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1. Administrative Data

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2. General Description

Echium Oil is a complex triglyceride of plant origin. It is produced by a combination of known extraction techniques used in the production of edible oils suitable for human consumption.

Seeds from the plant Echium plantagineum are crushed, solvent extracted using food grade hexane and the oil isolated by removing solvent by vacuum distillation. The level of residual solvent is below that stipulated by current food legislation.

Echium plantagineum and its products have not hitherto been used for human consumption to a significant degree within the Community. Echium plantagineum is a naturally occurring plant and has not been genetically modified.

Therefore Echium Oil falls under category (e) of Article 1(2) of Regulation (EC) No 258/97 [Ref. 2] and SCF class 2.2 [Ref. 3].

3. Identification of essential information requirements

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 [Ref. 3]:

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

4. Consultation of structured schemes

I. Specification of the novel food

Echium oil is a complex triglyceride obtained by extracting the oil from the seeds of Echium plantagineum. An optimum temperature continuous flow extraction process using food grade hexane is employed to obtain the oil, for which approval is sought in this application. The product specification for Ambient Temperature Solvent Extracted Echium oil can be found in Appendix 3.

Kings have used oil extracted from this method in all of the analyses that will follow in this section. The fact the Echium plantagineum is in the early stage of commercial development means that only two production batches were available for use (1124 and 1124-A). These two batches represent the entirety of Kings 1999 production. The lipid profiles from these two batches can be found in Appendix I / Table 1.

Supporting Data

In order that the results be based on more sample numbers, Croda Oleochemicals have kindly allowed Kings to use data obtained by testing their Super Refined® Oil. While the production process differs to that of unrefined Echium oil, the oil is derived from the same species of plant and, therefore, we believe that the data serves a useful purpose in supporting claims made from data based on tests performed on the 1999 samples.

The values for the level of each fatty acid found in Echium oil are based on data obtained from the following sources:

- Lipid profiles of natural oils from seed accessions from Scotia (Appendix 1 / Table 2), Croda (Appendix 1 / Table 3) and Kings (Appendix 1 / Table 4).
- Lipid profiles for Super Refined ® Echium plantagineum oil (Crossential SA14) from Croda. (Appendix 1 / Table 5)
- Lipid profiles from 1999 production batches of Kings ambient temperature solvent extracted unrefined Echium plantagineum oil. (Appendix 1 / Table 1)

It can be seen from these data that the lipid constituents of Echium plantagineum do not vary significantly between the natural oil, the Super Refined ® and the unrefined oil. It is concluded that, because these data are based on samples from several different years covering a number of variables such as weather and geographical location of the crop, it provides an accurate reflection of likely parameters for each fatty acid.

A full lipid profile for the two 1999 production batches are included in Appendix I(Table 5). All fatty acids at a percentage composition of 0.1 and above have been identified conclusively. All the fatty acids have been accounted for and so it can be assumed that cyclopropenoid, epoxy or other unusual fatty acids are absent from the oil.

Due to the undesirable nature of cyclopropenoid and epoxy fatty acids, tests were performed to confirm their absence from Echium oil. A GC-MS analysis was carried out, the results of which can be found in Appendix 11. They show that in the two production samples from the 1999 harvests neither cyclopropenoid nor epoxy fatty acids were found to be present in the oil.

Erucic acid is found in small quantities in Echium oil, typically at levels of less than 0.5%. It can be seen in Appendix I (Table 3) that the majority of samples from the 1999 harvest had an Erucic acid content of seed of 0.1%. The product specification (Appendix 3) stipulates an upper limit of 1%.

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.

The levels of unsaponifiable matter in the two 1999 production samples 1124 and 1124-a were 1.1% and 1.2% respectively. The unsaponifiable matter is a natural component of the oil and could contain hydrocarbons, sterols and other non-fatty compounds. These levels are not untypical for unrefined oils.

To confirm the absence of any harmful or unusual unsaponifiable matter in Echium oil an analysis was performed. The identity of the two components, campesterol and ß sitosterol, was deduced by their retention times in Gas Chromatography compared to known standards. We could not attempt the identification of any other components in the unsaponifiables as known standards are not readily available. The product specification stipulates an upper limit of 2% for unsaponifiable matter. These results can be found in Appendix 12.

Tests to determine the levels of heavy metals; arsenic, lead, iron and copper were performed. Levels of these heavy metals were all found to be below the lowest detectable level. Previous results gathered by Croda on a Super Refined ® oil show the levels of heavy metals to be less than 10ppm. Copies of the contract laboratory's test report can be found in Appendix 5. The product specification (Appendix 3) stipulates that the heavy metal content must be below the lowest detectable limit, which varies between substances.

A peroxide value of 10 maximum is included on the product specification. The peroxide value of 1999 production batches 1124 and 1124-A were 2.65 and 2.01 respectively.

Pyrrolizidine alkaloids are known to occur in certain species of the family Boraginaceae and have been isolated from Echium plantagineum [Ref. 4][Ref. 5]. Pyrrolizidine alkaloids are of concern because they cause acute and chronic liver disease [Ref. 6]. In addition to the liver they may damage the lung, kidney and other organs, they also possess mutagenic, teratogenic and carcinogenic properties [Ref. 6]. Chronic liver disease was observed at dietary levels of 2ppm with the pyrrolizidine alkaloid monocrotaline [Ref. 6]. A "no-effect" level of 1ppm in the diet has been hypothesised for mono-gastric animals such as pigs, poultry and rats [Ref. 6].

Pyrrolizidine alkaloids are not lipophillic and, therefore, would not be expected to be present in any substantial quantity in the oil. Tests carried out on several samples of both Super Refined ® and unrefined Echium oil shows that levels of Pyrrolizidine Alkaloids were either extremely low (<15ng/g) or below the lowest detectable level of 4ng/gram.

The product specification stipulates that pyrrolizidine alkaloids should not be greater than 15ng/gram which is considered to be well below levels that would cause harm [Ref.6]. There are other plants that are known to contain pyrrolizidine alkaloids in their foliage, one such example being Borago officinalis. Borage oil is currently widely used as health supplement.

Cytochrome C allergens have been isolated from the pollen of Echium plantagineum [Ref7]. In a rural area of Australia 60% of subjects with respiratory allergy were found to give positive skin test reactions to Echium plantagineum pollen extract and a similar number gave positive radioallergosorbent test (RAST) test [Ref8].

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 absorbance at 595nm of the coloured product of the reaction of protein and Bradford Reagent was measured. Cytochrome C allergens isolated from the pollen of echium plantagineum were characterised as proteins with a molecular weight of 12,800 [7].

It can be reasonably assumed that the maximum Cytochrome C allergen content is no more than the total protein content. Using standards, a total protein content of less than 19.85µg/gram of SuperRefined oil was determined. However problems associated with the low solubility of Echium oil in the aqueous reagent meant that the aqueous reagent phase and Echium oil (dissolved in hexane) phase had to be mixed vigorously before measurement of the sample solution using UV spectrometry at a wavelength of 595nm. This is not ideal and therefore a visual inspection of the sample, standards and blank was carried out. A total and recordable protein content (and therefore a Cytochrome C allergen content) of less than 1ppm in the SuperRefined oil and less than 2ppm in the unrefined oil was determined by this method.

With the recent review and subsequent revision of the “long term arrangements for the extension of use (2000)” the Pesticide Safety Directive, in consultation with the Advisory Committee on Pesticides and relevant Government Departments, have now included echium (see appendix 8). This development has two significant consequences, firstly it legally allows the field application of any pesticide with “on label” approval for use in oilseed rape (i.e. has a MAFF approval number and the product label on the pesticide container refers to oilseed rape). Secondly it demonstrates that all relevant agencies are confident that the extrapolation of safety data supplied to gain approval for use of these products on oilseed rape is valid and that their use on echium poses no threat to the consumer, environment or sprayer operator.

The field production of echium is not heavily reliant on pesticide applications, indeed it can achieve satisfactory yields with no pesticide usage (depending on climatic conditions and crop rotation). However, there are areas where selective and appropriate use of pesticides will enhance both crop yield and quality.

The first easily definable area is that of herbicides and the requirement to control weeds. As a matter of principle all growers adopt all measures of Integrated Crop Management possible to minimise weed seed burdens and develop “stale seedbed” practises as much as possible. This in practical terms means that as many weeds as possible are controlled prior to the planting of the Echium crop so that the final seedbed has a minimal number of potential weeds that may grow i.e. it is “stale”. There are times when

this is not sufficient and selective use of herbicides will be required, it must be stated that any herbicides used will be trialled before hand to assess both efficacy and residue levels to reaffirm the confidence shown by the agencies mentioned above.

The second easily definable area is that of disease prevention, in particular powdery mildew and sclerotinia; both of these have the potential to cause significant loss of both yield and quality. Again fungicides will be applied in line with all aspects of ICM and on a strictly “as required” basis. As these applications will be made by strictly following the product label guidance then there is no more risk attached to applying them to echium as to oilseed rape. More detailed information on the toxicology of these pesticides can be obtained from the PSD.

The simultaneous occurrence of gamma-linolenic acid (cis-6, 9, 12-octadecatrienoic acid) and stearidonic acid (cis-6, 9,12,15-octadecatetraenoic acid) has so far only reported in seed oils of the Boraginaceae, Primulaceae and Saxifragaceae families [Ref. 10].

Kings was attracted to the Boraginaceae family since we had accumulated several years’ experience growing Borago officinalis. Borage officinalis does not, however, contain Stearidonic Acid and so Kings started to examine other members of the Boraginaceae family.

The genus Echium contains about 30 species distributed across Europe, the Mediterranean region, Madeira, the Canaries and the Azores [Ref 11].

The main sources of viable germplasm for cultivation are producers of ornamental flower seed. Kings selected one such source for seed multiplication in the UK.

The species plantagineum can be distinguished from other members of this genus by the presence of two exserted stamens, distinctly bifid stigmae, and ovate basal leaves with prominent lateral veins [Ref. 11]. Corolla up to 18-30mm, blue becoming pink through purple, subglabrous, hairy on veins and margins only [Ref. 11].Effect of the production process applied to the novel food

II. Effect of the production process on the Novel Food

The seed is extracted at John K King & Sons Ltd extraction facility in Lincoln. This facility is used to process other seeds that currently have food approval status in the EC, including Borage and Evening Primrose Oil and for this reason the production process is not considered to be novel.

Seed can be received in either bulk or bulk bags, depending on requirement, and is transported to the silos using an air handling system. This air system has been purposely designed to reduce any risk of possible contamination between the various seed types that can be handled, as it is a totally self-cleaning system.

To ensure a successful extraction of the oil, the Echium seed is first cracked which, by gently 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 a continuous flow extractor where it is brought into contact with food grade hexane solvent. As the seed is conveyed through the extractor fresh solvent is washed through it in a counter current direction, resulting in the solvent becoming increasingly enriched with oil. 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 Echium seed is gently bathed in hexane over a period of ninety minutes in order to remove the highest yields possible of good quality oil. The extraction process is carried out at room temperature in an oxygen free atmosphere. The oil enriched solvent then passes through a distillation system where the solvent is removed from the oil to leave less than 1 part per million in the oil.

A flow diagram of this production process is presented in Appendix 8.

It should be noted that this production process differs from standard hexane extraction in that the whole process is performed at ambient temperature of up to 35^{o}c. Another difference is that a ‘continuous flow’ hexane is employed. The end result is that the quality of unrefined oil is higher than refined oil due to the presence of natural anti-oxidants that are retained in the oil.

Rigorous quality assurance and control procedures are followed before, during and after the production process to ensure that the product meets specification.

Prior to delivery, representative samples of seed are forwarded to the fully accredited laboratory at the Lincoln Extraction facility for Lipid profile and Acid Value analysis. If seed is of unsuitable quality it will not be despatched for oil extraction.

During the production process continuos checks are performed on the Moisture and Hexane level of the oil. If oil is found to be out of specification adjustments are made to the procedure to ensure that the bulk oil remains within specification.

Once production is completed the oil is tested against the product specification by the fully accredited laboratory at Lincoln. In the event that the oil is found to be out of

specification it will not be released by Quality Control. It will either be rejected outright or it could be re-processed until it passes the product specification.

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

ECHIUM OIL is a complex triglyceride oil obtained by extracting the seeds of Echium plantagineum.

Echium plantagineum has not been genetically modified.

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

The Boraginaceae are a large plant family with approximately 100 genera and 2500 species that are widely distributed throughout the Northern Hemisphere [Ref. 10]. The family is well known to herbalists and gardeners because it includes many ornamental and medicinal plants [Ref. 10].

The genus Echium contains about 30 species distributed across Europe, the Mediterranean region, Madeira, the Canaries and the Azores [Ref. 11].

Echium plantagineum is an erect biennial 20-60cm high, softly hairy, with one or many flowering stems [Ref. 11]. The basal leaves are ovate with prominent lateral veins and soft appressed setae [Ref. 11]. The cauline leaves are oblong to lanceolate, the uppermost being more or less cordate at the base [Ref. 11]. Inflorescence, usually branched [Ref. 11]. Calyxes are 7-10mm at anthesis and up to 15mm in the fruit [Ref. 11]. Corolla 18-30mm infundibuliform blue becoming pink through purple, hairy on veins and margins only [Ref. 11]. Two stamens exserted from corolla tube, the remaining stamens included or only slightly exserted [Ref. 11]. The stigmae are distinctly bifid [Ref. 11]. Echium plantagineum is also known by the common names of Purple Vipers Bugloss, Paterson's Curse and Salvation Jane.

Echium plantagineum and its products have not hitherto been used for human consumption to a significant degree within the Community. Human exposure to the plant does occur by ingesting honey produced by bees foraging on wild Echium. Evidence is available on the effects in animals of ingestion of Echium plantagineum.

Echium plantagineum occurs over significant areas of farmland in Australia [Ref. 6]. The young growth is eaten readily by livestock [Ref. 6]. The plant is considered a weed in good pastures while on poor country it is considered as a reserve fodder [Ref. 5]. Measurements of herbage dry matter content, nitrogen content and digestibility of Echium plantagineum indicate that it would be nutritious forage for grazing animals [Ref. 13]. However the presence of pyrrolizidine alkaloids in the plant means that there is a risk that grazing animals will be poisoned [Ref. 6]. The level of pyrrolizidine alkaloids is normally between 0.1-0.3% of the dry weight of the whole plant but levels as high as 0.9% have been reported [Ref. 14]. Field evidence strongly indicates that horses, pigs and to a lesser extent sheep are all affected [Ref. 6].

Experimental evidence includes an unpublished study in which young pigs were fed 15% Echium plantagineum in the diet [Ref. 6]. All developed the typical chronic liver damage within 5 months and one animal died within 4 months [Ref. 6]

Echium plantagineum was fed as the sole diet to crossbred sheep with or without a history of previous access to the plant in a pen feeding trial [Ref. 15]. Compared to a control group receiving a diet of Lucerne chaff and oats, sheep on the Echium diet lost weight and several animals died [Ref. 15]. Histological examination produced evidence of excessive copper accumulation in the liver and biochemical evidence of liver toxicity and was usually accompanied by pyrrolizidine alkaloid damage [Ref. 15]. It was concluded that Echium plantagineum alone was not suitable fodder for sheep [Ref. 15].

There was no mortality involving pyrrolizidine alkaloid poisoning in crossbred sheep grazing pasture for 19 months where Echium plantagineum constituted a considerable portion of the available forage [Ref. 16]. Histological evidence of moderately severe liver damage associated with high liver copper concentrations was found in at least one sheep [Ref. 16]. Sheep on the Echium plantagineum diet were significantly lighter and grew less wool compared with sheep on Echium free pasture [Ref. 16].

Young rats fed 40% Echium plantagineum for two weeks suffered 70% mortality within 5-13 weeks [Ref 17]. Young rats fed 20% Echium plantagineum for alternate two-week periods with a control feed had 50% mortality in 21 weeks [Ref. 17]. Adult rats fed Echium plantagineum continuously all died within 7-16 weeks at the 40% level and 37-40 weeks at the 20% level [Ref. 17]. The rats died with a mixture of acute and chronic liver damage [Ref. 17]. Tumours, 3 benign and I malignant, of a type observed in carcinogenesis experiments with other pyrrolizidine alkaloids developed in survivors of the study on adult rats fed 20 % Echium plantagineum [Ref. 6]. The number of tumours was below the significance level [Ref. 6].

Echium plantagineum is known to secrete nectar that is gathered by bees and it is used extensively by apiarists [Ref. 6]. It is estimated that honey from Echium plantagineum constitutes about 10-15% of total Australian production [Ref. 6]. The honey is sold mainly as blends with other honey. Honey prepared from Echium plantagineum has been shown to contain between 0.27 – 0.95ppm alkaloids [Ref. 18]. The possible intakes of pyrrolizidine alkaloids from this source are considered to be very low [Ref. 6]

All the toxicological findings reported are consistent with pyrrolizidine alkaloid poisoning. Pyrrolizidine alkaloids are not oil soluble (lipophillic) and therefore they would not be expected to be present in Echium plantagineum oil. 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. The tests determined that pyrrolizidine alkaloids were either not detectable or were present at extremely low levels (<15ng/g) in the oil.

IX Anticipated intake/ extent of use of the novel food

Anticipated use:
ECHIUM OIL is a vegetable oil rich in both omega-6 and omega-3 polyunsaturated fatty acids. It is anticipated that as a result it will be incorporated into dietary supplements and functional foods.

The potential for functional foods is estimated to grow to 5% of the world food market [Ref. 19]. So far omega-3 rich oils have been incorporated into breakfast cereals, milk, margarine, spreads, bread, cheese, yoghurt, cocoa, soft drinks, tea, confectionery, cookies and infant foods [Ref. 19][Ref. 20][Ref. 21]. Omega-3 enriched products are currently marketed in Japan, Korea, Taiwan and Europe including the United Kingdom and Scandinavia [Ref. 21].

In addition a docosahexaenoic acid enriched product has been fed to chickens in order to produce DHA enriched eggs [Ref. 19][Ref. 20]. The eggs are marketed in Germany, Spain, Portugal, Belgium, Luxembourg, Norway and Andorra [Ref. 19].

Dietary supplements of omega-6 and omega-3 fatty acids are normally offered in the form of gelatine capsules or oral emulsions. The addition of Vitamin E to these formulations is recommended in accordance with guidance offered by the UK committee on medical aspects of food policy [Ref. 22]. Vitamin E has been demonstrated as preventing the oxidation of polyunsaturated fatty acids (omega-3 and omega-6). A dose of 400-800IU of Vitamin E per day is recommended dependent on the total amount of polyunsaturated fatty acids in the supplement [Ref. 23].

We do not intend to sell Echium oil direct to consumers. Echium oil will be sold to food and health food manufacturers throughout Europe as an alternative to existing oils and fats rich in omega-6 or omega-3 polyunsaturated fatty acids.

Several oils containing triglycerides rich in omega-6 fatty acids are currently available on the market include 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.

We consider that the main application for Echium oil will be as a dietary supplement. We also envisage that Echium oil will be used as a source of essential fatty acids in other application areas including sports drinks, nutritional bars and dairy products such as milk and yoghurts. Echium oil will be marketed as possessing the benefits of both omega-3 and omega-6 essential fatty acids.

Anticipated intakes:
North Americans, Europeans and people of other industrialised nations are estimated to consume fats and oils at a level of 42% or more of their daily calories [Ref. 24]. The average number of calories consumed per person per day is 2500 [Ref. 24]. Since 1

gram of fat produces 9 calories this amounts to 110 grams of fat per person per day [Ref. 24].

Levels of fat consumption reported for developed countries include, Denmark 160 grams per day, New Zealand 155 grams per day, United Kingdom 142 grams per day and Canada 142 grams per day [Ref. 24]. The 1979 figures for the United states estimated fat consumption to be around 168 grams per day of which 34% was saturated, 40% monounsaturated and 15% polyunsaturated [Ref. 24].

The average western diet contains lower quantities of omega-3 than omega-6. Data from 1985 on the US national food supply indicates a level of 50mg per capita per day of eicosapentaenoic acid and 80mg per capita per day for docosahexaenoic acid [Ref. 22]. The dietary intake of total omega-3 fatty acids in the United Kingdom was estimated to be 250mg per capita per day in 1992 [Ref. 21].

In comparison a diet in which Echium oil provided all the fat content would consist of 11.1% saturated, 17.3% monounsaturated and 70.4% polyunsaturated fatty acids of which 43.5% would be omega-3 fatty acids. However it is highly unlikely that Echium oil would represent the sole source of dietary fat.

Although the potential for functional foods is estimated to grow to 5% the current market share is low [Ref. 19][Ref. 20]. In particular a yellow table spread enriched with 3% omega-3 fatty acids has been on the British market for over two years but only has a market share of 0.3% [Ref. 20].

An analysis of seventeen brands of encapsulated fish oil products purchased in the USA, UK and Canada during 1984-1988 identified eicosapentaenoic acid levels of between 80 - 302mg/g and docosahexaenoic acid levels of between 78 - 254mg/g [Ref. 25].

A similar analysis of encapsulated evening primrose oil products identified gamma linolenic acid levels of between 1.9 – 10.5 expressed as percentage weight of total fatty acids and linoleic acid levels of between 60.1 – 75.8 [Ref. 26].

An omega-3 / 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 intake. The daily intake of 2 gelatine- based capsules contains in addition 20mg of vitamin E (as D alpha tocopheryl acetate). The daily intake of 1 teaspoon (5ml) of oral emulsion based on high oleic acid sunflower oil contains in addition 10mg vitamin E (as DL-alpha tocopheryl acetate) The omega-3 / omega-6 fatty acid blend is provided by combining evening primrose oil and a marine fish oil.

In comparison 500mg capsules based solely on Echium oil would provide 58mg of gamma linolenic acid and 64mg of stearidonic acid.

We do not intend to sell Echium oil direct to consumers. Echium oil will be sold to food and health food manufacturers throughout Europe as an alternative to existing oils and fats rich in omega-6 or omega-3 polyunsaturated fatty acids. We consider that the main application for Echium oil will be as a dietary supplement. This will be in capsule form with a likely level of consumption of between 1000mg and 250mg per day.

We also envisage that Echium oil will be used as a source of essential fatty acids in other application areas including sports drinks, nutritional bars and dairy products such as milk and yoghurts. Echium oil would be added to such products at very low levels, less than 500mg per item.

XI Nutritional information on the novel food

Total fat & oil consumption:
Dietary fat is essential for health and the FAO/WHO expert consultation on fats and oils in human nutrition have recommended that fat should constitute between15% - 35% of energy intake [Ref. 27]. Adequate dietary fat intakes are considered particularly important prior to and during pregnancy and lactation [Ref. 27]. The FAO/WHO joint expert consultation recommended that women of reproductive age should consume at least 20% of their energy from fat [Ref. 27]. A calorific fat intake of approximately 20% is normally used clinically in hospitalised patients who are infected or at risk of becoming so [Ref. 28]. It is recommended that saturated fat should not exceed 10% of energy [Ref. 27].

It is intended that Echium oil will replace existing fats and oils in food and therefore total fat consumption should not increase. The amount of saturated fat may be reduced depending on the fat or oil that Echium oil is intended to replace.

Echium oil contains on average 11.1% of saturated fatty acids. 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 [Ref. 29]. The level of saturated fatty acids in herring oil is 26.1% and in mackerel is 27.5% [Ref. 29].

Omega-6 fatty acids:
About 1% of daily calories (an average of 3 grams) linoleic acid is enough to relieve the symptoms of deficiency of this essential fatty acid and therefore represents a minimum daily requirement [Ref. 24].

The optimum dose of linoleic acid is considered to be between 3-6% (9-18 grams on average) [Ref. 24]. The FAO/WHO expert consultation on fats and oils in human nutrition has recommended that linoleic acid should provide between 4-10% of energy [Ref. 27].

Echium oil contains on average 15.4% of linoleic acid and 11.5% of its metabolite gamma linolenic acid. 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 levels vary greatly in edible vegetable oils from 0% for safflower oil to approximately 10% for evening primrose oil and approximately 20% for borage oil [Ref. 29].

Omega-3 fatty acids:
The daily requirement and optimum dose of alpha linolenic acid is not known [Ref. 24]. A level of 0.54% of daily calories was required to reverse symptoms of alpha linolenic acid deficiency in a 6 year old girl [Ref. 24].

An optimum dose is hypothesised for alpha-linolenic acid of 6 grams per day [Ref. 24]. It is estimated that 95% of affluent people would benefit from dietary supplementation with omega-3 fatty acids [Ref. 24].

Echium oil contains on average 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 [Ref. 29]. 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 [Ref. 30]. Blackcurrant seed oil is an exception in that it contains 11.4% of alpha-linolenic acid and 3.02% of stearidonic acid.

Omega-6: omega-3 ratio:
The ^{6} Desaturase step is considered to be the rate limiting step in the conversion of the essential fatty acids to their more highly unsaturated metabolites (gamma-linolenic acid and stearidonic acid). Incorporation of high levels of linoleic or alpha-linolenic acid does not seem to raise the levels of their corresponding metabolites [Ref. 30]. However administration of those metabolites of linoleic and alpha linolenic acid usually raises the levels of that metabolite and its elongation products in human plasma [Ref. 30].

Dietary supplementation with oils rich in linoleic acid, such as safflower oil, did not increase omega-6 fatty acid content of human milk [Ref. 30]. Whereas oils rich in gamma linolenic acid such as evening primrose oil and black currant seed oil increased the levels of di-homo gamma linolenic acid in human milk two fold [Ref. 30].

The occurrence of eicosapentaenoic acid in the liver and plasma was two fold higher for rats whose diet was supplemented with the ethyl ester of stearidonic acid than with the ethyl ester of alpha-linolenic acid [Ref. 31].

In a comparison of various combinations of omega-3 and omega-6 methyl ester mixtures it was demonstrated that gamma-linolenic acid and its metabolites were incorporated more favourably into liver phospholipids than stearidonic acid and its metabolites [Ref. 32]. Switching the omega-6 content from linoleic to gamma-linolenic increased the omega-6: omega-3 ratio two fold [Ref. 32]. Whereas switching the omega-3 content from alpha linolenic to stearidonic acid decreased the omega-6: omega-3 ratio by 30% [Ref. 32].

The enzymes that convert omega-6 and omega-3 fatty acids are slower by a factor of four in the case of omega-3 fatty acids [Ref. 24]. However, detailed kinetic analysis of prostaglandin biosynthesis from omega-6 and omega-3 fatty acids indicated a four-fold difference in favour of omega-6 [Ref. 33].

A ratio of linoleic to alpha-linolenic acid of between 5:1 and 10:1 is recommended in the diet [Ref. 27]. The FAO/WHO expert consultation on fats and oils in human nutrition has recommended that linoleic acid should provide between 4-10% of energy [Ref. 27]. Therefore alpha-linolenic acid should provide between 0.4%-2% of energy depending on the amount of linoleic acid in the diet.

The average western diet contains lower quantities of omega-3 than omega-6. The dietary intake of total omega-3 fatty acids in the United Kingdom was estimated to be 250mg per capita in 1992 which represents only 0.09% of dietary energy [Ref. 21]. Analysis of the diet of healthy 40 year old men in Edinburgh indicated that linoleic acid intake was low but still represented 3% of energy levels [Ref. 30]. It is estimated that 95% of affluent people would benefit from dietary supplementation with omega-3 fatty acids [Ref. 24].

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.

XII Microbiological information on the novel food

Echium oil is an anhydrous system and therefore will not support microbiological growth. In addition the processes used in manufacturing Echium will act to filter out any microbial organisms. The absence of microbiological contamination has been confirmed by testing a sample of the oil; a copy of results appears in Appendix 13.

Echium oil is extracted from seed in a totally sealed environment achieved either by vacuum or by nitrogen capping. The Lincoln Solvent Extraction Facility operates in accordance to Good Manufacturing Practice (GMP) and so it is highly unlikely that microbiological contamination could occur during the production process. In addition, the moisture level of the oil is constantly checked throughout the production process to ensure that levels remain below 1000ppm, further reducing the possibility of microbiological contamination

XIII Toxicological information on the novel food

1.0 Component fatty acids:

The lipid profile for Echium oil is similar to that of borage oil and blackcurrant seed oil [Appendix 9]. Both borage oil and blackcurrant oil are widely used as ingredients of cosmetics, pharmaceuticals, foods and food supplements [Ref. 34][Ref. 35].

The major fatty acids found in Echium oil are as follows:

1.1 Palmitic acid

Palmitic acid is the most widely occurring saturated fatty acid and is present in most commercial oils [Ref. 29]. It is found in large quantities in fish oils (10-30%) and tropical fats such as coconut oil (6.9%), palm kernel oil (6.5–11%) and palm oils (32–59%) [Ref. 24][Ref. 29]. Echium oil contains on average 7.1% palmitic acid.

1.2 Stearic acid

Stearic acid is found in abundance in tallow (5-30%), cocoa butter (30-36%) and shea nut butter (44%) [Ref. 24] [Ref. 29]. Echium oil contains on average 4.0% stearic acid.

1.3 Oleic acid

Oleic acid is the most widely occurring natural fatty acid and is found in practically all lipids [Ref. 29]. It is found in large quantities in olive oil (43.7-83%), almond oil (65-70%) and peanut oil (37.9%) oils [Ref. 24]. Oleic acid is also produced endogenously in the body [Ref. 24][Ref. 29]. Echium oil contains on average 17.3% oleic acid.

1.4 Linoleic acid (LA)

Linoleic acid is found in safflower oil (75.3%), sunflower oil (68.5%), soybean oil (53%) and sesame oil (45%) [Ref. 24][Ref. 29]. Echium oil contains on average 15.4% linoleic acid.

1.5 Alpha linolenic acid (ALA)

Linolenic acid is the major fatty acid found in plant leaves, stems and roots and other photosynthetic organisms [Ref. 29]. Flaxseed is one of the richest sources of ALA with over 50%, Chia and kukui (candlenut) contain about 30%, hemp seed around 20% [Ref. 24]. Pumpkinseed oil may have up to 15%, canola oil up to 10% and walnut oil between 3-11% [Ref. 24]. Soybean oil normally contains 5-7% [Ref. 24]. Echium oil contains on average 30.7% ALA.

1.6 Gamma linolenic acid (GLA)

The richest source of GLA is borage oil (20%) followed by black currant seed oil (15%) and evening primrose oil (9%) [Ref. 24]. Echium oil contains on average 11.5% GLA.

1.7 Stearidonic acid (SA)

Stearidonic acid is found in most fish oils such as sardine oil, herring oil and pilchard oil [Ref. 36][Ref. 37]. The most well known plant source of stearidonic acid is black currant seed oil [Ref. 24]. Echium oil contains on average 12.8% stearidonic acid.

2.0 Omega-6 & omega-3 fatty acids:

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 acid (EFA) is a term used to describe fatty acids that are needed in order to manufacture body lipids, biological membranes and hormone like substances such as prostaglandins. EFA cannot themselves be synthesised in the body and therefore must be obtained from the diet [Ref. 38][Ref. 39]. 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. 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 [Appendix 10] [Ref. 39].

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.

A correct balance between the various eicosanoids is required in order to maintain good health. The ratio of omega-6: omega-3 in the body is about 1:1 in the brain, 5:1 in fat tissue and 4:1 in other tissues [Ref. 24]. The levels of the eicosanoids can vary during different stages in the development of the body, with age and during the menstrual cycle. In addition the activity of ^{6} desaturase, an enzyme system involved in the metabolism of essential fatty acids, 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 undesirably low levels of EFA metabolites [Ref. 40]. In this situation fatty acids such as gamma-linolenic acid and stearidonic acid become conditionally essential. 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.

2.1 Summary of efficacy of Essential Fatty Acid's in Disease Management.

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 have either a preventative or beneficial management effect in Cardiovascular disease, osteoporosis, diabetes, arthritis and numerous skin disorders.

5. Evaluation and Conclusion by the applicant

Echium oil is a complex triglyceride obtained by extracting the oil from Echium Plantagineum. This oil has not hitherto been widely consumed in the European Community but recent research into its fatty acid composition have led to an interest in utilising Echium oil as an ingredient for dietary supplements and other nutritional products.

The production process employed to extract the oil from Echium Plantagineum is not novel; the same process is currently used to process several lipids with food approval status in the EU, such as Evening Primrose, Borage 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. A summary of the production process can be found in Section 2 of this document.

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 currently consumed for food use in the EU. Oleic acid, for example, is found in Olive oil whilst Stearidonic Acid is found in most fish oils. A full breakdown of the fatty acid components of Echium oil can be found in Section XIII of this application.

Echium oil also contains very small levels of Erucic Acid, which has been shown to exhibit anti-nutritional properties. Erucic Acid is typically found at levels of 0.l% in Echium oil although it sometimes can be slightly higher. The product specification stipulates an upper limit of 1%, which is considerably lower than the 5% upper limit that EU regulations currently stipulate for food products.

Echium oil contains many minor constituents which are not unusual in plant derived oils. The product specification of Echium oil contains an upper limit of 2% for the unsaponifiable content. The unsaponfiable content contains a mixture of sterols, hydrocarbons and other non-fatty acid compounds and analysis shows (see Appendix 11) that the unsaponifiable content of Echium oil does not contain any unusual or unknown compounds. Tests have also been carried out to prove the absence of cyclopropenoid and epoxy fatty acids and heavy metals such as Arsenic and Lead.

Pyrrolizidine alkaloids are known to occur in certain species of the family Boraginaceae and have been isolated from Echium plantagineum [Ref4][Ref5]. Pyrrolizidine alkaloids are of concern because they cause acute and chronic liver disease [ref6]. In addition to the liver they may damage the lung, kidney and other organs and they also possess mutagenic, teratogenic and carcinogenic properties [Ref6].

Tests were carried out (see Appendix 6) to determine whether or not pyrrolizidine Alkaloids were present in Echuim oil samples. Pyrrolizidine Alkaloids are not lipophillic and, therefore, would not be expected to be present in the oil in any great quantity. Two of the four samples were found to be below the lowest detectable limits of 4 ng/g, whilst two other samples recorded results of 9ng/g and 11ng/g.

The product specification stipulates that Pyrrolizidine Alkaloids should not be greater than 15ng/gram which is considered to be well below levels that would cause harm [Ref.6]. There are other species that are known to contain pyrrolizidine alkaloids in their foliage, one such example being Borago officinalis. Borage oil is currently widely used as health supplement.

Cytochrome C allergens have been isolated from the pollen of Echium plantagineum [Ref7]. The filter process used in the processing of Echium, however, 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. A total and recordable protein content (and therefore a Cytochrome C allergen content) of less than 1ppm in the SuperRefined oil and less than 2ppm in the unrefined oil was ascertained by this method.

Echium oil is a vegetable oil rich in both Omega-3 and Omega-6 polyunsaturated fatty acids. It is anticipated that as a result it will be incorporated into dietary supplements and functional foods.

The functional food market is rapidly growing and it has been suggested that there is potential for it to grow to 5% of the world food market. Echium oil, as a rich source of essential fatty acids, is likely to be used as an ingredient in sports drinks, nutritional bars and dairy products. Echium oil would be added to these products at very low levels, typically less than 500mg per item.

The main uptake of Echium oil is likely to be for use as a dietary supplement. Dietary supplements of omega-3 and omega-6 fatty acids are normally offered in the form of gelatine capsules or oral emulsions. Echium oil has the potential to replace existing sources of these fatty acids such as Borage, Evening Primrose and Blackcurrant Seed Oil, consumed for their omega-6 fatty acid content, and tuna, sardine and menhaden oil, consumed for their omega-3 fatty acid content. When used in capsule form the likely level of consumption will be between 1000mg and 250mg per day.

Echium oil, due to its inherently high production cost, is highly unlikely to be utilised as a replacement for cooking oil s such as Canola, Sunflower and Olive oil. We, therefore, consider that the proportion of an average person’s daily fat intake derived from Echium oil will be very small.

In conclusion, we consider that Echium oil shares many characteristics with oils derived from both plants and fish that are currently consumed for food purposes and that these characteristics pose no toxicological or anti-nutritional threat to consumers. Furthermore, the production of Echium oil, from the growing of the crop to the extraction of oil, complies with all current food legislation and, again, can be considered to be as safe as reasonably possible. 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.

We believe that the main areas of concern relating to Echium oil is the fact that Echium plantagineum is known to contain pyrrolizidine alkaloids and an allergen to cytochrome C in the leaves and external seed coating. The possibility exists therefore that these may also be present in the oil. Tests have been carried out that show that both pyrrolizidine

alkaloids and cytochrome C allergen are either absent in the oil or are present in such negligible quantities as to be well below accepted 'no-effect' levels."

Summary of Echium Oil Food Approval Application

Specification of food approval application

Echium oil is a complex triglyceride obtained by extracting the oil from the seeds of Echium plantagineum. An optimum temperature continuous flow extraction process using food grade hexane is employed to obtain the oil.

The product specification for Ambient Temperature Solvent Extracted Echium oil can be found at the end of this summary.

The values for the level of each fatty acid found in Echium oil are based on data obtained from samples grown in several different years covering a number of variables such as weather and geographical location of the crop. Therefore, it is considered that they provide an accurate reflection of likely parameters for each fatty acid.

Due to the undesirable nature of cyclopropenoid and epoxy fatty acids, tests were performed to confirm their absence from Echium oil. A GC-MS analysis was carried out, which determined that in the two production samples from the 1999 harvests neither cyclopropenoid nor epoxy fatty acids were found to be present in the oil.

Erucic acid is found in small quantities in Echium oil, typically at levels of less than 0.5%. The product specification stipulates an upper limit of 1%.

The levels of unsaponifiable matter in the two 1999 production samples 1124 and 1124-a were 1.1% and 1.2% respectively. The unsaponifiable matter is a natural component of the oil and could contain hydrocarbons, sterols and other non-fatty compounds. These levels are not untypical for unrefined oils. Analysis has confirmed that no unusual or unknown compounds are present within the unsaponifiable matter of Echium oil.

Tests to determine the levels of heavy metals; arsenic, lead, iron and copper were performed. Levels of these heavy metals were all found to be below the lowest detectable level. The product specification stipulates that the heavy metal content must be below the lowest detectable limit, which varies between substances.

A peroxide value of 10 maximum is included on the product specification. The peroxide value of 1999 production batches 1124 and 1124-A were 2.65 and 2.01 respectively.

Pyrrolizidine alkaloids are known to occur in certain species of the family Boraginaceae and have been isolated from Echium plantagineum. Pyrrolizidine alkaloids are of concern because they cause acute and chronic liver disease. In addition to the liver they may damage the lung, kidney and other organs, they also possess mutagenic, teratogenic and carcinogenic properties.

Pyrrolizidine alkaloids are not lipophillic and, therefore, would not be expected to be present in any substantial quantity in the oil. Tests carried out on several samples of both Super Refined ® and unrefined Echium oil shows that levels of Pyrrolizidine Alkaloids were either below the lowest detectable limit of 4ng/g or were very low (<15ng/g).

The product specification stipulates that pyrrolizidine alkaloids should not be greater than 15ng/gram, which is considered to be well below levels that would cause harm [C C J Culvenor, Patersons Curse and toxic alkaloids]. There are other plants that are known to contain pyrrolizidine alkaloids in their foliage, one such example being Borago officinalis. Borage oil is currently widely used as health supplement.

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 absorbance at 595nm of the coloured product of the reaction of protein and Bradford Reagent was measured. Cytochrome C allergens isolated from the pollen of echium plantagineum were characterised as proteins with a molecular weight of 12,800.

It can be reasonably assumed that the maximum Cytochrome C allergen content is no more than the total protein content. Using standards, a total protein content of less than 19.85µg/gram of SuperRefined oil was determined. However problems associated with the low solubility of Echium oil in the aqueous reagent meant that the aqueous reagent phase and Echium oil (dissolved in hexane) phase had to be mixed vigorously before measurement of the sample solution using UV spectrometry at a wavelength of 595nm. This is not ideal and therefore a visual inspection of the sample, standards and blank was carried out. A total and recordable protein content (and therefore a Cytochrome C allergen content) of less than 1ppm in the SuperRefined oil and less than 2ppm in the unrefined oil was determined by this method.

With the recent review and subsequent revision of the “long term arrangements for the extension of use (2000)” the Pesticide Safety Directive, in consultation with the Advisory Committee on Pesticides and relevant Government Departments, have now included echium (see appendix 8). This development has two significant consequences, firstly it legally allows the field application of any pesticide with “on label” approval for use in oilseed rape (i.e. has a MAFF approval number and the product label on the pesticide container refers to oilseed rape). Secondly it demonstrates that all relevant agencies are confident that the extrapolation of safety data supplied to gain approval for use of these products on oilseed rape is valid and that their use on echium poses no threat to the consumer, environment or sprayer operator.

The taxonomy of Echium Plantagineum is:

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

Effect of the production process on the Novel Food

The seed is extracted at John K King & Sons Ltd extraction facility in Lincoln. This facility is used to process other seeds that currently have food approval status in the EC,

including Borage and Evening Primrose Oil and for this reason the production process is not considered to be novel.

Seed can be received in either bulk or bulk bags, depending on requirement, and is transported to the silos using an air handling system. This air system has been purposely designed to reduce any risk of possible contamination between the various seed types that can be handled, as it is a totally self-cleaning system.

To ensure a successful extraction of the oil, the Echium seed is first cracked which, by gently 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 a continuous flow extractor where it is brought into contact with food grade hexane solvent. As the seed is conveyed through the extractor fresh solvent is washed through it in a counter current direction, resulting in the solvent becoming increasingly enriched with oil. 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 Echium seed is gently bathed in hexane over a period of ninety minutes in order to remove the highest yields possible of good quality oil. The extraction process is carried out at room temperature in an oxygen free atmosphere. The oil enriched solvent then passes through a distillation system where the solvent is removed from the oil to leave less than 1 part per million in the oil.

Once production is completed the oil is tested against the product specification by the fully accredited laboratory at Lincoln. In the event that the oil is found to be out of specification it will not be released by Quality Control. It will either be rejected outright or it could be re-processed until it passes the product specification.

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

The Boraginaceae are a large plant family, of which Echium plantagineum is a member, with approximately 100 genera and 2500 species that are widely distributed throughout the Northern Hemisphere. The family is well known to herbalists and gardeners because it includes many ornamental and medicinal plants.

The genus Echium contains about 30 species distributed across Europe, the Mediterranean region, Madeira, the Canaries and the Azores.

Echium plantagineum and its products have not hitherto been used for human consumption to a significant degree within the Community. Human exposure to the plant does occur by ingesting honey produced by bees foraging on wild Echium. Evidence is available on the effects in animals of ingestion of Echium plantagineum.

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. Measurements of herbage dry matter content, nitrogen content and digestibility of Echium plantagineum indicate that it would be nutritious forage for grazing animals. However the presence of pyrrolizidine alkaloids in the plant means that there is a risk that grazing animals will be poisoned.

The level of pyrrolizidine alkaloids is normally between 0.1-0.3% of the dry weight of the whole plant but levels as high as 0.9% have been reported. Field evidence strongly indicates that horses, pigs and to a lesser extent sheep are all affected.

Echium plantagineum was fed as the sole diet to crossbred sheep with or without a history of previous access to the plant in a pen feeding trial. Compared to a control group receiving a diet of Lucerne chaff and oats, sheep on the Echium diet lost weight and several animals died Histological examination produced evidence of excessive copper accumulation in the liver and biochemical evidence of liver toxicity and was usually accompanied by pyrrolizidine alkaloid damage. It was concluded that Echium plantagineum alone was not suitable fodder for sheep.

Young rats fed 40% Echium plantagineum for two weeks suffered 70% mortality within 5-13 weeks. Young rats fed 20% Echium plantagineum for alternate two-week periods with a control feed had 50% mortality in 21 weeks. Adult rats fed Echium plantagineum continuously all died within 7-16 weeks at the 40% level and 37-40 weeks at the 20% level. The rats died with a mixture of acute and chronic liver damage. Tumours, 3 benign and I malignant, of a type observed in carcinogenesis experiments with other pyrrolizidine alkaloids developed in survivors of the study on adult rats fed 20 % Echium plantagineum. The number of tumours was below the significance level.

Echium plantagineum is known to secrete nectar that is gathered by bees and it is used extensively by apiarists. It is estimated that honey from Echium plantagineum constitutes about 10-15% of total Australian production. The honey is sold mainly as blends with other honey. Honey prepared from Echium plantagineum has been shown to contain between 0.27 – 0.95ppm alkaloids. The possible intakes of pyrrolizidine alkaloids from this source are considered to be very low.

All the toxicological findings reported are consistent with pyrrolizidine alkaloid poisoning. Pyrrolizidine alkaloids are not oil soluble (lipophillic) and therefore they would not be expected to be present in Echium plantagineum oil. 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. None or very small quantities of alkaloids were detected in the crude or refined oils, all results being below 15ng/g (equivalent to 0.015ppm)

Anticipated intake/ extent of use of the novel food

Anticipated use:
ECHIUM OIL is a vegetable oil rich in both omega-6 and omega-3 polyunsaturated fatty acids. It is anticipated that as a result it will be incorporated into dietary supplements and functional foods.

We do not intend to sell Echium oil direct to consumers. Echium oil will be sold to food and health food manufacturers throughout Europe as an alternative to existing oils and fats rich in omega-6 or omega-3 polyunsaturated fatty acids.

Several oils containing triglycerides rich in omega-6 fatty acids are currently available on the market include 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.

We consider that the main application for Echium oil will be as a dietary supplement. We also envisage that Echium oil will be used as a source of essential fatty acids in other application areas including sports drinks, nutritional bars and dairy products such as milk and yoghurts. Echium oil will be marketed as possessing the benefits of both omega-3 and omega-6 essential fatty acids.

Anticipated intakes:
North Americans, Europeans and people of other industrialised nations are estimated to consume fats and oils at a level of 42% or more of their daily calories. The average number of calories consumed per person per day is 2500. Since 1 gram of fat produces 9 calories this amounts to 110 grams of fat per person per day.

The average western diet contains lower quantities of omega-3 than omega-6. Data from 1985 on the US national food supply indicates a level of 50mg per capita per day of eicosapentaenoic acid and 80mg per capita per day for docosahexaenoic acid. The dietary intake of total omega-3 fatty acids in the United Kingdom was estimated to be 250mg per capita per day in 1992.

An omega-3 / 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 intake. The daily intake of 2 gelatine- based capsules contains in addition 20mg of vitamin E (as D alpha tocopheryl acetate). The daily intake of 1 teaspoon (5ml) of oral emulsion based on high oleic acid sunflower oil contains in addition 10mg vitamin E (as DL-alpha tocopheryl acetate) The omega-3 / omega-6 fatty acid blend is provided by combining evening primrose oil and a marine fish oil.

In comparison 500mg capsules based solely on Echium oil would provide 58mg of gamma linolenic acid and 64mg of stearidonic acid.

We consider that the main application for Echium oil will be as a dietary supplement. This will be in capsule form with a likely level of consumption of either 500mg or 250mg per day. We also envisage that Echium oil will be used as a source of essential fatty acids in other application areas including sports drinks, nutritional bars and dairy products such as milk and yoghurts. Echium oil would be added to such products at very low levels, less than 500mg per item.

Nutritional information on the novel food

Total fat & oil consumption:
Dietary fat is essential for health and the FAO/WHO expert consultation on fats and oils in human nutrition have recommended that fat should constitute between15% - 35% of energy intake. It is intended that Echium oil will replace existing fats and oils in food and therefore total fat consumption should not increase. The amount of saturated fat may be reduced depending on the fat or oil that Echium oil is intended to replace.

Echium oil contains on average 11.1% of saturated fatty acids. 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 [Ref. 29]. The level of saturated fatty acids in herring oil is 26.1% and in mackerel is 27.5% [Ref. 29].

Omega-6 fatty acids:
About 1% of daily calories (an average of 3 grams) linoleic acid is enough to relieve the symptoms of deficiency of this essential fatty acid and therefore represents a minimum daily requirement. Echium oil contains on average 15.4% of linoleic acid and 11.5% of its metabolite gamma linolenic acid.

Omega-3 fatty acids:
The daily requirement and optimum dose of alpha linolenic acid is not known [Ref. 24]. A level of 0.54% of daily calories was required to reverse symptoms of alpha linolenic acid deficiency in a 6 year old girl. Echium oil contains on average 30.7% of alpha-linolenic acid and 12.8% of its metabolite stearidonic acid.

Omega-6: omega-3 ratio:
The ^{6} Desaturase step is considered to be the rate limiting step in the conversion of the essential fatty acids to their more highly unsaturated metabolites (gamma-linolenic acid and stearidonic acid). Incorporation of high levels of linoleic or alpha-linolenic acid does not seem to raise the levels of their corresponding metabolites [Ref. 30]. However administration of those metabolites of linoleic and alpha linolenic acid usually raises the levels of that metabolite and its elongation products in human plasma [Ref. 30].

Dietary supplementation with oils rich in linoleic acid, such as safflower oil, did not increase omega-6 fatty acid content of human milk. Whereas oils rich in gamma linolenic acid such as evening primrose oil and black currant seed oil increased the levels of di-homo gamma linolenic acid in human milk two fold.

A ratio of linoleic to alpha-linolenic acid of between 5:1 and 10:1 is recommended in the diet. The FAO/WHO expert consultation on fats and oils in human nutrition has recommended that linoleic acid should provide between 4-10% of energy. Therefore alpha-linolenic acid should provide between 0.4%-2% of energy depending on the amount of linoleic acid in the diet.

The average western diet contains lower quantities of omega-3 than omega-6. The dietary intake of total omega-3 fatty acids in the United Kingdom was estimated to be 250mg per capita in 1992 which represents only 0.09% of dietary energy. Analysis of the diet of healthy 40 year old men in Edinburgh indicated that linoleic acid intake was low but still represented 3% of energy levels. It is estimated that 95% of affluent people would benefit from dietary supplementation with omega-3 fatty acids.

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Echium oil is extracted from seed in a totally sealed environment achieved either by vacuum or by nitrogen capping. The Lincoln Solvent Extraction Facility operates in accordance to Good Manufacturing Practice (GMP) and so it is highly unlikely that microbiological contamination could occur during the production process. In addition, the moisture level of the oil is constantly checked throughout the production process to ensure that levels remain below 1000ppm, further reducing the possibility of microbiological contamination

Toxicological information on the novel food

Component fatty acids:
The lipid profile for Echium oil is similar to that of borage oil and blackcurrant seed. Both borage oil and blackcurrant oil are widely used as ingredients of cosmetics, pharmaceuticals, foods and food supplements.

The major fatty acids found in Echium oil are Palmitic, Stearic, Oleic, Linoleic, Alpha-Linolenic and Gamma-Linolenic acids. These fatty acids are all widely found in natural oils currently consumed for food use in the EC.

Omega-6 & omega-3 fatty acids:

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 acid (EFA) is a term used to describe fatty acids that are needed in order to manufacture body lipids, biological membranes and hormone like substances such as prostaglandins. EFA cannot themselves be synthesised in the body and therefore must be obtained from the diet. 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. 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.

Summary of efficacy of Essential Fatty Acid's in Disease Management.

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 have either a preventative or beneficial management effect in Cardiovascular disease, osteoporosis, diabetes, arthritis and numerous skin disorders.

Evaluation and Conclusion by the applicant

Echium oil is a complex triglyceride obtained by extracting the oil from Echium Plantagineum. This oil has not hitherto been widely consumed in the European

Community but recent research into its fatty acid composition have led to an interest in utilising Echium oil as an ingredient for dietary supplements and other nutritional products.

The production process employed to extract the oil from Echium Plantagineum is not novel; the same process is currently used to process several lipids with food approval status in the EU, such as Evening Primrose, Borage 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.

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 currently consumed for food use in the EU.

Echium oil also contains very small levels of Erucic Acid, which has been shown to exhibit anti-nutritional properties. The product specification stipulates an upper limit of 1%, which is considerably lower than the 5% upper limit that EU regulations currently stipulate for food products.

Echium oil contains many minor constituents which are not unusual in plant derived oils. The product specification of Echium oil contains an upper limit of 2% for the unsaponifiable content. The unsaponfiable content has been analysed and it was shown contain a mixture of sterols, hydrocarbons and other non-fatty acid compounds. Tests have also been carried out to prove the absence of cyclopropenoid and epoxy fatty acids and heavy metals such as Arsenic and Lead.

Pyrrolizidine alkaloids are known to occur in certain species of the family Boraginaceae and have been isolated from Echium plantagineum. Pyrrolizidine alkaloids are of concern because they cause acute and chronic liver disease. In addition to the liver they may damage the lung, kidney and other organs and they also possess mutagenic, teratogenic and carcinogenic properties.

Tests were carried out to determine whether or not pyrrolizidine Alkaloids were present in Echuim oil samples. Pyrrolizidine Alkaloids are not lipophillic and, therefore, would not be expected to be present in the oil in any great quantity. Two of the four samples were found to be below the lowest detectable limits of 4 ng/g, whilst two other samples recorded results of 9ng/g and 11ng/g.

The product specification stipulates that Pyrrolizidine Alkaloids should not be greater than 15ng/gram which is considered to be well below levels that would cause harm. There are other species that are known to contain pyrrolizidine alkaloids in their foliage, one such example being Borago officinalis. Borage oil is currently widely used as health supplement.

Cytochrome C allergens have been isolated from the pollen of Echium plantagineum. The filter process used in the processing of Echium, however, 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. A total and recordable protein content (and therefore a Cytochrome C allergen content) of

less than 1ppm in the SuperRefined oil and less than 2ppm in the unrefined oil was ascertained by this method.

Echium oil is a vegetable oil rich in both Omega-3 and Omega-6 polyunsaturated fatty acids. It is anticipated that as a result it will be incorporated into dietary supplements and functional foods.

Echium oil, as a rich source of essential fatty acids, is likely to be used as an ingredient in sports drinks, nutritional bars and dairy products. Echium oil would be added to these products at very low levels, typically less than 500mg per item.

The main uptake of Echium oil is likely to be for use as a dietary supplement. Dietary supplements of omega-3 and omega-6 fatty acids are normally offered in the form of gelatine capsules or oral emulsions. When used in capsule form the likely level of consumption of between 1000mg and 250mg per day.

Echium oil, due to its inherently high production cost, is highly unlikely to be utilised as a replacement for cooking oil s such as Canola, Sunflower and Olive oil.

In conclusion, we consider that Echium oil shares many characteristics with oils derived from both plants and fish that are currently consumed for food purposes and that these characteristics pose no toxicological or anti-nutritional threat to consumers. Furthermore, the production of Echium oil, from the growing of the crop to the extraction of oil, complies with all current food legislation and, again, can be considered to be as safe as reasonably possible. 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.

We believe that the main areas of concern relating to Echium oil is the fact that Echium plantagineum is known to contain pyrrolizidine alkaloids and an allergen to cytochrome C in the leaves and external seed coating. The possibility exists therefore that these may also be present in the oil. Tests have been carried out that show that both pyrrolizidine alkaloids and cytochrome C allergen are either absent in the oil or are present in such negligible quantities as to be well below accepted 'no-effect' levels."

John K King & Sons Limited

PRODUCT SPECIFICATION

Product: Echium oil Cold Temperature Extracted

Appearance: A clear yellow to green-yellow free-flowing oil, free from foreign matter and imiscible with water.

Taste and Odour: Characteristic of oil – natural, bland taste and smell with no trace of rancidity or other abnormality organoleptically.

Tocopherols: Tocopherols added at request of customer.

Analytical Specifications:

Specific Gravity at 20oC	**0.915-0.925 g/ml**
Peroxide Value	**Not greater than 10.0 mEq 02 / kg oil.**
Acid Value	**Not greater than 4.0 mg KOH/g oil**
Trans Acids (Trans)	**Not more than 2%**
Non-Saponifiable Matter:	**Not more than 2%**
Heavy Metals	**Lead <0.1mg/kg Arsenic <0.1mg/kg Copper <0.05ppm Iron <1ppm**
Anisidine Value	**Not more than 20**
Moisture Content:	**Should be less than 0.1%**

SUMMARY OF ECHIUM OIL FOOD APPLICATION

Agrochemicals	**Should not be detected**
Residual Solvent	**< 1ppm**
Pyrrolizidine Alkaloids	**Not more than 15ng/g (0.015ppm)**

Fatty Acid Composition

Carbon Length	Name	%
C16:0	Palmitic Acid	6-8
C18:0	Stearic Acid	3-5
C18:1	Oleic Acid	15-19
C18:2	Linoleic Acid	14-18
C18:3 (n-3)	Alpha-Linolenic Acid	28-33
C18:3 (n-6)	Gamma-Linolenic Acid	9-12
C18:4	Stearidonic Acid	10.5-14
C22:1	Erucic Acid	Max 1

Revision Number:	**0000075/2**	**Date:**	**28/07/00**
Issued by:	**Phil Nicholls**	**Approved by:**	**Darren Keeler**
Reason for Revision:	**Added parameter for Erucic Acid. Reduced limits for Arsenic and Lead levels.**		

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Appendix 1

Lipid profiles of Echium Plantagineum.

Appendix 1 – Lipid profiles from trials work

Table 1: Lipid profiles of optimum temperature extracted Echium oil.

Year	% oil[2]	Lipid constituents[2]						
		16.0	**18.0**	**18.1**	**18.2**	**18.3[3]**	**18.3[4]**	**18.4[5]**
1999		6.5	3.7	14.7	17.5	30.5	10.5	12.8
		6.6	3.7	15.3	15.9	28.7	10.7	13.1
Mean		**6.55**	**3.7**	**15.0**	**16.7**	**29.6**	**10.6**	**12.95**

Notes
1. Data provided by John K King & Sons Ltd, Lincoln
2. The first number represents the number of carbon atoms in the fatty acid analysed, the second number represents the number of double bonds. Fatty acids not determined are left as blank entries.
3. Alpha-Linolenic acid (ALA)
4. Gamma-Linolenic acid (GLA)
5. Stearidonic acid (SA)

Table 2: Lipid profiles of oil extracted from Echium plantagineum[1]

Year	% oil[2]	Lipid constituents[3]							
		16.0	**18.0**	**18.1**	**18.2**	**18.3[4]**	**18.3[5]**	**18.4[6]**	
1991	28.0				14.0	37.0	9.1	14.6	
1992	24.7				14.7	38.8	10.2	7.5	
	23.1				13.1	34.9	9.8	16.2	
	23.9				14.9	37.2	10.5	9.4	
1993	25.5							14.4	
	22.5							15.9	
	28.9							14.5	
	28.0							12.8	
	27.0							13.8	
	25.3							14.3	
	27.2						9.5	15.4	
	24.8						9.2	11.7	
1995	24.6							11.2	
	28.6							10.8	
	37.8							11.6	
	35.3							9.9	
	39.8							11.7	
	33.4							12.0	
	41.5							11.5	
	29.0							11.8	
	28.4							10.8	
1996	29.9	6.4	3.6	14.5	13.8	36.0	9.7	14.9	
	28.7	6.6	2.6	13.6	14.8	37.3	10.0	13.8	
1997	24.6						12.1	11.7	
Mean	**28.8**	**6.5**		**3.1**	**14.1**	**14.2**	**36.9**	**10.0**	**12.6**
± SE	1.06	1.41		2.19	0.45	0.29	0.54	0.30	0.45

Notes

1. Data kindly provided by Scotia Plant Technology Centre, Writtle College, Chelmsford, Essex
2. The percentage of oil in the seed on a dry weight basis
3. The first number represents the number of carbon atoms in the fatty acid analysed, the second number represents the number of double bonds. Fatty acids not determined are left as blank entries.
4. alpha-Linolenic acid (ALA)
5. gamma-Linolenic acid (GLA)
6. Stearidonic acid (SA)

Each row relates to the lipid profile for a single representative sample of unrefined oil extracted from echium plantagineum seed. Where there is more than one sample for a single year this relates to different seed accessions. The size of the seed accession varies from a few grams to several kilograms.

Appendix 1 – Lipid profiles from trials work (Continued)

Table 3: Lipid profiles of oil extracted from Echium plantagineum[1]

Year	% oil[2]	Lipid constituents[3]						
		16.0	**18.0**	**18.1**	**18.2**	**18.3[4]**	**18.3[5]**	**18.4[6]**
1995	21.0	7.6	3.8	16.7	16.0	29.9	11.9	12.3
	26.3	7.4	4.1	18.9	16.6	28.5	11.4	11.3
1996	32.1	6.9	3.6	17.5	16.8	31.1	10.8	11.5
	26.1	7.3	4.1	17.2	16.2	30.5	10.9	11.9
	28.3	7.4	4.2	18.0	16.0	30.3	10.3	11.7
	30.9	7.1	3.6	15.8	16.5	33.6	9.8	11.9
	27.0	7.3	3.8	16.7	17.8	31.8	10.2	10.6
	32.4	6.3	3.5	17.5	14.0	32.0	11.2	13.9
Mean	**25.5**	**7.2**	**3.8**	**17.3**	**16.2**	**31.0**	**10.8**	**11.9**
± SE	3.61	0.14	0.09	0.33	0.38	0.54	0.24	0.34

Notes

7. Data kindly provided by Croda Universal Ltd, Hull, East Yorkshire
8. The percentage of oil in the seed on a dry weight basis
9. The first number represents the number of carbon atoms in the fatty acid analysed, the second number represents the number of double bonds. Fatty acids not determined are left as blank entries.
10. alpha-Linolenic acid (ALA)
11. gamma-Linolenic acid (GLA)
12. Stearidonic acid (SA)

Each row relates to the lipid profile for a single representative sample of unrefined oil extracted from echium plantagineum seed. Where there is more than one sample for a single year this relates to different seed accessions. The size of the seed accession varies from a few grams to several kilograms.

Table 4: Lipid profiles of oil extracted from Echium plantagineum[1]

Year	% oil[2]	Lipid constituents[3]							
		16.0	**18.0**	**18.1**	**18.2**	**18.3[4]**	**18.3[5]**	**18.4[6]**	**22.1**
1999		6.7	3.6	15.3	14.6	33.0	10.9	13.7	0.1
		6.7	3.8	15.2	15.6	31.5	11.3	13.1	0.1
		6.5	3.9	16.2	14.7	31.2	11.3	13.7	0.1
		6.3	4.0	16.9	14.9	31.6	10.8	13.4	0.1
		6.5	3.8	15.9	15.1	32.5	10.7	13.3	0.1
		6.7	3.5	14.7	14.7	33.0	10.7	14.1	0.1
		6.6	3.9	15.6	15.9	31.8	10.5	12.9	0.6
Mean	**25.5**	**7.2**	**3.8**	**17.3**	**16.2**	**31.0**	**10.8**	**11.9**	**0.17**
± SE		015	0.18	0.72	0.50	0.74	0.31	0.41	0.18

Notes

1. Data provided by John K. King & Sons Ltd, Coggeshall
2. The percentage of oil in the seed on a dry weight basis
3. The first number represents the number of carbon atoms in the fatty acid analysed, the second number represents the number of double bonds. Fatty acids not determined are left as blank entries.
4. alpha-Linolenic acid (ALA)
5. gamma-Linolenic acid (GLA)
6. Stearidonic acid (SA)

Each row relates to the lipid profile for a single representative sample of unrefined oil extracted from echium plantagineum seed. Each line represents a different crop grown in the UK in 1999 from the same seed accession.

Table 5: Lipid profiles of Crossential SA14 / Super Refined ® Echium plantagineum oil[1]

Year	% oil[2]	Lipid constituents[2]						
		16.0	**18.0**	**18.1**	**18.2**	**18.3[3]**	**18.3[4]**	**18.4[5]**
1996		7.3	4.2	19.0	16.5	27.8	11.6	11.1
		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
		6.8	3.8	15.9	14.5	33.2	11.1	13.8
Mean	**25.5**	**7.1**	**4.0**	**17.3**	**15.4**	**30.7**	**11.5**	**12.8**
± SE		0.10	0.13	0.86	0.59	1.43	0.21	0.67

Notes

1. Data provided by Croda Universal Ltd. Hull, East Yorkshire
2. The first number represents the number of carbon atoms in the fatty acid analysed, the second number represents the number of double bonds. Fatty acids not determined are left as blank entries.
3. alpha-Linolenic acid (ALA)
4. gamma-Linolenic acid (GLA)
5. Stearidnic acid (SA)

Each row relates to the lipid profile for a single representative sample of Super Refined ® echium plantagineum oil (Crossential SA14). Where there is no more than one sample for a single year this relates to different laboratory batches of the Super Refined ® oil,

Appendix 2

Test method for the analysis of lipid profile.

QCP/02 APPENDIX 2 Method No CU-RM-014
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CRODA UNIVERSAL ANALYTICAL METHOD

FATS & OILS RAW MATERIAL INTAKES

DETERMINATION OF THE COMPOSITION OF FATTY ACIDS PRESENT BY GAS LIQUID CHROMATOGRAPHY

1.0 DEFINITION

1.1 The composition expresses the percentage weight of lipids that elute over a specific range during a given gas chromatographic program.

2.0 PRINCIPLE

2.1 A triglyceride is converted to it's corresponding methyl esters, these are then analysed by gas chromatography.

3.0 APPARATUS

3.1 Flasks, 100ml and 250ml

3.2 Air Condenser for the above

3.3 Sand Bath

3.4 Measuring cylinders, 25ml

4.0 REAGENTS

4.1 Methanol - Anhydrous Grade

4.2 10 % w/v methanolic solution of sulphuric acid (GP) prepared by adding carefully, with stirring, 100g of concentrated sulphuric acid to 1000ml of dried methanol.

4.3 5% w/v methanolic potassium hydroxide solution prepared by dissolving 50g of potassium hydroxide (GP) in 1000ml of dried methanol.

4.4 n-Heptane (GP Grade)

4.5 Saturated sodium chloride solution (GP Grade)

Q.A.
UNCONTROLLED DOCUMENT

Issue No.....3.....
Replaces.....4.4.98.....
Date.....5.4.03.....
Signed.....

APPENDIX 2

5.0 PROCEDURES

PREPARATION OF METHYL ESTERS

5.1 Weigh 2-3g of the oil into the 100ml flask. Add 25ml of methanolic potassium hydroxide solution and reflux the mixture for 30 minutes on a sand bath.

5.2 To this solution add 25ml of methanolic sulphuric acid solution and reflux for 30 minutes.

5.3 Remove the flask from the sand bath, add 10ml of n-Heptane and then fill the flask with saturated salt solution such that the heptane solution of the methyl esters fills the neck of the flask.

GLC ANALYSIS OF METHYL ESTERS

5.4 The methyl esters prepared as above are then analysed by gas chromatography by an appropriate method. Two typical methods are outlined below.

5.5 PACKED G.C ANALYSIS

G.C type	:=	Unicam PU4550 or similar machine
Column length	:=	6 ft glass
diameter	:=	¼" OD
Packing	:=	20% diethylene glycol succinate on Chromosorb WAW
Carrier gas	:=	Nitrogen
Detector	:=	Flame ionisation
Temperature		
Oven	:=	200°C isothermal
Injector	:=	250°C
Detector	:=	300°C
Injector Volume	:=	0.3µl

5.6 CAPILLARY G.C ANALYSIS

G.C. type	:=	Perkin Elmer 8600 or similar machine
Column length	:=	60 metres
diameter	:=	0.36 mm

Q. A. UNCONTROLLED DOCUMENT

Issue No. [illegible]
Replaces [illegible]
Date 24.4.95
Signed [signature]

Column type	:=	J & W / DB-23 0.15µm film thickness or similar column
Carrier gas	:=	Helium 1.0ml/minute
Detector	:=	Flame ionisation
Temperature		
Oven	:=	160 - 230°C at 6C°minute
Injector	:=	250°C
Detector	:=	300°C
Injection volume	:=	0.2µl
Injection split ratio	:=	150 to 1

5.7 The above two methods will encompass all normal G.C requirements. The packed column method is used for general routine analysis and where a detailed examination is required the capillary column method should be used.

6.0 REFERENCES

6.1 Paul Speight (Croda Universal Ltd) - 2/11/92

6.2 Test Methods for Fatty Acids (AFAD) 1968 method 10

6.3 American Oil Chemists Society (AOCS) 1989 method Ce 1-62

6.4 Standard Methods for the Analysis of Oils, Fats and Derivatives (IUPAC) 1979 methods 2.301 and 2.302

Appendix 3

Product specification for Echium Oil

PRODUCT SPECIFICATION

Product: Echium oil Cold Temperature Extracted

Appearance: A clear yellow to green-yellow free-flowing oil, free from foreign matter and imiscible with water.

Taste and Odour: Characteristic of oil – natural, bland taste and smell with no trace of rancidity or other abnormality organoleptically.

Tocopherols: Tocopherols added at request of customer.

Analytical Specifications:

Specific Gravity at 20oC	**0.915-0.925 g/ml**
Peroxide Value	**Not greater than 10.0 mEq 02 / kg oil.**
Acid Value	**Not greater than 4.0 mg KOH/g oil**
Trans Acids (Trans)	**Not more than 2%**
Non-Saponifiable Matter:	**Not more than 2%**
Heavy Metals	**Lead <0.1mg/kg Arsenic <0.1mg/kg Copper <0.05ppm Iron <1ppm**
Anisidine Value	**Not more than 20**
Moisture Content:	**Should be less than 0.1%**

Agrochemicals	**Should not be detected**
Residual Solvent	**< 1ppm**
Pyrrolizidine Alkaloids	**Not more than 15ng/g (0.015ppm)**

Fatty Acid Composition

Carbon Length	Name	%
C16:0	Palmitic Acid	6-8
C18:0	Stearic Acid	3-5
C18:1	Oleic Acid	15-19
C18:2	Linoleic Acid	14-18
C18:3 (n-3)	Alpha-Linolenic Acid	28-33
C18:3 (n-6)	Gamma-Linolenic Acid	9-12
C18:4	Stearidonic Acid	10.5-14
C22:1	Erucic Acid	Max 1

Revision Number:	**0000075/2**	**Date:**	**28/07/00**
Issued by:	**Phil Nicholls**	**Approved by:**	**Darren Keeler**
Reason for Revision:	**Added parameter for Erucic Acid. Reduced limits for Arsenic and Lead levels.**		

Appendix 4

Test results for protein analysis of production batches of Echium Oil

01-AUG-2000 14:13 FROM 01405863294 TO 901522691748 P.01/01

CRODA

Croda Oleochemicals
Healthcare
Cowick Hall Snaith
Goole East Yorkshire
DN14 9AA England
Tel +44 (0)1405 860551
Fax +44 (0)1405 860205

Fax to Attn: Phil Nicholls
Kings
Fax no 01522 691748
From Graeme Kenny

Total pages 1 (including this) If you do not receive all the pages, please telephone immediately

Date 01/Aug/2000

RE: Echium Oil Test Results

Dear Mr Nicholls

This fax is to confirm the Cytochrome Allergen test results carried out by us on your behalf.

The results are as follows:

We carried out the analysis on Batch 1124-A and Batch 1124 supplied by Kings and found both to have less than 20 micrograms per gram of cytochrome allergen.

This can also be expressed as less than 20ppm or less than 0.002%.

Our Analytical manager, Keith Southwell, carried out the tests.

Thanks and regards

G. Kenny

Graeme Kenny

TOTAL P.01

Appendix 5

Test results for heavy metal analysis of production batches of Echium Oil

International Laboratory Services Ltd
Shardlow Business Park
London Road Shardlow
Derbyshire DE72 2GD
Telephone: 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 OEL
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

UKAS TESTING
No. 1039

International Laboratory Services Ltd
Shardlow Business Park
London Road Shardlow
Derbyshire DE72 2GD
Telephone: 01332 793000
Fax: 01332 799044/792010
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

UKAS TESTING
No. 1039

17/11 '98 10:48 CRODA R&D - CRODA RAWCLIFFE NO.056 002

BRETBY ANALYTICAL CO... LTD

Certificate of Analysis

CRODA LEEK LTD
Barnfield Road
Leek
Staffordshire
ST13 5QJ

Our Reference 44/154431
Date Received 30/10/98
Date Completed 10/11/98
Your Reference 48879

Sample: 391 SR Echium Seed Oil SR3946 BP4/744

Test Description	Ref No.	391
Heavy Metals BP Limit Test	BP008 P	< 10PPM
Copper ppm	IP009M.P	< 0.1
Iron ppm	IP010M.P	11.4

M F Scott
BSc MSc CBiol MIBiol FIMgt FIFST

T K Madden
BSc MSc MChemA EurChem CChem FRSC
Registered Analytical Chemist
Registered Professional Water Chemist

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

The suffix letter P indicates the test method is not NAMAS accredited

Page 1 of 1

Bretby Business Park, Ashby Road, Bretby, Burton upon Trent, Staffordshire DE15 0QD

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

M F Scott
BSc MSc CBiol MIBiol FIMgt FIFST

T K Madden
BSc MSc MChemA EurChem CChem FRSC
Registered Analytical Chemist
Registered Professional Water Chemist

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

The suffix letter P indicates the test method is not NAMAS accredited

Page 1 of 1

Bretby Business Park, Ashby Road, Bretby, Burton upon Trent, Staffordshire DE15 0QD
Tel: 01283 552770 · Fax: 01283 552743

Appendix 6

Test results for pyrrolizidine alkaloid analysis of production batches of Echium Oil

29/09/2000 09:38 JOHN K KING → 901522691'48
01376562218

Chemical Laboratory Dr Hermann Ulex Nachf.

John K. King & Sons Limited
Coggeshall, Colchester, Essex

CO6 ITH United Kingdom.

Analysis Report No. 02 28 02

Echinium Oil / Prod. No. 66
Batch no. L1/00033/1124/A
Sample Point : Pallecon / 21" 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 – Acetyline intermedine
Inner standard : monocrotaline

Result :

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

Chemisches Laboratorium Dr. Hermann Ulex Nachf.

Stammhaus gegr. Andreae 1763 als "Apothecke am Stubbenfunk " am Orde des Brauerknechtgrabens

Inhaber: Hans-Joachim Mierendorff

Royal Soc.f.Food a.Drug Res.London/Glasgow (RSFDR) • Intern.Soc.f. Residue-Anal.Wash.,D.C./Palo Alto U.A. (ISRA)
Deutsche Ges.f. Fettw. e.V. Münster/Westf.(DGF)

Chem. Laboratorium Dr.H.Ulex Nachf., Glasmoorstr.23, 22851 Norderstedt

Chemisches Handelslaboratorium
Meßstelle für Radioaktivität
Accreditation EN 17025 in Progress

Glasmoorstraße 23
22851 Hamburg-Norderstedt
Tel. : +49-40-529 587- 0
Fax : +49-40-529 587-33
Mobil: +49-172-655 73 10
eMail: Dr.Ulex@gmx.de

Hamburg-Norderstedt,
den 6. März 2000

Firma
John K. King & Sons Limited
Coggeshall, Colchester, Essex

CO6 ITH United Kingdom

Untersuchungsbericht No. 02 28 01

Echinum Oil / Prod. No. 66
Batch No. LI/00033/1124
Sample Point: Pallecon / 21. Febr. 00

Sie übersandten uns o.g. Probe zur Bestimmung des Gesamtgehaltes an Pyrrolizidinalkaloiden (PA) vom Retronecintyp.

Probenaufarbeitung:
Die Probe wurde in Gegenwart von Ethanol und Schwefelsäure mit Zinkpulver mehrere Stunden bei 40° C nach der Methode:
"Bestimmung von Pyrrolizidinalkaloiden durch Dünnschichtchromatographie in Samenölen von Borago off.L. : H.-J. Mierendorff, Fat Sci. technol. 97 No. 1 (1995) 33-37"
hydriert zur Reduktion der N-oxyde der PA zu freien Alkaloiden.
Diese wurden durch mehrfache Säure/Basen-Extraktion unter definierten Bedingungen extrahiert und aliquote Anteile zur HPTLC-Analyse gebracht.

DC-Bedingungen:
Platte: Kieselgel KG 60 E. Merck (No. 5583)
Laufmittel 1: Aceton, p.a., Merck
Laufmittel 2: Chloroform, Methanol, Ammoniak 80 / 19 / 1
Kammer: gesättigt
Detektion nach Mattocks (spezifisch für PA vom Retronecin-/Heliotridin-Typ)
Referenzsubstanzen: 7-Acetyllycopsamin / 7-Acetylintermedin
Innerer Standard: Monocrotalin

Resultat:

9 ± 2ng Pyrrolizidinalkaloide / g Substanz (NG u d B: 5 ng/g)

Institut für Pharmaanalytik Dr. Hermann Ulex Nachf. Fachlaboratorium II Glasmoorstr. 23 22851 Hamburg-Norderstedt

C:\Office50\TXT\Pharm\Div-phar\pyrrolizidinalkpes.sdw

HRA 43 936 AG. Hamburg
USt.-ID Nr.: DE 134 361 103

Chemisches Laboratorium Dr. Hermann Ulex Nachf.

Stammhaus gegr. Andreae 1763 als "Apputecke am Stubbenhuk " am Orde des Brauerknechtgrabens

Inhaber: Hans-Joachim Mierendorff

Royal Soc.f.Food a.Drug Res.London/Glasgow (RSFDR) • Intern.Soc.f. Residue-Anal.Wash.,D.C./Palo Alto C.A. (ISRA)
Deutsche Ges. f. Fettw. e.V. Münster/Westf.(DGF)

Chem. Laboratorium Dr.H.Ulex Nachf., Glasmoorstr.23, 22851 Norderstedt

Chemisches Handelslaboratorium
Meßstelle für Radioaktivität
Accreditation EN 17025 in Progress

Glasmoorstraße 23
22851 Hamburg-Norderstedt
Tel. : +49-40-529 587- 0
Fax : +49-40-529 587-33
Mobil: +49-172-655 73 10
eMail: Dr.Ulex@gmx.de

Hamburg-Norderstedt,
den 6. März 2000

Firma
John K. King & Sons Limited
Coggeshall, Colchester, Essex

CO6 ITH United Kingdom

Untersuchungsbericht No. 02 28 02

Echinum Oil / Prod. No. 66
Batch No. LI/00033/1124/A
Sample Point: Pallecon / 21. Febr. 00

Sie übersandten uns o.g. Probe zur Bestimmung des Gesamtgehaltes an Pyrrolizidinalkaloiden (PA) vom Retronecintyp.

Probenaufarbeitung:
Die Probe wurde in Gegenwart von Ethanol und Schwefelsäure mit Zinkpulver mehrere Stunden bei 40° C nach der Methode:
"Bestimmung von Pyrrolizidinalkaloiden durch Dünnschichtchromatographie in Samenölen von Borago off.L. : H.-J. Mierendorff, Fat Sci. technol. 97 No. 1 (1995) 33-37"
hydriert zur Reduktion der N-oxyde der PA zu freien Alkaloiden.
Diese wurden durch mehrfache Säure/Basen-Extraktion unter definierten Bedingungen extrahiert und aliquote Anteile zur HPTLC-Analyse gebracht.

DC-Bedingungen:
Platte: Kieselgel KG 60 E. Merck (No. 5583)
Laufmittel 1: Aceton, p.a., Merck
Laufmittel 2: Chloroform. Methanol, Ammoniak 80 / 19 / 1
Kammer: gesättigt
Detektion nach Mattocks (spezifisch für PA vom Retronecin-/Heliotridin-Typ)
Referenzsubstanzen: 7-Acetyllycopsamin / 7-Acetylintermedin
Innerer Standard: Monocrotalin

Resultat:
11 ± 2ng Pyrrolizidinalkaloide / g Substanz (NG u d B: 5 ng/g)

Institut für Pharmaanalytik Dr. Hermann Ulex Nachf. Fachlaboratorium II Glasmoorstr. 23 22851 Hamburg-Norderstedt

C:\Office50\TXT\Pharm\Div-phar\pyrrolidinalkpos.sdw

HRA 43 936 AG. Hamburg
USt.-ID Nr.: DE 134 361 103

Batch PP4 /744

Chemisches Laboratorium Dr. Hermann Ulex Nachf.

Firma
Bamfield Road

Leek ST135GJ

Messstelle für Radioaktivität
22851 Hamburg-Norderstedt
Tel. +49-40-526 [illegible] - 0
Fax +49 40-529 [illegible]
Mobil: +49-172-455 73 10
eMail: DrUlexHmb@aol.com

Hamburg-Norderstedt
den 06 November 1998
Ulex-No.: 8X 30 02

ATTEST

Betr **Echinum seed oil**
C 392 Purchase order No 48880

Sie übersandten uns o.g. Probe zur Bestimmung des Gesamtgehaltes an Pyrrolizidinalkaloiden (PA) vom Retronecintyp.

Probenaufarbeitung:
Die Probe wurde in Gegenwart von Ethanol und Schwefelsäure mit Zinkpulver mehrere Stunden bei 40° C nach der Methode
"Bestimmung von Pyrrolizidinalkaloiden durch Dünnschichtchromatographie in Samenölen von Borago off.L. H.-J. Mierendorff, Fat Sci. technol. 97 No. 1 (1995) 33-37"
hydriert zur Reduktion der N-oxyde der PA zu freien Alkaloiden.
Diese wurden durch mehrfache Säure/Basen-Extraktion unter definierten Bedingungen extrahiert und aliquote Anteile zur HPTLC-Analyse gebracht.

DC-Bedingungen:
Platte: Kieselgel KG 60 E Merck (No. 5583)
Laufmittel 1: Aceton p.a. Merck
Laufmittel 2: Chloroform Methanol Ammoniak 80 / 19 / 1
Kammer: gesättigt
Detektion nach Mattocks (spezifisch für PA vom Retronecin-Heliotridin-Typ)
Referenzsubstanzen: 7-Acetyllycopsamin / 7-Acetylintermedin
Innerer Standard: Monocrotalin

Resultat:
Keine PA nachweisbar, d.h. pro 100 µl Analysenlösung entsprechend
10g Öl < 20 ng PA
entsprechend (bei einem mittleren Respons von ca. 50-60%)

< 4 ng PA / g Öl (4ng/g) (Nachweisgrenze)

Institut für ... Dr. Hermann Ulex Nachf. Fachlabo-ratorium II ... Hamburg-Norderstedt

Fachlaboratorium I
Pharmazeutische Lebensmittelchemie
[illegible]

Fachlaboratorium II
Lebensmittelchemie und Pharmakognosie
[illegible]

Fachlaboratorium III
Institut für Umwelt- und Klinikhygiene
[illegible]

[illegible]

Batch PP4/886

Chemisches Laboratorium Dr. Hermann Ulex Nachf.

Stammhaus gegr. Andreae 1763 als "Apptecke am Stubbenhuk" am Orte des Brauerknechtgrabens

Inhaber: Hans-Joachim Mierendorff

Royal Soc. f. Food a. Drug Res. London ... • Intern. Soc. f. Residue-Anal. ... (ISRA)
Deutsche Ges. f. Fettw. e.V. Münster/Westf. (DGF)

Chem. Laboratorium Dr. H. Ulex Nachf., Gaensoorst 23 22851 Norderstedt

Firma
Croda Leek Ltd
Barnfield Road

Leek ST135QJ

Chemisches Handelslaboratorium
Meßstelle für Radioaktivität
Giesmoorstraße 23
22851 Hamburg-Norderstedt
Tel. : +49-40-529 587- 0
Fax : +49-40-529 587-33
Mobil: +49-172-655 73 10
eMail: Dr.Ulex@gmx.de

Hamburg-Norderstedt,
den 2. August 1999
Ulex-No.: 97 23 01

ATTEST

Betr.: **Echium Oil**
C547

Sie übersandten uns o.g. Probe zur Bestimmung des Gesamtgehaltes an Pyrrolizidinalkaloiden (PA) vom Retronecintyp.

Probenaufarbeitung:
Die Probe wurde in Gegenwart von Ethanol und Schwefelsäure mit Zinkpulver mehrere Stunden bei 40° C nach der Methode:
"Bestimmung von Pyrrolizidinalkaloiden durch Dünnschichtchromatographie in Samenölen von Borago off.L. ; H.-J. Mierendorff, Fat Sci. technol. 97 No. 1 (1995) 33-37"
hydriert zur Reduktion der N-oxyde der PA zu freien Alkaloiden.
Diese wurden durch mehrfache Säure/Basen-Extraktion unter definierten Bedingungen extrahiert und aliquote Anteile zur HPTLC-Analyse gebracht.

DC-Bedingungen:
- Platte: Kieselgel KG 60 E. Merck (No. 5583)
- Laufmittel 1: Aceton, p.a., Merck
- Laufmittel 2: Chloroform, Methanol, Ammoniak 80 / 19 / 1
- Kammer: gesättigt
- Detektion nach Mattocks (spezifisch für PA vom Retronecin-/Heliotridin-Typ)
- Referenzsubstanzen: 7-Acetyllycopsamin / 7-Acetylintermedin
- Innerer Standard: Monocrotalin

Resultat:
Keine PA nachweisbar, d.h. pro 100 µl Analysenlösung entsprechend
10g Öl < 20 ng PA
entsprechend (bei einem mittleren Respons von ca. 50-60%)

< 4 ng PA / g Öl (4ng/g) (Nachweisgrenze)

Institut für Pharmaanalytik Dr. Hermann Ulex Nachf. — Fachlaboratorium II — Hamburg-Norderstedt

c:\office5\...\...alkaloide\...pyrrolizidinalk.sdw

Fachlaboratorium I: ...
Fachlaboratorium II: Industrie- und Pharmalaboratorium ...
Fachlaboratorium III: Institut für Umwelt- und Rückstandsanalytik ...

HRA 43 936 AG Hamburg
USt-ID Nr.: DE 134 161 103

Batch PP4/887

Chemisches Laboratorium Dr. Hermann Ulex Nachf.

Inhaber: Hans-Joachim Mierendorff

Chem. Laboratorium Dr. H. Ulex Nachf. · Glasmoorstraße 23 · 22851 Norderstedt

Chemisches Handelslaboratorium
Meßstelle für Radioaktivität
Glasmoorstraße 23
22851 Hamburg-Norderstedt
Tel. : +49-40-529 587- 0
Fax : +49-40-529 587-33
Mobil: +49-172-655 73 10
eMail: Dr.Ulex@gmx.de

Firma
Croda Leek Ltd.
Barnfield Road

Leek ST135QJ

Hamburg-Norderstedt,
den 2. August 1999
Ulex-No.: 97 23 02

ATTEST

Betr.: **Echium Oil**
C548

Sie übersandten uns o.g. Probe zur Bestimmung des Gesamtgehaltes an Pyrrolizidinalkaloiden (PA) vom Retronecintyp.

Probenaufarbeitung:
Die Probe wurde in Gegenwart von Ethanol und Schwefelsäure mit Zinkpulver mehrere Stunden bei 40° C nach der Methode:
"Bestimmung von Pyrrolizidinalkaloiden durch Dünnschichtchromatographie in Samenölen von Borago off.L.: H.-J. Mierendorff, Fat Sci. technol. 97 No. 1 (1995) 33-37"
hydriert zur Reduktion der N-oxyde der PA zu freien Alkaloiden.
Diese wurden durch mehrfache Säure/Basen-Extraktion unter definierten Bedingungen extrahiert und aliquote Anteile zur HPTLC-Analyse gebracht.

DC-Bedingungen:
Platte: Kieselgel KG 60 E. Merck (No. 5583)
Laufmittel 1: Aceton, p.a., Merck
Laufmittel 2: Chloroform, Methanol, Ammoniak 80 / 19 / 1
Kammer: gesättigt
Detektion nach Mattocks (spezifisch für PA vom Retronecin-/Heliotridin-Typ)
Referenzsubstanzen: 7-Acetyllycopsamin / 7-Acetylintermedin
Innerer Standard: Monocrotalin

Resultat:
Keine PA nachweisbar, d.h. pro 100 µl Analysenlösung entsprechend
10g Öl < 20 ng PA
entsprechend (bei einem mittleren Respons von ca. 50-60%)

< 4 ng PA / g Öl (4ng/g) (Nachweisgrenze)

Fachlabor-atorium II

c:\office50\... .sdw

Fachlaboratorium I:
Allgemeines Handelslaboratorium

Fachlaboratorium II:
Industrie und Pharmalaboratorium

Fachlaboratorium III:
Institut für Umwelt- und Rückstandsanalytik

HRA 43 956 AG Hamburg

Batch PP4/886

Chemisches Laboratorium Dr. Hermann Ulex Nachf.

Stammhaus gegr. Anfrage 1763 als "Apothecke am Stubbenhuk" am Orde des Brauerknechtgrabens

Inhaber: Hans-Joachim Mierendorff

Royal Soc. f. Food a. Drug Res. London (Engl.) - RSFDR - Assoc. Soc. f. Residue Anal. Wash., D.C./Publ. AOAC (USA) ISRA)
Deutsche Ges. f. Fettw., e.V. Münster/Westf. (DGF)

Chemisches Laboratorium Dr. H. Ulex Nachf. Glasmoorstr. 23 22851 Norderstedt

Firma
Croda Leek Ltd
Barnfield Road

Leek ST135OJ

Chemisches Handelslaboratorium
Meßstelle für Radioaktivität
Glasmoorstraße 23
22851 Hamburg-Norderstedt
Tel. : +49-40-529 587- 0
Fax : +49-40-529 587-33
Mobil. +49-172-655 73 10
eMail: Dr.Ulex@gmx.de

Hamburg-Norderstedt,
den 2. August 1999
Ulex-No.: 97 23 01

ATTEST

Betr.: **Echium Öl**
C547

Sie übersandten uns o.g. Probe zur Bestimmung des Gesamtgehaltes an Pyrrolizidinalkaloiden (PA) vom Retronecintyp.

Probenaufarbeitung:
Die Probe wurde in Gegenwart von Ethanol und Schwefelsäure mit Zinkpulver mehrere Stunden bei 40° C nach der Methode:
"Bestimmung von Pyrrolizidinalkaloiden durch Dünnschichtchromatographie in Samenölen von Borago off.L. ; H.-J. Mierendorff, Fat Sci. technol. 97 No. 1 (1995) 33-37"
hydriert zur Reduktion der N-oxyde der PA zu freien Alkaloiden.
Diese wurden durch mehrfache Säure/Basen-Extraktion unter definierten Bedingungen extrahiert und aliquote Anteile zur HPTLC-Analyse gebracht.

DC-Bedingungen:
Platte: Kieselgel KG 60 E. Merck (No. 5553)
Laufmittel 1: Aceton, p.a., Merck
Laufmittel 2: Chloroform, Methanol, Ammoniak 80 / 19 / 1
Kammer: gesättigt
Detektion nach Mattocks (spezifisch für PA vom Retronecin-/Heliotridin-Typ)
Referenzsubstanzen: 7-Acetylycopsamin / 7-Acetylintermedin
Innerer Standard: Monocrotalin

Resultat;
Keine PA nachweisbar, d.h. pro 100 µl Analysenlösung entsprechend
10g Öl < 20 ng PA
entsprechend (bei einem mittleren Respons von ca. 50-60%)

< 4 ng PA / g Öl (4ng/g) (Nachweisgrenze)

Institut für Pharmazie Dr. Hermann Ulex Nachf. Fachlaboratorium II Glasmoorstr. 23 Hamburg-Norderstedt

c.office@chemisches-laboratorium.de

Batch 8404/5572

Chemisches Laboratorium Dr. Hermann Ulex Nachf.

Firma
England

Hamburg-Nordenbek,
den 12. Oktober 1999
Ulex-No.: 89 29 01

ATTEST

Betr. Echium Seed Oil
Sample No. C267 Purchase order No. 48807

Sie übersandten uns o.g. Probe zur Bestimmung des Gesamtgehaltes an Pyrrolizidinalkaloiden (PA) vom Retronecintyp.

Probenaufarbeitung:
Die Probe wurde in Gegenwart von Ethanol und Schwefelsäure mit Zinkpulver mehrere Stunden bei 40° C nach der Methode,
"Bestimmung von Pyrrolizidinalkaloiden durch Dünnschichtchromatographie in Samendien von Borago off. L." H.-J. Mierendorff Fat Sci. technol. 97 No. 1 (1995) 33-37"
hydriert zur Reduktion der N-oxyde der PA zu freien Alkaloiden.
Diese wurden durch mehrfache Säure/Basen-Extraktion unter definierten Bedingungen extrahiert und aliquote Anteile zur HPTLC-Analyse gebracht.

GC-Bedingungen:
Platte: Kieselgel 60 F (Merck (No. 5583)
Laufmittel 1: Aceton, p.a., Merck
Laufmittel 2: Chloroform, Methanol, Ammoniak 80 / 19 / 1
Kammer: gesättigt
Detektion nach Maddock (see Fish for PA vom Retronecin/Heliotridin-Typ)
Referenzsubstanzen: 7-Acetylycopsamin / 7-Acetylintermedin
Innerer Standard: Monocrotalin

Resultat:
Keine PA nachweisbar, d.h. pro 100 µl Analysenlösung entsprechend 10g Öl < 20 ng PA
entsprechend bei einem mittleren Respons von ca. 50-60%)

< 4 ng PA / g Öl (4ng/g) (Nachweisgrenze)

Chem. Lab. Dr. Ul.

Appendix 7

Pesticides Safety Directive ' Long term arrangements for extension of use (2000)'

PESTICIDES SAFETY DIRECTORATE
Mallard House, Kings Pool, 3 Peasholme Green, York YO1 7PX
Switchboard: 01904 640500 GTN: 5138
Direct Dial: 01904 45 Fax: 01904 45
International: (+44) 1904 45 International Fax: (+44) 1904 45
Email: f.j.daly@psd.maff.gov.uk

To: **All MAFF Approval Holders**
cc: **Interested Parties for Information**

Our Ref: AAHL/23/99
PRD 2224

Date: 13 December 1999

Dear Sir/Madam

THE REVISED LONG TERM ARRANGEMENTS FOR EXTENSION OF USE (2000)

Since 1 January 1990 'The Long Term Arrangements for Extension of Use' have been in place to permit many professional pesticide products to be used for additional specific minor uses, subject to adherence to certain conditions. These arrangements were subsequently reviewed in December 1994, with a requirement for a further review by the 31 December 1999. The Pesticides Safety Directorate (PSD) has undertaken this review in consultation with the Advisory Committee on Pesticides and Government Departments responsible for the approval of pesticides. The new arrangements are attached at Appendix II and are valid until 31 December 2004.

PSD has considered whether the current extrapolations are still valid, relevant and in line with currently accepted EU residues data extrapolations. New extensions of use requested by growers have been considered and the restrictions and guidance have been refined and clarified. The amendments/additions that have been made are detailed in Appendix I.

As this review has resulted in various amendments to the arrangements, it is important that the new arrangements are read thoroughly before proceeding with pesticide uses that were previously allowed under 'The Revised Long Term Arrangements for Extension of Use' (1995).

The continuation of the Long Term Arrangements for Extension of Use will ensure that the UK industry has access to essential pesticides, whilst ensuring that risks to the operator, consumer and wildlife/environment are not increased.

Users are reminded that use of a pesticides under these arrangements is at all times undertaken at the user's choosing and the user accepts the commercial risk to the crop. The conditions of use relating to the approved label recommendations and/or specific off-label approval apply to the extension of use, unless superseded by the terms of these arrangements.

INVESTOR IN PEOPLE

Appendix I

AMENDMENTS:

(i) Red chard, white chard and yellow chard have been added to the extrapolation from spinach to beet leaves in 'Section V: Crops Used Partly or Wholly For Human or Animal Consumption.'

(ii) The extrapolation to *Miscanthus spp* (Elephant Grass) from cereals/grass/maize has been added to 'Section I: Non-edible crops and Plants', as follows:

'Subject to the specific restrictions for extension of use set out above, herbicides approved for use on cereals, grass and maize may be used on commercial agricultural and horticultural holdings on *Miscanthus spp* (Elephant grass). Applications must not be made after the crop is 1 metre in height. The crop or products of the crop must not be used for food or feed.'

(iii) Oilseed rape to *Echium vulgare/Echium plantaginium* has been added to 'Section V: Crops Used Partly or Wholly For Human or Animal Consumption', with a restriction that the extrapolation is for crops grown as an oilseed only and does not apply to seed treatments.

(iv) The extrapolation in 'Section V: Crops Used Partly or Wholly For Human or Animal Consumption' from cereals to grass seed crops has been amended as follows:

The phrase 'Treated crops must not be grazed or cut for fodder' has been amended to read: 'Treated crops must not be grazed or cut for fodder until 90 days after treatment'.

The phrase 'Use of chlormequat-containing products is not permitted' has been added.

(v) A section clarifying crops that are considered to be equivalent has been added to 'Section V: Crops Used Partly or Wholly For Human or Animal Consumption', as follows:

Cobnuts and filberts are synonymous with hazelnuts
Navy beans are synonymous with French beans.
Picking pea/shelling pea/non-edible podded pea are synonymous with vining peas
Linola and flax are synonymous with linseed.
Durum wheat is considered to be wheat.

(vi) The 'Farm Forestry and Rotation Coppicing' section now includes reference to 'reclaimed brownfield sites'.

(vii) The extrapolation 'Rye and triticale from wheat and barley (Treatments applied before second node detectable stage only)' has been amended to read:

'Barley to rye and triticale for treatments applied before first spikelet of inflorescence just visible'.
'Wheat to rye and triticale.'

Appendix II

THE LONG TERM ARRANGEMENTS FOR EXTENSION OF USE (2000)

Please note that these extensions of use are at all times done at the user's choosing, and the commercial risk is entirely theirs.

SPECIFIC RESTRICTIONS FOR EXTENSION OF USE UNDER THESE ARRANGEMENTS

To ensure that the extension of use does not increase the risk to the operator, the consumer or the environment, **the following conditions MUST be followed** when applying pesticides under the terms of this scheme:

GENERAL RESTRICTIONS

1. These arrangements apply to label and specific off-label recommendations for use of ONLY products approved for use as Agricultural/Horticultural pesticides.

2. All safety precautions and statutory conditions relating to use (which are clearly identified in the statutory box on product labels) MUST be observed. If extrapolation from a specific off-label is to be used then in addition to all safety precautions and statutory conditions relating to use specified on the product label, all conditions relating to use specified on the Notice of Approval for the specific off-label use MUST be observed.

3. Pesticides MUST only be used in the same situation (outdoor or protected) as that specified on the product label/specific off-label Notice of Approval for the use on which the extrapolation is to be based, specifically:

   Pesticides must not be used on protected crops, i.e. crops grown in glasshouses, poly tunnels, cloches or polythene covers or in any other building, unless the product label/specific off-label Notice of Approval specifically allows use under protection on the crop on which the extrapolation is to be based. Similarly, pesticides approved only for use in protected situations must not be applied outdoors.

   PLEASE NOTE: Unless specifically restricted to outdoor crops only, pesticides approved for use on tomatoes, cucumbers, lettuce, chrysanthemum and mushrooms are assumed to be approved for use under protection. **For all other uses, if the label/specific off-label Notice of Approval does not specify a situation, then only extrapolation to an outdoor use is permitted.**

APPLICATION METHOD RESTRICTIONS

4. The method of application must be as stated on the pesticide label and in accordance with the relevant codes of practice and requirements under COSHH 1994 (Control of Substances Hazardous to Health).

petal fall) unless otherwise permitted. Applications of such pesticides must also not be made when flowering weeds are present or where bees are actively foraging.

8. If there is an aquatic buffer zone restriction set for the on-label/off-label use, then where appropriate, users are also obliged to conduct a Local Environmental Risk Assessment for Pesticides (LERAP) for the extension of use.

9. All reasonable precautions MUST be taken to safeguard wildlife and the environment.

EXCLUSIONS

10. The following uses are NOT PERMITTED under these arrangements.

(a) Aerial applications

(b) Use in or near water (in or near water includes drainage channels, streams, rivers, ponds, lakes, reservoirs, canals, dry ditches, areas designated for water storage).

(c) Use in or near coastal waters.

(d) Use of rodenticides and other vertebrate control agents.

(e) Use on land not intended for cropping, land not intended to bear vegetation, amenity grassland, managed amenity turf and amenity vegetation (this includes areas such as paths, pavements, roads, ground around buildings, motorway verges, railway embankments, public parks, turf, sports fields, upland areas, moorland areas, nature reserves, etc.).

EXTENSIONS OF USE

I. NON-EDIBLE CROPS AND PLANTS

(a) Subject to the SPECIFIC RESTRICTIONS FOR EXTENSION OF USE set out above, pesticides approved for use on any growing crop may be used on commercial agricultural and horticultural holdings and in forest nurseries on the following crops and plants:

(i) hardy ornamental nursery stock, ornamental plants, ornamental bulbs and flowers and ornamental crops grown for seed where neither the seed nor any part of the plant is to be consumed by humans or animals;

(ii) forest nursery crops prior to final planting out.

(b) Subject to the SPECIFIC RESTRICTIONS FOR EXTENSION OF USE set out above, pesticides approved for use on any growing edible crop may be used on commercial agricultural and horticultural holdings on non-ornamental crops grown for seed where neither the seed nor any part of the plant is to be consumed by humans or animals. This extrapolation EXCLUDES use on

If hand held or broadcast air assisted use is required see paragraphs 5 and 6 respectively of the SPECIFIC RESTRICTIONS FOR EXTENSION OF USE.

IV HOPS *(Humulus spp.)*

Subject to the SPECIFIC RESTRICTIONS FOR EXTENSION OF USE set out above, pesticides may be used on commercial agricultural and horticultural holdings on the following hop plants grown in the circumstance below:

(a) Mature stock or mother plants which are kept specifically for the supply of propagation material.

(b) Propagation of hop planting material- propagules prior to final planting out

(c) "Nursery hops". First year plants not taken to harvest that year, in their final planting out position

PLEASE NOTE:

For a - c above, treated hops must NOT be harvested for human or animal consumption (including idling) within 12 months of treatment.

If hand held or broadcast air assisted application is required, users must comply with paragraphs 5 and 6 respectively of the SPECIFIC RESTRICTIONS FOR EXTENSION OF USE.

V. CROPS USED PARTLY OR WHOLLY FOR HUMAN OR ANIMAL CONSUMPTION.

Subject to the SPECIFIC RESTRICTIONS FOR EXTENSION OF USE set out above, pesticides may be used on commercial agricultural or horticultural holdings on the crops listed in TABLE ONE and TWO below in the first column if they have been approved for use on the crop(s) listed opposite them in the second column.

HOWEVER, BEFORE USING ANY OF THE FOLLOWING EXTRAPOLATIONS (TABLES ONE AND TWO), THE USER MUST FIRST OBSERVE THE FOLLOWING:

(a) **It is the responsibility of the user to ensure that the proposed use does not result in any statutory UK Maximum Residue Levels (MRLs) being exceeded. MRLs are set out in statutory instrument No. 1985 of 1994: 'The Pesticides (Maximum Residue Levels in Crops, Food and Feeding Stuffs) Regulations 1994' (The Stationery Office, ISBN 0-11-044985-1) and any subsequent updates.**

(b) **These extrapolations DO NOT APPLY in the following situations:**

Column 1: Minor use	Column 2: Crops on which use is approved	Additional special conditions
B. FRUIT CROPS		
Almond, Chestnut, Walnut, Hazelnut	Apple or cherry or plum	For herbicides used on the orchard floor ONLY
Almond, Chestnut, Walnut, Hazelnut	Products approved for use on two of the following: almond, chestnut, hazelnut and walnut	
Quince, Crab apple	Apple or pear	
Nectarine, Apricot	Peach	
Blackberry, Dewberry Rubus species (e.g. tayberry, loganberry)	Raspberry	
Whitecurrant, Bilberry, Cranberry	Blackcurrant or redcurrant	
Redcurrant	Blackcurrant	
C. VEGETABLE CROPS		
Parsley root	Carrot or radish	
Fodder beet, Mangel	Sugar beet	
Horseradish	Carrot or radish	
Parsnip	Carrot	
Salsify	Carrot or celeriac	
Swede	Turnip	
Turnip	Swede	
Garlic, Shallot	Bulb onion	
Aubergine	Tomato	
Squash, Pumpkin, Marrow, Watermelon	Melon	

Column 1: Minor Use	Column 2: Crops on which use is approved.
Mustard, Sunflower, Honesty, Sesame, Linseed, Evening primrose, Poppy (grown for oilseed), Borage (grown for oilseed) Canary flower e.g. *Echium vulgare/Echium plantagineum* (grown for oilseed)	Oilseed rape

VI. CLARIFICATIONS:

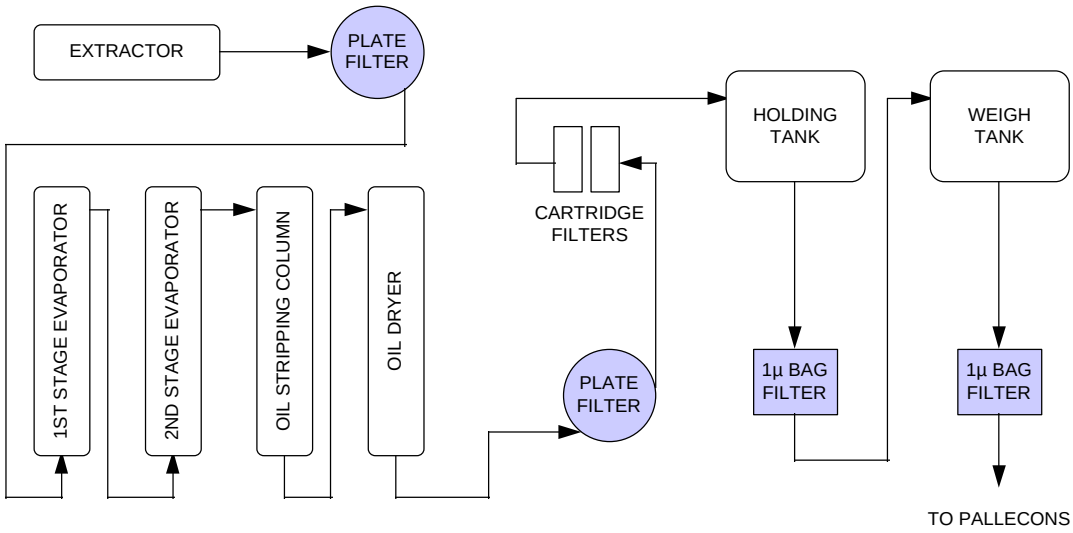
Under these arrangement the following crops are considered to be synonymous or equivalent and as such, uses on crops in Column 1 can be read across to uses in Column 2.

Column 1:	Column 2: equivalent
Hazelnut	Cobnuts, Filberts
French bean	Navy bean
Vining pea	Picking pea, Shelling pea, Non-edible podded pea
Linseed	Linola, Flax
Wheat	Durum wheat

Appendix 8

Schematic diagram for extraction process

Process Overview



Appendix 9

Comparison of lipid profiles of Echium Oil with Borage Oil and Blackcurrant Seed Oil

The anti-inflammatory activity of Echium oil has been investigated along with a number of other oils rich in essential fatty acids (see Appendix 11). Lipid profiles were determined for each of the oils prior to testing. For comparison the lipid profiles of borage oil, blackcurrant seed oil and echium oil (crossential SA14).

Fatty Acid	Borage Oil	Blackcurrant Oil	Echium Oil
C16:0	9.98	6.92	7.01
C16:1	0.39	0.20	0.19
C18:0	3.39	1.40	3.67
C18:1	16.37	11.76	16.41
C18:2 (LA)	38.79	44.68	14.96
C18:3 (ALA)	0.49	11.44	28.98
C18:3 (GLA)	20.68	16.27	11.83
C18:4 (SA)	0.13	3.02	12.99
C20:0	0.23	-	0.39
C20:1	3.83	0.86	0.68
C22:1	2.46	-	0.13
C24:1	1.23	-	0.14
Others	2.03	3.45	2.68

Appendix 10

Biochemical pathways for Omega-3 and Omega-6 fatty acids

OMEGA-6:

9,12-Octadecadienoic acid (linoleic acid)

↓ ^{6} Desaturase

6,9,12-Octadecatrienoic acid (*gamma*-linolenic acid)

↓ Elongase

8,11,14-Eicosatrienoic acid (dihomo-*gamma*-linolenic acid)→Prostaglandin E_1

↓ ^{5} Desaturase

5,8,11,14-Eicosatetraenoic acid (arachidonic acid) →Prostaglandin E_2 + Leukotriene B_4

↓ Elongase

Docosatetraenoic acid

↓ ^{4} Desaturase

Docosapentaenoic acid

OMEGA-3:

9,12,15-Octadecatrienoic acid (*alpha*-linolenic acid / ALA)

↓ ^{6} Desaturase

6,9,12,15-Octadecatetraenoic acid (stearidonic acid / SA)

↓ Elongase

8,11,14,17-Eicosatetraenoic acid

↓ ^{5} Desaturase

5,8,11,14,17-Eicosapentaenoic acid →Prostaglandin E_3 + Leukotriene B_5

↓ Elongase

7,10,13,16,19-Docosapentaenoic acid

↓ ^{4} Desaturase

4,7,10,13,16,19-Docosahexaenoic acid →Prostaglandin E_3 + Leukotriene B_5

Appendix 11

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	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 x 0.22mm BPX70 column. Our own-prepared laboratory standards, containing malvelic and sterculic acid were used to determine retention values.

Results

	Lot 1124	Lot 1124a
% Oxirane oxygen (indicative 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 undoubtably originated from slight oxidation of the oil.

Signatories: M H JEE Date: 30th August 2000

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Appendix 12

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

Croda Universal Ltd

Technical report
No. 5857

Date issued 11/9/00

For internal circulation only (not to be distributed to third parties)

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% ß sitosterol.

Other sterols were present but they could not be identified.

Distribution

K Nutbrown
D A Parker
N Wilson
Dr B T Hatton (hard copy)
B Holmes(hard copy)
Dr C Temple-Heald
P G Speight (hard copy)

K Coupland

Keywords

Echium Oil

Unsaponifiables

Sterols

Further action

Further work will be done on request.

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 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 ß 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 %	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 ß 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 ß sitosterol in the Echium oils is: -

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

4.0 Discussion

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

Sterol species	% of the total sterols
Brassicasterol	0 to 1.6
Campesterol	25 to 30
ß Sitosterol	22 to 42
Δ5 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 ß 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% ß sitosterol.

Other sterols were present but they could not be identified.

P Speight

Appendix 13

Test Results for determining Microbiological Activity in Echium Oil

Croda

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 Glycerides 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

Two herbicides are used in the growth of Echium Seed Oil (Crossential SA14). Theoretically 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
Reports
Other

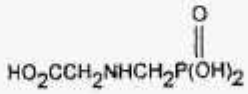
1.0 Introduction

Croda Oleochemicals are to submit Crossential SA14 for novel food approval. Information Services are preparing a dossier, which requires information to state if the material could be contaminated with either agrochemicals or micro-organisms.

2.0 Possible Pesticides

After consultation with Dave Coupland (Croda's agrochemicals expert) and Kevin Peacock, information showed that the farmer responsible for growing the crop had used only two "pesticides" on the land which could have come into contact with the Echium Seed Oil. The two herbicides which had been used in the growth of the plant were:-

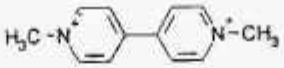
2.1 Glyphosphate (N-(phosphonomethyl) Glycine)



This herbicide is very insoluble in common organic solvents e.g. acetone, ethanol and xylene and hence is unlikely to be soluble in fat.

Environmental fate – usually stated as not being metabolised in plants but there is some evidence of metabolism in certain plants. The principle metabolite is aminomethylphosphoric acid.

2.2 Paraquat (1,1-dimethyl-4,4-bipyridium)



Also a herbicide and practically insoluble in most organic solvents and unlikely to be soluble in fat.

Environmental fate – on plant surfaces photochemical degradation occurs. Degradation products, which have been isolated, include 1-methyl-4-carboxy-pyridium chloride and methylamine hydrochloride.

3.0 Analysis of Glyphosphate and Paraquat

To prove that Crossential SA14 had not been contaminated with the two herbicides it was proposed that an external analyst be contacted to carry out the analysis. Bratby Analytical consultants were contacted. After being asked to analyse for the two herbicides in Crossential SA14 they stated that they could do the analysis but due to the insolubility of both herbicides and their metabolites in fat it would be highly unlikely to find anything. Over a period of years they have never seen these species in a fatty glycerine sample matrix. It was deemed unnecessary to carry out any analysis.

4.0 Possible Microbiological Contamination

A sample was submitted to the Rawcliffe Bridge microbiology lab for a total plate count. The tests revealed no evidence of microbiological contamination.

It should be noted that testing an isolated sample for micro-organisms does not on its own prove that Croda will be able to produce this material free from microbiological contamination. As microbiological contamination can come from three main sources:-

i) Raw materials (including the packaging)
ii) Environmental
iii) Personnel

To gain novel load approval it should be demonstrated through documentation that contamination at Croda Leek in the production of this material is unlikely to occur (GMP type documentation should suffice with information about the housekeeping practices in place).

5.0 Conclusions

5.1 It is impossible for the two herbicides mentioned in this report to be found in Crossential SA14.
5.2 No evidence of any microbiological contamination can be found in the sample of Crossential SA14 submitted.
5.3 Leek's production documentation should give evidence of housekeeping practices in place that will prevent microbiological contamination of products occurring.

6.0 References

6.1 The Pesticide Manual 10th Edition (The Royal Society of Chemistry).
6.2 Practical Microbiology for the Cosmetics Industry (The Cosmetic Toiletry and Perfumery Association).

G M Atkinson

G M Atkinson
/jw

Croda Chemicals Ltd

Cowick Hall Snaith Goole
North Humberside DN14 9AA UK
Telephone 0405 860551
Telex 57601 Fax 0405 860205

CBS

Crodaspec test method

No G038-1

Date 3 April 1993

Total aerobic bacteria

1.0 Definition

The total aerobic bacteria plate count is the number of bacteria present in the test sample under the conditions of the determination.

2.0 Principle

A known mass of sample is dissolved in a suitable solvent, filtered through a membrane and cultured on nutrient agar. An estimate of total count of aerobic bacteria is obtained from a colony count after 3 or 5 days if the growth is poor.

3.0 Scope

The plate count method may be applied to give an overall picture of the extent of contamination due to viable microorganisms. For a viable count to be statistically correct between 5 and 300 colonies per plate should be achieved.

4.0 Revision Changes

4.1 Change of nutrient broth.

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Revision 1

Croda Chemicals Ltd

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Crodaspec test method

No G038-1

Date 3 April 1993

5.0 Apparatus

5.1 Sterile, single vent, disposable Petri dishes (10).
5.2 Cotton wool plugged sterile blow out pipettes (1 ml).
5.3 Beaker (250 ml).
5.4 Incubator set at 30°C ± 1°C
5.5 Autoclave (at 121°C and 15 psi).
5.6 Lamina flow cabinet.
5.7 Balance (to 2 decimal places).
5.8 Butane gas burner.
5.9 Aluminium foil.
5.10 Conical flask (250 ml).
5.11 Non absorbent cotton wool.
5.12 Membrane filter holder (sterile plugged).
5.13 Membrane filters (0.45 micron pore, type WCN sterile).
5.14 Buchner funnel.
5.15 Vacuum pump.
5.16 Test tubes (3).
5.17 Microscope (40 x magnification).

6.0 Reagents

6.1 Distilled water (fresh daily).
6.2 Nutrient agar.
6.3 Nutrient broth (Difco).
6.4 Isopropyl myristate.
6.5 99% Ethanol (IMS 74OP).
6.6 Hycolin solution (2%).

7.0 Procedure

7.1 Disinfect the lamina flow cabinet and all work areas with 2% Hycolin solution.

7.2 Preparation of agar plates.

7.2.1 Weigh nutrient agar (4.6 g ± 0.01 g) into a 250 ml conical flask.

7.2.2 Add distilled water (200 ml) to the flask.

7.2.3 Plug the flask with non absorbent cotton wool and cover with aluminium foil.

7.2.4 Place the flask in the autoclave at 121°C and 15 psi for 15 minutes.

7.2.5 Allow to cool to approximately 60 - 70°C in the lamina flow cabinet and then pour approximately 20 ml of the medium into 10 Petri dishes.

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Crodaspec test method

No G038-1

Date 3 April 1993

7.2.6 Using the butane burner carefully flame the surface of each plate to remove any air bubbles formed.

7.2.7 Allow the plates to set and then store them upside down at 30°C for 24 hours until they are dry.

7.3 Preparation of the broth.

7.3.1 Prepare nutrient broth (250 ml) according to the manufacturers instructions.

7.3.2 Warm on a hot plate to dissolve the broth and then pour into a 250 ml bottle.

7.3.3 Plug the bottle with non absorbent cotton wool and cover with aluminium foil.

7.3.4 Place the bottle in the autoclave at 121°C and 15 psi for 15 minutes.

7.3.5 Allow to cool to room temperature in the lamina flow cabinet.

7.4 Sample preparation

7.4.1 Sterilise the filtration unit with the top plugged with non absorbent cotton wool wrapped in cotton in aluminium foil.

7.4.2 Dissolve the test sample (1 g ± 100 mg) in a suitable sterile solvent (9 ml) in each of three capped test tubes using a 1 ml sterile pipette for liquids or a spatula flamed in 99% ethanol for solids

7.4.2.1 Use distilled water for water soluble samples and isopropyl myristate for water insoluble samples.

7.4.3 With the aid of tweezers (sterilised by flaming in 99% ethanol) remove a membrane from its wrapping and place it on the sterile membrane filter holder.

7.4.4 Attach the Buchner funnel to the holder.

7.4.5 Flame the rim of the test tube, turn on the vacuum and as quickly as possible remove the plug from the funnel, pour in the sample and replace the plug.

7.4.6 When all the sample has filtered (15 - 60 seconds) flame the rim of the Letheen broth bottle and pour in approximately 20 ml to rinse the membrane. Replace the funnel plug.

7.4.7 When the filtration is complete turn off the vacuum pump and remove the membrane using sterile tweezers.

7.4.7.1 The funnel may be used again if the funnel is kept in a sterile place.

7.4.8 Place the membrane on the agar plate.

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Crodaspec test method

No G038-1

Date 3 April 1993

7.4.9 Repeat the process for the remaining two tubes of sample.

7.4.10 Incubate the nutrient agar plates at 30°C.

7.4.11 Examine the plates using a microscope after 48 and 72 hours and count any colonies arising.

7.4.12 Report the average number of colonies obtained after 72 hours.

8.0 Notes

8.1 After use all petri dishes and filter papers should be sterilised by autoclaving before disposal.

9.0 Health and safety

9.1 Refer to the health and safety data sheet of the test sample.

9.2 Hycolin contains synthetic phenolic derivatives. May cause irritation to eyes and skin. Exposure limit not assigned.

9.3 Nutrient broth is not a hazardous material. Exposure limit not assigned.

9.4 99% Ethanol (IMS 74 OP) is intoxicating if inhaled or ingested. Irritating to eyes. If ingested in undiluted form has a severe drying effect on mucous membranes of mouth and throat. Can be damaging if splashed in eyes. Exposure limit is 1900 mg/m³.

9.5 Isopropyl myristate may be harmful if ingested in quantity. Irritating to eyes. Can be absorbed through the skin. Exposure limit not assigned.

10.0 References

10.1 None.

11.0 Approval

Compiled by	Position	Signature	Date
M R Harrison	Technical Director	M. R. Harrison	4/5/93

Authorised by	Position	Signature	Date
K Backhouse	Assistant Chemist	K.B.	19/5/93
T J Bateman	QC Manager	T.J.B.	17/5/93.

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Crodaspec test method

No G038-2

Date 2 June 1993

Yeasts and moulds

1.0 Definition

The yeasts and moulds plate count is the number of yeasts and moulds present in the test sample under the conditions of the determination.

2.0 Principle

A known mass of sample is dissolved in a suitable solvent, filtered through a membrane and cultured on potato dextrose agar. An estimate of total count of yeasts and moulds is obtained from a colony count after a minimum of three days.

3.0 Scope

The plate count method may be applied to give an overall picture of the extent of contamination due to viable yeasts and moulds. For a viable count to be statistically correct between 5 and 300 colonies per plate should be achieved.

4.0 Revision Changes

4.1 Change of nutrient broth.

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Crodaspec test method

No G038-2

Date 2 June 1993

5.0 Apparatus

5.1 Sterile, single vent, disposable Petri dishes (10).
5.2 Cotton wool plugged sterile blow out pipettes (1 ml).
5.3 Beaker (250 ml).
5.4 Incubator set at 30°C ± 1°C.
5.5 Autoclave (at 121°C and 15 psi).
5.6 Lamina flow cabinet.
5.7 Balance (to 2 decimal places).
5.8 Butane gas burner.
5.9 Aluminium foil.
5.10 Conical flask (250 ml).
5.11 Non absorbent cotton wool.
5.12 Membrane filter holder (sterile plugged).
5.13 Membrane filters (0.45 micron pore, type WCN sterile).
5.14 Buchner funnel.
5.15 Vacuum pump.
5.16 Test tubes (3).
5.17 Microscope (x 40 magnification).

6.0 Reagents

6.1 Distilled water (fresh daily).
6.2 Potato dextrose agar.
6.3 Potato broth (Difco).
6.4 Isopropyl myristate.
6.5 99% Ethanol (IMS 74OP).
6.6 Hycolin solution (2%).

7.0 Procedure

7.1 Disinfect the lamina flow cabinet and all work areas with 2% Hycolin solution.

7.2 Preparation of agar plates.

7.2.1 Weigh the potato dextrose agar (7.8 g ± 0.01 g) into a 250 ml conical flask.

7.2.2 Add distilled water (200 ml) to the flask.

7.2.3 Plug the flask with non absorbent cotton wool and cover with aluminium foil.

7.2.4 Place the flask in the autoclave at 121°C and 15 psi for 15 minutes.

7.2.5 Allow to cool to approximately 60 - 70°C in the lamina flow cabinet and then pour approximately 20 ml of the medium into 10 Petri dishes.

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Crodaspec test method

No G038-2

Date 2 June 1993

7.2.6 Using the butane burner carefully flame the surface of each plate to remove any air bubbles formed.

7.2.7 Allow the plates to set and then store them upside down at 30°C for 24 hours until they are dry.

7.3 Preparation of the broth.

7.3.1 Prepare potato dextrose broth (250 ml) according to manufacturers instructions.

7.3.2 Warm on a hotplate to dissolve the broth and then pour into a 250 ml bottle.

7.3.3 Plug the bottle with non absorbent cotton wool and cover with aluminium foil.

7.3.4 Place the bottle in the autoclave at 121°C and 15 psi for 15 minutes.

7.3.5 Allow to cool to room temperature in the lamina flow cabinet.

7.4 Sample preparation

7.4.1 Sterilise the filtration unit with top plugged with non absorbent cotton wool wrapped in aluminium foil.

7.4.2 Dissolve the test sample (1 g ± 100 mg) in a suitable sterile solvent (9 ml) in each of three capped test tubes using a 1 ml sterile pipette for liquids or a spatula flamed in 99% ethanol for solids

7.4.2.1 Use distilled water for water soluble samples and isopropyl myristate for water insoluble samples.

7.4.3 With the aid of tweezers (sterilised by flaming in 99% ethanol) remove a membrane from its wrapping and place it on the sterile membrane filter holder.

7.4.4 Attach the Buchner funnel to the holder.

7.4.5 Flame the rim of the test tube, turn on the vacuum and as quickly as possible remove the plug from the funnel, pour in the sample and replace the plug.

7.4.6 When all the sample has filtered (15 - 60 seconds) flame the rim of the Letheen broth bottle and pour in approximately 20 ml to rinse the membrane. Replace the funnel plug.

7.4.7 When the filtration is complete turn off the vacuum pump and remove the membrane using sterile tweezers.

7.4.7.1 The funnel may be used again if it is kept in a sterile place.

7.4.8 Place the membrane on the potato dextrose agar plate.

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Crodaspec test method

No G038-2

Date 2 June 1993

7.4.9 Repeat the process for the remaining two tubes of sample.

7.4.10 Incubate the potato dextrose agar plates at 30°C.

7.4.11 Examine the plates using a microscope after 48 and 72 hours and count any colonies arising.

7.4.12 Report the average number of colonies obtained after 72 hours.

8.0 Notes

8.1 After use all petri dishes and filter papers should be sterilised by autoclave before disposal.

9.0 Health and Safety

9.1 Refer to the health and safety data sheet of the test sample.

9.2 Letheen broth is not a hazardous material. Exposure limit not assigned.

9.3 Potato dextrose agar is not a hazardous material. Exposure limit not assigned.

9.4 99% Ethanol (IMS 74OP) is intoxicating if inhaled or ingested. Irritating to eyes. If ingested in undiluted form has a severe drying effect on mucous membranes of mouth and throat. Can be damaging if splashed in eyes.

9.5 Isopropyl myristate is irritating to eyes. Frequent or prolonged contact with skin may cause dermatitis.

10.0 References

10.1 None.

11.0 Approval

Compiled by	Position	Signature	Date
M R Harrison	Technical Director	M. R. Harrison	2/6/93

Authorised by	Position	Signature	Date
K Backhouse	Assistant Chemist		3/6/93
T J Bateman	QC Manager		3/6/93

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Crodaspec test method

G35

No G038-3

Date 18 March 1993

Gram negative bacteria

1.0 Definition

The Gram negative bacteria plate count is the number of Gram negative bacteria present in the test sample under the conditions of the determination.

2.0 Principle

A known mass of sample is dissolved in a suitable solvent, filtered through a membrane and cultured on MacConkey's agar. An estimate of total count of aerobic bacteria is obtained from a colony count after a minimum of three days.

3.0 Scope

The plate count method may be applied to give an overall picture of the extent of contamination due to viable microorganisms. For a viable count to be statistically correct between 5 and 300 colonies per plate should be achieved.

4.0 Revision Changes

4.1 The temperature of incubation is changed to 30°C.

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Crodaspec test method

No G038-3

Date 18 March 1993

5.0 Apparatus

5.1 Sterile, single vent, disposable Petri dishes (10).
5.2 Cotton wool plugged sterile blow out pipettes (1 ml).
5.3 Beaker (250 ml).
5.4 Incubator set at 30°C ± 1°C.
5.5 Autoclave (at 121°C and 15 psi).
5.6 Lamina flow cabinet.
5.7 Balance (to 2 decimal places).
5.8 Butane gas burner.
5.9 Aluminium foil.
5.10 Conical flask (250 ml).
5.11 Non absorbent cotton wool.
5.12 Membrane filter holder (sterile plugged).
5.13 Membrane filters (0.45 micron pore, type WCN sterile).
5.14 Buchner funnel.
5.15 Vacuum pump.
5.16 Test tubes (3).
5.17 Microscope (x 40 magnification).

6.0 Reagents

6.1 Distilled water (fresh daily).
6.2 MacConkey's agar (Difco).
6.3 Letheen broth.
6.4 Isopropyl myristate.
6.5 99% Ethanol (IMS 74OP).
6.6 Hycolin solution (2%).

7.0 Procedure

7.1 Disinfect the lamina flow cabinet and all work areas with 2% Hycolin solution.

7.2 Preparation of agar plates.

7.2.1 Weigh the MacConkey's agar (10.0 g ± 0.01 g) into a 250 ml conical flask.

7.2.2 Add distilled water (200 ml) to the flask.

7.2.3 Plug the flask with non absorbent cotton wool and cover with aluminium foil.

7.2.4 Place the flask in the autoclave at 121°C and 15 psi for 15 minutes.

7.2.5 Allow to cool to approximately 60 - 70°C in the lamina flow cabinet and then pour approximately 20 ml of the medium into 10 Petri dishes.

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Crodaspec test method

No G038-3

Date 18 March 1993

7.2.6 Using the butane burner carefully flame the surface of each plate to remove any air bubbles formed.

7.2.7 Allow the plates to set and then store them upside down at 30°C for 24 hours until they are dry.

7.3 Preparation of the broth.

7.3.1 Prepare MacConkey broth (250 ml) according to the manufacturers instructions.

7.3.2 Warm on a hotplate to dissolve the broth and then pour into a 250 ml bottle.

7.3.3 Plug the bottle with non absorbent cotton wool and cover with aluminium foil.

7.3.4 Place the bottle in the autoclave at 121°C and 15 psi for 15 minutes.

7.3.5 Allow to cool to room temperature in the lamina flow cabinet.

7.4 Sample preparation

7.4.1 Sterilise the filtration unit with the top plugged with non absorbent cotton wool wrapped in aluminium.

7.4.2 Dissolve the test sample (1 g ± 100 mg) directly into a suitable sterile solvent (9 ml) in each of three capped test tubes using a 1 ml sterile pipette for liquids or a spatula flamed in 99% ethanol for solids

7.4.2.1 Use distilled water for water soluble samples and isopropyl myristate for water insoluble samples.

7.4.3 With the aid of tweezers (sterilised by flaming in 99% ethanol) remove a membrane from its wrapping and place it on the sterile membrane filter holder.

7.4.4 Attach the Buchner funnel to the holder.

7.4.5 Flame the rim of the test tube, turn on the vacuum and as quickly as possible remove the plug from the funnel, pour in the sample and replace the plug.

7.4.6 When all the sample has filtered (15 - 60 seconds) flame the rim of the Letheen broth bottle and pour in approximately 20 ml to rinse the membrane. Replace the funnel plug.

7.4.7 When the filtration is complete turn off the vacuum pump and remove the membrane using sterile tweezers.

7.4.7.1 The funnel may be used again if it is kept in a sterile place.

7.4.8 Place the membrane on the MacConkey's agar plate.

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Crodaspec test method

No G038-3

Date 18 March 1993

7.4.9 Repeat the process for the remaining two tubes of sample.

7.4.10 Incubate the MacConkey's agar plates at 30°C.

7.4.11 Examine the plates using a microscope after 48 and 72 hours and count any colonies arising.

7.4.12 Report the average number of colonies obtained after 72 hours.

8.0 Notes

8.1 After use all petri dishes and filter papers should be sterilised by autoclave before disposal.

9.0 Health and Safety

9.1 Refer to the health and safety data sheet of the test sample.

9.2 Letheen broth is not a hazardous material. Exposure limit not assigned

9.3 MacConkey's agar is not a hazardous material. Exposure limit not assigned.

9.4 96% Ethanol is intoxicating if inhaled or ingested. Irritating to eyes. If ingested in undiluted form has a severe drying effect on mucous membranes of mouth and throat. Can be damaging if splashed in eyes. Exposure limit is 1900 mg/m³.

9.5 Isopropyl myristate may be harmful if ingested in quantity. Irritating to eyes. Can be absorbed through the skin. Exposure limit not assigned.

10.0 References

10.1 None.

11.0 Approval

Compiled by	Position	Signature	Date
M R Harrison	Technical Director	M. R. Harrison	19/3/93

Authorised by	Position	Signature	Date
K Backhouse	Assistant Chemist		3/6/93
T J Bateman	QC Manager		3/6/93

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Revision 1

Appendix 14

Method for determining protein levels in oil samples

14-SEP-2000 14:13 FROM 01405863294 TO 901522691748 P.01/02

CRODA

Fax to Attn: Phil Nichols
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Fax no 01522 691748

From Graeme Kenny

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Total pages 1 (including this) if you do not receive all the pages, please telephone immediately

Date 14/Sep/2000

RE: Test Method

Dear Mr Nichols

The following is a draft copy of the test method. When it is on a Croda template and finished off I will send you another copy. I hope this will do for now.

Thanks and regards

Graeme Kenny

DETERMINATION OF THE CYTOCHROME C ALLERGEN CONTENTIN VEGETABLE OILS

1.0 Definition

The cytochrome C allergen content expresses the maximum content (%) of cytochrome C allergen present in vegetable oils.

2.0 Principle

Cytochrome C allergen is a protein. The total protein content present in a sample is determined by spectrophotometry using Bradfords Reagent to form a reagent – protein complex which absorbs visible light at 595nm (blue). The total protein content is equivalent to the maximum cytochrome C allergen content assuming all protein present in the sample is in the form of cytochrome C allergen.

3.0 Apparatus

3.1 Analytical balance, capable of weighing to four decimal places
3.2 Volumetric flasks, 10.0ml
3.3 Gilson autopipettes, 0 – 1.00ml
3.4 Disposable test tubes (20ml)
3.5 Hitachi model U-2000 spectrophotometer
3.6 Disposable cuvettes (3ml)

4.0 Reagents

4.1 Distilled water, Analytical grade
4.2 Hexane, Analytical grade
4.3 Bradford Reagent (Sigma Diagnostics)
4.4 Protein standards (Sigma Diagnostics)

5.0 Procedure

5.1 Preparation of protein standard solutions

5.1.1 To prepare protein standard solutions of concentration 1.0, 3.0, 5.0, 7.0 and 10.0µg/ml dilute accurately 0.1, 0.3, 0.5, 0.7 and 1.0 ml of a 10g/dL (equivalent to 100µg/ml) protein standard solution in 10.0ml distilled water, respectively.

5.2 Preparation of sample solutions

5.2.1 Accurately weigh 1.00g (±0.05) of sample oil into a 10ml volumetric flask and dilute in hexane

5.3 Analysis of sample and standard solutions

5.3.1 Accurately dispense 5.00ml of each standard solution and sample solution in to a disposable test tube. To separate test tubes add 5.00ml of distilled water (referred to as standard solution blank) and 1.00ml hexane (referred to as sample solution blank).

5.3.2 To each test tube accurately dispense 5.00ml Bradford Reagent and shake every five minutes for a period of thirty minutes.

5.3.3 After 30 minutes zero the spectrophotometer with distilled water. Determine the absorbance at 595nm of each protein standard and the absorbance for the standard solution blank (distilled water). Use distilled water as the reference solution in double beam spectrophotometers. Determine the blank corrected absorbances by subtracting the absorbance measured for the blank from the absorbance measured for each standard solution.

5.3.4 Determine the absorbance at 595nm for the water layer of the sample solution and the absorbance for the water layer of the sample solution blank (hexane). Determine the blank corrected absorbance by subtracting the absorbance measured for the blank from the absorbance for the sample solution.

6.0 Calculation of Results

6.1 Plot a calibration graph of protein concentration (µg/ml) vs. blank corrected absorbance.

6.2 If the absorbance of the sample solution is less than that for the 1µg/ml protein standard solution, the maximum concentration is less than 0.001 % (10ppm)

6.3 If the absorbance is greater than 1µg/ml use the calibration graph to determine the protein concentration equivalent to the absorbance measured for the sample solution.

6.4 Calculate the maximum cytochrome C allergen content using the following equation :

Maximum cytochrome C allergen content = protein concentration * (10/oil weight) * 0.0001