

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

CONJUGATED LINOLEIC ACID (CLA)-RICH OIL

Issue

The Irish Competent Authority has prepared an initial opinion on an application for the authorisation of CLA-rich oil derived from safflower seed (*Carthamus tinctorius* L) as a novel food ingredient (NI) under the Novel Food Regulation (EC) No. 258/97. The Committee is asked whether it agrees with the conclusions of the Irish CA or whether it has any further comments or objections to make on the application. The Committee's advice will form the basis for the UK's formal response.

Introduction

1. On 27 May 2008, the European Commission forwarded the Irish Competent Authority's (CA) initial opinion on an application made by Lipid Nutrition BV under Article 4(1) of Regulation (EC) 258/97, for the authorisation of Clarinol™, a modified oil rich in CLA, derived from safflower seed, as a novel food ingredient. Under the time scales set out in the regulation, the UK and other Member States have until 25 July 2008 to provide comments and/or reasoned objections to the initial opinion.
2. The Irish Initial Assessment Report is attached as **Annex 1**. The full dossier provided by the applicant is attached as **Annex 2** (restricted).

Background

3. This application from Lipid Nutrition B.V is for the placing on the market of CLA-rich oil derived from safflower oil. The NI has been previously consumed in the EU as a food supplement in weight management products since 1996, however its use in food products is considered novel and requires a pre-market safety assessment.
4. In accordance with the European Commission Regulation 258/97, the applicant considers that the NI can fall under two sub categories described under Article 1(2), (c) a complex novel food from non-GM source or (e) which is a food or food ingredient consisting of or isolated from plants or animals. This corresponds to class 2.1 under Commission Recommendation 97/518/EC, which sets out the

guidelines for novel food applications. The requirements for a submission for this class are as follows:

I	Specification of the NF	X
II	Effect of the production process applied to the NF	X
III	History of the organism used as the source of the NF	X
IV	Effect of the genetic modification on the properties of the host organism	-
V	Genetic stability of the GMO	-
VI	Specificity of expression of novel genetic material	-
VII	Transfer of genetic material from GM microorganisms	-

VIII	Ability to survive in and colonise the human gut	-
IX	Anticipated intake/extent of use of the NF	X
X	Information from previous human exposure to the NF or its source	X
XI	Nutritional information on the NF	X
XII	Microbiological information on the NF	X
XIII	Toxicological information on the NF	X

5. The key issues for consideration are presented below under these headings.

I. Specification of the novel food

Annex 2, p.5-13

6. The NI is made from the processing of safflower oil and primarily consists of isomers of CLA (78%), mainly a 50:50 mixture of the *c9, t11* and the *t10, c12* isomers. The specification of the NI is provided in the dossier (Annex 2 p.3, Table I. 1-1). The NI also contains free fatty acids, trans fatty acids, together with mono- and diglycerides. The isomeric composition of the NI is viewed to be important for the perceived health benefits that are attributed to its consumption.
7. Results obtained from the analysis of three non-consecutive production batches of the NI and a representative sample of the starting material were provided. The levels of various contaminants including heavy metals, dioxins, polyaromatic hydrocarbons, pesticides and aflatoxins were within legal limits. Fatty acid profiles of crude safflower oil and three batches of the NI are presented in Table I.3.1-1. The analyses demonstrate that all batches of the NI are within specification and although the specification includes an upper limit of 2% limit trans fatty acids, the levels in all three batches were below 1%. The sterol profiles for crude safflower and three batches of the NI were determined by GC analysis (Table I.3.2-1) and were similar to that of other commonly consumed vegetable oils, including sesame seed, borage, evening primrose and olive oils.
8. The applicant provided a representative example of a stability study data in Table I.5-1 for a batch of the NI stored under nitrogen at 25°C for 42 months. The NI is

stable for at least 36 months if stored dry in the unopened original packaging at a temperature between 10-20°C and away from strong odours and direct sunlight. The Irish CA accepted that the composition and stability data did not give cause for concern.

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

Annex 2, p.14-19

9. The applicant provided an overview of the production process in Fig. II.1-1 which is based on processes that are reported to be commonly employed by the fats and oils industry. The raw material is food grade safflower oil rich in linoleic acid ($\geq 76\%$) and containing very few other polyunsaturated fatty acids. A series of isomerisation, washing, distillation and esterification steps are used to create the crude NI which is then bleached and deodorised at 90-95°C under a nitrogen atmosphere. The NI is then dosed with a suitable antioxidant in accordance with GMP and stored in air-tight drums.
10. Only minor traces of lipases (commercial enzymes preparations used in the production) or other proteins (from safflower) are present in the final product. The applicant provided the results of analysis of protein content for crude safflower oil and three production batches of the NI in Table III.1.1-1. Results show that all contain less than 30 mg/kg protein (range 9-28 mg/kg).
11. The NI is produced in accordance with GMP and in compliance with EU Hygiene Legislation (regulation 1852/2004). In addition the manufacturing site has a valid and independently certified HACCP system in place. Procedures are in place to analyse the raw materials, intermediates and products throughout the production process against the target specifications. If the deviation from specification is marginal the material is reprocessed however if the deviation is significant, the material is discarded.

III. History of the source organism

Annex 2, p. 20-21

12. Safflower (*Carthamus tinctorius L.*) is a member of the Compositae or Asteraceae family and has been cultivated as a source of food oil throughout the world. The seeds contain 25 to 30% oil with a linoleic acid content of about 75% which is the highest known content of this fatty acid. Safflower oil is widely available throughout the world where it is used as a replacement for, or in combination with, sunflower oil in food products such as spraying oils for snacks and crackers, margarines, mayonnaise and salad dressings.

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

Annex 2, p.22-27

13. The NI is intended to be added into foods such as beverages, cereal products, meal replacements (nutritionally complete drinks in liquid or powder form)¹, milk and milk products and dry weight beverages (including chocolate and malted hot drinks). It is intended that the foods to which the NI is added will be labelled, marketed and priced at a premium compared to commodity foods. There is no daily recommended intake level for CLA, but a level of 1.5 g of CLA per serving is proposed by the applicant so that, if consumers choose to do so, they can conveniently obtain 3.0 g of CLA by consuming 2 servings of the target foods. In separate information provided by the applicant to the Irish CA the applicant advised that the 3g per day figure is based on the levels required to achieve the perceived health benefits.
14. The applicant has estimated the potential intake of the NI based on the proposed use-levels and food consumption data collected by the UK Food Standards Agency Dietary Survey Programme (DSP). A summary of the estimated total intake of CLA (g/person/day) from all food-uses in the EU by UK population is provided in Tables IX.2-1 and Table IX.2-2.
15. The percentage of users among all age groups was greater than 61.1% for those food products in which CLA is currently proposed for use, whilst young people (4-10 years) had the greatest percentage of users at 73%. Male adults were determined to have the greatest mean and 97.5th percentile all-person (0.74 and 4.30 g/person/day) and all-user intakes (1.21 and 5.75 g/person/day) of CLA. On a body-weight basis, children were identified as having the highest mean and 97.5th percentile all-person (30.92 and 154.45 mg/kg body weight/day) and all user intakes (50.61 and 179.48 mg/kg body weight/day).
16. The applicant recognises that it is possible for individuals who intentionally seek CLA-containing foods to consume more than 2 servings per day. However the applicant considers that long term consumption at this level is unlikely because the theoretical calculations above indicate that intake of more than 3g CLA per day is associated with consumption of the target foods at a level at or above 90th percentile. The applicant therefore contends that consumers who choose to supplement their diet with CLA may experience a short period of increased intake; it is unlikely that they will maintain this high level of consumption of the target foods over a long period of time. The applicant also notes that the relatively narrow range of foods to be fortified with the NI would also be a factor in making it difficult for many consumers to eat more than 2 servings a day.
17. The applicant provided information from a published Post-Market Surveillance (PMS) study conducted in 2006 in Spain following the launch of a range of products supplemented with CLA-rich oil, in ignorance of the requirement for

¹ The dossier incorrectly describes these as "food supplements "

evaluation under (EC) 258/97. The study interviewed 1,233 consumers of 3 CLA-rich oil fortified products (milk, yoghurt and orange juice, each containing 1.5 g CLA per serving). 93% consumed up to 2 servings a day of yoghurt. For juice and milk products the percentage of consumers eating up to 2 servings per day was 88 and 87% respectively. When all categories were combined, approximately 6% consumed 3 servings per day, 3% consumed 4 servings per day and less than 1% consumed more than 4 daily servings.

18. CLA will be added to food products intended for normal adults to assist in their weight management regimes, and the applicant anticipates that this should minimise consumption by pregnant and breastfeeding women, who are specifically advised by the medical profession not to diet. It is also anticipated that where the NI is added to foods, it will normally replace/partially replace existing fat in the products. Where this is not the case, CLA will contribute no more than 5g of fats (45 kcal) to the daily diet of an adult.
19. The Irish CA was content with the applicant's proposal to label products containing the NI as not being suitable for pregnant, lactating women and children less than 5 years.

X. Information from previous human exposure to the novel food or its source

Annex 2, p.26-29

20. The applicant provided background intakes of CLA isomers occurring naturally in foods like milk and meat in Table X.1-1. The highest level reported (1 g/day) was in a Hare Krishna community in Australia and this was attributed to their high intake of ghee and butter consumption. The most abundant isomer in dietary CLA is the *c9, t11* isomer which accounts for more than 90% of CLA intake in the diet whereas the NI consists of approximately equal proportions of *c9, t11* isomer and the *t10, c12* isomer. However the applicant states that because background intakes are low, they do not significantly affect the intakes from the intended food uses of CLA-rich oil (section IX).
21. Commercial CLA-rich oil has also been available on the EU market since 1995, in food supplements that typically deliver a dose of up to 3g of CLA per day. The applicant stated that there has been no consumer complaint reported or documented for any adverse effect, but does not detail whether there is a mechanism for the recording of such effects. The Secretariat notes however that adverse reactions (most commonly diarrhoea, nausea and dyspepsia) were reported as part of the Spanish PMS study (see IX above) in around 2% of the study population. The report authors did not follow up these reports to determine whether the NI was the likely cause of the adverse effects.

22. The Irish CA noted that there was established consumption of various CLA isomers in the EU diet, both as natural constituents of existing products and of supplements containing the NI.

XI. Nutritional information on the novel food

Annex 2, p.31-33

23. The applicant states that for the purposes of nutrition labelling, the NI is considered to be 100% fat. Of this approximately 7% is saturates, 12% is monounsaturates and 10% is polyunsaturates. The energy value of the NI is 9 Kcal/g.

24. The NI has been identified as being helpful for weight-management products by reducing the amount of body fat and increasing lean muscle mass and will therefore be added to foods intended for normal people as part of their weight management and weight loss regimes. The applicant intends to label the NI in accordance with general labelling requirements to state that such products should only be consumed as part of a healthy and balanced diet. The Irish CA notes that consumption of the recommended amount of the NI would lead to an increase of 5g fat or 45 Kcals/day to the diet of an average adult.

XII. Microbiological information on the novel food

Annex 2, p.34-35

25. The applicant provides a summary of the microbiological product analyses for three non-consecutive production batches of CLA-rich oil in Table XII.1-1. No microbiological contamination was observed for any of these production batches.

26. The applicant is of the view that because the NI is a water-free material, with specified water content of less than 0.1%, it would not support microbial growth. The Irish CA accepted this view.

XIII. Toxicological information on the novel food

Annex 2, p.36-111

27. As the NI is also currently being considered under EU Health Claims legislation, Members are asked to limit any comments to those of toxicological significance, and not comment on the validity of claims that might be made in relation to perceived health benefits. The Secretariat is of the view, however, that the safety assessment requires special attention to be paid to the implications of the postulated mechanisms for the weight loss effect that is attributed to consumption of CLA. Annex 3 comprises three published papers reviewing mechanisms of action and a meta-analysis of the effect of CLA in body fat in humans. (The Secretariat can provide copies of any additional published studies on request).

28. The applicant highlights that, when studying levels of fat in the diet, laboratory animals (particularly rodents) do not have the same level of adipose tissue and

therefore the ability to store fat as humans, and effects seen in rodents such as fat deposition in various organs are of limited relevance to human exposure. The applicant is also of the view that a safety assessment should focus on the relevant isomeric forms of CLA, namely the 50:50 mixture of *c*9, *t*11 and *t*10,,*c*12 isomers, as this reflects the composition of the NI. The dossier therefore gives higher priority to human clinical studies.

29. The general metabolic fate of CLA is comparable to that of any triglycerides as described by the applicant (Section XIII.2.1). Studies on rabbits, pigs, rats and humans demonstrate that CLA is well absorbed across the gastrointestinal mucosa, like most fatty acids and CLA is distributed in tissues around the body particularly in plasma lipids and milk and adipose tissue. The applicant describes that the metabolism of CLA isomers is very similar to that of linoleic acid and is metabolised by two distinct pathways, desaturation and oxidation. The metabolites are extensively excreted from the body in expired air and lesser amounts in urine and faeces.
30. The applicant summarises subchronic, chronic and reproductive and developmental toxicity studies undertaken with with CLA in Table XIII.3.7-1. (Annex 2, p 53-55)
31. The applicant describes a 13-week oral study in male and female Wistar rats which were administered either a high-fat (15% w/w safflower oil) or low-fat (7% w/w safflower oil) basal diet (Annex 2, p 46-48). Test groups received a high fat basal diet supplemented with 1%, 5% or 15% Clarinol G-80 (79% CLA 50:50 mixture). The authors reported the no-observed–adverse-effect-level (NOAEL) to be 5% Clarinol G-80, equivalent to be 2,433 and 2,728 mg/kg body weight/day for males and females respectively. The Irish CA highlights that the changes in lipid metabolism toxicity and fatty acid changes in the liver are due to the animals² not being able to cope with large changes in fat metabolism and being poor models for studying the effects of CLA, as already noted by the applicant.
32. The applicant described studies conducted on the mothers and offspring of pigs and rats fed on a diet of 0.25% to 5% CLA. The studies did not identify any adverse effects, although a significant uptake of CLA in maternal mammary gland and milk was reported in the rat studies.
33. The mutagenic potential of Clarinol G-80 (containing 79% CLA isomers) was tested in a bacterial mutagenicity assay at concentrations of 5000 µg/plate and was reported to not be mutagenic. Clarinol G-80 tested on human peripheral blood lymphocytes (concentrations up to 300 µg/l) did not induce chromosome aberrations.

² The initial assessment report refers to "the mouse", although the study was carried out in rats.

34. The applicant also provided information on an 18 month study of rats fed 1% CLA. The incidences of pituitary or testicular tumours, prostatitis, or lymphoma were not significantly different between groups. The applicant states that based on this study, the structure and metabolic fate of CLA it is not anticipated that the NI would represent an increased carcinogenic risk. This was further supported by the results of the mutagenicity study described above.
35. Additional *in vitro* and animal studies: A number of studies indicate CLA may have effects on cardiovascular disease, insulin sensitivity, maternal milk fat deposition and biomarkers of oxidation. However the applicant suggests that conclusions should only be drawn in light of the entire database of these studies. This is because numerous studies have shown inter-species variation in cardiovascular risk markers, the majority of data also demonstrate positive effects of CLA on inflammatory markers and insulin resistance effects have been demonstrated to be transient.
36. The Irish CA initially raised concerns that the absence of a robust chronic toxicity study may limit the assessment of the NI. However they accepted the applicant's view that the majority of EU-authorized novel foods currently on the market, including oils, had not been subjected to chronic pre-clinical studies. The Irish CA also agreed with the applicant that greater significance should be placed on human studies as animals are poor models for studying the effects of CLA on body fat.
37. **Allergenicity:** The applicant considers that the risk of allergenicity from the NI is considered to be low (Annex 2, p 111). Due to the high temperature, highly lipophilic environment and the purification steps in the production process, no significant proteins (and therefore potential allergens) are expected in the final product. No allergy-inducing proteins are reported to be associated with safflower oil, which is a recognized international food commodity oil. There have been no reports of allergenicity in over 30 clinical trials conducted on the NI.
38. **Clinical studies:** The applicant provided a summary of the clinical studies conducted on CLA in Table XIII.5-1-4 (Annex 2, p 58-88) and concentrates its discussion on the safety of the 50:50 mixtures. These studies evaluated a range of safety parameters as well as the potential effects of CLA on cardiovascular parameters (e.g. lipid metabolism, markers of inflammation and markers of oxidative stress), insulin sensitivity and glucose and milk fat deposition.
39. The Irish CA notes that no safety concerns were raised by any of the clinical studies that were based on CLA intakes up to a maximum of 6g per day. The initial opinion notes that the level of adverse events in test subjects was similar to that seen in placebo groups. Side effects were mainly due to gastrointestinal changes and some investigators suggested this could be due to the large

numbers of gelatine capsules administered in most of the trials. A 2 year study of healthy males and females for the effect of CLA supplementation on body fat did not identify any significant changes to a range of clinical chemistry variables and no safety issues were noted. In addition there were no convincing data that showed CLA has a consistently negative influence on lipids.

40. Human trials demonstrated a wide variation in changes of oxidative markers such as isoprostanes both before and after CLA administration. The Irish CA notes that while urinary excretion of isoprostanes is elevated in conditions associated with oxidation and inflammation, the use of such a surrogate marker of oxidative stress requires careful consideration of factors such as natural variation in isoprostane excretion and the accuracy of the measurement techniques. There is also still some debate on whether increased isoprostane is directly linked to oxidative stress, or due to other factors such as enhanced availability of substrate or altered catabolism. The Irish CA highlights a study provided by the applicant which concludes that CLA intake in humans may impair the breakdown of isoprostanes, rather than increase their production, thereby resulting in higher excretion.
41. The Irish CA notes that the while individual isomers of CLA may result in an increase in insulin resistance, the 50:50 mixture of isomers (c9, t11 and t10, c12) present in the NI appears to have a neutral if not beneficial effect. CLA also appeared to have no effect on the parameters of blood coagulation or platelet function so is not expected to pose a bleeding risk.
42. The Irish CA is of the view that the information presented on the effects of CLA on milk fat in lactating women and the consequences for breast-fed children are conflicting and are therefore of limited use. Their medical expert concludes that the conflicting results evident in some of the studies presented may reflect differences in study duration, cohort composition, study settings, and most importantly, supplement composition and the choice of an appropriate control fat. The expert did not identify consistent clinical evidence of adverse health effects related to CLA consumption but advised that lactating women should avoid the consumption of foodstuff containing CLA, based on the limited evidence available. The applicant therefore intends to advise pregnant or lactating women and children less than 5 years of age not to consume these products. In addition those on any form of medication would be advised on the label that they should consult their physician before consuming CLA-containing products.
43. The Irish CA accepted that the data presented on chronic toxicity, carcinogenicity are in line with similar data for other novel food applications and are considered adequate in light of the greater reliance on clinical studies. The Irish CA concludes that the NI is safe for consumption provided the product specifications

are adhered to, the limitation of the range of foodstuff is maintained and the advisory/warning labels are applied consistently.

Committee Action Required

44. The Committee is asked whether it agrees with the initial opinion from the Irish CA that CLA-rich oil produced by Lipid Nutrition BV should be granted authorisation as a novel food ingredient and whether it wishes to make any additional comments on the application.

Secretariat

July 2008

Annexes attached:

Annex 1 – Irish Competent Authority's initial assessment report on CLA.

Annex 2 - Application dossier submitted by Lipid Nutrition BV for the approval of CLA-rich oil as a novel food ingredient. (**RESTRICTED**)

Annex 3 - Published papers on CLA including, The biologically active isomers of conjugated linoleic acid (M. W. Pariza *et al.*, Progress Lipid Research, 2001), Efficacy of CLA for reducing fat mass: a meta-analysis in humans (Whigham *et al.*, The American Journal of Clinical Nutrition, 2007) and Perspective on the Safety and effectiveness of CLA (M. W. Pariza, American Journal of Clinical Nutrition, 2004)

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Irish Competent Authority's Initial Assessment Report

**Secretariat
June 2008**

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

RESTRICTED

Application dossier submitted by Lipid Nutrition BV for the approval of CLA-rich oil as a novel food ingredient.

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Published papers on CLA:

- p.1 The biologically active isomers of conjugated linoleic acid (M. W. Pariza *et al.*, Progress Lipid Research, 2001)
- p.5 Efficacy of CLA for reducing fat mass: a meta-analysis in humans (Whigham *et al.*, The American Journal of Clinical Nutrition, 2007)
- p.8 Perspective on the safety and effectiveness of CLA (M. W. Pariza, American Journal of Clinical Nutrition, 2004)

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