



120th Avenue NE
Bellevue
WA
980005

Felicia B. Satchell
Director
Division of Standards and Labeling Regulations
Office of Nutritional Products, Labeling, and Dietary Supplements (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD, 20740-3835

June 18, 2002

Dear Felicia Satchell,

Re: Premarket Notification for a New Dietary Ingredient

Further to my telephone conversation with Rhonda Kane, I have made amendments to our Premarket Notification for Echium Oil.

In compliance with Dietary Supplement Health and Education Act of 1994 and in accordance with 21 CFR 190.6, Pharmax LLC hereby makes its official Premarket Notification for a new dietary ingredient, Echium oil. Accordingly, please find five (5) copies of this Notification enclosed.

Please be advised as follows:

1. The name and address of the distributor of the dietary supplement that contains a new dietary ingredient:

**Pharmax LLC
120th Avenue NE,
Bellevue
WA 980005**

2. The name of the new dietary ingredient that is the subject of the premarket notification:

Echium plantagineum Seed Oil

3. A description of the dietary supplement or dietary supplements that contain the new dietary ingredient:

- 1) Soft gel gelatine capsule 1000mg Echium oil.**
- 2) 300ml of liquid fatty acid nutritional supplement containing Echium Oil**

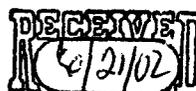
i) the level of the new dietary ingredient is:

- 1) 1000mg Echium oil per capsule**
- 2) 300ml of Echium oil per bottle**

ii) the conditions of use recommended or suggested in the labelling of the dietary supplement:

Suggested Use:

- 1) One (1) or two (2) capsules to be taken daily as a dietary supplement, preferably at mealtimes, or as directed by a healthcare provider.**
- 2) 5ml of oil to be taken per day**





4. The history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labelling of the dietary supplement, will be reasonably be expected to be safe:

See Safety Evaluation of Echium Oil (Section 6 of Premarket Notification)

Enclosed, please find documentation that establishes that the dietary ingredient, Echium Oil, will reasonably be expected to be safe when used under the conditions suggested on the label. This documentation includes Certificates of Analysis, Product Specification Sheets and a safety evaluation of Echium oil.

Please could you confirm receipt of this Premarket Notification.

Thank you for your time and attention to this matter. If you have any questions or comments, please do not hesitate to contact the undersigned.

Yours faithfully,

Pharmax LLC

Cathryn E. Wood

**Cathryn E Wood
Technical Scientist**

cathryn.cultech@btinternet.com

Tel: 01792 472110

Fax: 01792 472466

**Unit 3
Christchurch Road
Baglan Industrial Park
Port Talbot
SA12 7BZ
United Kingdom**

Premarket Notification for a New Dietary Ingredient, Echium Oil.

Author: Cathryn E. Wood



Date of original notification: 7th May 2002
Date of amended notification: 17th June 2002

RECEIVED
16/06/02
Rec'd 5 Copies¹



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¹ These companies have kindly allowed us to include their Specification Sheet and Certificate of Analysis for Echium Oil



1. Administrative Data

1.1 The name and address of the applicant and distributor of the dietary supplement that contains a new dietary ingredient:

Name of applicant: **Pharmax LLC**
Address of applicant: **120th Avenue NE,
Bellevue
WA 980005
USA**
Tel: 425-467-8054
Fax: 425-467-9112
Email: hq@pharmaxllc.com
Contact: Pablo Abrahams (secondary contact)

1.2 The name and address of the contract packers of the dietary supplement that contains a new dietary ingredient:

Name of contract packagers : **Cultech Limited**
Address: **Unit 3
Christchurch Road
Baglan Industrial Park
Port Talbot
SA12 7BZ
United Kingdom**
Tel: +44 (0) 1792 472110
Fax: +44 (0) 1792 472466
Email: cathryn.cultech@btinternet.com
Contact: Cathryn Wood (Primary Contact)

1.3 The name and address of the supplier of the Echium Oil.

Product: **CROSSENTIAL SA14**
Company: **Croda Leek Limited
Barnfield Road Industrial Estate
Leek
Staffordshire
T13 5QJ**
Tel: + 44 (0) 1538 380400
Fax: + 44 (0) 1538 280401

1.4 The name and address of the manufacturer of the Echium oil Capsules

**Eurocaps Ltd.
Crown Business Park
Dukes Town
Tredegar
Gwent
NP22 4EF**
Tel: +44 (0) 1495 308900
Fax: +44 (0) 1495 308990



- 2 The name of the new dietary ingredient that is the subject of the premarket notification:

Echium plantagineum Seed Oil

3. A description of the dietary supplement or dietary supplements that contain the new dietary ingredient:

- 1) **Soft gel gelatine capsule 1000mg Echium oil.**
- 2) **300ml of liquid fatty acid nutritional supplement containing Echium Oil**

(see appendix 5)

- 3.1 The level of the new dietary ingredient is:

- 1) **1000mg Echium oil per capsule**
- 2) **300ml of Echium oil per bottle**

- 3.2 The conditions of use recommended or suggested in the labelling of the dietary supplement:

Suggested Use:

- 1) **One (1) or two (2) capsules to be taken daily as a dietary supplement, preferably at mealtimes, or as directed by a healthcare provider.**
- 2) **5ml of oil to be taken per day**

The dietary supplements containing echium oil will contain clear label statements stating:

“If pregnant, breast-feeding or taking prescriptive medications, use this product under medical supervision.”



Additional Information

1. **What is CROSSENTIAL SA14?**
Super-refined Echium plantagineum Seed Oil
2. **Who are the consumers of the dietary supplements containing echium oil?**
The consumers of the dietary supplements containing echium oil are individuals of both sexes over 12 years of age.
3. **Are there any limits on duration of use?**
No.
4. **Are there any subgroups of the population who should not take this supplement?**
No. There are no known limitations on use of the oil by any population because essential fatty acids are required for optimal health by every population group. However, pregnant and lactating women will be advised to take the supplement under the supervision of their healthcare provider. Due to the potential that bleeding time might be prolonged by omega-3 fatty acids (this association has not been established), individuals on blood thinning or anti-coagulant therapy will be advised to monitor clotting time and prothrombin time regularly if using these supplements.
4. **What is the purpose of dietary supplements containing echium oil?**
 - 1) Provision of Omega-3 fatty acids
 - 2) Specific provision of stearidonic acid as a precursor to eicosapentaenoic acid (EPA).
 - 3) Specific provision of gamma-linolenic acid (GLA).

The dietary supplements containing Crossential SA14 oil will be used as a source of essential fatty acids of the omega-3 (alpha-linolenic and stearidonic acids) and omega-6 (linoleic and gamma-linolenic acids) families. It is recommended as a dietary supplement of essential fatty acids.

5. **Are these dietary supplements containing echium oil intended to be used therapeutically?**
No.
No claims will be made about the function or efficacy of this ingredient. Dietary supplements containing the new ingredient, echium oil are not intended to mitigate, treat, diagnose or cure any disease in man or animal. No claims will be made about the therapeutic benefits of echium oil. Pharmax LLC understand that in order to make health claims, they would need to undergo a different evaluation process. This premarket notification is for a new dietary ingredient for inclusion in dietary supplements only. The discussion regarding the health benefits of fatty acids in echium oil is to support the use and safety of echium oil only.

2. Echium plantagineum Seed Oil: Background Information

Echium oil is a complex triglyceride oil obtained by extracting the seeds of *Echium plantagineum*. *Echium plantagineum* is a naturally occurring plant and has not been genetically modified.

The oil is produced by a combination of known extraction techniques used in the production of edible oils suitable for human consumption.

Taxonomy: (Guil-Guerrero JL et al, 2000).

Division: Spermatophyta

Subdivision: Angiospermae

Class: Dicotyledonae

Family: Boraginaceae

Genus: *Echium*

Species: *plantagineum*

The Boraginaceae 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 (Guil-Guerrero JL et al. 2000). *Echium plantagineum* is also known by the common names of Purple Vipers Bugloss, Paterson's Curse and Salvation Jane.

Why Echium oil?

Echium oil offers high levels of both omega-6 (43.5%) and omega-3 (26.9%) fatty acids in a single vegetable oil of plant origin.

Essential fatty acids cannot be synthesised in the body and therefore must be obtained from the diet (Simopoulos, 1991). A number of diseases exhibit deficiencies in the various essential fatty acids and this has led to considerable research into the pharmacological effects of omega-3 and omega-6 fatty acids.

Essential fatty acids and their derivatives have been shown to benefit health in cardiovascular disease, osteoporosis, diabetes, arthritis and numerous skin disorders.

The advantage of Echium oil compared to other oils is that it contains stearidonic acid, an omega-3 fatty acid that is not present in borage or flaxseed oil. Although the high levels of EFAs and GLA in Echium oil are of great interest, it is the stearidonic acid (C18: 4n-3) content that makes it unique among plant seed oils.

Echium oil contains between 12 and 14% stearidonic acid, compared to 2% found in the only other available commercial plant source. In addition, the conversion rate of dietary stearidonic acid C18:4 to eicosapentaenoic acid (EPA) (C20:5) is twice as much as that of α -linolenic acid (C18:3) *in vivo* (Yamasaki et al. 1992).

α -linolenic acid is an omega-3 fatty acid found in flaxseed oil. EPA and docosahexaenoic acid (DHA) are the two fatty acids that have been found to be most beneficial in fish oil supplements.

These unique properties have led to the interest in using Echium oil as an ingredient for dietary supplements and other nutritional products.

3. Certificate of Analysis for Echium plantagineum Seed Oil
Croda Chemicals Ltd¹ (CROSSENTIAL SA14)

CRODA

Croda Leek Limited
Barnfield Road Industrial Estate
Leek
Staffordshire
ST13 5QJ
Tel 01538 380400 Fax 01538 380401

Certificate of Analysis

This material has been manufactured and tested with a quality management system registered by BSI to the International Standards BS EN ISO 9001 or BS EN ISO 9002 (depending on the site)
This document has been produced by an electronic data management system, a signature is not necessary

Customer details

Customer Ref.
Inspection Lot 040000021459
C of A Printed. 18.04.2002
Croda Order No.
Croda Del. No.
Quantity. 0.000
QA Contact.
Fax No.

Batch Details

Product Name: CROSSENTIAL SA14
Product Code: SR03959/0022/L32
Cust. Product Name:
Cust. Product Code:
Batch No. 0000032964
Date of Test: 26.03.2002
Specification: REVIEWED 24.10.2001
Manufactured at: LEEK
Date of manufacture: 24.02.2002

Quality Control Results

Analytical Test Method No.	Characteristic	Specification Limit		Value	Unit	Status
		Lower	Upper			
	Addendum 00			Fail	-	F
TMA001	ACID VALUE	0.000	2.000	0.000	mg KOH/g	P
TMA010	PEROXIDE VALUE	0.00	5.00	9.30	meq02/kg	F
	Important: value outside the specifications					
TMB027	COLOUR	0.0000	200.0000	35.0000	HAZEN	P
TMB031	APPEARANCE (FORM)	LIQUID		Pass	-	P
TMB031	APPEARANCE (COLOUR)	PALE YELLOW		Pass	-	P
TMB067	ANTIOXIDANT	800.0000	1200.0000	992.0000	ppm	P
TMB078	ANISIDINE VALUE	0.00	20.00	3.60		P
TMB086	C18:4	10.0000	100.0000	12.7000	%	P

Batch Status: Fail (70 QSc)

Certificate valid until: 26.03.2003 (Material should then be retested to confirm further period of validity)

Confirmed by

CRODA

Croda Leek Limited
Barnfield Road Industrial Estate
Leek
Staffordshire
ST13 5QJ
Tel 01538 380400 Fax 01538 380401

Certificate of Analysis

This material has been manufactured and tested with a quality management system registered by BSI to the International Standards BS EN ISO 9001 or BS EN ISO 9002 (depending on the site)
This document has been produced by an electronic data management system, a signature is not necessary

Customer details

Customer Ref.
Inspection Lot 040000021459
C of A Printed. 18.04.2002
Croda Order No.
Croda Del. No.
Quantity. 0.000
QA Contact.
Fax No.

K Southwell

4. Croda Chemicals Ltd. Material Specifications for Echium plantagineum Seed Oil

CRODA

Croda Chemicals Ltd
Cowick Hall Snaith Goole
East Yorkshire DN14 9AA England
Tel +44(0)1405 860551 Fax +44(0)1405 860205
VAT Registration no GB 168 6045 44

Selling Specification

Date: 18.04.2002

Page: 1 of 2

Your Fax:

Material Name: CROSSENTIAL SA14
Material Code: SR03959/0022/L32
Chemical Name: Echium Plantagineum Seed Oil
Specification: REVIEWED 24.10.2001

Analy. Test Method No.	Characteristic	Specification Limits		Units
		Lower	Upper	
TMA001	ACID VALUE	0.000	2.000	mg KOH/g
TMA010	PEROXIDE VALUE	0.00	5.00	meqO2/kg
TMB027	COLOUR	0.0000	200.0000	HAZEN
TMB067	ANTIOXIDANT	800.0000	1200.0000	ppm
TMB078	ANISIDINE VALUE	0.00	20.00	
TMB086	C18:4	10.0000	100.0000	%
TMB031	APPEARANCE (FORM)	LIQUID		
TMB031	APPEARANCE (COLOUR)	PALE YELLOW		

Period of validity of Certificate of Analysis for material stored in unopened containers and stored in cool dry conditions: 365 days

If you agree to accept this specification please complete the following section and return to us at the address above.

5. Safety Data Sheets

5.1 Croda Chemicals Ltd. ¹

5.2 VSP BV¹

5.3 Biodroga¹

Date of first issue: 16/08/99 Revision Number: 0 Date: 0/00/00

1. Identification Code: SR3959

Commercial Name: Crossential SA14

Emergency Telephone Number: 01405 863400

2. Composition Vegetable glycerides derived from
Echium Seed Oil (Super Refined)
CAS Registry Number: 84988-87-4
EINECS Number: 284-877-7
Risk Phrases: None
Safety Phrases: None

3. Hazards Not Classified as dangerous according to
Directive 67/548/EEC or its amendments

4. First aid measures

Eye contact: Flush eye with water or standard eye wash solution.
Seek medical advice should irritation occur and
persist.

5. Fire-fighting measures: Combustible but presents no particular
hazard.

Extinguishing Media: Treat as an oil fire with CO₂, foam,
dry chemical or skilled use of water
spray.

Protective equipment for firefighters: Standard

6. Accidental release measures: Absorb onto inert material then
scrape up.

7. Handling and storage: Store in sealed containers under normal
cool, dry warehouse conditions.

Crossential SA14

SR3959

8. Exposure control and personal protection:

In accordance with good industrial practice handle using standard eye protection.

9. Physical and Chemicals Properties

(Test Method)

Physical form:	Liquid
Colour:	Pale yellow
Odour:	-
pH of aq. soln.	-
Boiling point:	-
Melting point:	-
Viscosity:	-
Flash point:	>100 Deg C
Flammability solid/gas:	N/A
Autoflammability:	N/A
Explosive properties:	N/A
Oxidizing properties:	N/A
Vapour pressure:	-
Density:	-
Water solubility:	Insoluble
Other solubility:	-
Bulk density:	-

Partition coefficient octanol/water: -

Explosion limits: N/A

10. Stability and reactivity

Thermal decomposition: Stable under normal conditions of use.

Hazardous Reactions: None under normal conditions of use.

11a. Toxicological Data

Oral:	LD50 expected to be >2000 mg/kg (Rat). Essentially non-toxic.
Dermal:	Expected to be non-toxic by dermal route.
Inhalation:	N/A

Crossential SA14

SR3959

11b. Toxic effects

Skin: Expected to be essentially non-irritating.

Eye: May be slightly irritating.

12. Ecological Information

Biodegradation: Expected to be biodegradable

Fish Toxicity: No data

Bacterial Toxicity: No data

WGK Class: WGK 0, Self-Classification

13. Disposal conditions: Dispose of according to a recognised method of chemical waste disposal.

14. Transport Information
UN Name: Not Assigned

UN Number: N/A

IMDG Code/Class: Non Hazardous

IMDG Code Page No: N/A

ICAO/IATA (Air) Class: Non Hazardous

Packing Group: N/A

RID/ADR Class: Non Hazardous

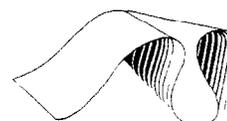
ADNR Class: Non Hazardous

15. Regulatory Information

Occupational Exposure Limits: N/A

16. Other Information:

SAFETY DATA SHEET



VSP

VEGETABLE SEEDOIL PRODUCTS

1. Identification of the substance and of the supplying company

Trade name Echum Oil
Manufacturer/Supplier Vegetable Seedoil Products
PO Box 45. 3244 ZG Nieuwe-Tonge, Holland
Phone No. (31) 187 657077
Telefax (31) 187 657088
Contact person Hanneke Bouman or Peter Clough

2. Composition/information on ingredients

Technical name Vegetable Oil
INCI name Vegetable Oil
CTFA name N/A
CAS-No N/A

3. Hazard identification

Non hazardous

4. First aid measures

Eye contact Flush with water minimum 15 mins. seek medical attention if persistent irritation.

5. Fire fighting measures

Extinguishing media Foams and Carbon dioxide
Unsuitable Extinguishing media Water

6. Accidental release measures

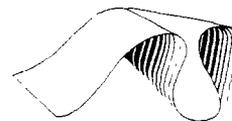
Personal protection May cause slipperiness on floors
Environmental protection Handle as food, keep out of sewers and natural waterways
Decontamination Collect the spill using an absorbing substance
Wash residue with mild soapy water
Store in container for deposit at approved site

7. Handling and storage

Handling No special precautions necessary
Storage Store cool, dry at normal humidity and away from products with a strong or foreign odour

8. Exposure controls - personal protection

Personal protective equipment not necessary, goggles or safety glasses recommended



VSP

VEGETABLE SEEDOIL PRODUCTS

9. Physical and Chemical properties

<i>Form</i>	Liquid
<i>Colour/Taste</i>	Yellow and odourless
<i>pH</i>	Not applicable
<i>Melting point</i>	< 0°C
<i>Flash point</i>	> 290°C
<i>Fire point</i>	> 320°C
<i>Explosion hazards</i>	Not applicable
<i>Density</i>	900 kg/m ³
<i>Solubility</i>	Not soluble in water. Soluble in acetone/hexane

10. Stability and Reactivity

<i>Stability</i>	Rancidity hastened by increased temperature, light and oxygen. Avoid strong oxidising agents.
<i>Hazardous decomposition products</i>	Akro Olein at extreme heating
<i>Hazardous reactions</i>	May ignite at high temperature close to flash point

11. Toxicological information

<i>Acute toxicity</i>	Non toxic. Food grade product
<i>Effects on overexposure</i>	No adverse effects known

12. Ecological information

<i>Biodegradation</i>	Fully biodegradable, closed bottle test (OECD 301 D)
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13. Disposal considerations

<i>Residue for disposal</i>	Deposit according to local regulations
<i>Contaminated containers</i>	Disposal according to local regulations

14. Transport information

<i>Classification</i>	Not classified in RID/ADR - ADNR - IMDG - ICAO/IATA DGR.
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15. Regulatory information

<i>Legislation</i>	According to EC and local legislation
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16. Other information

The product is a refined food grade vegetable oil.

The information herein is given in good faith but no warranty expressed or implied is made.

SAFETY DATA SHEET

ECHIUM OIL

1	<u>Description:</u>	This fully refined oil is a clear yellow liquid obtained through extraction.
1.1.	<u>Form:</u>	Liquid, oil
1.2	<u>Color:</u>	Light yellow, clear
1.3	<u>Odor:</u>	Mild, typical
2	<u>Physical and safety related technical data</u>	
2.1	<u>Solubility in water:</u>	Insoluble (see 2.7)
2.2	<u>Flash point:</u>	Greater than 200#F
2.3	<u>Recommended extinguishing media:</u>	CO ₂ , foam or dry chemical
2.4.	<u>Unusual fire or explosion hazards:</u>	None known
2.5	<u>Hazardous decomposition products:</u>	None known
2.6	<u>Special fire fighting procedures:</u>	None known
2.7	<u>Further information:</u>	The oil is soluble in ethanol, miscible with ether, chloroform or with oils and fats

SAFETY DATA SHEET

ECHIUM OIL

3 **Labeling for user and transport purposes**

Hazard symbol: None required

Risk phrases: None required

Safety phrases: None required

4 **Storage and handling**

Technical precautions: Keep container tightly closed and store in a cool (10°C) and dry place. Protect the oil against high temperature and light exposure. Use nitrogen atmosphere to avoid oxidation

5 **Personal protection:**

Respirators: None required

Eye contact: Flush thoroughly with copious amounts of running water. No further precaution necessary after removal of the product

6 **Toxicological Data:** Not demonstrable

7 **Ecological Data:** No data

8 **Waste disposal:** This product, if disposed as shipped, is not a hazardous waste. Consult state or local officials for proper disposal method

6 Safety Evaluation of Echium Oil

- 6.1 Echium Oil: Manufacturing Process
- 6.2 Echium Oil: Components
- 6.3 Echium Oil: Product Formulation
- 6.4 Echium Oil: Toxicological Information
- 6.5 Echium plantagineum: Human Exposure
- 6.6 Echium Oil: Microbial information
- 6.7 Echium Oil: Other Oils
- 6.8 Echium Oil: Conclusion
- 6.9 References

6.1 Echium Oil: Manufacturing Process

Seeds from the plant *Echium plantagineum* are crushed, the solvent is extracted using food grade hexane and the oil is isolated by the removal of the solvent by vacuum distillation.

The production process used to extract oil from *Echium plantagineum* seeds is an established method that is currently used to process several edible oils, such as Evening Primrose Oil, Borage Oil and Wheat Germ oil. It is therefore considered that the production process employed will not have any detrimental effect on the suitability or safety of using Echium oil for human consumption purposes.

6.2 Echium Oil: Components

Echium oil contains many constituents that are common to plant-derived oils. Its component fatty acids include significant levels of Palmitic, Stearic, Oleic, Linoleic, Alpha-Linolenic, Gamma Linolenic and Stearidonic acid. All of these fatty acids are found, in varying degrees, in either vegetable or fish oils that are consumed regularly throughout the USA. Oleic acid is found in olive oil, whilst Stearidonic acid is found in most fish oil.

6.2.1 Component fatty acids

The lipid profile for Echium oil is similar to that of borage oil and blackcurrant seed oil (see table below). Both borage oil and blackcurrant oil are widely used as ingredients of cosmetics, pharmaceuticals, foods and food supplements.

Table 1. The major fatty acids found in Echium oil²

Fatty acid		Echium seed oil (%)	Borage seed oil (%)	Blackcurrant seed oil (%)
Palmitic acid	16:0	7.01	9.98	6.92
Stearic acid	18:0	3.67	3.39	1.40
Oleic acid	18:1	16.41	16.37	11.76
Linoleic acid (LA)	C18:2	14.96	38.79	44.68
Alpha linolenic acid (ALA)	C18:3	28.98	0.49	11.44
Gamma linolenic acid (GLA)	C18:3	11.83	20.68	16.27
Stearidonic acid (SA)	C18:4	12.99	0.13	3.02

² Data obtained from J K Kings & Sons Ltd.

6.3 Echium Oil: Product Formulation

Typical of dietary supplements containing omega-3 and omega-6 fatty acids, Echium oil will be marketed in the form of gelatine capsules or liquid oils.

The Echium oil gelatine capsules and liquid oil will include vitamin E and mixed tocopherols. The addition of Vitamin E to formulations of fatty acids is recommended in accordance with guidance offered by the UK committee on medical aspects of food policy (Simopoulos AP 1991). It has been demonstrated that Vitamin E can prevent the oxidation of polyunsaturated fatty acids (omega-3 and omega-6).

6.4 Echium Oil: Toxicological Information

Echium oil contains minor constituents that are common in plant derived oils.

Gel permeation chromatography (GPC) analysis of a laboratory sample of Super Refined oil batch CW/014 identified the levels of triglyceride as 99.7%, and oligomer as 0.3% of the oil. The oligomer may be defined as oxidised triglyceride, this is not a natural component of the oil. Oxidation of the triglyceride is initiated during processing and storage and is kept to a minimum by storing in drums under nitrogen.

6.4.1 Cyclopropenoid and epoxy fatty acids

Tests have been performed to confirm the absence of cyclopropenoid and epoxy fatty acids from Echium oil. A GC-MS analysis was carried out on behalf of J K Kings & Sons, Ltd., the results of which can be found in appendix 1³. They show that in the two production samples, neither cyclopropenoid nor epoxy fatty acids were found to be present in the oil.

6.4.2 Erucic acid

As in borage oil, erucic acid (C22:1) is found in small quantities in Echium oil, at levels of less than 0.5%. Erucic acid is typically found at levels of 0.1% in Echium oil. The majority of samples from the 1999 harvest had an erucic acid content of seed of 0.1%. The product specifications from suppliers of Echium oil stipulate an upper limit of 1%.

6.4.3 Unsaponifiable Matter

To confirm the absence of any harmful or unusual unsaponifiable matter in Echium oil, an analysis was performed. The unsaponifiable content contains a mixture of sterols, hydrocarbons and other non-fatty acid compounds. Analysis shows (appendix 2) that the unsaponifiable content of Echium oil does not contain any unusual or unknown compounds⁴. The product specification of Echium oil contains an upper limit of 2% for the unsaponifiable content.

6.4.4 Heavy Metals

Tests to determine the levels of heavy metals; arsenic, lead, iron and copper have been performed. Levels of these heavy metals were all found to be below the lowest detectable level (arsenic: <0.50mg/kg; lead: <1.00mg/kg; iron: <1.0mg/kg; copper: <0.2 mg/kg). Previous results gathered by Croda on a Super Refined oil show the levels of heavy metals to be less than 10 ppm (appendix 3). The product specification stipulates that the heavy metal content must be below the lowest detectable limit.

³ Data kindly supplied by J K Kings & Sons, Ltd

⁴ Data kindly supplied by Croda Universal Ltd.

6.4.5 Peroxidation

A maximum peroxide value of 5 is stipulated on the product specification.

6.4.6 Pyrrolizidine alkaloids

Pyrrolizidine alkaloids are known to occur in species of the Boraginaceae family and have been isolated from both *Echium plantagineum* and *Borago officinalis* plants (Culvenor CCJ, 1956). *Borago officinalis* is used to make borage oil.

Pyrrolizidine alkaloids are of concern because they are known to cause liver disease (Curran JM et al. 1996). However, pyrrolizidine alkaloids are not oil soluble (lipophilic) and would not be expected to be present in *Echium plantagineum* seed oil.

During the extraction process, pyrrolizidine alkaloids are removed to meet the required limits of 15ng/g in *Echium* oil, which is a similar level to borage oil. The product specification stipulates that the level of pyrrolizidine alkaloids should not be greater than 15ng/gram.

An analysis of the alkaloid content of the crude and refined oil and the *Echium plantagineum* meal has been carried out. The meal contained 0.1mg/g total alkaloids. In the *Echium* oil, tests determined that pyrrolizidine alkaloids were either not detectable or were present at extremely low levels (<15ng/g).

The product specification of *Echium* oil stipulates that pyrrolizidine alkaloids should not be greater than 15ng/gram which is equivalent to 0.015ppm and is well below levels that would cause harm. A "no-affect" level of 1ppm in the diet has been proposed for mono-gastric animals such as pigs, poultry and rats (Culvenor CCJ et al. 1981).

There are a number of plants that are known to contain pyrrolizidine alkaloids in their foliage, including *Borago officinalis* (Culvenor CCJ, 1956). Borage oil is currently widely used as a health supplement.

6.5 *Echium plantagineum*: Human Exposure

Human exposure to this plant occurs during ingestion of honey produced by bees foraging on wild *Echium*. *Echium plantagineum* is known to secrete nectar that is gathered by bees and it is used extensively by apiarists (Culvenor CCJ et al, 1981). It is estimated that about 10-15% of Australian honey is from *Echium plantagineum*. (Culvenor CCJ et al, 1981). The honey is sold mainly as blends with other honey. Honey prepared from *Echium plantagineum* has been shown to contain between 0.27-0.95 ppm alkaloids (Culvenor CCJ et al 1981). The possible intake of pyrrolizidine alkaloids from this source is considered to be very low (Culvenor CCJ et al 1981).

Evidence is available on the effect of *Echium plantagineum* ingestion in animals. *Echium plantagineum* grows over significant areas of farmland in Australia. The young plants are eaten readily by livestock. The plant is considered a weed in good pastures, while on poor country it is considered a reserve fodder. Measurements of herbage dry matter content, nitrogen content and digestibility of *Echium plantagineum* indicate that it would be nutritional forage for grazing animals.

All the toxicological findings reported in animals are consistent with pyrrolizidine alkaloid poisoning. The pyrrolizidine alkaloids are not oil soluble and are found in very low quantities in *Echium* and borage oil.

Cytochrome C allergens have been isolated from the pollen of *Echium plantagineum*. However, the filter process used in the processing of *Echium* will act to remove any pollen or particulate plant debris in the oil. To confirm the absence of Cytochrome C allergens in the oil, a total protein test has been performed

using Bradford reagent. The total and recordable protein content (and therefore maximum possible Cytochrome C allergen content) was less than 1ppm in the Super Refined oil and less than 2ppm in the unrefined oil.

6.6 Echium Oil: Microbial information

Echium oil is an anhydrous system and will therefore not support microbiological growth. In addition, the manufacturing processes used to produce Echium oil will filter out any microbial organisms. The microbial analysis of Echium oil samples has confirmed the absence of microbiological contamination (appendix 4).

6.7 Echium Oil: Other Oils

Several oils containing triglycerides rich in omega-6 fatty acids are currently available on the market including blackcurrant seed oil, borage oil, evening primrose oil, soybean oil and safflower oil. Additionally oils containing triglycerides rich in omega-3 fatty acids currently available on the market include herring oil, mackerel oil, menhaden oil, sardine oil and tuna oil.

Echium oil will be marketed as possessing the benefits of both omega-3 and omega-6 essential fatty acids. The fatty acid profile of Echium is substantially similar to other oils of this type. Many plants from the borage family have been used to produce oils for nutritional supplementation.

Table 2. Fatty acid Profiles of Echium oil, Borage oil and Blackcurrant seed oil.

Fatty acid		Echium seed oil	Borage seed oil	Blackcurrant seed oil
Palmitic acid	16:0	7.01%	9.98%	6.92%
	16:1	0.19%	0.39%	0.20%
Stearic acid	18:0	3.67%	3.39%	1.40%
Oleic acid	18:1	16.41%	16.37%	11.76%
Linoleic acid (LA)	C18:2	14.96%	38.79%	44.68%
Alpha linolenic acid (ALA)	C18:3	28.98%	0.49%	11.44%
Gamma linolenic acid (GLA)	C18:3	11.83%	20.68%	16.27%
Stearidonic acid (SA)	C18:4	12.99%	0.13%	3.02%
	C20:0	0.39%	0.23%	-
	C20:1	0.68%	3.83%	0.86%
	C22:1	0.13%	2.46%	-
	C24:1	0.14%	1.23%	-
	Others	2.68%	2.03%	3.45%

Echium Oil contains 11.1% of saturated fatty acids on average. This compares to levels of saturated fatty acids in omega-6 rich vegetable oils of 8.3% blackcurrant seed oil, 13.6% borage oil, 9% evening primrose oil, 16% soybean oil and 10.1% safflower oil (Guil-Guerrero JI et al, 2000).

	% saturated fatty acids	Alpha-Linolenic Acid	Stearidonic acid	Total omega-3 fatty acid content	Total omega-6 fatty acid content
Echium Oil	11.1	30.7	12.8	26.9	43.5
Blackcurrant seed oil	8.3	11.4	3.02		
Borage oil	13.6				
Evening primrose oil	9				
Soybean oil	16				
Safflower oil	10.1				
Herring oil	26.1			7.46	
Mackerel oil	27.5			19.83	

An omega-3 and omega-6 fatty acid blend which is currently marketed (Efamarine™) in the form of capsules and an oral emulsion provides 68mg of gamma linolenic acid, 34mg of eicosapentaenoic acid and 22mg docosahexaenoic acid per daily dose. The daily intake of 2 gelatine-based capsules also contains 20mg docosahexaenoic acid and 20mg of vitamin E (as D alpha tocopheryl acetate). The omega-3 and omega-6 fatty acid blend is created by combining evening primrose oil with a marine fish oil.

In comparison, Echium oil as a single source would provide 116mg of gamma linolenic acid and 128mg of stearidonic acid in 1000mg capsules.

6.7.1 Omega-6 fatty acids

The optimum intake of omega-6 fatty acids for adults (both males and females over 6 years of age) is considered to be between 6-11 grams per day (Simopoulos AP, 1991).

Echium oil contains about 15.4% of linoleic acid and 11.5% of gamma linolenic acid (Guil-Guerrero JL et al. 2000). Omega-6 rich vegetable oils such as blackcurrant seed oil, borage oil, evening primrose oil, soybean oil and safflower oil all provide significantly higher levels of linoleic acid. Gamma linolenic acid content varies greatly between edible vegetable oils from 0% for safflower oil to approximately 10% for evening primrose oil and approximately 20% for borage oil (Guil-Guerrero JL et al. 2000).

6.7.2 Omega-3 fatty acids

Concern has been expressed about an increased risk of bleeding after intake of omega-3 PUFA, but the risk of bleeding seems to be very low and is considered less than after intake of aspirin (Schmidt & Dyerberg, 1994).

The daily requirement and optimum dose of alpha linolenic acid is not known. The recommended intake of omega-3 fatty acids for adults (both males and females over 6 years of age) is thought to be approximately 1-2 grams per day (Simopoulos AP, 1991).

Echium oil contains an average of 30.7% of alpha-linolenic acid and 12.8% of its metabolite, stearidonic acid. In comparison, the total omega-3 fatty acid content of fish oils is 7.46% in herring oil and 19.83% in mackerel oil (Guil-Guerrero JL et al. 2000). Although vegetable oils on the market such as corn oil and sunflower oil contain high levels of omega-6 fatty acids, they usually have very low levels of omega-3 fatty acids (Simopoulos AP, 1999). Black currant seed oil is an exception in that it contains 11.4% of alpha-linolenic acid and 3.02% of stearidonic acid.

Echium oil offers high levels of both omega-6 (43.5%) and omega-3 (26.9%) fatty acids in a single vegetable oil of plant origin.

6.8 Echimium Oil: Conclusions

Echimium oil as a single source shares many characteristics with oils derived from both plants and fish that are currently consumed for food purposes. Echimium oil offers high levels of both omega-6 (43.5%) and omega-3 (26.9%) fatty acids in a single vegetable oil of plant origin. These characteristics pose no toxicological or anti-nutritional threat to consumers.

The production of Echimium oil, from the growing of the crop to the extraction of oil, can be considered safe. Other areas of possible concern, that would relate to all oil products, such as peroxide value, unsaponifiable matter content and heavy metal content, have an upper limit stipulated in the product specification.

Tests have shown that both pyrrolizidine alkaloids and Cytochrome C allergens are either absent in Echimium oil or are present at such negligible quantities that are well below accepted "no affect" levels. Pyrrolizidine alkaloids are also present in *Borago officinalis*, which is used to make borage oil: a supplement that is currently freely available on the market.

Echimium oil is considered to be substantially equivalent to existing oils and fats on the market that are rich in essential fatty acids. We therefore conclude that Echimium oil poses no risk as a new ingredient for dietary supplements.

6.9 References

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7. List of Appendices

- 1. Test results for analysis of Cyclopropanoid and Epoxy fatty acids in Echium oil.^{5,6}**
- 2. Test results for analysis of unsaponifiable matter in production batches of Echium oil.^{5,6}**
- 3. Test results for heavy metal analysis of production batches of Echium oil.^{5,6}**
- 4. Test results for determining microbial activity in Echium oil.^{5,6}**
- 5. Product Formulation Sheets**
 - 5.1. Echium Oil Capsules**
 - 5.2. Liquid fatty acid nutritional supplement containing Echium Oil**
- 6. Test results for pyrrolizidine alkaloid analysis of Echium Oil.^{5,6}**

⁵ Permission to use these documents was kindly given by J K Kings & Sons. Ltd and Croda Universal Ltd

⁶ Each certificate of analysis is accompanied by a legible translation of the text

Appendix 1

**Test results for analysis of Cyclopropenoid and Epoxy fatty acids in
Echium oil.**



Certificate of Analysis

Contact:	Phil Nicholls	Report No.	308014454
Company:	John K King & Sons Ltd	Your Ref.	-
Address:	The Silo Skillingthorpe Road Lincoln LN6 0EL	Received	10/8/00
		Page No.	1 of 1

Analysis of Unrefined Echium Oil

Methods

Perox Acids were determined by A.O.A.C. Method Cd 9-57

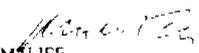
Cyclopropane fatty acids were determined by conversion to methyl esters by reaction with sodium methoxide in methanol, followed by extraction and analysis by GC on a 60m x 0.22mm HPX70 column. Our own-prepared laboratory standards, containing malvalic and sterculic acid were used to determine retention values.

Results

	Lot 1124	Lot 1124a
% Oxirane oxygen (indicative of peroxy acids)	0.14	0.28
Cyclopropane fatty acids (%)	N.D.	N.D.

N.D. = None detected (detection limit about 0.02% under these conditions)

The values obtained for oxirane oxygen were very low, and undoubtedly originated from slight oxidation of the oil.

Signatories:  Date: 30th August 2000
M.H. JEF

These results relate only to the samples tested and do not guarantee the bulk of the material to be of equal quality. RSSI cannot be held liable in respect of the use to which the information is put.

READING SCIENTIFIC SERVICES LTD
The Lord Zuckerman Research Centre
Whitelights, PO Box 234 Reading RG6 6L A UK
Tel: 0118 968541 (Int: +44 118 968541)
Fax: 0118 968937 (Int: +44 118 968937)

Certificate of Analysis

Contact:	Phil Nichols	Report No:	308014454
Company:	John K. King & Sons Ltd	Your ref:	-
Address:	The Silo	Received:	10.8.00
	Skillingthorpe Road	Page No:	1 of 1
	Lincoln		
	LN6 0EL		

Analysis of Unrefined Echium Oil

Methods

Epoxy Acids were determined by A.O.A.C. Method Cd 9-57.

Cyclopropene fatty acids were determined by conversion to methyl esters by reaction with sodium methoxide in methanol, followed by extraction and analysis by GC on a 60m by 0.22mm BPX70 column. Our own-prepared laboratory standards, containing malvelic acid and sterculic acid were used to determine retention values

Results

	Lot 1124	Lot 1124a
% Oxirane oxygen (indicate of epoxy acids)	0.14	0.28
Cyclopropane fatty acids (%)	N.D.	N.D

N. D = none detected (detection limit about 0.02% under these conditions)

The values obtained for oxirane oxygen were very low, and undoubtedly originated from slight oxidation of the oil.

Signatories:

M H JEE

Date: 30th August 2000

These results relate only to the sample(s) used and do not guarantee the bulk of the material to be of equal quality
RSSI staffs were not responsible for the making of samples
RSSI cannot be held liable in respect of the use to which the information is put.

Reading Scientific Services Ltd.
The Lord Zuckerman Research Centre
Whiteknights, PO Box 234, Reading RG6 6LA, UK
Tel: 0118 9868541 (Int: +44 0118 9868541)
Fax: 0118 9868932 (Int: +44 0118 9868932)

Appendix 2 Test results for analysis of unsaponifiable matter in production batches of Echium oil.

Croda Universal Ltd		Technical report No 5857
For internal circulation only (not to be distributed to third parties)		Date issued 11/9/00
Subject To comment on the sterols present in 2 samples of Echium oil		
Author(s) P. G. Speight : P. Champkin		
Report directed to N. Wilson	Requested by K. Coupland	
Department Laboratory	Other references None	
Section Technical Service		
Abstract The two samples of Echium oil were both found to contain 0.18% campesterol and 0.18% β sitosterol. Other sterols were present but they could not be identified.		Distribution K Nulbrow D A Panner N Wilson Dr B T Hatton (hard copy) B Holmes (hard copy) Dr C Temple-Heald P G Speight (hard copy) K Coupland
Further action Further work will be done on request		Keywords Echium oil Unsaponifiables Sterols
Signature		Date completed 11/9/00

Echium oil.

TSE 5857

1 0 Introduction

1 1 In July we received 2 samples of Echium oil from K Coupiand. The samples were labelled '1124' and '1124-A'

We were asked to comment on the sterols present in these 2 samples

This report has been delayed for about a month due to the works shut down

2 0 Experimental

2 1 The analysis was carried out by first determining the unsaponifiables and then the subsequent analysis of these unsaponifiables by capillary gas chromatography

2 2 The identity of the two components, campesterol and β sitosterol, was deduced by their retention times compared to known standards. We could not attempt the identification of any other components in the unsaponifiables as known standards are not readily available

3 0 Results

3 1 The Echium oils had the following analysis-

	Sample 1124	Sample 1124-A
Unsaponifiable content %	4	3
Sterol analysis by GC	% of each component in the unsaponifiables	
Component A	2	2
Component B	3	3
Component C	4	5
Component D	13	14
Thought to be campesterol		
Component E	13	14
Thought to be β sitosterol		
Component F	12	12
Component G	4	5
Component H	2	2
Minor components	20	20
Total components eluting in the sterol region	73	75
Other components that did not elute in the sterol region	27	25
Total	100%	100%

Echium oil

TSE 5857

1.0. Introduction

1.1 In July we received 2 samples of Echium oil from K Coupland. The samples were labelled "1124" and "1124-A"

We were asked to comment on the sterols present in these 2 samples.

This report has been delayed for about a month due to the works shut down.

2.0. Experimental

2.1. The analysis was carried out by first determining the unsaponifiables and then the subsequent analysis of these unsaponifiables by capillary gas chromatography.

2.2. The identity of the two components, campesterol and β sitosterol, was deduced by their retention times compared to the known standards. We could not attempt the identification of any other components in the unsaponifiables as known standards are not readily available.

3.0 Results

3.1 The Echium oils had the following analysis-

	Sample 1124	Sample 1124-A
Unsaponifiable content %	1.4	1.3
Sterol analysis by GC		
	% of each component in the unsaponifiables	
Component A	2	2
Component B	3	3
Component C	4	5
Component D Thought to be campesterol	13	14
Component E Thought to be β sitosterol	13	14
Component F	12	12
Component G	4	5
Component H	2	2
Minor components	20	20
Total components eluting in the sterol region	73	75
Other components that did not elute in the sterol region	27	25
Total	100%	100%

3.2 The level of campesterol and β sitosterol in the Echium oils is -

	Sample 1124	Sample 1124-A
Campesterol	13% of 1.4% = 0.18%	14% of 1.3% = 0.18%
β Sitosterol	13% of 1.4% = 0.18%	14% of 1.3% = 0.18%

4.0 Discussion

4.1 Echium is part of the Boraginaceae family and a typical profile of sterols found in Boraginaceae are as follows

Sterol species	% of the total sterols
Brassicasterol	0 to 1.6
Campesterol	25 to 30
β Sitosterol	22 to 42
Δ^5 Avenasterol	15 to 28
24-methyl cholesterol	15 to 20

Extracted from Physical and Chemical Characteristics of Oils, Fats and Waxes. This is an AOCS publication

4.2 We could not attempt the identification of any other components in the unsaponifiables apart from campesterol and β sitosterol as known standards are not readily available

5.0 Conclusion

5.1 The two samples of Echium oil were both found to contain about 0.18% campesterol and 0.18% β sitosterol

Other sterols were present but they could not be identified

P Speight

3.2. The level of campesterol and β sitosterol in the Echium oils is: -

	Sample 1124	Sample 1124-A
Campesterol	13% of 1.4% = 0.18%	14% of 1.3% = 0.18%
β Sitosterol	13% of 1.4% = 0.18%	14% of 1.3% = 0.18%

4.0. Discussion

4.1. Echium is part of the Boraginaceae family and a typical profile of sterols found in Boraginaceae are as follows: -

Sterol species	% of the total sterols
Brassicasterol	0 to 1.6
Campesterol	25 to 30
β sitosterol	22 to 42
Δ^5 Avenasterol	15 to 28
24-methyl cholesterol	15 to 20

Extracted from Physical and Chemical Characteristics of Oils, Fats and Waxes. This is an AOCS publication.

4.2 We could not attempt the identification of any other components in the unsaponifiables apart from campesterol and β sitosterol, as known standards are not readily available.

5.0 Conclusion

5.1 The two samples of Echium oil were both found to contain about 0.18% campesterol and 0.18% β sitosterol.

Other sterols were present but they could not be identified.

Appendix 3

Test results for heavy metal analysis of production batches of Echium oil.



International Laboratory Services Ltd
 2500000 Business Park
 Lough Road, Antrim,
 Co. Down BT1 2JL
 Telephone: 01232 390000
 Fax: 01232 390444/390010
 Website: www.lslabtest.co.uk

Report No: E0069/01
 Issue No: 1
 Date: 22/07/00
 Order No: 10032

John K King of Lincoln
 The Silc
 Skellingthorpe
 Lincoln LN6 0EL
 FAX: DARRAN KEENE

TEST REPORT

Lab Ref No: 10005185

Sample received on 09/03/00 Samples tested on 10/03/00

Description: ECHIUM OIL 0000000 BATCH LI/000333/11241 PALM OIL
 7.5 00

Std Test Method	Determination	Results	Units
10.86	Nitrogen	0.04	%
10.14	Protein (N x 6.25)	0.1	%
10.16	Fat profile	See page 2	
10.21	Arsenic	< 0.50	mg/kg
10.19	Copper	< 1.0	mg/kg
10.26	Iron	< 1.0	mg/kg
10.22	Lead	< 1.0	mg/kg



International Laboratory Services Ltd.
Shardlow Business Park
London Road
Shardlow
Derbyshire D72 2GD

Tel: 01332 793000
Fax: 01332 799044 / 792010
Website: www.ils-limited.co.uk

Report No: E0068/57
Issue No: 1
Date: 22/03/00
Order No: P0032

John K King of Lincoln
The Silo
Skellingthorpe
Lincoln LN6 0EL
FAO DARREN KEELER

TEST REPORT

Lab Ref No : 10005186

Sample received on 09/03/00

Samples Tested on 10/03/00

Description: ECHIUM OIL 0000066 BATCH LI/ 00033/ 1124/ PALLECON
7.3.00

Std Test Method	Determination	Results	Units
30.86	Nitrogen	0.04	%
30.14	Protein (N x 6.25)	0.3	%
30.16	Fat Profile	See page 2	
30.23	Arsenic	<0.50	mg/kg
30.29	Copper	<0.2	mg/kg
30.26	Iron	<1.0	mg/kg
30.22	Lead	<1.00	mg/kg



International Laboratory Services Ltd
Dunton Business Park
Dunton Road - Shefflow
Dorchester DT12 0LJ
Telephone: 0132 793000
Fax: 0132 799044
Website: www.is-limited.co.uk

Report No: E0068/56
Issue No: 1
Date: 22/03/00
Order No: P0042

John K King of Lincoln
The Site
Skellingthorpe
LINCOLN LN6 0EL
FAO DARREN KESLER

TEST REPORT

Lab Ref No: 10005185

Sample received on 09/03/00 Samples tested on 10/03/00

Description: ECHIUM OIL 0000007 BATCH L1/000033-0124JA PALLETON
7.000

Std Test Method	Determination	Results	Units
30 07	Nitrogen	0.12	%
30 14	Protein (N x 6.25)	n.d.	%
30 16	Fat Profile	See page 2	
30 23	Arsenic	< 0.50	mg/kg
30 24	Copper	< 0.2	mg/kg
30 25	Iron	< 1.0	mg/kg
30 22	Lead	< 1.0	mg/kg



International Laboratory Services Ltd.
Shardlow Business Park
London Road
Shardlow
Derbyshire D72 2GD

Tel: 01332 793000
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Website: www.ils-limited.co.uk

Report No: E0068/56
Issue No: 1
Date: 22/03/00
Order No: P0032

John K King of Lincoln
The Silo
Skellingthorpe
Lincoln LN6 0EL
FAO DARREN KEELER

TEST REPORT

Lab Ref No: 10005185

Sample received on 09/03/00

Samples Tested on 10/03/00

Description: ECHIUM OIL 0000066 BATCH LI/ 00033 / 1124 / A PALLECON
7.3.00

Std Test Method	Determination	Results	Units
30.86	Nitrogen	0.13	%
30.14	Protein (N x 6.25)	0.8	%
30.16	Fat Profile	See page 2	
30.23	Arsenic	<0.50	mg/kg
30.29	Copper	<0.2	mg/kg
30.26	Iron	<1.0	mg/kg
30.22	Lead	<1.00	mg/kg

BRETBY

Certificate of Analysis

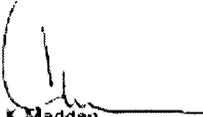
CRODA LEEK LTD
Barnfield Road
Leek
Staffordshire
ST13 5QJ

Our Reference 33/144499
Date Received 13/08/98
Date Completed 19/08/98
Your Reference PQ 48978

Sample. Sample No. C336

Sample Description	Copper mg/litre IP009W.P	Iron mg/litre IP010W.P	BP Heavy Metals BP008.P
1	<0.1	4.8	<10ppm

M F Scott
BSc MSc CBiol MRBiol FIMgt FIFST


T. K. Madden
BSc MSc MChemA EneChem CChem FASC
Registered Analytical Chemist
Registered Professional Water Chemist

D. K. Buckley
MSc FIMgt FIFST CChem FASC
Registered Analytical Chemist
Qualified Person

The entire letter Processed via our method & not REBAS software

Page 1 of 1

Bretby Business Park, Ashby Road, Bretby, Burton upon Trent, Staffordshire DE13 0GD
Tel: 01293 552776 Fax: 01293 552747

Bretby

Certificate of Analysis

CRODA LEEK LTD
Barnfield Road
Leek
Staffordshire
ST13 5QJ

Our reference: 33/144499
Date Received: 13/08/98
Date completed: 19/08/98
Your reference: PO 48978

Sample: Sample no. C336

Sample Description	Copper mg/ litre IP009W.P	Iron mg/litre IP010W.P	BP Heavy Metals BP008.P
1	<0.1	4.6	<10ppm

MF Scott

TK Madden

DK Buckley

Page 1 of 1

Appendix 4 Test results for determining microbial activity in Echium oil.



Technical report

Computer index number 6883

Name: Graham Atkinson
Author's reference: R49/GMA/211
Laboratory: Analytical Services Department

Date: 23 April 1998
Enquiry no: CSE 954
Customer: internal
Country: -

Keywords

Crossentral SA 14 Vegetable Glycerides derived from Echium Seed Oil Herbicide analysis, Glyphosate Paraquat, Microbiological contamination

Objective

To discover if it is possible for Crossentral SA 14 to be contaminated with any herbicides, pesticides or microorganisms as Crossentral SA 14 is to be submitted for novel food approval

Abstract

Two herbicides are used in the growth of Echium Seed Oil (Crossentral SA 14). Theoretically it will be impossible to find levels of the Herbicides, Glyphosphate or Paraquat in Crossentral SA 14 due to the insolubility of the herbicides in fats or organic solvents. A leading Analytical Consultants Laboratory has confirmed this.

No evidence of microbiological contamination was found in the sample of Crossentral SA 14 submitted.

Further action

None

Distribution

KC KL (Leak) KB KVP DC ARB JAN PM IM File

References

Expt Nos
Reports
Other

Technical report

Computer index number **6883**

Date 23 April 1998

Author	Graham Atkinson	Enquiry no.	CSE 954
Author's reference	R49/ GMA/ 211	Customer	Internal
Laboratory	Analytical Services Department	Country	-

Keywords:

Crossential SA14, Vegetable Glyceride derived from Echium Seed Oil, herbicide analysis, Glyphosphate, Paraquat, Microbiological contamination.

Objective

To discover if it is possible for Crossential SA14 to be contaminated with any herbicides, pesticides or microorganisms as Crossential SA14 is to be submitted for novel food approval.

Abstract

The herbicides are used in the growth of Echium Seed Oil (Crossential SA14). Thereoetically it will be impossible to find levels of the Herbicides Glyphosphate or Paraquat in Crossential SA14 due to the insolubility of the herbicides in fats or organic solvents. A leading Analytical Consultants Laboratory has confirmed this.

No evidence of microbiological contamination was found in the sample of Crossential SA14 submitted.

Further action:

None

Distribution

KC KL (Leek) KB KVP DC ARB JAN PM IM File

References: Expt nos.

Repeats

Other

Appendix 5 Product Formulation Sheets

5.1.1 Product Formulation Certificate for Echium oil Capsules

5.1.2 Product Development Proposal for Echium oil capsules

5.2 Product Development Proposal for a liquid fatty acid nutritional supplement containing Echium Oil

PRODUCTION FORMULATION CERTIFICATE

PRODUCT NAME: ECHIUM OIL SOFT GELS

BULK CODE:

DATE: 18-04-02

CAPSULE SIZE: SOFT GELATIN

TRIAL NO: 1

EXTRA NOTES: BEING MANUFACTURED BY EUROCAPS. CULTECH TO PROVIDE ECHIUM OIL FREE ISSUE. EUROCAPS TO PROVIDE REST.

INGREDIENT	RAW MAT CODE	MG PER CAP	LABEL CLAIM PER CAP	EXCIPIENT CODE
Echium plantagenum oil	N/A	1000mg		
Vitamin E oil	N/A			
Mixed Tocopherols	N/A			
CAPSULE FILL:				
Gelatin (Soft)				4
TOTAL WEIGHT:				

Excipient Codes:

1. Bulking Agent
2. Flow Agent
3. Drying Agent
4. 2 piece hard shell Vegetarian capsule
5. Oil Carrier

Company	Name	Date
Cultech Ltd		
Solo Nutrition		

Product Development Proposal

<u>Ref</u>	<u>Product Name</u>	<u>Product ref</u>
3922	Echium oil 300ml + Nat Vit E + Mix Tocs	TBA
<u>Customer</u>		
Cultech	Amber glass bottle	

Material ID	MaterialName	LabelClaim
999999	Echium oil FOC CUL	300 ml
987010	d alpha tocopherol 1000 iu/g	
987022	d a.b.d.g tocopherol	

Prepared By Cathryn Wood

Date 01/05/2002

Approved By
Job Title

Date:

Appendix 6

Test results for pyrrolizidine alkaloid analysis of Echium Oil

25/07/2006 14:18:11 JOHN K KING - 90152491416
21376562719

Chemical Laboratory Dr Hermann Ulex Nachf.

John K King & Sons Limited
Coggeshall, Colchester, Essex

CO6 1TH United Kingdom.

Analysis Report No. 02 28 02

Echinium Oil / Prod. No. 66
Batch no. L1'00033/1124/A
Sample Point: Pallecon / 21st Febr. 00

You sent us the sample referred to above for determination of the total content of pyrrolizidine alkaloids (PA) of retronecine type

Processing of the sample

- The sample was hydrated in the presence of ethanol and sulphuric acid with zinc powder for several hours at 40 ° C according to the method
"Determination of pyrrolizidine alkaloids by thin film chromatography in seed oils of Borago off. L.
H. J. Mierendorff, Fat Sci. Technol. 97 No. 1 (1995) 33 - 37"
for the reduction of N oxides of PA to free alcohols
These were extracted by multiple acid / bases extraction under defined conditions and the aliquot proportions were subjected to HPTLC analysis

DC conditions

- Plate: silica gel KG 60 E Merck (No. 5583)
Running agent 1: Acetone, p. a. Merck
Running agent 2: Chloroform-methanol, ammonia 80 / 19 / 1
Chamber: Saturated
Detection in accordance with Mattocks (specifically for PA of retronecine / heliotridine type)
Reference substances: 7-acetyl lycopsamine / 7 - Acetylne intermedine
- Inner standard: monocrotaline

Result:

11 ± 2ng pyrrolizidine alkaloids / g substance (N.O. u. d. B. 5 ng / g)

29/09/2000 09:38

John K King; - 901522691748
01376562219

Chemical laboratory Dr Hermann Ulex Nachf

John K King & Sons Limited
Coggeshall, Colchester, Essex

CO6 1TH United Kingdom

Analysis Report No. 02.28.02

Echium Oil/Prod. No. 66
Batch No. L1/00033/1124/A
Sample Point: Pallecon / 21st Febr. 00

You sent us the sample referred to above for determination of the total content of pyrrolizidine alkaloids (PA) of retronecine type.

Processing of the sample:

The sample was hydrated in the presence of ethanol and sulphuric acid with zinc powder for several hours at 40°C according to the method:

"Determination of pyrrolizidine alkaloids by thin film chromatography in seed oils of Borage off. L. : H. - J. Mierendorff. Fat Sci. technol. 97 No. 1 (1995) 33 – 37. "

for the reduction of N oxides of PA to free alcohols.

These were extracted by multiple acid / bases extraction under defined conditions and the aliquot proportions were subjected to HPTLC analysis.

DC conditions:

Plate : silica gel KG 60 E Merck (No. 5583)

Running agent 1: Acetone. p. a. Merck

Running agent 2: Chloroform, methanol, ammonia 80 / 19 / 1

Chamber: saturated

Detection in accordance with Mattocks (specifically for PA of retronecine / heliotridine type)

Reference substance: 7 acetyl lycopsamine / 7-Acetyl intermediate

Inner standard: monocrotaline

Results

11±2ng pyrrolizidine alkaloids/g substance (NQ u d B : 5ng/g)