

Mr Andreas Klepsch
European Commission
By email

12 July 07

Reference: NFU 634

Dear Mr Klepsch,

INITIAL OPINION: REFINED ECHIUM OIL

On 11 August 2006, the UK Competent Authority accepted an application from Croda Chemicals Europe Ltd for the authorisation for Refined Echium Oil as a novel food ingredient, in accordance with Article 4 of regulation (EC) 258/97. The Advisory Committee on Novel Foods and Processes (ACNFP) reviewed this application and their opinion is attached. I apologise for the delay in submitting this opinion as the ACNFP's evaluation was extended while we obtained additional information from the applicant.

In view of the ACNFP's opinion, the UK Competent Authority considers that Refined Echium Oil meets the criteria for acceptance of a novel food defined in Article 3(1) of regulation 258/97.

I am copying this letter and the ACNFP's opinion to the applicant.

Yours sincerely,
(By email only)

Shuhana Begum

For the UK Competent Authority

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

INITIAL OPINION ON AN APPLICATION UNDER THE NOVEL FOODS REGULATION FOR REFINED ECHIUM OIL DERIVED FROM *Echium plantagineum* AS A FOOD INGREDIENT

Applicant: Croda Chemicals Europe Ltd.

Responsible Person: David Parker

EC Classification: 2.2

Introduction

1. An application was submitted to the Food Standards Agency in August 2006 by Croda Chemicals Europe Ltd. for the authorisation of refined echium oil as a novel food ingredient. A copy of the application was placed on the Agency's website for public consultation.
2. Echium oil is a vegetable oil rich in omega-6 and omega-3 polyunsaturated fatty acids and is obtained by refining oil extracted from the seeds of *Echium plantagineum*, which is a member of the *Boraginaceae* family. The applicant proposes to market their refined echium oil as a novel food ingredient in a range of food products (including milk and yoghurt-based drinks, breakfast cereals and nutrition bars) and in food supplements.
3. The application for authorisation of refined echium oil was prepared pursuant to Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients. The applicant's refined echium oil has been classified as a complex novel food from non-GM sources (class 2.2).

I. Specification of the novel food

Information on this aspect is provided on p. 3-12 of the application dossier

4. The novel ingredient (NI) is obtained from the seeds of *Echium plantagineum* using a solvent chromatographic technique and is rich in stearidonic acid (STA; cis-6, 9, 12, 15-octadecatetraenoic acid).
5. The NI is a pale yellow fully refined oil and the applicant has provided the following proposed specification:

Stearidonic acid content	Not less than 10% w/w of total fatty acids
Trans fatty acids	not more than 2% w/w of total fatty acids
Unsaponifiable content	not more than 2%
Acid value	not more than 5mg KOH/g
Peroxide value	not more than 5 meq O ₂ /kg
Lead	not more than 0.1 mg/kg
Protein content (total nitrogen)	not more than 20 µg /ml

6. Compositional data were provided on three batches of the NI, the raw material and a blend of the NI which confirmed that the NI is produced consistently to meet the above specification.
7. The applicant has quality control procedures in place to ensure that that the NI meets the stated specification. If the NI does not meet this specification it will not be released. The applicant has stated that the standard site procedures for reprocessing are;
 - If the material failure is considered marginal, for example in terms of colour, then it is reprocessed. The material is either re-refined or blended with another batch of material and then re-refined to generate a product that is in specification.
 - If the failure of specification is significant, and it is not possible to remedy by reprocessing, the material will be discarded.
8. Unsaponifiable matter from both the raw material and three production batches of the NI was investigated using GC analysis which demonstrated that the NI contains between 0.80 and 0.87% (1.08% in the crude oil). Independent analysis has confirmed that the trans fatty acid content is below 2g/100g oil (% w/w).
9. The applicant also provided details on the sterol content of the NI, which was compared with traditional counterparts such as borage, blackcurrant, evening primrose and safflower. The applicant was therefore, of the view that this demonstrated that the sterol profile of the NI is within the range of other commonly consumed oil. For example, the levels of campesterol in the NI ranged from 23-28% compared to 25-30% in borage oil.
10. Approved agrochemical products could potentially be used at the production cycle of *Echium plantagineum* for weed control or as a pre-harvest desiccant. Analysis confirmed that no residues of pesticides are detectable in the NI.
11. Analysis of both the crude oil and the NI confirms that heavy metals such as arsenic and cadmium are all below detection limits. The NI complies with EU contaminants legislation, which specifies an upper limit of 0.1mg/kg for lead in vegetable oil. Analytical data have been provided to demonstrate that the levels

of dioxins, furans, dioxin-like PCB's and PAH's are all below the maximum permitted levels (Annex A appendix 1, parts G and H).

12. The NI is stabilised with approved antioxidants, which have been added in accordance with Directive 95/2/EC on food additives other than colours and sweeteners. The applicant has measured oxidation of the oil using the peroxide value (PV) and the p-anisidine value (p-AV), which are measures of the extent of oxidation in materials containing unsaturated fatty acids such as the NI and ensures that it is produced to a set specification.
13. In addition the applicant tested the oxidative stability of the NI using an automated test system (Rancimat) and compared the results with traditional counterparts. The NI was studied under identical conditions to other vegetable derived oils with the exception of a higher temperature. The reaction kinetics for oxidation indicated that an induction time in the region of 2.5 hours would be obtained at 100 °C, which would be comparable to other oils.

Discussion: The Committee noted the applicant's proposed specification for the NI.

II. Effect of the production process applied to the novel food

Information on this aspect is provided on p. 14-17 of the application dossier

14. The *E. plantagineum* crop used to produce the NI is to be grown under contract in the UK. The production process is patented and involves cracking the echium seeds and extraction using hexane, followed by a series of distillation and filtration steps. The residual level of hexane in the oil is less than 1mg/kg, consistent with the requirements of EC legislation on extraction solvents (Directive 88/388/EEC).
15. The NI is processed in a batch-wise manner using a commercial scale chromatographic technique developed by the applicant to achieve high purity natural oils.
16. The production process of the NI has been independently assessed and certified in accordance with HACCP, which is in place throughout the production process.

Discussion: The Committee was satisfied that the applicant's proposed production process for the NI did not give cause for concern.

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

Information on this aspect is provided on p.18-20 of the application dossier

17. The NI is derived from the seeds of *E. plantagineum*, which is a member of the *Boraginacea* family. The *Boraginacea* family is a large plant family with approximately 100 genera and 2,500 species, which are widely distributed and well known to herbalists.
18. *E. plantagineum* is also known by its common names of Purple Vipers Bugloss, Paterson's Curse and Salvation Jane. It is an erect, biennial, soft hairy plant with

one or many flowering stems. *E. plantagineum* is widespread throughout Australia and is eaten readily by livestock.

Discussion: The Committee noted that current consumption of *E. plantagineum* as a food is very limited.

IX. Anticipated intake/extent of use of the novel food

Information on this aspect is provided on p.23-28 of the application dossier

19. The applicant intends that the NI will be used as an ingredient in a variety of products.

20. A complete list of products and levels at which refined echium oil will be added (expressed in terms of Stearidonic acid (STA), which comprises not less than 10% of the oil) can be found below. According to the applicant these use levels are largely based on the delivery of approximately 200mg of STA per day. The products will not be restricted locally and there are no plans to target a particular consumer group. However, the applicant anticipates that products containing the NI will be primarily consumed by vegetarians as an alternative to flax oil, borage oil and other existing sources of omega-3 fatty acids.

1. Summary of the proposed food uses and use levels (expressed as STA) for Refined Echium Oil		
2. Food Category	3. Food use	4. Maximum Use Level (mg STA/100g)
5. Dairy products	6. Milk	7. 75
	8. Cheese	9. 250
	10. Fromage frais	11. 250
	12. Yoghurt	13. 75
14. Dairy analogues	15. Soy products	16. 250
	18. Imitation milk products	17. 750 in cheese analogues 19. 250
20. Fats and dressings	21. Spreadable fats and dressings	22. 750
23. Grain based products	24. Breakfast cereals	25. 625
	26. Nutrition bars	27. 500
	28. Bread products	29. 200
30. Meal replacements	31. Meal replacement beverages	32. 250
33. Sauces	34. Savoury sauces	35. 500 (200 in pasta sauces)
36. Fruit juice products	37. Fruit juices	38. 75
	39. Fruit smoothies	40. 75
	41. Ready-to-drink soft drinks (not low calorie)	42. 75
	43. Ready-to-drink soft drinks (low calorie)	44. 75
45. Dietary foods for special medical purposes	46. In accordance with the particular nutritional requirements of the persons for whom products are intended	
47. Food supplements		48. 500 (mg STA per daily dose as

	recommended by the manufacture)
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21. Based on these proposed use levels the applicant has estimated the daily intake of STA using the data from the National Dietary Nutrition Survey (NDNS) of 1992/3 for children aged 1.5-4.5, 1997 for young people aged 4-18 and 2000/1 for adults aged 18-64. A summary of the estimated intake for different age groups can be found below:

From Table IX.a-2 Summary of the Estimated Daily Intake of STA from Refined Echium Oil from All Proposed Food Categories in the U.K. by Population Group (NDNS Data)							
Population Group	Age (Years)	% Users	Actual # of Total Users	All-Person Consumption (mg/day)			
				Mean	Percentile		
					90	95	97.5
Children	1½ to 4½	98.8	1628	719	1053	1216	1354
Young People	4 to 10	99.6	834	860	1234	1371	1561
Female Teenagers	11 to 18	97.8	436	805	1265	1403.	1594
Male Teenagers	11 to 18	99.5	414	1056	1647	18723	2076
Female Adults	16 to 64	94.3	903	866	1325	1507	1692
Male Adults	16 to 64	95.0	728	1124	1751	19312	2189

22. The applicant's estimates of mean daily intake vary between 719 mg/person for children to 1124 mg/person for male adults and the high level daily intake (97.5th centile) varies between 1354 mg/person for children and 2189 mg/person for male adults. The applicant has explained that the highest level exposure to the NI (the 97.5th percentile of estimated intake in male adults) is equivalent to 11 servings of foods containing the NI, or approximately 2200 mg of STA. In practice this is an over-estimate and it is unlikely that these "worst case" intake levels will be achieved in practice as it is extremely unlikely that consumers will choose so many products containing the NI. (see paragraph 42 below)

23. The applicant was also asked to provide information on the EDI of the echium oil itself. (Note: the following estimates are based on consumers of the fortified products rather than the whole population, i.e. "users only" rather than "all person"). The applicant reported that the greatest mean and 97.5th percentile intakes of echium oil (on an absolute basis) are found in male adults, at approximately 9g and 17g person/day, respectively. On a body-weight basis, children were identified as having the highest intakes of any population group, with mean and 97.5th percentile all-user echium oil intakes of 0.4 and 0.8 g/kg body weight/day respectively.

Discussion: The Committee considered that the consumption of the NI at the proposed levels of incorporation in the different food categories did not raise any specific safety concerns.

XI. Nutritional information on the novel food

Information on this aspect is provided on p.29-33 of the application dossier

24. The NI contains fatty acids commonly found in the diet such as 6% palmitic acid, 3.5% stearic acid, 17.2% oleic acid, 18.6% linoleic acid, 29.5% alpha linoleic acid, 10.2% gamma linoleic acid and 12.6% stearidonic acid.
25. From a nutritional safety perspective the applicant considers that the NI is equivalent to existing oils and fats that are rich in essential fatty acids.
26. The applicant notes that animal and human studies have demonstrated that STA can be efficiently converted into eicosapentaenoic acid (EPA). EPA displaces arachidonic acid in platelet membranes, which results in an alteration in eicosanoid production in favour of platelet anti-aggregatory mediators. A combined daily intake of EPA and DHA in excess of 3g/day has been associated with a reduction in platelet aggregation and an increase in bleeding time and for this reason subjects receiving anti-coagulant therapy should avoid consuming foods rich in EPA and DHA. The applicant notes that the intake of refined echium oil may result in a decrease in triglyceride levels in healthy subjects with normal or low triglyceride levels. These effects have typically not been considered with other nutritional products known to reduce triglyceride levels (i.e. soy, fish oil and certain fibres).
27. In response to a request from the Committee, the applicant has explained that the maximum amount of EPA that might be theoretically produced from ALA and STA in the echium oil, even in high level consumers, is well below the 3 g/day threshold of EPA and DHA that has been set by other regulatory bodies for the prevention of changes to platelet function and bleeding time, and well below the amounts administered in studies assessing the effects of combined administration of anticoagulants and EPA / DHA. The applicant was of the view that at the proposed levels of use, the NI is not expected to increase the risk of bleeding in individuals receiving anticoagulant therapy
28. In its initial discussions, the Committee was concerned that the NI could be seen as an alternative to other sources of polyunsaturated fatty acids, although the nutritional value of STA is lower than that of other PUFAs such as EHA and DPA. Consumers might therefore be disadvantaged if they consumed products containing the NI in preference to products such as oily fish, for which a nutritional benefit has been established. The applicant explained that it proposes to label the NI as "refined echium (vegetable) oil". This, in the view of the applicant should distinguish it from fish oils which are the predominant source of DHA/EPA in the diet and are focussed on cardiovascular health. The applicant also stressed that echium oil is rich in the essential fatty acids alpha-linolenic acid (ALA, Omega-3) and d-gamma-linolenic acid (GLA, Omega-6) which are precursors for eicosanoids in the body. The applicant highlights that ALA is recognised as being of nutritional importance in its own right, and a number of Member States have set a daily recommended value of up to 2.2 g per day for total omega-3 fatty acids. The applicant also pointed out that a similar product, flax seed oil (linseed) oil, is widely available on the market and is included in many food supplements, including combinations with fish oils. The applicant

stated that the NI would be an alternative vegetable based source of essential omega-3 and omega-6 fatty acids, similar to flaxseed oil.

Discussion: The Committee was satisfied with the nutritional information provided for the NI and was content that the NI would not be nutritionally disadvantageous to consumers. Members also agreed that the product would be marketed in the same market sector as existing vegetable oils and would not be viewed as an alternative to fish oils.

XII. Microbiological information on the novel food

Information on this aspect is provided on p.34-35 of the application dossier

29. The NI is produced in an anhydrous system and will therefore not support microbial growth. Also the production of the NI includes a range of chromatographic techniques which work to filter any microbial organisms and the production is controlled through HACCP procedures.

30. In response to a request by the ACNFP the applicant confirmed that the HACCP certificate provided in the application, which was for the manufacture of fish oil concentrates and refined vegetable oil for use in animal feeds, is also applicable for food grade oil.

31. Microbiological analyses on the NI demonstrated the absence of microbiological contamination and are summarised below;

- Osmophilic yeast <10cfu/g
- Yeast <10cfu/g
- Moulds <10cfu/g
- Enterobacteria <10cfu/g
- *Staphylococcus aureus* <10cfu/g

Discussion: The Committee was of the view that the microbiological safety of the NI had been demonstrated and noted that the manufacturer's HACCP procedures are also applicable for food grade oil.

XIII. Toxicological information on the novel food

Information on this aspect is provided on p. 4-5 and p.26-46

32. Pyrrolizidine alkaloids and cytochrome C allergens are two known potentially toxic inherent constituents that are associated with the *Boraginacea* family.

33. The applicant does not anticipate that pyrrolizidine alkaloids (PA) will be present in the NI due to the fact that they are polar compounds and not expected to be carried over into the hexane-extracted oil and subsequently refined. The applicant

has carried out analysis to confirm that PA levels are below the limit of detection (limit of detection = 4µg PA/kg oil).

34. Cytochrome C allergens have been characterised as proteins with a molecular weight of 12,800 and the applicant notes that the chromatographic technique used to refine the NI will act to remove any pollen or particulate plant debris in the oil. The applicant analysed both the NI and the crude oil for protein content using the Bradford assay. The protein content of the crude oil was 210 µg /ml whilst the NI contained less than the limit of detection of 10µg /ml. The proposed specification for the NI allows a maximum protein content of 20µg /ml.
35. Echioleum has been extensively studied at both the whole plant and extracted oil levels. The applicant has identified that the critical risk factors pyrrolizidine alkaloid and cytochrome C are effectively absent in the NI. Using the data from paragraph 11, the maximum possible intake of PA would be less than 0.1 µg /day, assuming the "worst case" intake of 20g of the NI containing 4 µg of PA per kg. This is considerably lower than the doses of PA associated with toxicity (70-147 mg/day for infants and 570-1380 mg/day for adults).
36. Echioleum has also been associated with respiratory allergy (cytochrome C allergens) in the pollen of the plant. The protein content of the oil is reported to be <10µg /ml in all 3 batches tested (see paragraph 34 above). Assuming the worst case, i.e. that the oil contains 10µg /ml protein and that 100% of that protein is cytochrome C allergens, then the consumption of 20g of the oil would result in the intake of 200µg of allergenic protein. The applicant suggested that this is below what would be required to trigger an allergic reaction in a sensitive individual and, furthermore, it is likely that heat treatments during the manufacturing process will denature the protein so reducing its allergenicity.
37. The Committee accepted that it was very unlikely that serious allergic reaction, such as anaphylaxis, would result from the intake of this small amount of allergenic protein. The Committee nevertheless asked the applicant to provide further details of the protein composition to confirm that the cytochrome C allergen is not actually present at the level suggested by this worst case analysis. The protein was extracted from both crude and refined echium oil using a modified Olszewski *et al.* procedure where the crude oil was shown to contain 21.2µg protein/g and the refined oil contained 11.1µg protein/g. Analysis of the limit of detection for cytochrome C by gel electrophoresis and of the recovery from the extraction procedure led to the conclusion that the refined oil contained less than 3µg cytochrome C per kg of oil.
38. The metabolism of STA and STA-rich oil has also been studied to determine whether consumption of STA increases EPA levels in the bloodstream.
39. The applicant has detailed a series of feeding studies on both the echium oil itself and on stearidonic acid. The applicant has provided a summary of the oil profiles and dose levels of the echium oil used in these studies, and these are presented in the following table:

Study		Dose level	Results
Toxicology studies on Echium oil			
4 week dietary exposure in rats		Diets containing 5% sunflower, flaxseed, echium (containing 12.5% STA) and canola oils	<ul style="list-style-type: none"> No significant difference in body weight Echium oil may be useful for elevating EPA and DPA n-3 in the body.
12 week clinical study – Healthy young males	Part 1: Immune Function	9g of echium oil per day containing 1g of STA	<ul style="list-style-type: none"> No effect on immune function at 1g/day
	Part 2: Fatty acid composition in blood lipids and mononuclear cells	9g per day of 1 of the 7 oil blends. (Each oil blend consisted of palm oil, sunflower oil, EPA rich oil, borage oil and echium oil at various levels)	<ul style="list-style-type: none"> No significant effects were observed with each lipid fraction STA may be used as a precursor to increase the EPA content of human lipids
4 week clinical trial in asymptomatic subjects with mild to moderate hypertriglyceridemia		Subjects followed the US National Cholesterol Education Programme Step 1 – 15 g echium oil [supplied by the applicant](containing approx. 1.9 of STA) per day	<ul style="list-style-type: none"> No significant differences between baseline values of vital signs and clinical laboratory markers. Dietary plant oils rich in STA are metabolised to longer chain, more unsaturated (n-3) PUFA Oils appear to possess hypotriglyceridemic properties which are usually associated with fish oil
Toxicology studies on stearidonic acid			
<i>In vitro</i> study – Modification of liver fatty acid metabolism in mice by n-3 and n-6 delta 6-desaturase substrates and products		Mice fed a fat free semi-purified diet supplemented with 1% (w/w) fatty acid ethyl ester mixture	<ul style="list-style-type: none"> Competition for subsequent metabolic enzymes n-6 fatty acids derived from GLA are incorporated more favourably into liver phospholipids.
<i>In vivo</i> – comparison of the conversion rates of ALA and STA to longer polyunsaturated fatty acids in rats		Lipid free diet supplemented with lard (9% w/w) and either ALA ethyl esters (1%) or STA ethyl esters (1%)	<ul style="list-style-type: none"> STA found in liver lipid fraction in small amounts. Desaturation at C-6 is the rate limiting step in the conversion of ALA to EPA
3 week dietary exposure in rats		TAG mixtures containing 10% of STA, ALA or EPA	<ul style="list-style-type: none"> No differences in n-3 PUFA's were observed
7 week dietary exposure in mice		Ethyl esters of ALA, STA, EPA, DHA, CLA and GLA compared with oleic acid at a level of 3g/100g in the diets of APC.	<ul style="list-style-type: none"> No significant difference between prostaglandin levels or body weight STA and EPA attenuate tumorigenesis and this effect may be related in part to alterations in prostaglandin biosynthesis
3 week clinical study in humans		Encapsulated STA, ALA or EPA ingested in daily doses of 0.75g and then 1.5g	<ul style="list-style-type: none"> No consistent effect on lipopolysaccharide stimulated synthesis of prostaglandin E2 and thromboxane A2 No significant differences between groups

40. The applicant has concluded that refined echium oil is comparable in most respects to other plant oils used as foods but it contains a higher level of STA.
41. The applicant notes that none of the toxicity studies allow the setting of a No Observed Adverse Effect Level. However from a human nutritional safety perspective the applicant considers that the two most important clinical studies are those in which echium oil was consumed at levels resulting in up to 1.9g STA per day and for periods for up to 12 weeks. In these studies echium oil was found to have no significant effect on immune function, to decrease serum triglycerides and to have no effect on cholesterol.
42. In light of the information from these studies the applicant considers that 1.9g/person/day is a safe intake level of STA in humans, when consumed in the form of refined echium oil. Therefore, the proposed maximum use level of 200mg of STA per daily serving of various foods would allow for consumption of approximately 9-10 daily servings. (See paragraph 22).

Discussion: The Committee queried the level of protein present in the NI and requested additional information. Members reviewed the results of the additional studies carried out by applicant and were content that these provided the necessary reassurance there were no significant levels of protein present. The Committee also confirmed that they were content with the toxicological assessment carried out by the applicant on the NI which showed it is safe for human consumption at the proposed level of use.

Labelling

Information on this aspect is provided on p.i of the application dossier

43. Although the applicant initially proposed to describe the NI as STA (stearidonic acid)-rich oil *from *Echium plantagineum*" (where * may be used as a footnote), the Committee was of the view that the average consumer may not understand what STA is. The applicant therefore proposed that at a minimum the term "refined echium (vegetable) oil", will be included on the ingredient list of the final food and that, in addition to normal fat labelling requirements, the stearidonic acid content and total omega-3 fatty acid content will be included in the nutrition panel of the food.

Discussion: The Committee was content with the applicants proposed labelling of food products containing the NI and noted that any labelling concerning the nutrient content of foods containing the novel ingredient must comply with the relevant legislation.

CONCLUSION

44. The Advisory Committee on Novel Foods and Processes is satisfied by the evidence provided by Croda Chemicals Europe Ltd that the range of uses for its refined echium oil is acceptable, subject to the applicant's adherence to the proposed specification and the production parameters described above. The Committee also wishes to note that any foods containing this novel ingredient should be labelled in accordance with existing legislation and should not make claims that are likely to mislead consumers.

July 2007