APPLICATION FOR THE APPROVAL OF REFINED ECHIUM OIL (STEARIDONIC ACID-RICH OIL FROM *ECHIUM PLANTAGINEUM*)

- Final -

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Non-confidential

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Table of Contents

List of Appendices

List of Tables and Figures

APPLICATION FOR THE APPROVAL OF REFINED ECHIUM OIL (STEARIDONIC ACID-RICH OIL FROM *ECHIUM PLANTAGINEUM*)

EXECUTIVE SUMMARY

Crossential Super Refined™ Echium Oil is a stearidonic acid-rich oil of plant origin. It is obtained by Super Refining™ oil extracted from the seeds of *Echium plantagineum*. Super Refining™ is a patented commercial scale chromatographic technique developed to achieve high purity natural oils by removing polar impurities without altering the chemical composition.

Echium plantagineum (*E. plantagineum*) and its products have not hitherto been used for human consumption to a significant degree within the Community. *E. plantagineum* is a naturally occurring plant and has not been genetically modified. Crossential Echium Oil falls under category (e) of Article 1(2) of Regulation (EC) No 258/97 (European Parliament and the Council of the European Union, 1997) (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 the European Commission's Scientific Committee on Food (SCF) Recommendations (Commission Recommendation 97/618/EC) (Commission of the European Communities, 1997), Class 2.2 [complex (non-GM derived)] Novel Food. Intact plants, animals and microorganisms used as food as well as food components…the source of the NF has no history of food use in the Community.

The tradename "Crossential Super Refined™ Echium Oil" will be used for marketing the food ingredient to food manufacturers. The proposed name for labelling purposes on final foods as presented to the consumer is "STA (stearidonic acid)-rich oil *from *Echium plantagineum"* (where * may be used as a footnote). Analytical data has been provided to show that the Super Refined™ oil contains no detectable toxins, allergens or contaminants that are commonly associated with this species and the Boraginaceae family in general, a family which includes borage oil, which has been consumed for many years in the E.U. Specifically, analysis results have been presented for pyrrolizidine alkaloids, cytochrome C allergens (proteins), erucic acid, epoxy fatty acids, trans fatty acids, unsaponifiable matter, pesticides, heavy metals, dioxins and dioxin-like PCB's and PAH's. The specification has been well defined and batch manufacture results have been provided to show that the products can consistently be manufactured in accordance with defined chemical and physical parameters.

The production process for Crossential Super Refined™ Echium Oil comprises of a patented process that involves cracking of the echium seeds and extraction using hexane, followed by a series of distillation and filtration steps. The process has been independently assessed in

accordance with Hazards Analysis and Critical Control Points (HACCP) principles and shown to be tightly controlled and highly reproducible. The process itself is also naturally unsupportive to both microbial growth and to the survival of polar contaminants such as allergenic proteins.

Echium comes from the family of Boraginaceae, which is a large plant family with approximately 100 genera and 2500 species that are widely distributed throughout the northern hemisphere. The genus Echium contains about 30 species distributed across Europe, the Mediterranean region, Madeira, the Canaries and the Azores. *Echium plantagineum* is an erect biennial 20 to 60 cm high, softly hairy, with one or many flowering stems. In addition to cultivation in Europe, *Echium plantagineum* occurs over significant areas of farmland in Australia. The young growth is eaten readily by livestock. The plant is considered a weed in good pastures while on poor country it is considered as a reserve fodder. The echium crop used for the manufacture of Super Refined™ Echium oil is typically grown under contract in the United Kingdom. There are two components of the plant *E. plantagineum* that are of significance: pyrrolizidine alkaloids are known to occur in certain species of the family Boraginaceae and have been isolated from whole plant samples of *E. plantagineum*; and cytochrome C allergens are proteins contained in the pollen of *E. plantagineum*. Whilst both of these components are not detected in Crossential Super Refined™ Echium Oil, a review of their toxicity has been provided.

Super Refined™ Echium Oil has been proposed for use in food groups such as dairy products and dairy analogues, spreadable fats and dressings, grain-based products such as breakfast cereals, nutrition bars and bread products, savoury sauces, meal replacement beverages, and fruit-based beverages. The use levels are largely based on the deliverance of approximately 200 mg of STA in a daily serving. An assessment of the consumption of Super Refined™ Echium Oil, as measured by STA content proposed for use in the E.U. has been completed, based on the proposed use-levels and food consumption data collected as part of the United Kingdom (U.K.) Food Standards Agency's, Dietary Survey Programme (DSP). Of the individual population groups assessed, male adults were determined to have the greatest mean and 97.5th percentile all-user intakes of stearidonic acid from echium oil on an absolute basis, at 1,128 and 2,175 mg/person/day, respectively, while children had the lowest intakes of 719 and 1,351 mg/person/day, respectively (Table IX.a-2). Conversely, on a body weight basis, children were identified as having the highest intakes of any population group, with mean and 97.5th percentile all-user stearidonic acid from echium oil intakes of 51 and 103 mg/kg body weight/day, respectively. Overall, it can be seen that even for the highest intake scenario of male adults consumption of Super Refined™ Echium Oil, when measured as STA can be seen not to exceed the equivalent of 11 servings of food maximally dosed (200 mg of STA per daily serving), or approximately 2,200 mg of STA. Mean consumption for the same group is equivalent to approximately 5 to 6 daily servings of 200 mg STA (approximately 1,128 g per person per day), which would be more representative of upper level consumption in the practical sense. This is clearly a significant over-estimation of realistic intakes, as it would be extremely unlikely for a person to choose so many products, maximally dosed with Super Refined™ Echium Oil.

Crossential Super Refined™ Echium Oil is a rich, safe and suitable vegetarian source of a number of important fatty acids, notably stearidonic acid (C18:4 n-3). Stearidonic acid has been shown in human models to be a precursor of the long chain omega-3 fatty acid eicosapentaenoic acid (EPA). The lipid profile for Crossential Super Refined™ Echium Oil is similar to that of Borage oil and Blackcurrant oil.

The results of microbiological testing confirm the absence of pathogens, which, as discussed earlier, is to be expected from a product that has been solvent extracted, distilled and is of extremely low water activity.

From a toxicological perspective, as discussed earlier, echium is a well-defined member of the Boraginaceae family and has been studied extensively at both a whole plant and extracted oil level. Critical risk factors of pyrrolizidine alkaloid and cytochrome C allergen levels been identified, and extensive analysis has shown these contaminants are removed by the Super-Refining™ process. There are no new fatty acids introduced into the diet, with the most significant difference being the higher level of STA. The metabolism of STA and STA-rich echium oil have been extensively studied in both animal and human models, with expected significant effect of STA in increasing EPA levels [but not docosahexaenoic acid (DHA) levels] in the bloodstream. From a human nutritional safety perspective, the two most important clinical studies to date in echium oil were conducted at dose levels of STA of up to 1.9 g per person per day and for periods of up to 12 weeks. Echium oil was found to have no significant effect on immune function; to decrease serum triglycerides; and to have no significant effect on cholesterol, LDL-C or HDL-C concentrations. In both studies safety and tolerability were monitored with no significant adverse effects compared to baselines attributable to the echium oil. These data are largely supported by a human clinical study conducted with STA esters at a dose of 0.75g per day for 3 weeks followed 1.5 g STA per day for 3 weeks (James *et al.,* 2003).

At the proposed maximum demonstrated safe intake level in humans of 1.9 g per person per day, and a proposed maximum use level in the proposed food use groups of approximately 200 mg of STA per daily serving, this would represent approximately 9 to 10 daily servings. This is comparable to the highest intake scenario of male adults consumption of Super Refined™ Echium Oil, when measured as STA, which does not exceed the equivalent of 11 servings of food maximally dosed, or approximately 2200 mg of STA at the 97.5th and nearly double the mean consumption for the same group is approximately 5 to 6 daily servings of 200 mg STA (approximately 1,130 mg per person per day), would be more representative of upper level consumption in the practical sense.

1.0 ADMINISTRATIVE DETAILS

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2.0 GENERAL INTRODUCTION

2.1 Novel Classification

Crossential Super Refined™ Echium Oil is a stearidonic acid-rich oil of plant origin. It is obtained by Super Refining™ oil extracted from the seeds of *Echium plantagineum*.

Super Refining™ is a patented commercial scale chromatographic technique developed to achieve high purity natural oils by removing polar impurities without altering the chemical composition (Cade, 1989).

Echium plantagineum (*E. plantagineum*) and its products have not hitherto been used for human consumption to a significant degree within the Community. *E. plantagineum* is a naturally occurring plant and has not been genetically modified. Crossential Echium Oil falls under category (e) of Article 1(2) of Regulation (EC) No 258/97 (European Parliament and the Council of the European Union, 1997) (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 the European Commission's Scientific Committee on Food (SCF) Recommendations (Commission Recommendation 97/618/EC) (Commission of the European Communities, 1997), Class 2.2 [complex (non-GM derived)] Novel Food. Intact plants, animals and microorganisms used as food as well as food components…the source of the NF has no history of food use in the Community.

2.2 Previous Novel Foods Applications for Echium

Echium oil has been submitted to the UK Advisory Committee on Novel Foods and Processes (ACNFP) most recently in October 2000 when an application was made from John K King & Sons to approve echium oil as a novel food ingredient. It was proposed that echium oil would be used as a dietary supplement or as a source of essential fatty acids in

other products such as nutritional bars. This application was discussed at the 53rd meeting of the ACNFP, held on 31st January 2002, and following this meeting the Secretariat wrote to the Company requesting they conduct a human feeding study. The Company did not consider that a human feeding study would be a commercially viable option and withdrew their application for novel food clearance in March 2002 (ACNFP, 2002). In addition to this the Committee had concerns regarding the measurement and absence of cytochrome C allergen and epoxy fatty acids.

Croda's Crossential Echium Oil product is a downstream, further purified product using the same source material (*E. plantagineum*) as John K. King & Sons.

2.3 Dossier Requirements

According to the SCF Recommendations described in Section 2.2, the index to the structured schemes to be followed for each class of novel food identifies the following essential information requirements for novel foods assigned to SCF category 2.2:

I Specification of the novel food

II Effect of the production process applied to the novel food

III History of the organism used as the source of the novel food

IX Anticipated intake/extent of use of the novel food

XI Nutritional information on the novel food

XII Microbiological information on the novel food

XIII Toxicological information on the novel food

We present below our submission in this required format.

I SPECIFICATION OF THE NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to the specifications of the novel food (Commission of the European Communities, 1997):

a. "…is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?"

b. "Is the information representative of the novel food when produced on a commercial scale?"

c. "Is there an appropriate specification (including species, taxon *etc*. for living organisms) to ensure that the novel food marketed is the same as that evaluated?"

We will address each point in turn in this section.

A glossary is provided at the end of this document to provide explanation of abbreviated terms referred to in this dossier.

I.a Chemical Name

Crossential Super Refined™ Echium Oil is a stearidonic acid-rich oil produced from the seeds of *Echium plantagineum* using a solvent chromatographic technique.

I.a.1 Proposed Names

Echium oil

"STA (stearidonic acid)-rich oil from *Echium plantagineum"* (where * may be used as a footnote)

I.a.2 Trade Names

Crossential Super Refined™ Echium Oil

I.b Analytical Information

For analysis purposes the Echium Oil samples discussed below have been labelled as follows:

EAL 121A	Crude echium oil (raw material)
EAL 121B	Super-Refined™ Echium Oil Batch 160344 (Manufactured 02/2006)
EAL 121C	Super-Refined™ Echium Oil Batch 150601 (Manufactured 11/2005)
EAL 121D	Super-Refined™ Echium Oil Batch 154257 (Manufactured 12/2005)
EAL 121E	Blend of Super-Refined™ Echium Oil Batch numbers 160344, 150601 and 154257 in weight ratio 1:1:1

I.b.1 Potentially Toxic Inherent Constituents

Echium itself has been associated with 2 principle inherent constituents which are commonly associated with Boraginacae, pyrrolizidine alkaloids and cytochrome c allergens. The toxicity of these ingredients is discussed in detail in Section IIId. In addition to this standard parameters measured in the seed oil industry are also included, erucic acid (commonly associated with rape seed oil); epoxy fatty acids and trans fatty acids. Whilst generally not considered toxic, as such, the level and profile of the unsaponifiable fraction is also considered relevant. Analysis results for each constituent are provided in the section below.

I.b.1.1 Pyrrolizidine Alkaloids

Pyrrolizidine alkaloids are known to occur in certain species of the family Boriginaceae and have been isolated from *E. plantagineum* (Culvenor, 1956; WHO, 1989)]. The toxicity of Pyrrolizidine alkaloids is reviewed in Section III.d.1.1. Analysis of several plant samples of *E. plantagineum* from New South Wales revealed a total alkaloid content of about 0.3%. The maximum level of total alkaloid measured was 0.9% (Culvenor, 1985).

Pyrrolizidine alkaloids are polar therefore they would not be expected to be extracted by the hexane extraction solvent present in the oil and subsequent further refining. Analysis of Crossential Super-Refined™ Echium Oil has been performed. The results of this analysis show that batches EAL121A (crude echium oil) and EAL121E (a 1:1:1 composite of batches EAL 121B, EAL 121C and EAL 121D respectively) levels contain less than 4 µg PA/kg oil (this is the limit of detection). The certificates of analysis are provided in Appendix 1 (part A).

I.b.1.2 Cytochrome C Allergens (Proteins)

The chromatographic technique used in the Super Refined™ process will act to remove any pollen or particulate plant debris in the oil. Cytochrome C allergens isolated from the pollen of *E. plantagineum* have been characterised as proteins with a molecular weight of 12,800 (Matthews *et al.*, 1988).

It can be assumed that the maximum cytochrome C allergen concentration is equivalent to the total protein content. Analysis results for total protein are provided in Appendix 1 (part B) and are summarised in Table I.b.1.2-1 below. Ten percent emulsions of the oil samples were made in water. These were then centrifuged to clarify. The extracts were then analysed for protein content using the Bradford dye binding assay (Bradford, 1976). Measurements are relative to a Bovine Serum Albumin standard curve. It is shown that the Super Refining™ process significantly reduces the protein content to below detection limits and therefore minimises the allergenic potential.

Table I.b.1.2-1 Total Protein Analysis Results for Crossential Super-Refined™ Echium Oil Compared to Crude Echium Oil

Batch Number	EAL 121A (crude echium oil)	EAL 121B	EAL 121C	EAL 121D
Protein Content µg/mL	210	<10	<10	<10

1.b.1.3 Erucic Acid

Erucic acid (C22:1) levels were determined by GLC analysis. Assignment of the appropriate signal in the GLC trace was achieved by comparison of the retention time with known erucic acid standard. Further confirmation was provided by addition of erucic acid to an echium oil sample (EAL 121D). Values for EAL 121A, B, C and D are provided in Table 1.b.3-1 below with an average value of 0.5% of total fatty acids.

1.b.1.4 Epoxy Fatty Acids

Full lipid profiles for a number of production volume batches are included in Section I.c. All fatty acids at a percentage composition of 0.3% w/w total fatty acids and above have been identified conclusively. We have no data to suggest the presence of cyclopropenoid and epoxy fatty acids. These results are verified by independent analysis presented in Appendix

1 (part C) which concludes that "…these two cyclopropenoid fatty acids would have been detected readily if they were at 0.1% or more in the oils tested".

I.b.2.5 Trans Fatty Acids

Independent analysis results for trans fatty acids have been provided in Appendix 1 (part H). These results are all below 2 g/100 g oil (% w/w).

Table I.b.1.5-1 Trans Fatty Acids Analysis Results of Crossential Super Refined™ Echium Oil Compared to Crude Echium Oil

	Batch Number			
	EAL 121A (crude echium oil)	**EAL 121B**	**EAL 121C**	**EAL 121D**
Trans fatty acid content g/100 g	0.75	0.77	1.09	0.95

1.b.1.6 Unsaponifiable Matter

The unsaponifiable matter was isolated from the raw material echium oil and three Crossential SA14 production batches. The composition of the unsaponifiable matter was investigated by GLC.

Unsaponifiable Matter Identification

The unsaponifiable echium oil samples were dissolved in acetone and cholesterol was added as an internal standard (not naturally present in the samples in >0.5%) and GLC analysis performed. Sterol content and identification has been performed by an independent laboratory for crude echium oil, EAL 121A, and the certificate of analysis is provided as Appendix 1 (part D). By comparison with the known sterol content for EAL 121A, that of EAL B, C and D could be deduced. Known standards of ß-sitosterol and stigmasterol were available in the laboratory to further confirm the presence or absence of these sterols in samples of EAL 121B, C and D. Some lower molecular weight compounds (possible hydrocarbons) were also observed by GLC analysis but not in appreciable amounts (i.e. less than 5% composition). A significant proserving of mass is lost on injection and is assumed to be heavy molecular weight compounds that do not pass through the GLC column. The data obtained on the unsaponifiable matter is summarised in Table I.b.1.5-1 to Table I.b.1.5-3.

Table I.b.1.5-2 Total Unsaponifiable Matter Results from Echium Oil

	EAL 121A (crude echium oil)	**EAL 121B**	**EAL 121C**	**EAL 121D**
Unsaponifiable matter / % by weight	1.08	0.82	0.80	0.87

Table I.b.1.5-3 Analysis of Sterol Composition				
Sterol	**Composition[1] /%**			
	EAL 121A	**EAL 121B**	**EAL 121C**	**EAL 121D**
Campesterol	29.2	27.9	23.5	26.3
ß-Sitosterol	29.7	18.6	12.0	18.5
?-5-Avenasterol	16.6	18.0	9.3	14.1
24-Methylene-cholesterol	9.3	5.3	13.1	4.2

[1] Composition / % is raw data from GLC traces performed in the laboratory except EAL 121A which is based on data provided by RSSL.

Comparison to Traditional Counterparts

The sterol profile for echium oil is similar to that of traditional counterparts such as borage, blackcurrant, evening primrose and safflower (Table I.b.1.5-4, taken from Firestone D, ed. "Physical and Chemical Characteristics of Oils, Fats and Waxes", 1999).

Table I.b.1.5-3 Typical Sterol Profiles for Traditional Counterparts				
Sterol	**Borage**	**Blackcurrant**	**Safflower**	**Evening Primrose**
Cholesterol	-	0.2-0.7	0-0.5	-
Brassicasterol	0-0.16	-	-	-
Campesterol	25-30	7.2-10.4	9.2-13.0	8-9
Stigmasterol	-	0.5-1.0	6.5-9.6	-
ß-Sitosterol	22-42	70-85	40.2-49.8	87-90
?5-Avenasterol	15-28	2-3	2.1-4.0	4
?7-Stigmasterol	-	0.4-4.5	15.7-22.4	2
?7-Avenasterol	1	0.4-2	2.9-5.3	-
24-Methylene-cholesterol	15-20	-	-	-

I.b.2 External Contaminants

I.b.2.1 Pesticides

Approved agrochemical products could potentially be used at two stages within the production cycle of *E. plantagineum*, pre-drilling for weed control or as a pre-harvest desiccant.

The results of a pesticides screen conducted at an independent laboratory are presented as Appendix 1 (part E). No residues of the pesticides sought in the samples supplied, at or above the reporting limits quoted.

I.b.2.2 Heavy Metals

Heavy metals analysis results for crude echium oil and Crossential Super Refined™ Echium Oil are provided as Appendix 1 (part F). All results are below detection limits. For vegetable

oils EU Contaminants Legislation only specifies a limit of 0.1 mg/kg for lead and all batches are within this level (Commission Regulation 466/2001 [Commission of the European Communities, 2001).

Table I.b.2.2-1 Heavy Metal Analysis Results of Crossential Super Refined™ Echium Oil Compared to Crude Echium Oil

Heavy Metal Tested	Batch Number			
	EAL 121A (C1922-EL-1-004) Crude Echium Oil	EAL 121B (C2138/160344) Super Refined™	EAL 121C (C2140/150601) Super Refined™	EAL 121D (C2142/154257) Super Refined™
Lead mg/kg	<0.10	<0.10	<0.10	<0.10
Cadmium mg/kg	<0.01	<0.01	<0.01	<0.01
Mercury mg/kg	<0.005	<0.005	<0.005	<0.005
Arsenic mg/kg	<0.10	<0.10	<0.10	<0.10
Copper	<0.1	<0.1	<0.1	<0.1
Iron mg/kg	<0.1	<0.1	<0.1	<0.1
Nickel mg/kg	<0.1	<0.1	<0.1	<0.1
Tin mg/kg	Not tested	<0.2	<0.2	<0.2
Total Heavy Metals (expressed as Lead) mg/kg	<10	<10	<10	<10

I.b.2.3 Dioxins and Dioxin-like PCB's

Independent test results for dioxins, furans and dioxin-like PCB's are provided in Appendix 1 (part G). These results are summarised and compared to the respective E.U. maximum levels in Table I.b.2.3-1. All results are well below maximum permitted levels.

Table I.b.2.3-1 Dioxins and Dioxin-like PCB's Results Crossential Super Refined™ Echium Oil Compared to Crude Echium Oil

Test Parameter	Batch Number			
	EAL 121A (22/04/06)	EAL 121B (C2139/ 160344)	EAL 121C (C2141/ 150601)	EAL 121D (C2143/ 154257)
PCDD/PCDF - WHO TEQ with DL's (pg/g)	0.238	0.331	0.156	0.258
EU 199/2006* Guideline Maximum levels – sum of PCDD/PCDF (WHO-PCDD/F-TEQ) (pg/g)	0.75	0.75	0.75	0.75
Dioxin Like PCBS – WHO TEQ with D L's (pg/g)	0.127	0.105	0.0608	0.0595
Sum PCDD/PCDF/Dioxin-like PCBs –WHO TEQ with DLs (pg/g)	0.365	0.436	0.217	0.318
EU 199/2006* Guideline Maximum levels – sum of PCDD/PCDF/Dioxin-like PCBs (pg/g) (WHO-PCDD/F-PCB-TEQ)	1.5	1.5	1.5	1.5

* COMMISSION REGULATION (EC) No 199/2006 of 3 February 2006 amending Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs as regards dioxins and dioxin-like PCBs (Commission of the European Communities, 2006).

I.b.2.4 Polycyclic Aromatic Hydrocarbons (PAH's)

Polycyclic Aromatic Hydrocarbon (PAH) analysis results for batches 121A (crude echium oil) to 121D are presented in Appendix 1 (part J). All results are considered to be within the acceptable range by the analysis laboratory.

I.b.3 Nutrients

The fatty acid profiles of batches of Crossential Super Refined™ Echium Oil can be seen in Table 1.b.3-1 below, crude echium oil from recent production is also included for comparison. These results can also be compared to the profiles of the un-refined oil presented in Table 1.b.3-2 from several years' trial data. It is evident that the lipid constituents of the Super Refined™ Echium Oil are not significantly different from those of the crude oil. Although there are minor variations in oil composition from season to season due to changes in temperature, light intensity, *etc.*, the lipid profile of both the crude and Super Refined™ Echium Oil remains within the specified ranges. The information covers several years' trials work, and is therefore considered an accurate representation of this species. Each row relates to the lipid profile for a single representative sample of echium oil. Where there is more than one sample for a single year this relates to different laboratory batches of the oil.

Table 1.b.3-1 Fatty Acid Profiles of Batches of Crossential Super Refined™ Echium Oil Compared to Crude Echium Oil

Fatty Acid	**% Composition of total fatty acids (GLC analysis)**				
Batch/Description	**EAL 121B (160344)**	**EAL 121C (150601)**	**EAL 121D (154257)**	**Mean**	**EAL 121A (Crude echium oil)**
C16:0; palmitic acid (PA)	6.2	6.0	5.8	6.0	6.4
C18:0; stearic acid (SA)	3.8	3.5	3.3	3.5	3.8
C18:1*cis* n-9; Oleic acid (OA)	16.9	17.9	16.7	17.2	17.3
C18:2 n-6; linoleic acid (LA)	19.1	18.9	17.7	18.6	19.0
C18:3 n-3; Alphalinolenic acid (ALA)	29.4	29.3	29.8	29.5	29.3
C18:3 n-6; Gamma linolenic acid (GLA)	10.5	9.6	10.6	10.2	10.1
C18:4 n-3; stearidonic acid (STA)	12.5	12.5	12.7	12.6	12.3
C20:0; arachidic acid (ADA)	Trace	Trace	1.3	Trace-1.3	<0.3
C20:1 n-9; gondoic acid (GA)	0.8	0.8	0.8	0.8	<0.8
C22:0; behenic acid (BA)	<0.3	<0.3	0.3	<0.3	<0.3
C22:1 n-9; erucic acid (EA)	0.3	0.4	0.7	0.5	0.5
n-3 % total	41.9	41.8	42.5	42.1	41.6
(n-3)+(n-6) % total	71.5	70.3	70.8	70.2	70.7

Table 1.b.3-2 Fatty Acid Profiles of Un-Refined Echium Oil							
Year	**% Composition of total fatty acids (GLC analysis)**						
	C16:0	**C18:0**	**C18:1 n-9**	**18:2 n-6**	**18:3 n-3**	**18:3n-6**	**18:4n-3**
1996	7.3	4.2	19.0	16.5	27.8	11.6	11.1
1996	7.1	4.2	18.6	16.4	28.7	12.0	12.2
1997	7.1	3.7	15.8	14.3	33.1	11.2	13.9
1997	6.8	3.8	15.9	14.5	33.2	11.1	13.8
Mean	7.1	4.0	17.3	15.4	30.7	11.5	12.8

I.c Representative Commercial Scale Batch Data

Table I.c-1 Analytical Data for Production Batches of Crossential Super Refined™ Echium Oil				
Inspection Characteristic	**Specification**	**EAL 121B**	**EAL 121C**	**EAL121D**
Appearance (form)	Liquid	Liquid	Liquid	Liquid
Appearance (colour)	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Acid Value	0.00-2.00 mg KOH/g	0.14	0.16	0.17
Peroxide Value	0-5 meq O_2/kg	0.28	3.13	1.01
Specific Gravity @ 25ºC	0.9200-0.9300	0.9263	0.9279	0.9282
Refractive Index @ 25ºC	1.4700-1.4900	1.4815	1.4810	1.4805
Anisidine Value	0.0-20.0	1.2	5.73	2.5
Stearidonic Acid	>10.00%	12.42	12.01	12.89

I.c.1 Stability Data

Crossential Super Refined™ Echium Oil is stabilised with safe and suitable antioxidants that are added in accordance with the requirements of *Directive 95/2/EC (as amended) on food additives other than colours and sweeteners* (European Parliament and the Council of the European Union, 1995).

Measures of oxidation for the oil are peroxide value (PV) and p-anisidine value (p-AV). PV measures the products of primary oxidation, that is peroxides, and p-AV measures the products of secondary oxidation, that is aldehydes and ketones. These are ideal measures of the extent of oxidation in materials containing unsaturated fatty acids such as echium oil and the material is produced to a set specification.

In addition to this, Rancimat studies have been conducted in order to provide a measure of the stability of Super Refined™ Echium Oil over time. Samples of oil were exposed to a stream of air at elevated temperatures. Under these conditions the oxidation of the material is accelerated resulting in the formation of volatile carboxylic acids. The measured induction time is the time taken for the oil to be oxidised to the organic acids and represents the

oxidative stability of the oil. These studies have the advantage of providing a rapid (hours or days) measure of the stability of the oil, in comparison with shelf-life studies where the time taken to obtain results can be weeks or months.

The oxidative stability of Super Refined™ Echium Oil has also been investigated and compared with traditional counterparts using Rancimat studies and the results are presented in Table 1.c.1-1. Note that, immediately before Rancimat studies are performed the oil samples are analysed for p-AV and PV values. These are carried out to ensure that the oil has not undergone significant oxidation prior to analysis. Echium oil was studied under identical Rancimat conditions to the other vegetable-derived oils included in the study with the exception of a higher temperature (10 °C). Reaction kinetics for oxidation indicate that an induction time in the region of 2.5 h would be obtained at 100 °C, which is comparable to the other oils.

Table 1.c.1-1 Comparison of Oxidative Stability of Super Refined™ Echium Oil *vs*. Traditional Counterparts

Type of Oil	Temperature / °c	Average Induction Time / h
Borage Oil	100	4.13
Evening Primrose Oil	100	3.12
Safflower Oil	100	1.40
Super-Refined™ Echium Oil	110	1.25

The Rancimat method complies with national and international standards (AOCS official method Cd 12b-92 and ISO Standard 6886, 1996).

I.d Proposed Regulatory Specification

The proposed regulatory specification for Crossential Super Refined™ Echium Oil is presented in Table 1.d-1. In addition to standard measures of purity and oxidative stability employed in the fats and oils industry, it is also considered important that the level of residual protein is kept to a minimum to avoid the incidence of cytochrome c allergens.

Table I.d-1 Proposed Regulatory Specification for Crossential Super Refined™ Echium Oil

Test	Product Specification
Stearidonic acid content	Not less than 10% w/w of total fatty acids
Trans fatty acids	Not more than 2% (w/w of total fatty acids)
Acid value	Not more than 5 mg KOH/g
Peroxide value	Not more than 5 meq O_2/kg
Unsaponifiable Content	Not more than 2%
Lead	Not more than 0.1 mg/kg
Protein Content (total nitrogen)	Not more than 20 µg/mL

Once processing is complete the oil is tested against the product specification by the Quality Control laboratory at Croda. In the event that the oil is found to be out of specification it will not be released by Quality Control and the standard site procedure for the reprocessing of material is followed:

- If the failure of specification is significant, and it is not possible to remedy by reprocessing, the material will be discarded.
- If the material failure is considered marginal, for example in terms of colour, then it is reprocessed. The material is either re-super-refined, or blended with another batch of material and then re-super-refined to generate a product that is in specification.

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to the production process of the novel food (Commission of the European Communities, 1997):

a. "Does the novel food undergo a production process?"

b. "Is there a history of use of the production process for the food?" If no, "does the process result in a significant change in the composition or structure of the novel food compared to its traditional counterpart?"

c. "Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from use of the process?"

d. "Are the means identified for controlling the process to ensure that the novel food complies with its specification?"

e. "Has the process the potential to alter the levels in the novel food of substances with an adverse effect on public health?"

f. "After processing is the novel food likely to contain microorganisms of adverse public health significance?"

We will address each point in turn in this section.

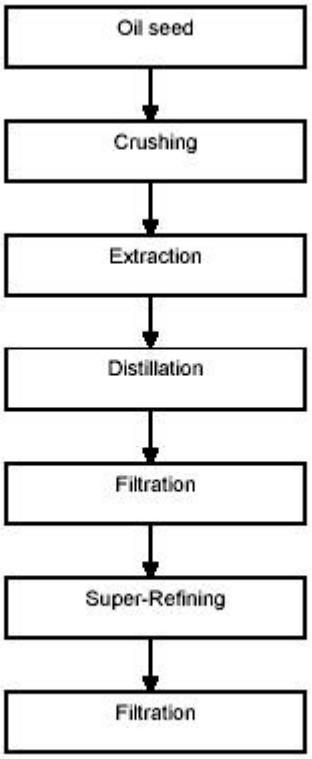
II.a Overview of Production Process

II.a.1 Process Before Super-Refining™

An overview of the production process is presented in Figure 1. The echium crop used for the manufacture of Super Refined™ Echium Oil is typically grown under contract in the United Kingdom. The echium seed is first cracked which, by rupturing the cell walls of the individual seeds, makes the oil more accessible. This is a standard procedure employed to prevent excessive degradation of product due to mechanical attrition. The Echium seed is then conveyed into an extractor system where it is brought into contact with food grade hexane solvent. The extraction process works on the principle that oil is soluble in a solvent, in this case food grade hexane that is a non-polar solvent. As the fatty acids on the triglyceride are also non-polar the oil is soluble in hexane. The extraction process is carried out at room temperature in an oxygen free atmosphere.

The oil enriched hexane then passes through a distillation system where the solvent is removed from the oil to leave less than 1 mg/kg of hexane in the oil, in accordance with the requirements of Directive 88/344/EC on extraction solvents (Council of the European Communities, 1988).

Figure II-1 Super-Refined™ Echium Oil Process Flow Diagram



II.a.2 The Super Refining™ Process

Crossential Super Refined ™ Echium Oil is processed using a commercial scale chromatographic technique developed by Croda to achieve high purity natural oils by removing polar impurities without altering the chemical composition (Cade, 1989). The process is conventionally operated in a batch-wise manner using 1 to 5% of an adsorbent earth such as montmorillonite clay. Earth treatment of natural oils is a standard process used by the edible oil industry to improve colour and odour. The Super Refining™ process extends this principle but is operated in a semi-continuous manner and employs larger quantities of adsorbent. The Super Refining™ process has been utilised on a commercial scale for over 10 years and is considered proprietary.

II.b History of Production Process

Crushing and hexane extraction are standard process techniques used in the oil processing industry. As explained above, earth treatment of natural oils is a standard process used by the edible oil industry to improve colour and odour.

II.c Potential Hazards

II.c.1 Toxicological

See Section I.b.1.

II.c.2 Nutritional

See Section I.b.3.

II.c.3 Microbiological

See Section XII

II.d Production Control

Croda's manufacturing site is independently certified to have a valid Hazards Analysis and Critical Control Points (HACCP) system in place (Appendix 2).

II.e Potential Effect on Public Health of Hazardous Substances

It is not anticipated that there would be hazardous substances produced by this process. Sections II and XII provide information on inherent toxicological constituents, external and microbiological contaminants. The non-polar solvent extraction system, supported by certified HACCP procedures acts to reduce potential hazards and regular analysis provide further reassurance.

II.f Potential Effect on Public Health of Hazardous Microorganisms

See Sections II.c.3 and 4.

III HISTORY OF THE ORGANISM USED AS THE SOURCE OF THE NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to the history of the source organism (Commission of the European Communities, 1997):

a. "Is the novel food obtained from a biological source, *i.e.*, a plant, animal or microorganism?"

b. "Has the organism used as the source of the novel food been derived using GM?"

c. "Is the source organism characterized?"

d. "Is there information to show that the source organism and/or foods obtained from it are not detrimental to human health?"

We will address each point in turn in this section.

III.a Source of Echium Oil

Echium comes from the family of Boraginaceae, which is a large plant family with approximately 100 genera and 2,500 species that are widely distributed throughout the northern hemisphere. The family is well known to herbalists and gardeners because it includes many ornamental plants.

The genus Echium contains about 30 species distributed across Europe, the Mediterranean region, Madeira, the Canaries and the Azores (Tutin *et al*., 1972).

E. plantagineum is an erect biennial 20 to 60 cm high, softly hairy, with one or many flowering stems. The basal leaves are ovate with prominent lateral veins and soft appressed setae. The cauline leaves are oblong to lanceolate, the uppermost being more or less cordate at the base. Inflorescence usually branched. Calyx 7 to 10 mm at anthesis, up to 15 mm in fruit. Corolla 18 to 30 mm in fundibuliform blue becoming pink through purple, hairy on veins and margins only. Two stamens exerted from corolla tube, the remaining stamens included or only slightly exerted. Stigmae distinctly bifid. *E. plantagineum* is also known by the common names of Purple Vipers Bugloss, Paterson's Curse and Salvation Jane.

In addition to cultivation in Europe, *E. plantagineum* occurs over significant areas of farmland in Australia. The young growth is eaten readily by livestock. The plant is considered a weed in good pastures while on poor country it is considered as a reserve fodder (Culvenor 1956). Measurements of herbage dry matter content, nitrogen content and digestibility of *E. plantagineum* indicate that it would be nutritious forage for grazing animals (Piggin 1977).

III.b GM Status of *Echium plantagineum*

Echium plantagineum is not a genetically modified organism.

III.c Characterization of *Echium plantagineum*

The taxonomy of the plant source of echium oil is as follows:

Taxonomy (Linnaeus):

Division:	Spermatophyta
Subdivision:	Angiospermae
Class:	Dicotyledonae
Family:	Boraginaceae
Genus:	Echium
Species:	plantagineum

III.d Information on Detrimental Health Effects

III.d.1 *Echium plantagineum*

There are two components of the plant *E. plantagineum* that are of significance. Pyrrolizidine alkaloids are known to occur in certain species of the family Boriginaceae and have been isolated from *E. plantagineum* (Culvenor, 1956; WHO, 1989). Analysis of several whole-plant samples of *E. plantagineum* from New South Wales revealed a total alkaloid content of about 0.3%. The maximum level of total alkaloid measured was 0.9% (Culvenor, 1985). Cytochrome C allergens are proteins contained in the pollen of *E. plantagineum*. Whilst both of these components are not detected in Crossential Super Refined™ Echium Oil, a review of their toxicity is provided below.

III.d.1.1 Animal Studies - Pyrrolizidine Alkaloids

The level of pyrrolizidine in Echium alkaloids is normally between 0.1 to 0.3% of the dry weight of the whole plant but levels as high as 0.9% have been reported (Seawright *et al.*, 1985). Field evidence strongly indicates that horses, pigs and to a lesser extent sheep are all affected (Culvenor, 1985).

Pyrrolizidine alkaloids are polar and therefore they would not be expected to be present in echium oil. An analysis of the alkaloid content of the crude and Super Refined™ oil and the *E. plantagineum* meal has been carried out. The meal contained 0.1 mg/g total alkaloids. No alkaloids were detected in the crude or Super Refined™ oils. The detection limit was <4 µg/g. To put this value into perspective, it has been estimated that honey from *E. plantagineum* constitutes about 10 to 15% of total Australian production. The honey is sold mainly as blends with other honey. Honey prepared from *E. plantagineum* has been shown to contain between 0.27 to 0.95 mg/kg alkaloids (Culvenor *et al.*, 1981). The possible intakes of pyrrolizidine alkaloids from this source are considered to be very low (Culvenor, 1985). A review of the toxicity of *E. plantagineum* pyrrolizidine alkaloids is provided below.

Toxicity and Metabolism of Pyrrolizidine Alkaloids (Cheeke, 1988)

Pyrrolizidine alkaloids (PA) are found mainly in plants of three families: boraginaceae, Compositae and Leguminosae. Diester Pyrrolizidine alkaloids common to Heliotropium and *Echium* spp. are metabolized in the ovine rumen to 1-methyl metabolites. Exposure to PA results in high concentrations of liver Cu, reduced liver Zn, and abnormal Fe metabolism with haematopoiesis markedly impaired. Pyrrolizidine alkaloid toxicity alters vitamin A metabolism in rats, depressing plasma and liver levels of vitamin A. Synthetic antioxidants in the diet confer protective activity in laboratory animals (*e.g.*, rats, mice) against PA toxicoses.

Up to 40-week Intermittent Dietary Exposure –Rat (Peterson and Jago, 1984)

Young rats fed 40% *E. plantagineum* (600 mg/kg pyryzolidine alkaloids) for 2 weeks suffered 70% mortality within 5 to 13 weeks. Young rats fed 20% *E. plantagineum* (300 mg/kg

pyrizolidine alkaloids) for alternate 2-week periods with a control feed had 50% mortality in 21 weeks. Adult rats fed *E. plantagineum* continuously all died within 7 to 16 weeks at the 40% level and 37 to 40 weeks at the 20% level. The rats died with a mixture of acute and chronic liver damage. Tumours, 3 benign and 1 malignant, of a type observed in carcinogenesis experiments with other pyrrolizidine alkaloids developed in survivors of the study on adult rats fed 20% *E. plantagineum*. The number of tumours was below the significance level.

4-month Dietary Exposure – Pig (Culvenor, 1985)

Experimental evidence includes a study by the New South Wales Department of Agriculture in which young pigs were fed 15% *E. plantagineum* in the diet. All developed the typical chronic liver damage within 5 months and 1 animal died within 4 months.

19-month Dietary Exposure (Field-grazing) – Sheep (Seaman et al., 1989)

A field grazing trial was undertaken to monitor the health and production of crossbred sheep grazing pasture where *E. plantagineum* constituted a considerable proserving of the available forage. The trial, conducted for 19 months over successive grazing seasons, demonstrated a significant difference in production, with sheep on the *E. plantagineum* pasture being lighter and growing less wool compared with sheep on Echium-free pasture. No mortalities involving pyrrolizidine alkaloid poisoning were recorded in sheep grazing *E. plantagineum*, although there was histological evidence of moderately severe liver damage associated with high liver copper concentrations in at least one sheep following the grazing of large quantities of the plant.

16-week Dietary Exposure (Pen-Feeding) – Sheep (Seaman and Dixon, 1989)

In a pen feeding trial fresh *E. plantagineum* was fed as the sole diet to crossbred sheep with or without a history of previous access to the plant. Control groups received a diet of lucerne chaff and oats. During the trial, sheep on the echium diet lost weight and deaths occurred with histological evidence of excessive copper accumulation, usually accompanied by pyrrolizidine alkaloid damage, in the liver and biochemical evidence of liver toxicity.

III.d.1.2 Allergenic Potential – Cytochrome C from Pollen

Cytochrome C allergens isolated from the pollen of *E. plantagineum* have been characterized as proteins with a molecular weight of 12,800 (Matthews *et al.*, 1988). The chromatographic technique used in Super Refining™ will act to filter out any pollen or particulate plant debris in the oil. To confirm the absence of cytochrome C allergens in the Super Refined™ oil a total protein test has been performed using Bradford Reagent (see Section II.b.1.2 above). A review of the allergenicity of *E. plantagineum* is presented below.

In vitro - Cytochrome C allergens isolated from the pollens of E. plantagineum (Matthews et al., 1988)

Cytochrome C allergens were isolated from extracts of the pollen of *E. plantagineum* by ion exchange chromatography, gel filtration and preparative isoelectric focusing. They were characterized by their absorption spectra, molecular weight, p*I* and amino acid composition. The cytochrome C bound specific IgE in the sera of hypersensitive patients by RAST.

In vivo – Human Clinical Study - Investigation of the involvement of Echium plantagineum in seasonal allergy, IgE antibodies to Echium and other weed pollens (Katelaris et al., 1982)

The possible allergenicity *E plantagineum*, was investigated in a rural area of Australia. Sixty-one subjects with respiratory allergy were studied. Positive skin test reactions to defatted ammonium bicarbonate extract of pollen were found in over 60% of subjects, and positive RAST tests in a similar number.

In vivo – Human Case Study – Allergic Rhinitus (Burdon and Burdon, 1983)

A case of allergic rhinitis, which occurred on exposure to *E. plantagineum* is described. Symptoms developed on exposure both to flowering and to dead, dried plants. Inhalational challenge tests with pure preparations of pollen and epidermal debris, including plant hairs, resulted in the symptoms and signs of allergic rhinitis.

IX ANTICIPATED INTAKE/EXTENT OF USE OF THE NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to the intake/extent of use of the novel food (Commission of the European Communities, 1997).

a. "Is there information on the anticipated uses of the novel food based on its properties?"

b. "Is there information to show anticipated intakes for groups predicted to be at risk?"

c. "Will introduction of the novel food be restricted geographically?"

d. "Will the novel food replace other foods in the diet?"

We will address each point in turn in this section.

IX.a Available Intake Information

The proposed food uses for Super Refined™ Echium Oil are detailed in Table IX.a-1 below. These use levels are largely based on the deliverance of approximately 200 mg of STA in a daily serving. More detail is available on the conventional food group uses in Table 3-1 of Appendix 3. The intake calculations are exclusive of food supplements and the PARNUTS categories of dietary foods for special medical purposes; and foods intended for use in energy-restricted diets for weight reduction, because it is assumed that by the definition these products would be taken as replacements for normal foods rather than together with them.

Table IX.a-1 Proposed Uses of Crossential Echium Oil

Use Group	Maximum Use Level of STA
Dairy products including milk and yoghurt-based drinks	250 mg/100 g or for cheese products 750 mg/100 g 75mg/100g for drinks
Dairy analogues	250 mg/100 g or for analogues to cheese products 750 mg/100 g
Spreadable fat and dressings	750 mg/100 g
Breakfast cereals	625 mg/100 g
Food supplements	500 mg per 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
Foods intended for use in energy-restricted diets for weight reduction	250 mg/meal replacement
Non-carbonated fruit-based drinks	75 mg/100 g
Nutrition Bars (Low-sugar, based on fruit, cereal and/or protein)	500 mg/100 g
Savoury Sauces	500 mg/100 g, or for pasta sauces 200 mg/100 g
Bread and Bread Products	200 mg/100 g

An assessment of the consumption of Super Refined™ Echium Oil, as measured by STA content proposed for use in the E.U. in foods such as dairy products and dairy analogues, spreadable fats and dressings, grain-based products such as breakfast cereals, nutrition bars and bread products, savoury sauces, meal replacement beverages, and fruit-based beverages has been completed, based on the proposed use-levels and food consumption data collected as part of the United Kingdom (U.K.) Food Standards Agency's, Dietary Survey Programme (DSP).

The mean and high-level (97.5th percentile) all-person and all-user intakes, as well as the percent of the population consuming the foods were calculated for each of the 6 population groups on a milligram per day and milligram per kilogram body weight per day basis. The following groups were included in the survey data:

children, ages 1½ to 4½ ;
young people, ages 4 to 10;
female teenagers, ages 11 to 18;
male teenagers, ages 11 to 18;
female adults, ages 16 to 64;
male adults, ages 16 to 64

The data used in this intake assessment is included in the U.K. National Diet and Nutrition Survey (NDNS), which consists of 4 different surveys for specific age groups. The surveys produce the most up-to-date data, which is used to evaluate food-use, patterns of food consumption, and nutritional status in the U.K. The data is contained in the form of 4- or 7-day weighed food records for individuals, and is selected using a stratified multi-stage random probability design. Private households were sampled throughout Great Britain in order to obtain the data, and this was completed using postal sectors (UKDA, 1995, 2001) or local authority wards (UKDA, 1991).

The data included in the NDNS were obtained from both individuals as well as whole households via 4- or 7-day weighted dietary intake records. Records were completed throughout all 4 seasons of the year in order to capture the variability in eating behaviours and patterns due to seasonal changes. Survey respondents were responsible for recording the dietary data; however, in the case of the children's survey, parents or guardians recorded the data. Dietary and Nutritional Survey of British Adults (DNSBA) 1986-1987 is composed of more than 2,190 7-day weighted dietary records for respondents aged 16 to 64, whereas NDNS 1992-1993 provides data for a total of 1,592 children aged 1½ to 4½ years. Seven-day records capturing the dietary records for approximately 1,700 youth aged 4 to 18 years is contained within the 1997 NDNS (UKDA, 1991, 1995, 2001). The dietary data obtained from the children's survey (4-day data) was weighted to 7-days in order to facilitate comparison with the adult and youth 7-day dietary survey data. Full details of the weighting method applied are provided in Appendix J of the report on the children's diet and nutrition survey (Gregory *et al*., 1995).

The individual proposed use-levels for STA from Super Refined™ Echium Oil employed in the current intake analysis are summarized in Table IX.a-1. The food type, and main and subsidiary food group classifications set forth in the NDNS reports were the basis of the MAFF food code list from with the representative food codes were chosen (UKDA, 1991, 1995, 2001). It should be noted that a given food code may not be associated with all 3 surveys; as with each new survey the food code list has been updated to reflect recent changes in the availability of some foods.

Estimates for the total daily intakes of STA from Super Refined™ Echium Oil from all proposed food-uses are provided in Table IX.a-2, while Table IX.a-3 provides total daily intakes on a per kilogram body weight per day basis. As would be expected for a 7-day survey, the percentage of users was high among all age groups evaluated in the current intake assessment; greater than 94.3% of the population groups consisted of users of those food products in which Super Refined™ Echium Oil is currently proposed for use (Table IX.a-

2). Young people had the greatest percentage of users at 99.6%. Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates.

Of the individual population groups, male adults were determined to have the greatest mean and 97.5th percentile all-user intakes of stearidonic acid from echium oil on an absolute basis, at 1,128 (equivalent to approximately 5 to 6 daily servings of 200 mg STA) and 2,175 mg/person/day (equivalent to approximately 11 daily servings of 200 mg STA), respectively, while children had the lowest intakes of 719 (approximately 3 to 4 servings of 200 mg STA) and 1,351 mg/person/day (approximately 7 servings of 200 mg STA), respectively (Table IX.a-2). Female teenagers had higher mean and 97.5th percentile intakes than children with values of 804 and 1,594 mg/person/day (approximately 4 and 8 servings, respectively of 200 mg STA); however, these intakes were lower than those reported by young people aged 4 to 10 years. Males had higher mean and 97.5th percentile intakes than women, and STA from Super Refined^{TM} Echium Oil intakes increased with age.

Table IX.a-2 Summary of the Estimated Daily Intake of STA from Super RefinedTM Echium Oil from All Proposed Food Categories in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption				All-User Consumption			
				Mean (mg)	Percentile (mg)			Mean (mg)	Percentile (mg)		
					90	95	97.5		90	95	97.5
Children	1½ to 4½	98.8	1628	719	1053	1216	1354	719	1053	1208	1351
Young People	4 to 10	99.6	834	860	1234	1371	1561	860	1245	1374	1561
Female Teenagers	11 to 18	97.8	436	805	1265	1403	1594	804	1271	1418	1594
Male Teenagers	11 to 18	99.5	414	1056	1647	1873	2076	1057	1647	1873	2091
Female Adults	16 to 64	94.3	903	866	1325	1507	1692	871	1326	1520	1693
Male Adults	16 to 64	95.0	728	1124	1751	1932	2189	1128	1752	1928	2175

Conversely, on a body weight basis, children were identified as having the highest intakes of any population group, with mean and 97.5th percentile all-user stearidonic acid from echium oil intakes of 51 and 103 mg/kg body weight/day, respectively, while female adults had the lowest mean and 97.5th percentile intakes, respectively, at 13 and 26 mg/kg body weight/day (Table IX.a-3). In contrast to the values reported on an absolute basis, the intakes observed on a per kilogram body weight/day basis decreased as the age of the survey respondents increased, although males still reported higher intakes than females.

Table IX.a-3 Summary of the Estimated Daily Per Kilogram Body Weight Intake of STA from Super Refined™ Echium Oil from All Proposed Food Categories in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption				All-User Consumption			
				Mean (mg)	Percentile (mg)			Mean (mg)	Percentile (mg)		
					90	95	97.5		90	95	97.5
Children	1½ to 4½	98.8	1,628	50	76	86	103	51	76	86	103
Young People	4 to 10	99.6	834	34	51	58	68	34	51	58	68
Female Teenagers	11 to 18	97.8	436	15	25	28	32	15	25	28	32
Male Teenagers	11 to 18	99.5	414	20	32	35	39	20	32	35	39
Female Adults	16 to 64	94.3	903	12	20	23	26	13	20	23	26
Male Adults	16 to 64	95.0	728	13	22	24	28	14	22	25	28.

Overall, it can be seen that even for the highest intake scenario of male adults consumption of Super Refined™ Echium Oil, when measured as STA can be seen not to exceed the equivalent of 11 servings of food maximally dosed, or approximately 2200 mg of STA. Mean consumption for the same group is equivalent to approximately 5 to 6 daily servings of 200 mg STA (approximately 1,128 g per person per day), which would be more representative of upper level consumption in the practical sense. This is clearly a significant over-estimation of realistic intakes, as it would be extremely unlikely for a person to choose so many products, maximally dosed with Super Refined™ Echium Oil. Safety endpoints are discussed in Section XIII, with the conclusion that Super Refined™ Echium Oil is safe to be consumed at levels of at least 1.9 g of STA per day. It is clear that realistically the estimated daily intakes presented above will not exceed this endpoint.

A full report on these intakes estimates is provided as Appendix 3.

IX.b At Risk Groups

Animal and human studies have demonstrated that STA can be converted efficiently into EPA (Miles *et al.*, 2004b; Surette *et al.,* 2004; Cleland *et al.,* 2005). EPA displaces arachidonic acid (AA) in platelet membranes, resulting in an alteration in eicosanoid production in favour of platelet anti-aggregatory mediators. Because 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. STA in echium oil is efficiently converted into EPA but not DHA; thus, as a conservative safety measure, it should be recommended that consumption of excessive amounts of echium oil by individuals receiving anticoagulant therapy be avoided.

In a 4-week study assessing the effects of echium oil on blood lipid profiles in 11 subjects with mild to moderate hypertriglyceridemia, the intake of approximately 1.9 g per day STA (administered as 15 g per day echium oil) resulted in a significant (21%; $P<0.05$) reduction from baseline in serum triacylglycerol concentrations (Surette *et al.,* 2004). Although,

echium oil may cause triglyceride levels to decrease in healthy subjects with normal or low triglyceride levels, these effects have typically not been considered with other nutritional products known to reduce triglyceride levels (*i.e.,* soy, fish oil, certain fibres).

IX.c Geographical Restrictions

There are no geographical restrictions anticipated, within the European Union for the introduction of this product.

IX.d Replacement of Other Foods in the Diet

It is anticipated that Super Refined^{TM} Echium Oil will be consumed for example by vegetarians as an alternative to fish oil, flax oil, borage (starflower) oil and other sources of omega-3 fatty acids.

XI NUTRITIONAL INFORMATION ON THE NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to nutritional information available on the novel food (Commission of the European Communities, 1997):

a. “Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?”

We will address each point in turn in this section.

XI.a Comparison of Lipid Profile to Traditional Counterparts

The lipid profile for Crossential Super Refined™ Echium Oil is similar to that of Borage oil and Blackcurrant oil (Table XI.a-1). Both borage oil and blackcurrant oil are widely used as ingredients of cosmetics, pharmaceuticals, foods and food supplements (Williams, 1976; Societe des Produits Nestle, 1983).

Table XI.a-1 Comparison Fatty Acid Profiles of Various Seed Oils

Fatty Acid	**% Composition of Total Fatty Acids (GLC)**		
Type of Oil	**Borage Oil**	**Blackcurrant Oil**	**Crossential Super – Refined™ Echium Oil**
C16:0;PA	10.0	6.9	6.0
C18:0;SA	3.39	1.4	3.5
C18:1*cis* n-9;OA	16.4	11.8	17.2
C18:2 n-6;LA	38.8	44.7	18.6
C18:3 n-3;ALA	0.5	11.4	29.5
C18:3 n-6;GLA	20.7	16.3	10.2
C18:4 n-3;STA	0.1	3.0	12.6
C20:0; ADA	0.2	-	Trace – 1.3
C20:1 n-9; GA	3.8	0.9	0.8
C22:1 n-9; EA	2.5	-	0.1

The major fatty acids found in Crossential Super Refined™ Echium Oil are as follows:

Palmitic acid

Palmitic acid is the most widely occurring saturated fatty acid and is present in most commercial oils (Gunstone *et al.*, 1986). It is found in large quantities in fish oils (10 to 30%) and tropical fats such as coconut (6.9%), palm kernel (6.5 to 11%) and palm (32 to 59%) oils (Gunstone *et al.*, 1986; Horrobin, 1990a,b). Crossential Super Refined™ Echium Oil contains on average 6.0% palmitic acid.

Stearic acid

Stearic acid is found in abundance in tallow (5 to 30%), cocoa butter (30 to 36%) and shea nut butter (44%) (Gunstone *et al.*, 1986; Erasmus, 1993). Crossential Super Refined™ Echium Oil contains on average 3.5% stearic acid.

Oleic acid (OA)

Oleic acid is the most widely occurring natural fatty acid and is found in practically all lipids (Gunstone *et al.*, 1986). It is found in large quantities in olive (43.7 to 83%), almond (65 to 70%) and peanut (37.9%) oils (Erasmus, 1993). Oleic acid is also manufactured in the body

(Gunstone *et al*., 1986; Erasmus, 1993). Crossential Super Refined™ Echium Oil contains on average 17.2% oleic acid.

Linoleic acid (LA)

Linoleic acid is found in safflower (75.3%), sunflower (68.5%), soybean (53%) and sesame (45%) oils (Gunstone *et al*., 1986; Erasmus, 1993). Crossential Super Refined™ Echium Oil contains on average 18.6% linoleic acid.

Alpha linolenic acid (ALA) (C18:3n-3)

Linolenic acid is the major fatty acid found in plant leaves, stems and roots and other photosynthetic organisms (Gunstone *et al.*, 1986). Flax seed is the richest source of ALA with over 50%; Chia and kukui (candlenut) contain about 30%, and hemp seed around 20% (Erasmus, 1993). Pumpkin seed oil may have up to 15%, canola up to 10% and walnut between 3 to 11% (Erasmus, 1993). Soybean oil normally contains 5 to 7% (Erasmus, 1993). Crossential Super Refined™ Echium Oil contains on average 29.5% ALA.

Gamma linolenic acid (GLA)

The richest source of gamma-linolenic acid (GLA) is borage oil (20%) followed by black currant seed oil (15%) and evening primrose oil (9%) (Erasmus, 1993). Crossential Super Refined™ Echium Oil contains on average 10.2% GLA.

Stearidonic acid (STA) (C18:4n-3)

STA is found in fish oils such as mackerel (2.5%), herring (1.1 to 2.8%), sardine (2.9%) and menhaden (0.8 to 3.6%) (Gunstone *et al.*, 1986). The most well-known plant source of stearidonic acid is black currant seed oil (3%) (Erasmus, 1993). Crossential Super Refined™ Echium Oil contains a minimum of 12.6% STA.

XI.b Nutritional Equivalence to Existing Foods

From a nutritional safety perspective Crossential Super Refined™ Echium Oil is considered to be substantially equivalent to existing oils and fats on the market, which are rich in essential fatty acids. Essential fatty acids is a term used to describe fatty acids which are needed in order to manufacture body lipids, biological membranes and hormone like substances such as prostaglandins but which cannot be synthesised in the body and therefore must be obtained from the diet (Brooks, 1984, Newton, 1996). Only two fatty acids are truly essential, linoleic acid and *alpha*-linolenic acid, the remaining polyunsaturated fatty acids are derived from these by a sequence of desaturation and elongation steps (Figure X1.a.1). Linoleic acid is the precursor for the omega-6 series of fatty acids which are found primarily in plant oils whereas *alpha*-linolenic acid is the precursor for the omega-3 series of fatty acids which occur mainly in green leafy vegetables and oily fish (Newton, 1996).

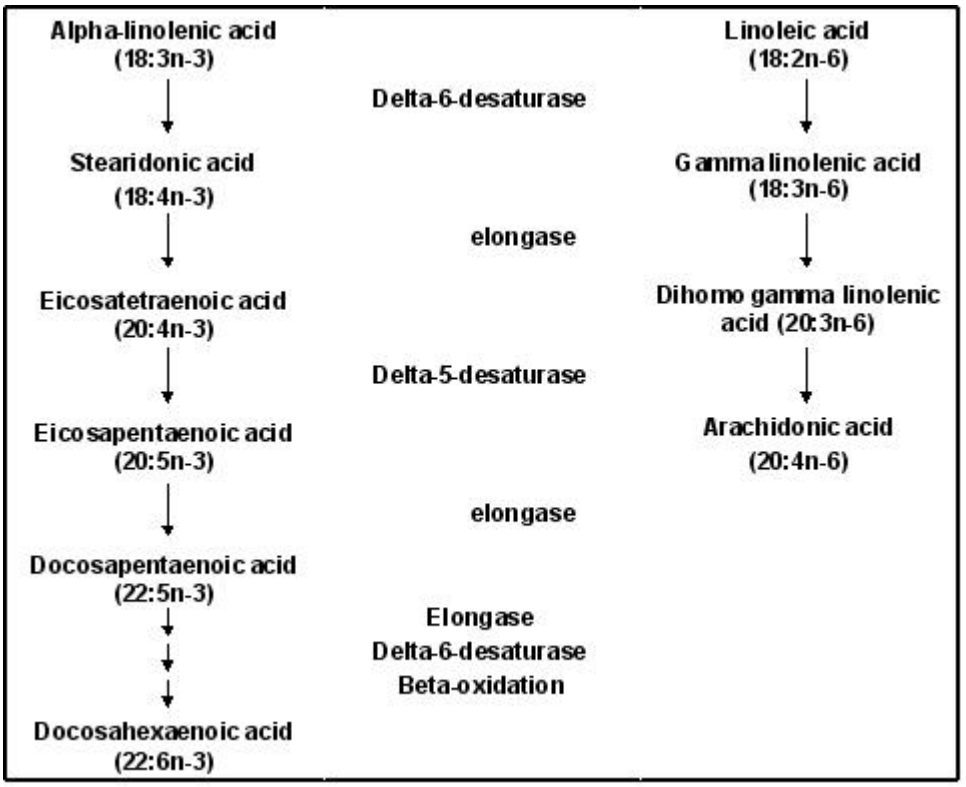


Figure X1.a-1 Elongation and Desaturation of n-3 and n-6 Polyunsaturated Fatty Acids

The n-3 and n-6 fatty acids compete for the same enzymes in the synthesis of their respective long-chain polyunsaturated fatty acids.

Both series of essential fatty acids are the starting materials for the manufacture of a group of complex hormone like compounds known collectively as eicosanoids, which include the prostaglandins, leukotrienes, prostacyclins and thromboxanes. The eicosanoids have profound physiological activity even at extremely low concentrations. They are implicated in the functions of the nervous, cardiovascular and immune systems and can also affect the function of both the endocrine and exocrine glands.

The correct balance between the various eicosanoids is required in order to maintain good health. The ratio of omega-6:omega-3 fatty acids in the body is about 1:1 in the brain, 5:1 in fat tissue and 4:1 in other tissues (Erasmus, 1993). The levels of the eicosanoids can vary during different stages in the development of the body, with age and during the menstrual cycle. Delta-6-desaturase is the rate-limiting step in the synthesis of long-chain polyunsaturated fatty acids; in addition, the activity of delta-6-desaturase is known to be inhibited by a number of factors, including diabetes, stress, excess saturated fats, high alcohol intake, smoking and viral infections. This can lead to deficiencies in the levels of the various essential fatty acids (Horrobin, 1995). The same enzymes are used to metabolise both the omega-3 and the omega-6 series of essential fatty acids and it is believed that the metabolites of *alpha*-linolenic acid will compete for these enzymes with the metabolites of linoleic acid.

Echium oil contains a relatively high amount of STA, which is the fatty acid that is normally synthesized from alpha-linolenic acid *via* the rate-limiting delta-6-desaturase enzyme. STA in echium oil has been shown to act as a precursor for the long chain omega-3 fatty acid eicosapentaenoic acid EPA in both animal and human studies (Miles *et al.*, 2004b; Surette *et al.,* 2004; Cleland *et al.,* 2005). Thus, like flaxseed oil, which contains alpha-linolenic acid at approximately 50% of total fatty acids, STA-rich echium oil may be an important terrestrial source of n-3 fatty acids. The role in the body of these polyunsaturated fatty acids and their various recommended daily levels in EU Member States are discussed in the recent European Food Safety Authority, NDA Panel Opinion related to nutrition claims concerning omega-3 fatty acids, monounsaturated fat, polyunsaturated fat and unsaturated fat (EFSA, 2005).

XII MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to microbiological information available for the novel food (Commission of the European Communities, 1997):

a. "Is the presence of any microorganisms or their metabolites due to the novelty of the product/process?"

We will address this point in the following section.

XII.a Presence of Microorganism/ their Metabolites

Crossential Echium Oil is an anhydrous system and therefore will not support microbiological growth. In addition the chromatographic technique used in Super Refining™ will act to filter out any microbial organisms. The absence of microbiological contamination has been confirmed by testing samples of the oil.

The results of independent microbiological analysis are presented in Appendix 1 (part J) and summarised in Table II.c-1 below. The results all show the absence of microbiological contamination, which is to be expected from a product which is solvent extracted, distilled and of extremely low water activity.

Table II.c.3-1 Microbiology Test Results

Sample Code	**EAL 121A (703-2006-00039551)**	**EAL 121B (703-2006-00039552)**	**EAL 121C (703-2006-00039553)**	**EAL 121D (703-2006-00039554)**
Osmophilic yeasts (cfu/g)	<10	<10	<10	<10
Yeast (cfu/g)	<10	<10	<10	<10
Moulds (cfu/g)	<10	<10	<10	<10
Enterobacteria (cfu/g)	<10	<10	<10	<10
Staphylococcus aureus (cfu/g)	<10	<10	<10	<10

The manufacturing plant for Super Refined™ Echium oil has an independently certified HACCP system in place (Appendix 2).

XIII TOXICOLOGICAL INFORMATION ON THE NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to toxicological information available on the novel food (Commission of the European Communities, 1997):

a. “Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?”

b. “Compared to the traditional counterpart, does the novel food contain any new toxicants or changed levels of existing toxicants?”

OR

c. “Is there information from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use?”

d. “Is there information which suggests that the novel food might pose an allergenic risk to humans?”

We will address each point in turn in this section.

XIII.a Toxicology Studies

XIII.a.1 Toxicology Studies on Echium Oil

The animal and human studies that have been conducted on echium oil itself are summarised in Table XIII.a.1-1. It is important to note that these studies have been conducted principally for research rather than pure safety purposes.

Table XIII.a.1-1 Summary of Oil Profiles and Dose Levels of Studies Using Echium Oil and Comparison to Crossential Echium Oil

Study	Fatty Acid Composition g/100g total fatty acids			
	Crossential Super Refined™ Echium Oil	**Cleland *et al.*, 2005 Rat**	**Miles *et al.*, 2004a,b Humans**	**Surette *et al.*, 2004- (Crossential Super Refined™ Echium Oil) Humans**
C16:0 Palmitic Acid	6.0	7.0	5.5	6.0
C18:0 Stearic Acid	3.5	3.1	2.2	3.7
C18:1 Oleic Acid	17.2	13.9	11.7	15.4
C18:2 Linoleic Acid	18.6	15.5	24.8	18.8
C18:3 (n-3) Alpha-Linolenic Acid	29.5	35.4	33.4	28.4
C18:3 (n-6) Gamma-Linolenic Acid	10.2	10.0	10.7	11.0
C18:4 Stearidonic Acid	12.6	12.5	11.7	12.5
Study Details				
Dose level Echium oil		5% in diet	8.3 g/day	15 g/day
Dose level of STA		0.6% in diet	1.0 g/day	1.9 g/day
Number of Subjects per group		4	8-12 & 8-12	11
Duration of Study (weeks)		4	12	4

XIII.a.1.1 Animal Studies

Whilst there have been no specific toxicology studies conducted in animals with echium oil, we have identified one study for nutritional research purposes, which may provide some insight into its metabolism in relation to conventional oil sources and therefore its relative safety.

4-week Dietary Exposure in the Rat (Cleland et al., 2005)

Six-week-old Dark Agouti rats (n=4 per group) were fed diets containing sunflower, flaxseed, echium and canola oils at 5% w/w in the diet for 4 weeks. The echium oil contained 12.5% STA. There was no statistically significant difference in weights between the groups either at

baseline of after the 4-week intervention. Following sacrifice the LC n-3 PUFA concentrations were measured in the heart and plasma phospholipids.

The relative potencies of the vegetable oils in raising cardiac EPA was flaxseed>echium>canola>sunflower. For elevation of docosapentaenoic acid (DPAn-3), the Echium and flaxseed diets were similar to each other and both were twice as effective as canola. For DHA, canola had the greatest effect in increasing levels followed by echium and flaxseed. The relative efficacy of each of the vegetable oils in elevating plasma phospholipid LC n-3 PUFA was the same scale as for cardiac. The authors concluded that echium may provide a useful oil for elevating EPA and DPA n-3 in the body. The apparent "superiority" of canola oil over flaxseed oil and echium oil in raising cardiac and plasma phospholipid levels of DHA may have stemmed from the relatively low level of alpha-linolenic acid in canola oil (9.7% *vs.* 35.4% and 56.5% in echium and flaxseed oil, respectively). As can be seen in Figure X.1.a, alpha-linolenic acid competes with EPA for delta-6 desaturase activity; consequently, it may limit the amount of DHA produced from EPA.

XIII.a.1.2 Human Clinical Studies

12-week Clinical Study – Healthy Young Males

Part 1: Immune Function (Miles et al., 2004a)

Subjects consumed 9 g of echium oil per day containing 1 g of STA for a period of 12 weeks as 1 of 7 oil blends, to determine the effects of EPA, STA, or GLA on immune outcomes. Neither STA nor its derivative 20:4n-3 appeared in peripheral blood mononuclear cells (PBMC). However, STA in combination with GLA (0·9 g/d) increased the proserving of EPA in PBMC. None of the treatments altered neutrophil or monocyte phagocytosis or respiratory burst, production of inflammatory cytokines by monocytes, T lymphocyte proliferation or the delayed-type hypersensitivity response. Production of cytokines by T lymphocytes increased in all groups, with no differences among them. There were no other effects on lymphocyte sub-populations. Plasma IgE concentration decreased in most groups, but not in the control group. The authors concluded that STA from echium oil can increase immune cell EPA status, but at 1 g/day does not affect human immune function.

Part 2: Fatty Acid Composition in Blood Lipids and Mononuclear Cells (Miles et al., 2004b)

The second part of this study set out to identify whether STA from echium oil can be used to increase the EPA content of plasma lipids and cells in humans and to understand more about the effects of increased consumption of GLA, STA, and EPA in humans. Healthy young males were randomised to consume 1 of 7 oil blends for a period of 12 weeks (9 g oil/day) (n = 8–12 subjects/group). Palm oil, sunflower oil, an EPA-rich oil, borage oil (rich in GLA), and echium oil were blended in various combinations to generate a placebo oil and oils providing approximately 2 g GLA + STA + EPA per day, but in different combinations. Blood was collected at 0, 4, 8, and 12 weeks and the fatty acid compositions of plasma triacylglycerols, cholesteryl esters and phospholipids and of PBMCs determined. Significant effects were observed with each lipid fraction. Neither STA nor its derivative 20:4n-3

appeared in any of the lipid fractions studied when STA (up to 1 g/day) was consumed. However, STA (1 g/day), in combination with GLA (0.9 g/day), increased the proserving of EPA in some lipid fractions, suggesting that STA-rich plant oils may offer a novel means of increasing EPA status. Furthermore, this combination tended to increase the dihomo-g-linolenic acid (20:3n-6; DGLA) content of PBMCs, without an increase in AA (20:4n-6) content. EPA consumption increased the EPA content of all lipid fractions studied. Consumption of GLA (2 g/day), in the absence of STA or EPA, increased DGLA content with a tendency to increase AA content in some fractions. This effect was prevented by inclusion of EPA in combination with GLA. Thus, this study indicates that STA may be used as a precursor to increase the EPA content of human lipids and that combinations of GLA, STA, and EPA can be used to manipulate the fatty acid compositions of lipid pools in subtle ways. Such effects may offer new strategies for manipulation of cell composition in order to influence cellular responses and functions in desirable ways.

Additional data has since been published regarding the adverse events monitoring for this study. Subjects visited the laboratory 4 times, at 4 weekly intervals and in the fasting state. On each occasion they gave a blood sample and completed a questionnaire that included questions about health problems that they had experienced during the previous 4 weeks. Of the 74 subjects who commenced the study, 68 completed it. The results are summarised in Table XIII.a.1-2, which indicates the number of subjects in each group who reported problems during each of the 4-week periods of the study, and the nature of those problems, and the number of subjects in each group who reported problems during the entire 12-week period. Overall 36 subjects (49%) reported a health problem during the period of the study. The most commonly reported problem was the common cold. This is not surprising given that the study ran from late winter, through spring and into early summer. Gastrointestinal upsets, usually mild diarrhoea, were also reported by some subjects. Problems were reported by subjects in all groups, including the placebo group, and there appeared to be no group-specific pattern of problems. Chi-squared analysis of the number of subjects reporting problems did not reveal any significant differences among the groups (Miles *et al*., 2006).

Table XIII.a.1-2 Self-Reported Health Problems in Male Volunteers - Supplementing Their Diet with Capsules Containing Different Oils (Miles *et al*., 2006).

Group	Placebo	EPA	Borage oil	Echium oil	Blend 1	Blend 2	Blend 3
Number of subjects starting study	10	11	12	8	11	11	11
Number of subjects completing study	9	10	12	8	9	10	10
Reasons for withdrawal	Diagnosed with hypertension	Could not comply due to work commitments	-	-	Diarrhoea; Not specified	Nausea and gastrointestinal upset	Could not comply due to work commitments

Table XIII.a.1-2 Self-Reported Health Problems in Male Volunteers - Supplementing Their Diet with Capsules Containing Different Oils (Miles *et al*., 2006).

Group	Placebo	EPA	Borage oil	Echium oil	Blend 1	Blend 2	Blend 3
Number of subjects reporting problems over the period 0 to 4 weeks	2/10 2 x Cold	3/11 Cold Eye infection Tooth infection	0/12	1/8 Cold	2/11 Cold Gastrointestinal upset	1/11 Cold	5/11 3 x Cold Gastrointestinal upset Migraines
Number of subjects reporting problems over the period 4 to 8 weeks	2/10 Gastrointestinal upset Eye infection	3/11 Cold Headaches Constipation Heartburn	3/12 2 x cold Bloating	1/8 Cough	1/10 Cold	2/11 2 x Headaches Fever Gastrointestinal upset	3/11 2 x Cold 2 x Gastrointestinal upset
Number of subjects reporting problems over the period 8 to 12 weeks	2/9 Cold Hay fever	2/10 Cold Constipation Hay fever	1/12 Cold	5/8 3 x Cold Hay fever Leg infection (treated with antibiotics)	1/9 Gastrointestinal upset	3/10 Sore throat Hay fever Urinary tract infection (treated with antibiotics)	3/10 2 x Cold Hay fever
Number of subjects reporting problems over the period 0 to 12 weeks	5	5	4	6	5*	4	7*
Total number of problems reported over the period 0 to 12 weeks	6	10	4	7	4	8	12

4-week Clinical Study Asymptomatic Subjects with Mild to Moderate Hypertriglyceridemia (Surette et al., 2004)

The objective of this study was to investigate the effect of dietary echium oil (supplied by Croda), on tissue fatty acid content and serum triacylglycerol concentrations in hypertriglyceridemic humans. Asymptomatic subjects with mild-to-moderate hypertriglyceridemia were enrolled in an open-labelled study. Subjects (men and non-pregnant women =20 years old) underwent a 4-week lead-in period and were then instructed to follow the US National Cholesterol Education Program Step 1 diet. Subjects (n =11) whose serum triacylglycerol concentrations remained between 3.4 and 5.1 mmol/L (300 and 450 mg/dL) were instructed to consume 15 g of echium oil (approximately 1.9 g STA) daily for 4 weeks. During the treatment period, serum triacylglycerol concentrations decreased by 21%, or 0.87 ±0.26 mmol/L (mean ± SD) compared with baseline ($P < 0.05$); 8 of 11 subjects had a decrease in serum triacylglycerols ranging from 13 to 52% with a decrease from baseline of 30%, or 1.26 ±0.41 mmol/L (mean ± SD). There were no significant changes in

any other clinical laboratory variables. Concentrations of long-chain (n-3) PUFA, including EPA, increased (P< 0.05) in plasma and neutrophils when subjects consumed echium oil. Baseline and post-supplementation serum total cholesterol, LDL-C, or HDL-C concentrations did not differ significantly from baseline. Safety and tolerability were also measured throughout the study. During the course of the trial, 6 adverse events were reported. Cold symptoms occurred in 2 subjects and were assessed to be unrelated to the echium oil. Sinus headache and foot pain were also reported and found to be unrelated to the echium oil. Muscle pain in arms, and cough were reported and were rated as unlikely to be related to diet supplementation with echium oil. There were no significant differences between baseline values of vital signs and clinical laboratory markers (Table XIII.a.1-3). The authors concluded that dietary plant oils rich in STA are metabolised to longer-chain, more unsaturated (n-3) PUFA. These oils appear to possess hypotriglyceridemic properties typically associated with fish oils.

Table XIII.a.1-3 Summary of Clinical Laboratory Measurements for 4-Week Clinical Trial Conducted by Surette *et al*. (2004)*

	Baseline (day 0)	Visit 5 (day 28)
Glucose (mg/dL)	99.3 ± 3.2	102.1 ± 3.0
Creatinine (mg/dL)	1.0 ± 0.05	1.0 ± 0.1
Sodium (mEq/L)	138.0 ± 0.5	138.0 ± 0.9
Potassium (mEq/L)	4.4 ± 0.1	4.3 ± 0.2
Calcium (mg/dL)	9.5 ± 0.1	9.4 ± 0.1
Alkaline Phosphatase (U/L)	65.9 ± 8.4	65.2 ± 4.9
SGOT (AST) (U/L)	32.9 ± 2.7	29.9 ± 2.9
SGPT (ALT) (U/L)	28.2 ± 3.9	27.5 ± 4.4
Total Bilirubin (mg/dL)	0.7 ± 0.04	0.6 ± 0.03
Albumin (g/dL)	4.1 ± 0.1	4.1 ± 0.1

* We are very grateful to Professor Floyd “Ski” Chilton of the Department of Physiology and Pharmacology at Wake Forest University School of Medicine, North Carolina, on behalf of the authors of this study, for the provision of this additional unpublished data.

XIII.a.2 Toxicology Studies on Stearidonic Acid (STA)

Table XIII.a.2-1 Summary of Oil Profiles and Dose Levels of Studies Using Stearidonic Acid Esters

	Fatty Acid Composition g/100 g total fatty acids			
Study	**Crossential Echium Oil**	**Ishihara *et al.*, 2002 – mice**	**Hansen Petrik *et al.*, 2000**	**James *et al.*, 2003**
C16:0 Palmitic Acid	7.2	4.4	23.5	Not available
C18:0 Stearic Acid	3.8	1.6	13.5	Not available
C18:1 Oleic Acid	17.3	66.3	27.0	Not available
C18:2 Linoleic Acid	16.2	14.9	18.7	Not available
C18:3 (n-3) Alpha-Linolenic Acid	31.0	0.6	1.77	Not available
C18:3 (n-6) Gamma-Linolenic Acid	10.8	0.1	ND	Not available
C18:4 Stearidonic Acid	11.9	10.0	11.31	Not available
Study Details				
Dose level oil blend		10% in diet	35% in diet	
Dose level of STA		1% in diet	3% in diet	Up to 1.5 g per day
Number of Subjects per group		7	9-10	15
Duration of Study (weeks)		3	7	3

XIII.a.2.1 Metabolic Studies

In vitro Study - Modification of liver fatty acid metabolism in mice by n-3 and n-6 delta 6-desaturase substrates and products (Huang et al., 1991)

The effects of dietary supplementation of either ALA or STA in combination with either LA or GLA (18:3(n-6)) on liver fatty acid composition in mice were examined. Essential fatty acid deficient male C57BL/6 mice were separated into 4 groups of 7 each and were fed a fat-free semi-purified diet supplemented with 1% (w/w) fatty acid methyl ester mixture (1:1), LA/ALA, LA/STA, GLA/ALA, or GLA/STA. After 7 days on the diets, fatty acid compositions in liver phosphatidylcholine and phosphatidylethanolamine fractions were analyzed. In groups fed STA (LA /STA) or GLA/STA as compared to those fed ALA (LA /ALA) or GLA/ALA, the levels of STA, EPA and DHA were increased, whereas those of DGLA and ARA were decreased. When GLA replaced LA as the source of n-6 acids, the levels of GLA, DGLA, ARA and DPA n-6 were increased, whereas those of STA and EPA were reduced. Replacing ALA by STA reduced the (n-6)/(n-3) ratio by approx. 30%, whereas replacing LA by GLA increased the (n-6)/(n-3) ratio by approximately 2-fold. These findings indicated that delta 6-desaturase products were metabolised more readily than their precursors. Both products also competed for the subsequent metabolic enzymes. However, the n-6 fatty acids derived from GLA were

incorporated more favourably into liver phospholipids than n-3 fatty acids derived from 18:4(n-3).

In vivo - Comparison of the conversion rates of ALA and STA to longer polyunsaturated fatty acids in rats (Yamazaki et al., 1992)

The conversion rate between ALA and STA was compared by feeding rats on a lipid-free diet supplemented with lard (9%, w/w) and ALA ethyl ester (1%) diet or on a diet containing lard (9%) and STA ethyl ester (1%). A lard (10%)-supplemented diet was used as the control diet. The fatty acid compositions of total phospholipids, triglycerides and free fatty acids of both liver and plasma were measured after 1 or 3 weeks on different diets. The molar ratio of EPA of most lipid fractions was about 2-fold higher in rats fed the STA-supplemented diet than in rats fed the ALA-supplemented diet. STA was found in the liver lipid fraction in only a very small amount, even in the STA supplemented groups. The authors suggested that desaturation at C-6 is the rate-limiting step in the conversion of ALA to EPA.

XIII.a.2.2 Animal Studies

3-week Dietary Exposure in the Mouse – Comparison of the effects of dietary alpha-linolenic, stearidonic, and eicosapentaenoic acids on production of inflammatory mediators in mice (Ishihara et al., 2002)

The effects of dietary stearidonic acid (18:4n-3) on inflammatory mediator release in whole blood and splenocytes was investigated in Balb/c mice, and the effects were compared with those of 2 other n-3 PUFA: ALA and EPA. TAG mixtures containing 10% of STA, ALA, or EPA as the respective sole n-3 PUFA were enzymatically synthesized. Diets containing synthesized TAG mixtures were fed to Balb/c mice for 3 weeks. Initial body weight, body weight gain, total food intake and relative liver weight were not different among the dietary groups. The release of prostaglandin F2 (PGE2) and tumour necrosis factor (TNF) were measured in whole blood and splenocytes stimulated with lipopolysaccharide. In whole blood, the production of TNF was suppressed by all dietary n-3 PUFA (ALA, STA, and EPA) as compared with the control diet, which contained TAG prepared from safflower oil. PGF2 production was not significantly changed. Differences among the n-3 PUFA (18:3n-3, 18:4n-3, and 20:5n-3) were not observed. In splenocytes, PGE2 production was suppressed by dietary n-3 PUFA, but TNF production was not. GLC analysis of plasma and splenocyte FA profiles showed an increase in the levels of 20:4n-3, 20:5n-3, and 22:6n-3 in mice fed the diet containing 18:4n-3.

7-week Dietary Exposure in the Mouse - Highly unsaturated (n-3) fatty acids, but not alpha-linolenic, conjugated linoleic or gamma-linolenic acids, reduce tumorigenesis in Apc(Min/+) mice (Hansen Petrik et al., 2000)

This study was undertaken to evaluate the antitumorigenicity of ethyl esters of ALA, STA, EPA, DHA, conjugated linolenic acid (CLA) and GLA compared with oleic acid at a level of 3 g/100 g in the diets of APC:(Min/+) mice and to determine whether any alterations in

tumorigenesis correspond to alterations in prostaglandin biosynthesis. Tumour multiplicity was significantly lower by approximately 50% in mice fed SDA or EPA compared with controls, whereas less pronounced effects were observed in mice fed DHA (P: = 0.15). ALA, CLA and GLA were ineffective at the dose tested. Although lower tumour numbers coincided with significantly lower prostaglandin levels in STA- and EPA-fed mice, ALA and DHA supplementation resulted in equally low prostaglandin levels, despite proving less efficacious with regard to tumour number. Prostaglandin levels did not differ significantly in the CLA and GLA groups compared with controls. The authors speculate that these results suggest that STA and EPA attenuate tumorigenesis in this model and that this effect may be related in part to alterations in prostaglandin biosynthesis. Repeated measures ANOVA showed no significant difference in weekly body weights among the dietary groups throughout the study. Food intake was not significantly different among the groups at each time point by one-way ANOVA. However, repeated measures ANOVA revealed a significant difference in intake over time. This was most likely due to the decline in food intake in the GLA group during the final 2 weeks, probably related to the increasing tumour burden toward the end of the study.

XIII.a.2.2 Human Clinical Studies

3-week Clinical Study in Normal Subjects - Metabolism of stearidonic acid in human subjects: comparison with the metabolism of other n-3 fatty acids. (James et al., 2003)

The objective of this study was to examine the ability of dietary STA to increase tissue concentrations of EPA and DHA in healthy human subjects and to compare the effectiveness of STA with that of the n-3 fatty acids ALA and EPA. Its effects on cytokine and eicosanoid synthesis and plasma lipids were also measured. Encapsulated STA, ALA, or EPA (as methyl ester) was ingested daily in doses of 0.75 g and then 1.5 g for periods of 3 weeks each by healthy male and postmenopausal female subjects (n = 15/group) in a double-blind, parallel-group design. Dietary STA increased EPA and DPA (n-3) concentrations but not DHA concentrations in erythrocyte and in plasma phospholipids. The relative effectiveness of the tested dietary fatty acids in increasing tissue EPA was 1:0.3:0.07 for EPA:STA:ALA. The test fatty acids had no consistent effect on the lipopolysaccharide-stimulated synthesis of prostaglandin E2, and thromboxane A2 synthesis during blood clotting was not affected by any of the test fatty acids. There were no significant differences between groups in concentrations of fasting triacylglycerol or of total, LDL, or HDL cholesterol.

XIII.a.3 Conclusions from Toxicology Studies

Echium is a well-defined member of the Boraginaceae family and has been studied extensively at both a whole plant and extracted oil level. Critical risk factors of pyrrolizidine alkaloid and cytochrome C allergen levels been identified, and extensive analysis has shown these contaminants are removed by the Super-Refining™ process. The composition of echium oil is well defined and comparable to other plant oils used as foods. There are no new fatty acids introduced into the diet, with the most significant difference being the higher

level of STA. The metabolism of STA and STA-rich echium oil have been extensively studied in both animal and human models, with expected significant effect of STA in increasing EPA levels (but not DHA levels) in the bloodstream. From a human nutritional safety perspective, the two most important clinical studies to date in echium oil were conducted at dose levels of STA of up to 1.9 g per person per day and for periods of up to 12 weeks. Echium oil was found to have no significant effect on immune function (Miles *et al.,* 2004a); to decrease serum triglycerides; and to have no significant effect on cholesterol, LDL-C or HDL-C concentrations (Surette *et al.*, 2004). In both studies safety and tolerability were monitored with no significant adverse effects compared to baselines attributable to the echium oil. These data are largely supported by a human clinical study conducted with STA esters at a dose of 0.75g per day for 3 weeks followed 1.5 g STA per day for 3 weeks (James *et al.,* 2003).

At the proposed maximum demonstrated safe intake level in humans of 1.9 g per person per day, and a proposed maximum use level in the proposed food use groups of 200 mg of STA per daily serving, this would represent approximately 9 to 10 daily servings. This is comparable to the highest intake scenario of male adults consumption of Super Refined™ Echium Oil, when measured as STA, which does not exceed the equivalent of 11 servings of food maximally dosed, or approximately 2,200 mg of STA at the 97.5th (Section IX). This is clearly a significant over-estimation of realistic intakes, as it would be extremely unlikely for a person to choose so many products, maximally dosed with Super Refined™ Echium Oil. Mean consumption for the same group is equivalent to approximately 5 to 6 daily servings of 200 mg STA (approximately 1,130 mg per person per day), which would be more representative of upper level consumption in the practical sense.

OVERALL CONCLUSIONS

Crossential Super Refined™ Echium Oil is a rich, safe and suitable vegetarian source of a number of important fatty acids, notably stearidonic acid (C18:4 n-3). The Super Refined™ oil contained no detectable toxins, allergens or contaminants that are commonly associated with this species and the Boraginaceae family in general, a family which includes borage oil, which has been consumed for many years in the EU. The production process has been shown to be tightly controlled and highly reproducible. For the proposed food uses the mean and 97.5th percentile intakes for the highest intake group (adult males) are equivalent to approximately 5 to 6 daily servings and 11 daily servings of 200 mg STA respectively (approximately 1,130 mg and 2,200 mg per person per day). The composition of Super Refined™ Echium Oil raises no concerns on a toxicological level and study data relies predominantly on human clinical studies that present STA from echium oil at levels up to 1.9 g per day. Clearly in the practical sense predicted intakes will not exceed this level.

GLOSSARY/LIST OF ABBREVIATIONS

(n-3) PUFA	Omega-3 polyunsaturated fatty acid
AA	Arachidonic acid
ACNFP	Advisory Committee on Novel Foods and Processes
ANOVA	Analysis of variance
C14:0; MA	Myristic acid
C16:0; PA	Palmitic acid
C16:1 n-9; POA	Palmitoleic acid
C18:0 SA	Stearic acid
C18:1 n-9; OA	Oleic acid
C18:2 n-6; LA	Linoleic acid
C18:2 n-6; CLA	Conjugated linoleic acid
C18:3 n-3; ALA	*alpha*-Linolenic acid
C18:3 n-6; GLA	*Gamma*-Linolenic acid
C18:4 n-3; STA	Stearidonic acid
C20:0; ADA	Arachidic acid
C20:1 n-9; GA	Gondoic Acid
C20:3 n-6; DGLA	Dihomo-*gamma*-linolenic acid
C20:4 n-3; STA	Stearidonic acid
C20:4 n-6; ARA	Arachidonic acid
C20:5 n-3; EPA	Eicosapentaenoic acid
C22:0; BA	Behenic acid
C22:1 n-9; EA	Erucic acid
C22:5 n-6; DPA n-6	Docosapentaenoic acid
C22:6 n-3; DHA	Docosahexaenoic acid
C24:1 n-9; NA	Nervonic acid
CAS	Chemical Abstract Service
CI-MS	Chemical ionization – Mass Spectroscopy
Cu	Copper
DNSBA	Dietary and Nutritional Survey of British Adults
E. plantagineum	*Echium plantagineum*
EI	Electron Impact Ionization
GLC	Gas Chromatography/Gas Liquid Chromatography
GLC-MS	Gas Liquid Chromatography - Mass Spectrometry
HDL-C	High-density lipoprotein cholesterol
IgE	Immunoglobulin E
LDL-C	Low-density lipoprotein cholesterol
NF	Novel Food
PA	Pyrrolizidine alkaloids
PAH	Paracyclic Aromatic Hydrocarbon
PBMC	Peripheral blood mononuclear cells
PGE2	Prostaglandin E2
RAST	RadioAllergoSorbent Test

SCF	European Commission's Scientific Committee on Food (now replaced by EFSA)
SD	Standard deviation
STA	Stearidonic Acid
TNF	Tumour necrosis factor
UK	United Kingdom
Zn	Zinc

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