Initial Assessment

Clarinol™ CLA-Rich Oil

Name of Applicant: Lipid Nutrition B.V.

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Novel Food Classification: 2.1.

Introduction

A novel food application was submitted to the Food Safety Authority of Ireland (FSAI) in October, 2007, for the authorisation of Clarinol™, an oil that is rich in conjugated linoleic acid (CLA) and derived from safflower seed. The dossier, submitted under *Article 4* of the novel food Regulation (EC) No. 258/97, was formally accepted by the FSAI on November 23rd, 2007, by letter to Mr. Jaap Kluifhooft of Lipid Nutrition B.V. and copied to Mr. Andreas Klepsch of the European Commission.

The application for authorisation of Clarinol™ as a novel food ingredient was prepared pursuant to Commission Recommendation 97/618/EC concerning the scientific aspects and the presentation of information in support of an application to market novel foods and novel food ingredients in the EU. The applicant makes a case that Clarinol™ could be considered in two of the sub-categories listed in *Article 1.2.* of the novel food Regulation: (c) "foods and food ingredients with a new or intentionally modified primary molecular structure"; and (e) "foods and food ingredients consisting of, or isolated from plants, and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating or breeding practices and having a history of safe food use". In order to assess its wholesomeness, Clarinol™ is considered in Class 2.1. by the applicant; "Complex novel food from non-GM source which has a history of food use in the community" (Commission Recommendation 97/618/EC).

CLA represents a family of positional and geometric isomers of linoleic acid that is naturally present at low levels in the EU diet in foods such as meat and dairy products. CLA preparations consisting of approximately equal proportions of the two isomers *c*9, *t*11 and *t*10, *c*12 are favoured from a potential health benefit perspective, while high levels of trans-fatty acids and certain other CLA isomers can have negative health implications. Naturally occurring CLA predominantly consists of the *c*9, *t*11 isomer (90%) whereas the beneficial 50:50 mixture of *c*9,*t*11 and *t*10,*c*12 isomers make up three quarters of Clarinol™ that is processed from safflower seed oil.

The applicant intends to add Clarinol™ to a range of foodstuffs including beverages, cereal products, food supplements and milk products. Target foods will contain 1.5g of CLA per serving and will be marketed at a price reflecting the significant production cost of CLA-rich oil. A novel food authorisation is required as CLA has a

history of consumption in supplement form but not in general foods in the EU prior to 1997 (Standing Committee, February 14, 2005).

Using the schemes set out in Commission Recommendation 97/618/EC, the information addressing the safety of CLA is set out as follows.

I. Specification of the novel food

Clarinol™ is an oil derived from safflower seed that is predominantly made up of isomers of CLA (≥78%). The isomeric composition of CLA is important in that certain combinations have potential health benefits while other isomers, along with trans-fatty acids can have deleterious health effects. The CLA content in Clarinol™ is primarily (≥74%) made up of equal proportions of two isomers (*c*9,*t*11 and *t*10,*c*12) and is reported to confer certain beneficial health effects. Though the chemical specification stipulates that CLA-rich oil consists of ≤2% of trans-fatty acids, batch test results indicate the actual levels are less than 1%.
Analysis of contaminants including heavy metals, dioxins, pesticides and aflatoxins were carried out by a reputable and accredited laboratory with the levels recorded within legal limits. The stability of CLA-rich oil can vary depending on various environmental conditions including temperature and light exposure but was greatest when stored at 25°C under nitrogen, where it was stable for greater than 42 months.

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

The manufacturing process for CLA-rich oil is diagrammatically represented in Figure II.1-1 of the application dossier and includes processes commonly used in the fats and oils industry. The process begins with refined safflower seed oil that is rich in linoleic acid. CLA-rich oil is produced by a series of enzymatic reactions that include hydrolysis, isomerisation and esterification. The quality of the final CLA-rich oil is enhanced through mechanical and chemical processes including distillation, bleaching and deodorisation. An independently certified HACCP system is in place backed up by quality assurance and quality control programmes. Samples are tested at all stages of production for adherence to specifications, with product being discarded in the event of unsatisfactory deviation from those specifications.

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

Carthamus tinctorius (Safflower) has been well characterised and has a considerable history of safe use in food around the world. Though an experimental GM safflower has been developed to produce human insulin in its seeds, the variety of safflower used for this product is non-GM. Safflower seed oil is used as cooking oil, in salad dressings and also in the colouring and flavouring of a variety of foods. Only the oil has a history of consumption as food in the EU.

IV. – VIII. Not applicable. No GMO involvement

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

The EFSA Scientific Panel on Dietetic Products, Nutrition and Allergies (Opinion on the presence of trans-fatty acids in foods and the effect on human health of the

consumption of trans-fatty acids, July 8, 2004) estimated the average natural dietary intake of CLA in the EU to be approximately 0.3 g/day. This estimate mirrors findings in Germany and Finland and leads the applicant to suggest that CLA intake estimates predicted for food fortified with CLA should not be significantly affected by CLA naturally occurring in food. The applicant intends using Clarinol^{TM} in a range of products including beverages, cereal products, food supplements and milk products at a level of 1.5g CLA per serving. Recommended daily intake levels have yet to be established for CLA, however, the applicant's preferred intake of 3g per day is based on the levels required to achieve the purported health benefits (separate information provided by the applicant). Foods containing Clarinol^{TM} are to be specifically targeted at healthy, overweight adults as part of a weight management programme. The premium cost associated with CLA-containing foods will reflect the production cost of Clarinol^{TM} and may also help to reduce any excessive or indiscriminate consumption of such foods.

The applicant used food consumption data from the UK's Food Standards Agency to predict the intake of Clarinol^{TM} through the consumption of the target foodstuffs. Of the individual population groups surveyed, male teenagers were estimated to have the greatest mean and 97.5th percentile all-user intakes (0.92 and 3.12 g/person/day, respectively) of CLA. The estimated intakes represent a "worst case scenario" based on theoretical calculations that would be applicable to UK citizens, but could also apply to citizens of many other EU Member States. The applicant envisages a typical consumption of 3g per day, but intakes as high as 4.5g per day would be possible in some instances. Post market monitoring in 2006 following the 2004 launch of a limited range of products containing CLA-rich oil in Spain indicated that an average of 3 - 6g per day of CLA was consumed by adults, with only a few gastrointestinal health effects reported. The Spanish data indicates that consumption was within the predicted intake levels deduced by this applicant.

The applicant identifies certain at-risk groups in relation to potential health hazards from CLA consumption. The evidence of possible health effects in relation to cardiovascular disease, insulin resistance and maternal milk-fat deposition are dealt with separately in this report.

The applicant argues that pregnant and breast-feeding women are specifically advised by the medical profession not to diet. This, along with the fact CLA will be added to food products intended for healthy, overweight adults to assist in their weight management regimes, should minimise consumption of these products by this population group.

In general, estimates of CLA intake by children from fruit juices and milk products, (particularly yogurt and soya milk) and also from the category "dry weight beverages for slimming purposes" were somewhat high. However, the statistical reliability of some of these estimates is low because of limited sample numbers. This together with the fact that children are not a target for these food products means that intakes in this population group are not of major concern.

During discussions with the applicant it was agreed that foodstuffs containing added Clarinol^{TM} would be labelled to advise pregnant or lactating women and children less than five years of age not to consume these products.

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

As mentioned in section III, safflower has a significant history of use as a food ingredient worldwide, though only seed oil in supplement form has been available in the EU. The applicant provides data from Germany, Finland, USA and Australia on CLA intake levels from natural dietary sources. The data from Germany and Finland confirms that normal dietary CLA consumption in those Member States is within range of the EFSA estimate of 0.3g per day. The relatively high levels (1,000mg/day) identified in Australia are possibly explained by certain sub-groups (e.g. Hare Krishna) consuming high levels of particular dairy products. Naturally occurring CLA is predominantly made up of the *c*9,*t*11 isomer while the Clarinol^{TM} CLA-rich oil consists primarily of equal proportions of *c*9,*t*11 and *t*10,*c*12 isomers.

XI. Nutritional information on the novel food

CLA-rich oil is 100% fat of which 7% is saturated, 12% is monounsaturated and 80% is polyunsaturated. CLA-rich oil has an energy value of 9 kcal/g and will be added to foods as part of a weight management programme for overweight individuals. The applicant intends that added CLA will replace, or partially replace existing fat and in any case would add approximately 5g of fat or 45 kcals/day to the diet of an average adult.

XII. Microbiological information on the novel food

CLA-rich oil is a water-free material (water <0.1%) and does not support significant microbial growth. The batch analyses carried out did not detect the presence of any microbial contaminants.

XIII. Toxicological information on the novel food

Laboratory animals (particularly rodents) are not suitable for studies on dietary lipids as they do not have the same level of adipose tissue, and therefore the same fat storage capacity as humans. For this reason the applicant makes the reasonable argument that the safety assessment of CLA in food should be based primarily on human studies rather than traditional pre-clinical experimental studies in animals.

Absorption, Distribution, Metabolism and Excretion (ADME)
The absorption, distribution, metabolism and excretion of CLA are all similar to those of other fatty acids, including linoleic acid. Animal studies, confirmed by human studies, demonstrate that CLA is absorbed across the intestinal mucosa and distributed in tissues around the body, with some preference shown to plasma lipids, as well as milk and adipose tissue. CLA is metabolised via oxidation and desaturation, with metabolites excreted from the body primarily through exhaled air (as CO_2), and to some extent in urine and faeces.

Toxicity
A limited amount of animal data relating to toxicity is provided in the application. The oral LD_{50} in rats is reported to be >3g/kg.

Subacute/Subchronic toxicity – A robust 13 week study in rats fed CLA (79% 50:50 mixture of *c*9,*t*11 and *t*10,*c*12) concluded that 5% of CLA in the diet represented the "no-observed-adverse-effect-level (NOAEL), equivalent to 2,433 and 2,728 mg/kg/day for males and females respectively. The applicant noted profound changes in lipid metabolism in the mouse, with toxicity and fatty acid change in the liver. However, as already noted the mouse cannot cope with large changes in fat metabolism and is thus a poor model for studying the effects of CLA.

Mutagenicity – CLA (Clarinol™ G-80) was not found to have any mutagenic potential when tested in a bacterial mutagenicity assay, nor did it produce chromosomal aberrations in human lymphocytes. The results of these studies indicate that CLA does not have genotoxic potential.

Chronic toxicity/Carcinogenicity – Classical carcinogenicity studies on CLA were not available, and the one long term study presented (an 18-month study in rats) examined the effects of a diet supplemented with 1% CLA (42% *c*9,*t*11 and 44% *t*10,*c*12). The long term study found no significant differences in tumour incidence between CLA fed rats and control animals which, the applicant argues, along with mutagenicity studies and knowledge of the structure and fate of CLA, suggests that CLA-rich oil does not pose a carcinogenicity risk. Effects on blood glucose were reported in this study, although the NOAEL was reported to be 1% CLA in the diet, the only dose tested.

Reproductive and Development Toxicity – A number of studies in rats and pigs did not identify any adverse effects on mothers or offspring that were on diets consisting of 0.25% to 2% CLA. Though significant uptake into the maternal mammary gland of CLA was reported for rats, there were no associated adverse effects.

Other toxicological studies – a number of toxicological studies have shown that CLA has effects on lipid metabolism, resulting in alterations in body composition and possible anti-atherogenic, anti-carcinogenic and immune modulatory effects

Initially there was some concern by FSAI that the absence of a robust chronic toxicity study may affect the quality of the safety assessment of the novel ingredient. However, during discussion with the applicant it was discovered that the majority of EU-authorised novel foods currently on the market, including a range of oils, had not been subjected to chronic pre-clinical studies. In addition, the preference for human over animal studies in assessing the safety of CLA was noted with respect to the significance of traditional pre-clinical studies.

Allergenicity

The risk of allergic reactions from the inclusion of Clarinol™ in foodstuffs is considered to be low as Safflower is not known to contain any endogenous allergenic proteins. Therefore it is not a surprise that there have been no reports of allergenic reactions associated with the use of Clarinol™ during the course of more than 30 clinical trials. In addition, the production process for Clarinol™ is relatively protein-unfriendly, resulting in CLA-rich oil with almost no residual protein from the original safflower or the enzymatic production process.

Clinical studies

Numerous clinical studies on various preparations of CLA were provided by the applicant and examined by a medical expert engaged by the FSAI. The original manuscripts referred to in the section on "Summary of Clinical Studies Conducted with 50:50 Mixtures" as well as other relevant publications were reviewed along with the supplementary expert reports.

No safety concerns were raised by any of the studies which were based on CLA intakes up to a maximum of 6g per day. Overall the level of adverse events in test subjects were similar to those reported in placebo groups, while reported side effects were largely due to gastrointestinal changes. A prolonged study (2yr) 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 arose.

Many of the studies focused on parameters that have a potential bearing on cardiovascular health, as reflected by changes in risk factors such as serum lipids. However, there was no convincing data that showed CLA has a consistently negative influence on lipids.

The urinary excretion of isoprostanes is elevated in conditions associated with oxidation and inflammation, though the potential 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 various isoprostane measurement techniques. However, it is still a matter of some debate whether increased isoprostane excretion is directly associated with oxidative stress, or due to other factors such as enhanced availability of substrate or altered catabolism. A number of studies provided by the applicant linked increased levels of isoprostane excretion to the consumption of CLA.The FSAI medical expert considers that current scientific knowledge does not support the extrapolation of these data to imply a direct association between CLA consumption and oxidative stress. This stance is supported by a recent study provided by the applicant and relating to increased excretion of isoprostanes due to CLA consumption. The study, which is currently in press, concludes that CLA intake in humans may impair the breakdown of isoprostanes rather than increase their production, thereby resulting in higher levels being excreted.

Many studies have addressed the effects of CLA on insulin resistance and sensitivity which can be associated with increased risk of vascular events. However, while the individual isomers of CLA may result in an increase in insulin resistance, the 50:50 mixture of isomers (*c*9,*t*11 and *t*10,*c*12) present in Clarinol^{TM} seems to have a neutral, if not beneficial effect.

There were no significant effects on immune or vascular functions as a result of the supplementation of the diets of healthy individuals with a 50:50 mixture of CLA isomers. CLA was also observed to have no effect on the parameters of blood coagulation or platelet function and thus would not be expected to pose a bleeding risk.

A number of studies have shown that CLA has the potential to alter body fat and lean body mass. CLA consumption was not found to be associated with adverse effects on components of bone or muscle turnover and though results varied, the overall impact on body fat and its distribution around the body was considered to be neutral or even beneficial.

The information available on the effects of CLA on milk fat in lactating women and the consequences for breast fed children are conflicting and thus of limited use. Therefore, in the absence of further studies it is prudent that lactating women avoid dietary CLA supplementation. The 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 concluded that with the exception of milk fat and possible concerns for lactating women, the clinical studies presented do not provide evidence of consistent adverse effects resulting from the consumption of the 50:50 mix of CLA isomers present in Clarinol^{TM}.

During discussions with the applicant it was agreed that foodstuffs containing added CLA would be labelled to advise pregnant or lactating women and children less than five years of age not to consume these products. In addition, the label would advise that that people on any form of medication should consult their physician prior to consuming CLA-containing products.

Conclusions

CLA-rich oil has been commercially available on the EU market in supplement form since 1995, with no adverse health effects reported. However, dietary supplements are consumed only by a certain proportion of the population, primarily adults who wish to improve their nutritional balance. Adding CLA-rich oil to a range of foodstuffs, as intended by the applicant, has the potential to expose many more people to this ingredient and thus a safety assessment is required under the novel food Regulation.

A number of conclusions can be drawn from this assessment:

1. Animals, particularly rodents are poor models for studying the effects of CLA on body fat, which led the applicant to assign more significance to clinical studies in humans rather than data from animal studies.

2. From the studies provided by the applicant, a medical expert engaged by the FSAI did not identify consistent clinical evidence of adverse health effects related to CLA consumption. While some of the studies provide conflicting evidence, on balance there is no new evidence that would raise any concerns for the safety of healthy people consuming CLA-containing foodstuffs as part of a weight-management programme. The medical expert advised that lactating women should avoid the consumption of foodstuffs with added CLA based on the limited evidence available.

3. The data presented on chronic toxicity, carcinogenicity and genotoxicity are in line with similar data for other novel food applications and considered adequate in light of the greater reliance on clinical studies.

CLA-fortified foodstuffs are to be targeted at healthy adults as part of a weight-management programme and will be marketed accordingly, at a premium price reflecting the production costs of CLA-rich oil. The relatively high theoretical intakes achieved by children through products such as yogurt and other dairy products, soya milk, and fruit juices will be addressed by the applicant through a system of advisory labelling. Potential concerns regarding CLA intake by pregnant and lactating women will be similarly addressed through a system of advisory labelling.

Recommendation

The FSAI is satisfied that the use of CLA (Clarinol^{TM}) in foodstuffs meets the criteria for novel food set out in *Article 3.1.* of the novel food Regulation. The FSAI does not have concerns about the safety of this ingredient provided the product specifications are adhered to, the limitation of the range of foodstuffs is maintained and that advisory/warning labels discussed with the applicant are applied consistently for the benefit of consumers.