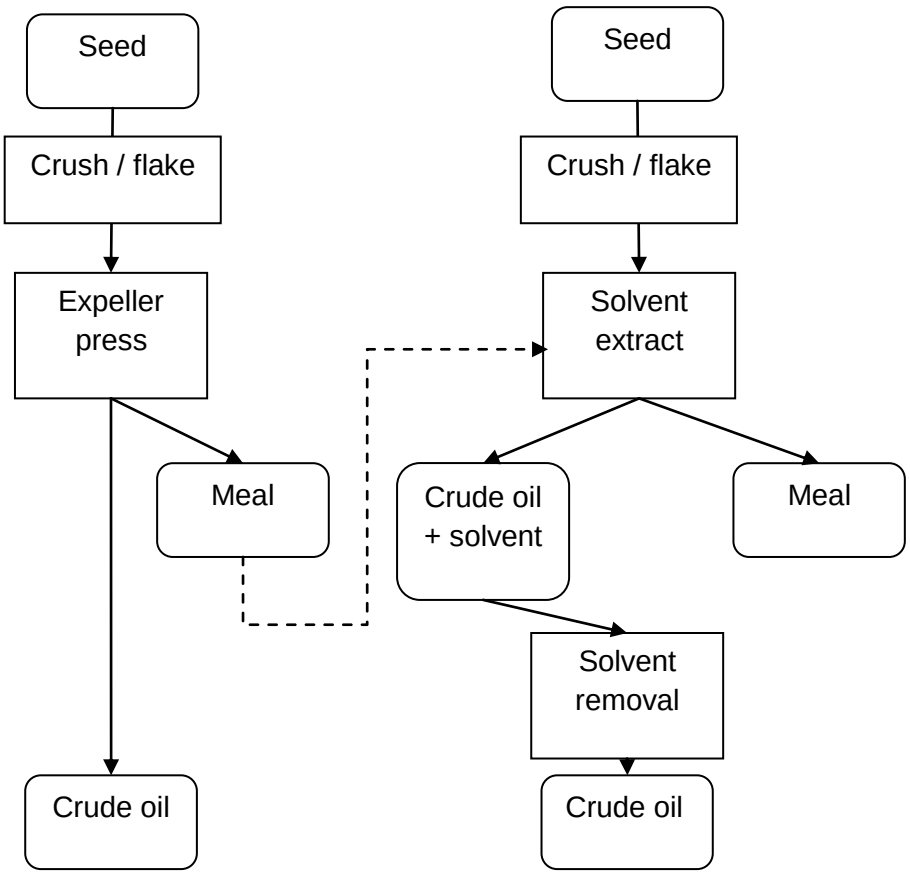

Figure 1 - Extraction process for Buglossoides oil

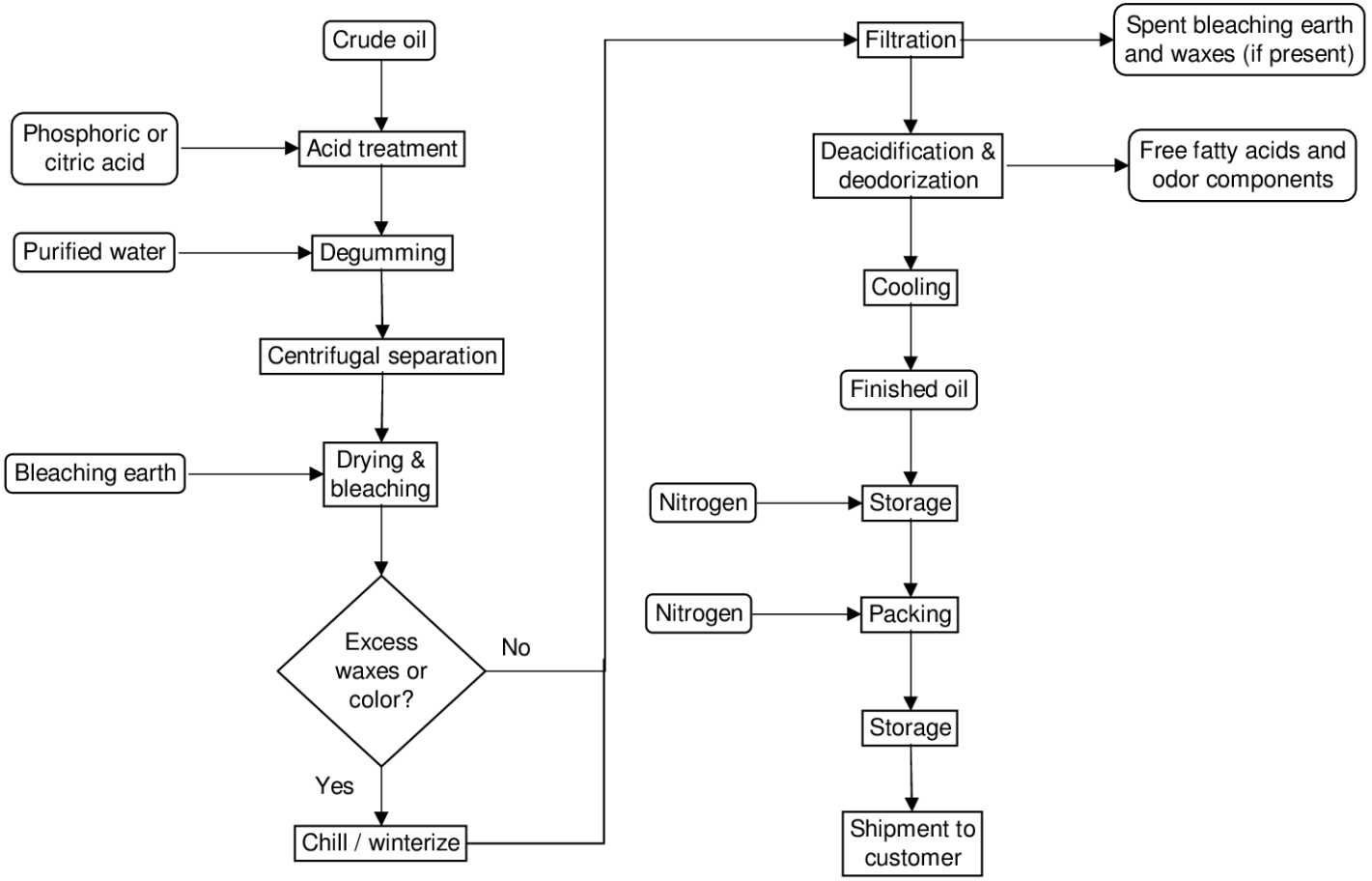

Figure 2 - Refining process for Buglossoides oil

After extraction, crude Echium oil may be refined by several methods, using processes which are standard in the edible oil industry. Buglossoides oil is also refined using similar processes, as indicated in Figure 2. The following additional procedures (which are all standard practice in edible oil processing in Europe) may also be applied if needed.

Sodium hydroxide may be added in order to neutralize free fatty acids in the oil and previously added citric or phosphoric acid. The free fatty acids are converted to sodium soaps which are then separated by centrifugation and discarded.

Citric acid may be added with the bleaching clay to act as a chelating agent to remove metals and improve the removal of any residual soaps.

Cellulosic filter aids - Filtration of the oil may be improved when cellulosic filter aids are added to the oil. The filter aids form a "filtering cake" on the screens or membrane of the filter equipment and assist in removal of impurities

Activated carbon - If oil contains high levels of chlorophyll, metals or other contaminants, activated carbon may be added to the oil (normally at the same time as the bleaching earth) to assist in their efficient removal. The activated carbon is removed by filtration.

Silica gel - Adding silica gel to the oil may assist in the removal of impurities such as soaps, phospholipids and trace metals. The spent gel is removed by filtration.

Antioxidants - To improve the stability of the finished oil, antioxidants may be added that are approved for use in the EU. The antioxidant protects and improves the stability of the oil by inhibiting oxidation. The quantity of antioxidant added will fall below the applicable regulatory maximum limit.

All Buglossoides oil processing follows current Good Manufacturing Practice (cGMP) and the applicant's factory operates in conformance with ISO 22000:2005 (which incorporates the HACCP system) (Appendix 2). The applicant believes that the physical and chemical effects of the processes applied to Buglossoides oil and to the three approved Echium oil products are broadly comparable and that any differences will not give rise to any differences in the composition of the final product.

The applicant will initially be producing and refining the oil in his own facilities. If the product is commercially successful, it is likely that some production will be outsourced to manufacturers who are able to meet the same or similar standards. However the seed, the intermediate products and the finished products will remain the property of the applicant.

B.4 Composition of final product

Full analytical data on three non-consecutive batches of Buglossoides oil which were manufactured using the processing method described above are shown in Appendix 1, together with data obtained from Echium oil extracted from consumer packs of Echiomega soft gelatine capsules marketed by Igennus Ltd., St. John's Innovation Centre, Cowley Road, Cambridge, CB4 0WS. The product was purchased by telephone mail order on 27 September 2012 (Appendix 2). The oil was removed from the capsules under a nitrogen atmosphere and sealed under nitrogen in a glass container. The commercial Echium oil analyses do not include assessment of external

contaminants, as these will reflect the history of the individual oil batch rather than any inherent differences between the two oil sources.

Further data on three batches of Echium oil, taken from the original novel food application (Croda 2006) are also included.

There are no significant differences in the chemical and physical analysis results between Echium oil and Buglossoides oil as shown in Table 4. The variation in peroxide value, p-anisidine value and oxidative stability index are indicative of the oxidation status of the particular sample of oil rather than reflecting any real difference between the two oil sources.

Table 5 shows the primary constituents of the two oils. These were not reported in the original Echium oil dossier but analysis of the commercial Echium oil sample shows that it is very similar in composition to Buglossoides oil.

B.5 Nutrient composition

The comparative fatty acid profiles are shown in Table 6. The same major fatty acids are present in both oils, but Buglossoides oil has a higher content of alpha-linolenic and stearidonic acids and a lower content of gamma-linolenic acid, linoleic and oleic acids. As a result, the proportion of (n-3) fatty acids and the proportion of (n-3) + (n-6) (total polyunsaturated) are both higher in Buglossoides oil. There are no fatty acids present in Buglossoides oil which are not also found in Echium oil.

The largest component in the unsaponifiable fraction in both oils comprises of sterols, although the level in the Buglossoides oil samples is lower than that found in the Echium oil, possibly as a result of differences in the refining process. The distribution of the different sterols is similar.

The level of tocopherols and tocotrienols was not recorded in the original Echium oil dossier, but tocopherols are a significant component of the unsaponifiable fraction in both oils. The concentrations found in Echium oil are greater than in Buglossoides oil and, while α, γ and δ-tocopherols are found in Echium oil, only γ-tocopherol was identified in Buglossoides oil at a significant concentration. Neither oil contains any tocotrienols at the level of detection of the analyses (Table 8).

C Nutritional value

The major nutritional value of both Buglossoides oil and Echium oil lies in their fatty acid content and specifically in their content of stearidonic acid (SDA). Since Buglossoides oil has a significantly higher content of stearidonic acid, it follows that an equivalent intake of SDA can be obtained from a smaller quantity of oil, thus reducing energy intake. Apart from this, the applicant does not consider that Buglossoides oil is significantly different from Echium oil.

D Metabolism

It is considered unlikely that there are any differences in the metabolism of Buglossoides oil relative to Echium oil given the similarity of their composition.

E Intended use

It is intended that Buglossoides oil will be used in the same products as Echium oil in such quantities as to provide up to the same maximum quantity of SDA, as summarised in Table 3. Since, as noted above, the SDA level is higher in Buglossoides oil this means that the amount of oil required will be lower. For instance, to provide the daily maximum quantity of 500mg SDA for a dietary supplement with Echium oil containing 14.7 % SDA (area %, commercial sample) would require approximately 3.8 grams of oil:

500 / (0.89 x 0.147) = 3,822

whereas to provide it from Buglossoides oil with 20.3% SDA would require approximately 2.8 grams of oil:

500 / (0.89 x 0.203) = 2,768g

A factor of 0.89 has been used for converting from fatty acid proportion (area %) to proportion of the total oil (w/w %). This factor allows for the presence of a glycerol moiety in the triglyceride molecule which is not included in the calculation of area %. It varies slightly according to the molecular weight of each fatty acid; in the above approximate example it has been calculated on the basis of linoleic acid.

This decrease in oil quantity consumed will substantially offset the increased content of α-linolenic acid (ALA) in Buglossoides oil, so that the overall consumption of this fatty acid is similar between the two oils:

Concentration of ALA in commercial Echium oil = 32.6 x 0.89 = 29.0 %w/w

Mean concentration of ALA in Buglossoides oil = 43.8 x 0.89 = 39.0 %w/w

3.8g of Echium oil contains 3.8 x 29.0% = 1.1 g ALA

2.8g of Buglossoides oil contains 2.8 x 39.0% = 1.1 g ALA

Thus, since the SDA and ALA consumption is the same, so is the omega-3 consumption.

All other significant fatty acids are present in Buglossoides oil at a lower proportion than in Echium oil. By a similar calculation to the above, it can therefore be shown that the increased proportion of total polyunsaturates in Buglossoides oil is more than offset by lower oil consumption level, giving an intake of 2.1g of polyunsaturates for Buglossoides oil as against 2.5g for commercial Echium oil.

Table 2 – Intended food uses

	Maximum level of stearidonic acid (SDA)	
Use group	Refined Echium Oil (Anonymous 2008)	Buglossoides oil
Milk-based products and drinkable yoghurt products delivered in a single dose	250 mg/100 g; 75 mg/100 g for drinks	250 mg/100 g; 75 mg/100 g for drinks
Cheese preparations	750 mg/100 g	750 mg/100 g
Spreadable fat and dressings	750 mg/100 g	750 mg/100 g
Breakfast cereals	625 mg/100 g	625 mg/100 g
Food supplements	500 mg/daily dose as recommended by the manufacturer	500 mg/daily dose as recommended by the manufacturer
Dietary foods for special medical purposes	in accordance with the particular nutritional requirements of the persons for whom the products are intended	in accordance with the particular nutritional requirements of the persons for whom the products are intended
Foods intended for use in energy-restricted diets for weight reduction	250 mg/meal replacement	250 mg/meal replacement

F Level of undesirable substances

A literature survey has been carried out in order to identify possible undesirable substances which may 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.

Species in the Boraginaceae have long been known to contain pyrrolizidine alkaloids which are toxic to both humans and livestock (Huizing & Malingré 1981, EFSA 2011). They are present in both *Echium plantagineum* (Croda 2006) and *Buglossoides* species (Roeder 1999).

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 synthesis them could be usefully elevated in crop species for the control of insect predators.

The roots of *Lithospermum erythrorhizon* Siebold & Zucc. have been commonly used in traditional Chinese medicine since at least the 16th century (Papageorgiou *et al*. 1999). The active component has been identified as shikonin, a naphthoquinone which has demonstrated wound healing, antitumour 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 3.

Table 3 – Shikonin analogues in *B. arvensis* and *Echium spp.*

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

n/r = 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.

Croda (2006) indicated two further potentially undesirable compounds associated with *Echium plantagineum* oil, namely cytochrome c allergens (proteins) and erucic acid. No other reports of undesirable compounds which may be present in Buglossoides oil have been discovered.

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

F.1 Inherent substances

Pyrrolizidine alkaloids

Pyrrolizidine alkaloids (PAs) are polar compounds which are extracted from the seed into the oil only to a limited degree and are reduced below the limit of detection by the refining process. In the original dossier on Echium oil Croda (2006) was unable to detect alkaloids in a merged sample of the finished product down to a level of 4 µg/kg. In the present application, with a more sensitive analysis, no PAs were found down to 1 µg/kg in either the commercial sample of Echium oil or in any of the individual Buglossoides oil samples (Table 5).

Cytochrome c allergens (proteins)

Cytochrome c has been reported as a respiratory allergen from echium pollen (Matthews *et al*. 1988) and Sharma *et al.* consider it to be an important respiratory allergen in the fungus *Curvularia lunata* (Sharma *et al.* 2010). 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 and 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, molluscs, 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 vapour 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 atomised 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 pressurised) 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.

As noted above, pollen from *Echium plantagineum* has been reported both as causing allergic respiratory reactions in susceptible individuals and as containing the cytochrome c allergen. No reports of allergic reactions to Buglossoides arvensis pollen are recorded in the Allergome database. 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 have been found in the literature. (It is possible that it is referring to the well known hepatotoxicity of comfrey leaves which is caused by pyrrolizidine alkaloids (EFSA 2011).) There is therefore no *a priori* reason to suppose that refined Buglossoides oil poses any significant allergenic risk.

Notwithstanding the above, 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.

A limit on the protein content of Echium oil of 20 µg/ml was included in the regulatory specification approved by the European Commission (Anonymous 2008). In the light of the above, TCI does not feel that such a limit is necessary for Buglossoides oil, but accepts that the committee may wish to impose one on the basis of substantial equivalence.

No protein was detected in any of the original three Croda samples (with a limit of detection of 10 µg/ml), or the commercial Echium oil sample or in any of the three Buglossoides oil samples with a limit of detection of 10 ppm using the Bradford assay (Bradford 1976) as shown in Table 5. This method has been deprecated by the ACNFP and so the analysis was repeated on the original Echium oil samples using more sensitive methods (ACNFP 2007). The oil was found to contain 11.1µg protein/g and it was determined that the refined oil contained less than 3µg cytochrome c per kg of oil, representing approximately 0.03% of total protein. Given the close relationship between the species and the similarity of the products, it would be surprising if cytochrome c were to be a substantial component of the protein in Buglossoides oil. The three Buglossoides oil samples were further analysed using a combustion/chemiluminescence method which again failed to find any protein at the level of detection (<10 ppm total N, equivalent to 62.5 ppm of protein).

Erucic acid

Diets rich in erucic acid have been reported to cause a transient accumulation of triacylglycerol (lipidosis) in the heart and other tissues of rats although this has not been reported in humans (FSANZ 2003). The level of erucic acid in the fat component of a product is controlled within the EU to a maximum of 5% of the total level of fatty acids (Anonymous 1976). Erucic acid normally associated with Brassica seed oils, but is found at low levels in both Echium oil and Buglossoides oil – typically 0.5% or less, and the level in Buglossoides oil is similar to that in Echium oil (Table 6).

Epoxy fatty acids

Epoxy fatty acids can be formed naturally in all polyunsaturated vegetable oils as some of the many chemical species resulting from oxidation. No epoxy fatty acids were found in the samples of Echium oil or Buglossoides oil down to a level of 0.1% of total fatty acids.

Trans fatty acids

Trans fatty acids are formed by isomerisation of unsaturated fatty acids from the natural cis form, normally as a result of hydrogenation in the manufacture of margarine or of exposure to high temperatures (Mensink & Katan 1990). Hydrogenation is not used in the manufacturing of Buglossoides oil, and the temperatures during refining are not sufficiently high to cause isomerisation. The level of trans fatty acids in Echium oil and Buglossoides oil is controlled at 2% in the specification (Table 1). The content of trans fatty acids in both oils were well below this limit (Table 5).

Unsaponifiable fraction

As noted in the Echium oil dossier, the unsaponifiable matter fraction is not normally considered to be toxic; it has been included in this section to provide easy and complete cross reference to the original Croda dossier.

The unsaponifiable fraction in the Buglossoides oil samples is smaller than that found in the Echium oil (Table 5). This may be a characteristic difference between the two oils, or a result of the

different refining processes employed. The applicant does not believe that the difference is significant, as a lower unsaponifiable content is generally a preferred characteristic of edible oils

Sterol content and composition have been determined for both Echium oil and Buglossoides oil (Table 5 and Table 7). The principal sterols present in both oils are campesterol and β-Sitosterol, with smaller proportions of other sterols. Of these, all were either found at a greater concentration in the Echium oil than in the Buglossoides oil or are found in other commonly consumed foods (Table 7).

No analyses were presented for tocopherols or tocotrienols (vitamin E) in the original Echium oil dossier. Analysis on the commercial sample of Echium oil showed a total content of 965 mg/kg of tocopherols and <10 mg/kg of tocotrienols, with the principal tocopherol being γ-tocopherol and significant quantities of α- and δ-tocopherol. In Buglossoides oil, only γ-tocopherol was found, and at a much lower level than in Echium oil. This may be due to inherent differences between the oils, or may be a consequence of the degree to which the individual oils have been refined, since refining can remove tocopherols from the oil.

F.2 Microbiology

Both Buglossoides oil and Echium oil are anhydrous systems which do not support microbiological growth and the production and refining process effectively sterilises and removes any possible contamination. As confirmation, no such contamination was found in any sample of either oil (Table 12).

F.3 External chemical contaminants

The degree to which external contaminants may be found in an oil sample will be determined by the history of exposure of the material from which it was made, from the crop in the field through to the finished product. Differences between individual batches are therefore much more likely to result from differences in exposure rather than innate differences and therefore no analyses for external contaminants have been performed on the commercial sample of Echium oil.

Pesticides

Approved agrochemical products could potentially be used during the life-cycle and post-harvest on both Echium and Buglossoides crops. No pesticide residues were found in the original Echium oil analyses or in any of the samples of Buglossoides oil (Table 9 and Appendix 2).

Metals

The results of elemental analysis on three batches of Buglossoides oil are presented in Table 9. No significant residues were detected.

Dioxins and Dioxin-like PCBs

Analysis results for dioxins and dioxin-like PCBs are presented in Table 10. All results are well below maximum permitted levels.

Polycyclic Aromatic Hydrocarbons (PAHs)

No significant concentrations of PAHs have been detected in the samples of Buglossoides oil (Table 11). In the Echium oil dossier, Croda stated "All results are considered to be within the acceptable range by the analysis laboratory." (Croda 2006).

G Other relevant data

G.1 Safety studies

The safety of Buglossoides oil has been assessed in two unpublished studies in mice (Surette 2013, Surette and Matar 2012) and one in salmon fry (Plante & Surette 2012).

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, colour 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. Tumour volume was measured on days 10, 14, 18, 22 and 27 post-injection. Tumour 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, colour 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 tumour 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 tumour growth, with tumour 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.

G.2 Labelling

The proposed name for labelling purposes on final foods as presented to the consumer is “Refined Buglossoides oil”.

G.3 Monitoring

The seed used for producing Buglossoides oil is produced under contract to the applicant or to seed production companies acting on his behalf. The terms of the contract allow for all crops to be visited by the applicant’s representatives at least once during the growing season. All harvested seed is tested for purity and moisture and crop identity prior to delivery. Buglossoides oil is manufactured in conformance with cGMP and with ISO 22000:2005 (which incorporates the 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 by either the in-house Quality Control laboratory or by external analysts working to similar standards and under the control of the applicant. 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.

In the event that a batch of oil is found to be out of specification after processing, it is not released by Quality Control and the following procedure for the reprocessing of material is followed:

- If the material failure is considered to be remediable, then the batch will be reprocessed using some or all of the methods described on page 6, or blended with another batch of material and then reprocessed in order to generate a product that will again be tested against the specification.
- If the failure is not considered to be remediable, the material will be disposed of in accordance with the appropriate regulations.

Definitions

ACNFP	Advisory Committee on Novel Foods and Processes
ALA	α-linolenic acid
AOCS	American Oil Chemists Society
Buglossoides	*Buglossoides arvensis* (L.) I.M.Johnst.
Buglossoides oil	Refined edible oil obtained from the seeds of *Buglossoides arvensis* (L.) I.M.Johnst.
cGMP	Current Good Manufacturing Practice
DGF	German Society for Fat Science (Deutsche Gesellschaft für Fettwissenschaft)
DNA	Deoxyribonucleic acid
Echium	*Echium plantagineum* L.
Echium oil	Refined edible oil obtained from the seeds of *Echium plantagineum* L.
FDA	Food and Drug Administration
HACCP	Hazard analysis and critical control points
hr	hour
ISO	International Organization for Standardization
LD_{50}	Median lethal dose
MFHPB	Microbiology Food Health Protection Branch (Canada)
MFLP	Microbiology Food Laboratory Procedure (Canada)
n-3	Omega-3 (fatty acid)
n-6	Omega-6 (fatty acid)
n/a	Not applicable
ND	Not detected
NLT	Not less than
NMT	Not more than
NR	Not reported
NS	Not specified
PAH	Polycyclic aromatic hydrocarbon
PAM	Pesticide Analytical Manual
PCB	Polychlorinated biphenyl
SDA	Stearidonic acid
USP	United States Pharmacopeia
w/w	Mass fraction (weight/weight)
WHO	World Health Organisation

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Appendix 1 – Summary of analytical results

Samples from three non-consecutive batches of manufactured Buglossoides oil were analyzed and the results are shown in Table 4 to Table 12.

Table 4 – Chemical and physical analyses

	Echium oil				Buglossoides oil			
Parameter	**EAL121B**	**EAL121C**	**EAL121D**	**Purchased**	**NZ00053 Batch 4**	**NZ00056 Batch 5**	**NZ00058 Batch 6**	**Analytical Method**
Appearance	Pale yellow liquid	Pale yellow liquid	Pale yellow liquid	Pale yellow liquid	Pale yellow liquid	Pale yellow liquid	Pale yellow liquid	In-house method AP-041
Odour	NR	NR	NR	Slight, characteristic	Slight, characteristic	Slight, characteristi	Slight, characteristi	In-house method AP-005
Colour	NR	NR	NR	1.4R; 12.0Y	0.4R; 4.2Y	0.8R; 8.7Y	0.6R; 7.1Y	AOCS Cc 13j-97
Refractive Index at 25°C	1.4815	1.4810	1.4805	1.4835	1.4867	1.4840	1.4861	AOCS Cc 7-25
Viscosity @25°C	NR	NR	NR	36.9	42.2	46.4	49.0	Brookfield Instrument Method
Iodine Value	NR	NR	NR	206	233	227	222	AOCS Cd 1b-87
Specific Gravity @ 25°C (g/ml)	0.9263	0.9279	0.9282	0.931	0.942	.935	.935	AOCS To 1a-64
Flash point (°C)	NR	NR	NR	230	187	176	185	AOCS Cc 9b-55
Cold test	NR	NR	NR	Pass	Pass	Pass	Pass	AOCS Cc 11-53
Peroxide value (meq O_2/kg)	0.28	3.13	1.01	4.64	2.03	1.55	1.22	AOCS Cd 8-53
p-Anisidine value	1.2	5.73	2.5	7.66	12.42	13.13	6.07	AOCS Cd 18-90
Oxidative Stability Index (hr @ 100°C)				0.5	0.30	0.33	0.31	AOCS Cd 12b-92
Acid Value (mg KOH/g)*	0.14	0.16	0.17	0.12	0.22	0.12	0.34	AOCS Ca 5a-40
Moisture (w/w%)	NR	NR	NR	0.05	0.09	0.02	0.07	AOCS Ca 2e-84
Residual solvent (ppm)**	NR	NR	NR	<1.0	n/a	n/a	<1.0	In-house GC/MS method

Refined Buglossoides Oil

* Acid Value = 1.99 x Free Fatty Acids %.
** Only applicable to solvent-extracted oils
*** These batches were cold pressed
NR – Not reported n/a – not applicable Data in columns 2-4 from Croda (2006)

Table 5 – Primary constituents

Analyte (%)	EAL121B	EAL121C	EAL121D	Purchased	NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6	Method
Triglycerides	NR	NR	NR	93.71	92.58	86.72	89.49	AOCS Cd 11c-93
Diglycerides	NR	NR	NR	2.92	6.11	2.00	2.48	
Monoglycerides	NR	NR	NR	4.13	2.33	3.72	3.93	
Glycerol	NR	NR	NR	<1.00	1.08	<1.0	<1.0	
Epoxy fatty acids	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	AOCS Ce 1h-05
Trans fatty acids	0.77	1.00	0.95	<1.0	<1.0	<1.0	<1.0	AOCS Ce 1h-05
Unsaponifiable matter	0.82	0.80	0.87	0.88	0.28	0.43	0.73	AOCS Ca 6a-40
Sterols (mg/kg)	NR	NR	NR	4770	2560	2160	2430	ISO 12228
Tocopherols & tocotrienols (mg/kg)	NR	NR	NR	965	546	258	390	DGF F-II 4a
Pyrrolizidine alkaloids (µg/kg)	<4 (merged sample)			<1	<1	<1	<1	In-house method
Protein	<10 µg/ml	<10 µg/ml	<10 µg/ml	<10 ppm	<10 ppm	<10 ppm	<10 ppm	Bradford protein assay (Bradford 1976)
Total N (protein/6.25)					<10 ppm	<10 ppm	<10 ppm	Antek 9000NS Analyzer (combustion/ chemiluminescence)

NR – Not reported
Data in columns 2-4 from Croda (2006)

Table 6 – Fatty acid composition

	% Composition of total fatty acids (GLC analysis - AOCS Ce 1h-05)								
	Refined Echium Oil (Croda 2006)				Echium purchased	Buglossoides oil			
	EAL121B	EAL121C	EAL121D	Mean		NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6	Mean
Myristic acid (14:0)	NR	NR	NR	NR	<0.1	0.0	0.0	0.0	0.0
Myristoleic acid (14:1)	NR	NR	NR	NR	<0.1	0.0	0.0	0.0	0.0
Palmitic acid (16:0)	6.2	6.0	5.8	6.0	6.6	5.2	5.3	5.2	5.2
Palmitoleic acid (16:1)	NR	NR	NR	NR	<0.1	0.1	0.0	0.1	0.1
Stearic acid (18:0)	3.8	3.5	3.3	3.5	3.3	1.8	1.9	1.8	1.8
Oleic acid (18:1 n-9))	16.9	17.9	16.7	17.2	14.5	7.6	7.6	7.5	7.6
Linoleic acid (18:2 n-6)	19.1	18.9	17.7	18.6	14.6	12.7	12.7	12.7	12.7
alpha-Linolenic acid (18:3 n-3)	29.4	29.3	29.8	29.5	32.6	44.0	44.0	43.5	43.8
gamma-linolenic acid (18:3 n-6)	10.5	9.6	10.6	10.2	11.6	6.4	6.2	6.3	6.3
Stearidonic acid (18:4 n-3)	12.5	12.5	12.7	12.6	14.7	20.5	19.7	20.8	20.3
Arachidic acid (20:0)	Trace	Trace	1.3	Trace - 1.3	<0.1	0.0	0.0	0.0	0.0

Gondoic acid (20:1 n-9)	0.8	0.8	0.8	0.8	0.7	0.7	0.8	0.8	0.8
Dihomolinoleic acid (20:2 n-6)	NR	NR	NR	NR	<0.1	0.0	0.0	0.0	0.0
Dihomolinolenic acid (20:3 n-3)	NR	NR	NR	NR	<0.1	0.0	0.0	0.0	0.0
Arachidonic acid (20:4 n-6)	NR	NR	NR	NR	<0.1	0.0	0.0	0.0	0.0
Behenic acid (22:0)	<0.3	<0.3	0.3	<0.3	<0.1	0.0	0.0	0.0	0.0
Erucic acid (22:1 n-9)	0.3	0.4	0.7	0.5	0.3	0.2	0.2	0.2	0.2
Lignoceric acid (24:0)	NR	NR	NR	NR	<0.1	<0.1	<0.1	<0.1	<0.1
Nervonic acid (24:1)	NR	NR	NR	NR	0.2	0.1	0.0	0.0	0.0
(n-3)% total	41.9	41.8	42.5	42.1	47.3	64.5	63.7	64.3	64.2
(n-3) + (n-6) % total	71.5	70.3	70.8	70.2	73.5	83.6	82.6	83.3	83.2

Method: AOCS Ch 2a-94, Ce 1f-96
NR = Not recorded
Values expressed as area %

Table 7 – Phytosterol content

Analyte (% sterols)	EAL121B	EAL121C	EAL121D	Purchased	NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6	Canola Oil (CODEX 2005)	Vegetable Oil Phytosterols* (Cargill 2000)	Arabica Coffee (Valdenebro *et al*. 1999)
Cholesterol	NR	NR	NR	0.2	0.7	0.6	0.7	ND – 1.3	0.36	1.2
Campesterol	27.9	23.5	26.3	30.4	37.1	39.4	38.7	5.0 – 13.0	23.58	15.4
Campestanol	NR	NR	NR	0.3	<0.1	2.3	1.8	NR	0.89	0.73
Stigmasterol	NR	NR	NR	1.1	0.4	0.4	0.5	0.2 – 1.0	23.24	18.9
Δ-7-Campesterol	NR	NR	NR	4.0	0.8	0.6	0.5	NR	0.71	0.6
Chlerosterol	NR	NR	NR	0.9	0.4	0.3	0.4	NR	NR	0.87
β-Sitosterol	18.6	12.0	18.5	26.9	47.2	43.1	42.8	45.1 – 57.9	42.27	52.7
Sitostanol	NR	NR	NR	0.4	0.5	0.7	0.7	NR	NR	2.41
Δ-5-avenasterol	18.0	9.3	14.1	18.3	7.3	5.5	7.8	2.5 – 6.6	0.82	2.84
Δ-5,24-Stigmasterol	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.6
Δ-7-Stigmastenol	NR	NR	NR	0.4	0.3	0.2	0.2	ND – 1.3	0.72	2.04
Δ-7-Avenasterol	NR	NR	NR	2.6	1.4	1.2	1.2	ND – 0.8	0.26	1.74

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Analyte (% sterols)	EAL121B	EAL121C	EAL121D	Purchased	NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6	Canola Oil (CODEX 2005)	Vegetable Oil Phytosterols* (Cargill 2000)	Arabica Coffee (Valdenebro *et al*. 1999)
24-Methylene-cholesterol	5.3	13.1	4.2	9.0	2.2	1.7	2.6	NR	NR	NR
Brassicasterol	NR	NR	NR	<0.1	<0.1	<0.1	<0.1	5.0 – 13.0	0.45	NR
** Δ 5,23 stigmastadienol	NR	NR	NR	1.8	0.2	2.7	1.1	NR	NR	NR
Δ 5,24 stigmastadienol	NR	NR	NR	3.5	1.5	1.3	1.0	NR	NR	0.6
Others	30	42.1	36.9	<0.1	<0.1	<0.1	<0.1	ND – 4.2	3.46	NR

Method: ISO 12228
ND = Not Detected.
* Mean results from five samples. This material has been notified as GRAS without objection (OFAS 2000).
** Δ 5,23 stigmastadienol is present in olive oil and is one 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).
Data in columns 2-4 from Croda (2006)

Table 8 – Tocopherol and tocotrienol content

Analyte (mg/kg)	Echium oil EAL121B, C & D	Purchased Echium oil	Codex soybean oil specification*	NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6
α-tocopherol	Not recorded	105	9 – 352	<10	<10	<10
β-tocopherol	Not recorded	<10	ND – 36	<10	<10	<10
γ-tocopherol	Not recorded	719	89 – 2307	535	258	390
δ-tocopherol	Not recorded	141	154 – 932	11	<10	<10
α-tocotrienol	Not recorded	<10	ND – 69	<10	<10	<10
β- tocotrienol	Not recorded	<10	Not specified	<10	<10	<10
γ- tocotrienol	Not recorded	<10	ND – 103	<10	<10	<10
δ- tocotrienol	Not recorded	<10	ND	<10	<10	<10
Total	Not recorded	965	600 – 3370	546	258	390

* CODEX 2005
ND = Not detected
Method: DGF FII-4a

Table 9 – Potential external contaminants

Analyte	EU Limit*	EAL121B	EAL121C	EAL121D	NZ00053 Batch 4	NZ0056 Batch 5	NZ0058 Batch 6	Method
Arsenic (mg/kg)	-	<0.10	<0.10	<0.10	<0.007	<0.007	<0.007	ICP-MS/ AOAC 993.14
Cadmium (mg/kg)	1.0[FS]	<0.01	<0.01	<0.01	<0.002	<0.002	<0.002	
Copper (mg/kg)	-	<0.1	<0.1	<0.1	<0.013	<0.013	<0.014	
Iron (mg/kg)	-	<0.1	<0.1	<0.1	<0.95	<0.94	1.16	
Lead (mg/kg)	0.1	<0.10	<0.10	<0.10	<0.007	<0.007	<0.007	
Mercury (mg/kg)	0.1[FS]	<0.005	<0.005	<0.005	<0.004	0.010	0.004	
Nickel (mg/kg)	-	<0.1	<0.1	<0.1	0.018	0.013	0.020	
Silver (mg/kg)	-	Not recorded	Not recorded	Not recorded	<0.07	<0.07	<0.07	
Tin (mg/kg)	200[CF]	<0.2	<0.2	<0.2	<0.03	<0.03	<0.03	
Total heavy metals as lead (mg/kg)	-	<10	<10	<10	<10	<10	<10	USP/NF 231
Pesticides	Various	ND	ND	ND	ND	ND	ND	FDA PAM 304 E3C5
Melamine (mg/kg)	2.5 total	Not recorded	Not recorded	Not recorded	<0.05	<0.05	<0.05	FDA LIB 4422
Cyanuric acid (mg/kg)		Not recorded	Not recorded	Not recorded	<0.25	<0.25	<0.25	

* Commission Regulation (EC) No 1881/2006 (Anonymous 2006)

[FS] Food supplements only [CF] Canned foods only ND – Not detected Data in columns 3-5 from Croda (2006)

Table 10 – Dioxins and dioxin-like PCBs

Analyte	EU Limit	EAL121B	EAL121C	EAL121D	NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6
PCDD/PCDF - WHO TEQ with DL's (pg/g)	0.75[1]	0.331	0.156	0.258	0.36	0.21	0.21
Dioxin Like PCBS – WHO TEQ with DL's (pg/g)		0.105	0.0608	0.0595	0.171	0.100	0.0990
Sum PCDD/PCDF/Dioxin-like PCBs – WHO TEQ with DLs (pg/g)	1.5[1]	0.436	0.217	0.318	0.531	0.31	0.309

[1] Commission Regulation (EC) No 1881/2006 (Anonymous 2006)
In-house method. Data in columns 3-5 from Croda (2006)

Table 11 – Polycyclic aromatic hydrocarbons (PAHs)

Analyte (µg/kg)	EU Limit*	NZ00053 Batch 4	NZ00056 Batch 5	NZ00058 Batch 6
acenaphthene		<1.0	<1.0	<1.0
acenaphthylene		<2.0	<2.0	<2.0
anthracene		<3.0	<3.0	<3.0
Benzo[a]anthracene		<2.0	<2.0	<2.0
benzo[a]pyrene	2.0	<2.0	<2.0	<2.0
benzo[b]fluoranthene		<3.0	<3.0	<3.0
Benzo[ghi]perylene		<3.0	<3.0	<3.0
benzo[k]fluoranthene		<4.0	<4.0	<4.0
chrysene		<1.0	<1.0	<1.0
dibenz[a,h]anthracene		<3.0	<3.0	<3.0
fluoranthene		<1.0	<1.0	<1.0
fluorene		<2.0	<2.0	<2.0
indeno[1,2,3-cd]pyrene		<3.0	<3.0	<3.0
naphthalene		<2.0	<2.0	<2.0
phenanthrene		<2.0	<2.0	2.3
pyrene		<1.0	<1.0	<1.0
Sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene	10	ND (<8.0)**	ND (<8.0)**	ND (<8.0)**

* Commission Regulation (EC) No 1881/2006 (Anonymous 2006)
** Sum of detection limits
In-house method QA049

Table 12 – Microbiological tests

Test (cfu/g)	EAL121B	EAL121C	EAL121D	Purchased	NZ00053 Batch 4	NZ0056 Batch 5	NZ00058 Batch 6
Total aerobic plate count	NR	NR	NR	<5	<5	<5	<5
Osmophilic yeast	<10	<10	<10	<5	<5	<5	<5
Yeasts	<10	<10	<10	<5	<5	<5	<5
Moulds	<10	<10	<10				
Enterobacteria	<10	<10	<10	ND	ND	ND	ND
Staphylococcus aureus	<10	<10	<10	ND	ND	ND	ND

ND = Not detected
Methods Used USP <61>, MFLP 43, MFHPB-22
Data in columns 2-4 from Croda (2006)

