

**Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies
on a request from the Commission related to
an application to market Enova oil as a novel food in the EU**

(Request N° EFSA-Q-2004-089)

(adopted on 2 December 2004)

SUMMARY

The Archer Daniel Midland Company (ADM) has submitted a request under the Novel Food Regulation (EC) N° 258/97 to market a novel oil, Enova oil, as a food ingredient. It is intended that the novel oil will be used as a cooking oil and as an ingredient for fat spreads/margarines, dressings for salads/mayonnaise, bakery products, yoghurt, health bars and health drinks. An initial assessment by the Dutch Competent Authority reached the conclusion that it was safe for human consumption. Some of the other Member States (MS) raised concerns and objections but with the exception of Spain, these were satisfied by further safety data supplied by ADM. The Commission has asked EFSA to provide a scientific opinion on the use of Enova oil as a food ingredient in view of the comments/objections raised by the MS and especially those of Spain. These concerns included the possible use of genetically-modified (GM) materials, details of the production process, the stability of the product, the *trans* fatty acid content, possible adverse health effects on potentially sensitive groups, possible impairment of liver and pancreas function and uptake of fat-soluble vitamins and micronutrients, health claims and post-launch monitoring schemes.

Enova oil contains at least 80% diacylglycerols, up to 20% triacylglycerols, up to 5% monoacylglycerols plus trace amounts of antioxidants. Oleic, linoleic and linolenic acids are the main fatty acid components of the oil and are derived mainly from food-grade soybean and rapeseed oils. The diacylglycerols are produced by esterification of the fatty acids with glycerol or monoacylglycerol in the presence of an immobilised lipase. A similar oil (Econa oil) has been on sale in Japan since February 1999 and Enova oil has been on sale in the US since January 2003.

The Panel based its assessment on the structured scheme in the Commission recommendations (1997). It accepted the applicant's view that although the source of fatty acids could be from both conventional and genetically-modified soybean and rapeseed oils the questions at issue related to the safety of the novel oil and were not an assessment of the genetic modification since the applicant would only source from GM crops that had been assessed to be as safe as conventional crops under the appropriate EU Directives and Regulations.

The applicant provided data on the specification of the novel oil, the effect of the production process, the history of the organisms (edible plant seeds) used as the source of the oil, the anticipated intake and extent of use of the novel food, information from previous human exposure, and nutritional, microbiological and toxicological information, as required by the structured scheme. The esterification step in which the lipase immobilised on a resin support was used, did not introduce potentially toxic compounds, nor additional protein into the oil and the heating applied during subsequent steps would be expected to denature any proteins,

including enzymes. The novel oil proved to be as stable as conventional oils to oxidation, low pH and repeated frying conditions. The applicant has calculated that from all possible sources of the oil, on the assumption that a person consumes all the products in one day, the average European would consume 51 g Enova oil/day equivalent to 0.7 g/kg body weight/day based on a 70 kg consumer. The Dutch Competent Authority calculated from TNO data that the 90th percentile of total fat intake in young men aged 19 to 22 years is 161 g/day. This population group consumes the most energy.

Enova oil and conventional (triacylglycerol) oils of comparable fatty acid composition were nutritionally equivalent with respect to energy value and digestibility and did not affect the absorption of fat soluble vitamins in diets.

The toxicological data do not indicate that Enova oil induces specific effects compared with conventional oils with a similar fatty acid composition.

The Panel concludes that the product is safe for human consumption but that in order for it not to be nutritionally disadvantageous to consumers, the *trans* fatty acid (TFA) content should be reduced to the level in the conventional vegetable oils that the novel oil is intended to replace. Vegetable oils and liquid margarines have a low proportion of TFA, usually below 1% (NDA, 2004). The Panel notes that the applicant does not intend to use this ingredient as a fat source in infant formulae and follow-on formulae.

KEY WORDS

Diacylglycerol oil, triacylglycerol, novel food ingredient, fatty acids, PUFAs, food safety.

INTRODUCTION

The Archer Daniels Midland Company (ADM) has requested approval to market a novel food Enova oil in Europe under the Novel Food Regulation (EC) N° 258/97. The novel oil consists mainly of diacylglycerols and differs from traditional oils that are mainly composed of triacylglycerols. The oil is manufactured from glycerol and fatty acids using a specific lipase. The fatty acids are obtained from common edible oils and the enzyme from a microorganism. The applicant proposes to use the oil to replace traditional fats and oils in cooking oils, spreadable fats, salad dressings, confectionery products, yoghurt, health bars and health drinks.

BACKGROUND

In April 2002, Archer Daniels Midland (ADM) submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to the Dutch authorities for placing on the market Enova oil as a food ingredient.

On 20 December 2002, the Dutch authorities forwarded to the Commission their initial assessment report of the product concerned, carried out by the Gezondheidsraad (NL), which had reached the conclusion that Enova oil would be safe for human consumption.

In accordance with Article 6(4) of the Novel Foods Regulation, the Commission forwarded the initial assessment report to Member States on 21 January 2003. Member States submitted their comments and/or presented reasoned objections within the 60 day period provided for in the authorisation procedure. In consequence, a Community Decision is now required and beforehand, it appears necessary to request a scientific opinion of the European Food Safety Authority, as there might be an effect on public health (Article 11 of Regulation (EC) N° 258/97).

The concerns raised by the Competent Authorities of Member States were:

- The classification of the novel oil by the applicant is subclass 1.1 which is defined as pure chemicals or simple mixtures from non-genetically-modified (GM) sources where the source of the novel food has a history of safe use in the EU. However the applicant intends to source its vegetable oils from both non-GM and GM crops e.g. soya and rapeseed.
- The documentation does not describe in detail the production process used to obtain the diacylglycerols.
- It must be ascertained whether the enzyme used in the process is totally inactivated.
- The applicant should provide data on the stability of the oil particularly with respect to the effects of changes to pH and exposure to light and air.
- The level of *trans* fatty acids is high (4%) and the oil would not be suitable for infant and follow-on formulae in view of the recommendation of the Scientific Committee on Food (SCF) to reduce levels to 3% of total fatty acids.
- The possibility should be considered that the novel food might have adverse health effects on sensitive population groups such as the elderly, children, pregnant or breast feeding women or persons suffering from various diseases.
- The increase in GPT and amylase in human subjects could indicate impaired liver and pancreas function.
- The absorption and bioavailability of fat soluble vitamins, micronutrients and carotenoids may be reduced compared with traditional oils.
- Health claims in labelling and advertising should not be allowed.
- A post-market monitoring scheme should be put in place.

In addressing these issues and considering the overall safety of the novel oil the Panel has used information from the original dossier supplied to the Dutch Competent Authority, the report of the Dutch Committee on the Safety Assessment of Novel Foods and comments and data supplied by the applicant to the Member States.

In May 1998 the Japanese government gave the Kao Corporation permission to market the novel oil, called Econa[®] oil, on the Japanese market and in December 2000 the oil was given

GRAS status (Generally recognised as safe) in the US where it is marketed by ADM and the Kao Corporation jointly under the name Enova[®] oil.

TERMS OF REFERENCE

In accordance with Article 29 (1) (a) of Regulation (EC) N° 178/2002, the European Commission requests the European Food Safety Authority to issue a scientific opinion on the use of Enova oil as a food/food ingredient in the context of Regulation (EC) N° 258/97.

The Authority is asked to specify whether the authorisation of Enova oil (diacylglycerol oil) as a food/food ingredient is likely to have an effect on public health and, in particular, to focus on the elements of a scientific nature in the comments/objections raised by the Member States (annex 3) to the Initial Assessment Report (annex 2).

As apparently all the questions/objections were answered to the satisfaction of the Member States except those raised by Spain, the additional assessment might focus largely on those issues which are still “open”.

ASSESSMENT

The applicant has proposed that the novel food should be placed in Class 1, sub-class 1.1, described in the SCF Guidelines in Part 1 of the European Commission Recommendation 97/618/EC as a simple mixture of chemical substances, derived from non-genetically-modified sources and that these sources have been previously used at a significant level for foods within the European Community. However the applicant has stated that the source material may be derived from GM crops as well as conventional (non-GM) ones. It is not the purpose of this Opinion to review safety data pertaining to particular GM events employed in the development of crop varieties but to concentrate on the safety of the novel product as it is intended to be used as a food/food ingredient. For this reason this Opinion will be an assessment of the safety data provided by the applicant to comply with the information required for novel foods of Class 1.1 i.e. information requirements I, II, III, IX, X, XI, XII and XIII as detailed in the following text. However the implications of using GM crop sources will be discussed later (section III and Discussion).

I. Specification of the novel food (NF)

The NF (Enova[®] oil) contains at least 80% diacylglycerols (DAG), up to 20% triacylglycerols (TAG), up to 5% monoacylglycerols (MAG), plus trace amounts (approximately 0.2%) of antioxidants. The DAG component is produced as a mixture of 1,3-DAG (approximately 70%) and 1,2-DAG (approximately 30%).

The fatty acid side chains, derived from edible plant oils, are esterified randomly into these positions. The main fatty acid components of DAG are oleic acid (C18:1) (20-65% by weight), linoleic acid (C18:2) (15-65%) and linolenic acid (C18:3) (about 15%) derived from either soybean (*Glycine max*) or rapeseed (*Brassica campestris*) oils. Saturated fatty acids (C16:0 and C18:0) are present at not more than 10%. Current levels of *trans* fatty acids are

2.8%. The NF also contains about 0.025% ascorbyl palmitate and about 0.075% mixed tocopherols. The phytosterol content ranged between 0.05 and 1.2% by weight.

The metals lead, arsenic, mercury, cadmium, copper, antimony, strontium and bismuth were not detected using inductively coupled argon plasma atomic emission spectrometry with a detection limit of 0.5 mg/kg. According to the applicant, additional analyses with a lower limit of detection have shown that the product complies with the maximum levels for heavy metals set in Regulation (EC) 466/2001 (0.1 mg/kg for lead) and the Codex Standard for named vegetable oils (0.1 mg/kg for lead and arsenic).

Data on the composition of five different lots produced between 1998 and 2000 showed the range of values to be within the aforementioned specification suggested by the applicant.

Measurements have been made on the stability of DAG oils (Econa oil in Japan by the Kao Company and Enova oil by ADM in the US) under various conditions. The stability of Econa oil was evaluated for one year while stored at room temperature in the light and in the dark. Parameters measured were peroxide values, changes in acidity, flavour, DAG content, moisture levels and colour. Comparisons were with conventional corn oil and a conventional oil made by the Kao Company. The results indicate that the stability of the DAG oil during the study was similar in terms of both oxidative and organoleptic qualities.

The stability of Enova oil is currently being assessed in the US in comparison with a standard salad oil composed of a mixture of soybean and rapeseed oils (7:3) in both opened and unopened bottles exposed to light at room temperature. Parameters being measured include DAG content, oxidative stability index, acid value, peroxide value, flavour and colour. At nine months the data indicate that the fatty acid composition of Enova oil is stable and in addition that all indicators of fat oxidation undergo similar relative changes in both Enova and the conventional oil.

Additional data have been supplied to show that Enova and a conventional oil when used to fry potatoes on three consecutive occasions showed the same stability to frying temperatures. Finally Enova oil may be exposed to changes in pH when used as an ingredient e.g. in a mayonnaise or salad dressing. In a mayonnaise at pH 4.1 the % DAG content remained stable for seven months and in a salad dressing at pH 3.8 for nine months. These were the time limits of the experiments.

II. Effects of the production process applied to the NF

Glycerols or monoacylglycerols, and fatty acids obtained from edible oils, all of food quality, are used in the production of the novel oil. The esterification process is carried out in the presence of an immobilised 1,3-specific lipase isolated from the fungus *Aspergillus oryzae* (Lipozyme IM[®] from Novo Nordisk, Denmark). This organism was genetically-modified by the introduction of the gene that codes for the lipase in another fungus *Rhizopus miehei*. The food industry's use of enzyme isolated from *Aspergillus oryzae* dates back to before 1958. The organism is not known to have any pathogenic properties. Lipozyme IM has been used in Europe by the food industry since 1993.

The support material for the immobilisation of the enzyme is a polymerised phenol-formaldehyde ion exchange resin functionalised with triethylenetetramine. It is produced

commercially by Rohn and Haas (France) under the trade name Duolite A568 for use in the food industry. To maximise the purity of the resin before its use and to avoid potential residues in the food material being processed, the resin is supplied pre-treated by extensive washing with dilute HCl and regenerated with 2-6% NaOH. The possible presence of resin residues in Enova oil under normal processing conditions was investigated by analysing the levels of potential compounds that could have been leaked or formed from the resin, viz formaldehyde, monophenols, dimethyl phenols, xylenes and chlorine. Retained oil samples from seven different production lots manufactured sequentially using the same resin were analysed. None of the substances tested for was detected in any of the oil samples at the detection limits (formaldehyde, 0.5 mg/kg; mono- and dimethyl phenols and xylenes, 5 mg/kg; and chlorine, 3 mg/kg). In theory the end product should not contain any lipase enzyme since during the production of the DAG the enzyme is immobilised. Using the principle of protein dye binding (Bradford, 1976) the average protein concentration from three lots of oil was 0.19 µg/mL. To produce the final product the crude DAG oil preparation undergoes a subsequent refining process that includes distillation during which a temperature of 200°C is reached for ten minutes, decolouration and deodorisation when the product is held at 230°C for approximately three hours. As a result of the heat treatments applied the end product will not contain any intact enzyme or other proteins. Mixed tocopherols at a level of 0.02% by weight and citric acid at a level of 0.0015% by weight are added.

III. History of the organism used as the source of the NF

The NF is derived from edible plant seed oils, primarily extracted from soybeans and rapeseed. Other commercial vegetable oils are also used. These oil sources have been used for many years as food sources for human populations around the world. The seeds are purchased on the world market and the oil may or may not come from GM plant varieties. According to the applicant the GM varieties used have been approved for human consumption by the governments of the US, the EU and other countries. Tests for the presence of DNA in the NF using PCR methods have been negative. The tests were developed to detect DNA sequences from Roundup Ready soybeans and rapeseed (Monsanto), herbicide tolerant oilseed (Agrevo) and SeedLink rapeseed (Aventis) and had a detection limit of less than 20 copies of the sequence.

IX. Anticipated intake and extent of use of the NF

On the basis that Enova contains at least 80% of DAG, only 70% of which is 1.3 DAG and 30% of total fat intake is in the form of vegetable oil (Leatherhead, 1999), then if all the vegetable oil in the diet is replaced by Enova oil 1.3 DAG from Enova oil would form about 17% of total fat intake.

The intended use of the NF will be as a non-hydrogenated cooking oil for home use and as a replacement for oil used in fat spreads/margarines, dressings for salads/mayonnaise, bakery products (bread, biscuits, croissants, pastries, cakes and crackers), yoghurt, health bars and health drinks. The NF will not be used in addition to already existing oils used in the EU but as a substitute for some of them.

In the holistic approach data compiled by the Leatherhead Food Research Association in the UK for 16 European countries (Austria, Belgium, Denmark, Finland, France, Germany,

Greece, Ireland, Italy, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the UK) indicate that the average added vegetable oil consumption in Europe is about 12 g/day (0.17 g/kg body weight/day) and that the highest oil consumption is in Greece with an average oil consumption of about 30 g/day (0.43 g/kg body weight/day). These data are based on European oil production information and must include therefore not only cooking oil but that used as an ingredient in processed foods. These are average values and take no account of intake distributions and maximum intakes within the population.

In the cumulative approach estimates of intakes of the various categories of foods in which DAG oil would be an ingredient are obtained from two databases and the amounts of DAG oil in each category summed to give a total intake.

Values for the consumption of fat spreads/margarine have been based on the Eurostat report 1998-99 that includes eight countries (Belgium, Denmark, Finland, France, The Netherlands, Spain, Sweden and the UK). The estimated average level of this food category is 27 g/person/day. The applicant states that the technological requirement for hard fats in these products limits the amount of DAG oil to a maximum of 64% and that the contribution of DAG oil as a constituent of spreads/margarine will be 17 g/person/day. Using a database produced by TNO, in The Netherlands the average consumption of foods in this category is about 22 g/person/day equivalent to a maximum of 14 g DAG oil/person/day.

For salad dressings/mayonnaise the Eurostat database estimates an intake of 14 g/person/day. This would be equivalent to 12 g/person/day DAG oil based on a 90% oil composition. However the TNO estimates in The Netherlands the consumption is much lower at 2 g/person/day equivalent to about 2 g/person/day DAG oil.

For bakery products the level of added oil can only be about 1% by weight. The European average consumption of bread and biscuits as determined by the Institute of European Food Studies (for five countries) is 125 g/person/day, equivalent to about 1 g/person/day of DAG oil. The TNO data include a more complete list of bakery products (bread, biscuits, croissants, pastries, cakes, crackers and cookies) and calculate about 173 g/person/day equivalent to about 2 g DAG oil/person/day.

For yoghurts the European average consumption is 55 g/person/day while in The Netherlands it is estimated to be 78 g/person/day. On the basis of 3% replacement of intrinsic fat in yoghurt with DAG oil this would equate to about 2 g of DAG oil/person/day.

There are no European data for the consumption of health bars. The average size of health bars is 40-60 g with a 6% fat replacement level and consumption of one bar per person daily. Similar considerations apply to health drinks and the applicant suggests 3 g of DAG oil/person/day based on the US volume size of 250-325 mL, 1% fat content and consumption of one can per person daily.

The average intake of cooking oil for the European and Dutch consumer based on TNO data is 12 g/person/day all of which could be DAG oil.

It is unlikely that a person consumes all the above products in one day. However the applicant has added the calculated values to give an estimate of 51 g DAG oil/person/day for the average European consumer and 38 g/person/day for Dutch consumers. These values are

equivalent to 0.7 g/kg body weight/day and 0.5 g/kg body weight/day based on a 70 kg consumer.

As pointed out earlier the data supplied by the applicant concern average values and take no account of maximum intakes. The Dutch Committee that made the first assessment of the submission calculated from TNO data that the 90th percentile of total fat intake in young men aged 19 to 22 years is 161 g/day. This population group consumes the most energy. However it is not realistic to suppose that the total fat content of the diet can be substituted by Enova oil. In addition, high-level consumption of fats and oils in European countries are above the estimates made by the applicant. The 95th and 97th percentile consumptions of fats and oils only among consumers are in the range of 65-90 g/day in Sweden and Italy, for example. Assuming all is DAG oil this results in an intake of up to 1.3 g/kg body weight/day from fats and oils only.

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

Europeans have consumed diacylglycerides and monoacylglycerides as part of a normal diet via plant oils and vegetable-based fats and from additives (emulsifiers). Additionally, Japanese consumers have used DAG oil since February 1999, and American consumers since February 2003.

It has been estimated that plant oils and vegetable fats contribute between 1-10% of DAG consumption as part of the human diet. Significant sources of DAG are olive oil with 5.5%, cottonseed oil 9.5% and palm oil 5.8%. Olive oil has been consumed extensively in Europe and particularly in the Mediterranean area.

According to the applicant, DAG and MAG have been considered GRAS substances in the US since 1964 and were approved by JECFA in 1974 as food additives. They have been used extensively as emulsifiers in a wide range of foods such as baked goods, carbonated drinks, confections, jams, jellies, dairy products, coffee whiteners and whipped toppings. In the US it was estimated that in 1989 the combined intake of DAG and MAG from their use as food additives was 3.6 g/person/day. An earlier study published in 1975 reported a consumption of between 1-10 g/person/day.

In the EU both DAG and MAG containing a higher amount of saturated fatty acids are approved food additives (E471, Directive 95/2/EC) and are used in a variety of foods.

In Japan between February 1999 and October 2003, 58,100 tonnes of DAG oil (96.8 million bottles) were sold.

This information confirms that there is a significant exposure history of DAG and the constituents of DAG oil by humans.

XI. Nutritional information on the NF

It is intended that Enova oil should be marketed to totally or partially replace traditional edible oils and as a replacement for oils added to a variety of foods and beverages and it is therefore important that consumers are not at a nutritional disadvantage if changing from the

traditional to the novel oil. The applicant concludes that it is unlikely that the consumption of Enova oil could have any nutritional disadvantage, mainly based on evidence on: a) energy value and digestibility of DAG versus TAG, b) metabolic features of DAG versus TAG, c) effects of consumption of DAG on the bioavailability of fat-soluble vitamins, and d) compositional information.

Energy value and digestibility

The applicant makes reference to a recent study in rats (Taguchi *et al.*, 2001) which showed that the energy values determined by a bomb calorimeter were found to be similar for the DAG (38.9 kJ/g) compared with 39.6 kJ/g for TAG oils of comparable fatty acid composition. In the same report digestibility was assessed in 2 groups of 8 male rats that were fed either 20 g DAG or TAG oil/100 g diet for 15 days. No significant differences between groups occurred in food consumption, body weight, fat intake, dried faecal mass, fat excretion, faecal fat content or fat absorption coefficients. Digestibility (absorption) of the DAG and TAG oils was calculated to be $96.3 \pm 0.4\%$ and $96.3 \pm 0.3\%$, respectively (Taguchi *et al.*, 2001).

Metabolic differences between 1,3-DAG and TAG

The applicant provides a brief overview of fat digestion and metabolism based on an intake consisting mainly of TAG or mainly of DAG. DAG (and thus also DAG oil or Enova oil) are metabolized by the body using conventional fat digestion pathways. However, there is an important structural difference (esterification sites of the fatty acids on the glycerol molecule) between the 1,3-DAG and the 1,2-DAG derived from TAG digestion, as both lingual and pancreatic lipase enzymes mainly hydrolyze fatty acids on the 1 and 3 positions in the glycerol molecule.

2-MAG is the building block for re-esterification in the intestinal cells and the formation of re-esterified TAG that is incorporated into chylomicrons (lipoprotein particles) which enter the blood as a fine emulsion via the lymph system. While 2-MAG is rapidly esterified to TAG the esterification of 1-MAG or 3-MAG is much slower. Thus, these MAG are more likely to undergo hydrolysis to glycerol and free fatty acids and more free fatty acids would go directly to the liver (through the portal vein) for oxidation than being esterified and packaged in chylomicrons (Mattson and Volpenhein, 1964; Small, 1991; Watanabe *et al.*, 2001a).

DAG oil (Enova oil) contains about 70% or more of DAG molecules esterified at 1 and 3 positions such that the main result of the lipase enzyme action is 1- (or 3-) MAG, glycerol and free fatty acids (Enova oil is comprised of 80% DAG). Under these conditions, the preferential metabolic fate of free fatty acids from DAG can be toward β -oxidation rather than deposition as body fat (Hara *et al.*, 1993; Murata *et al.*, 1994 and 1997; Katsuragi *et al.*, 1999; Taguchi *et al.*, 2000; Watanabe *et al.*, 2001a). Also as a consequence of this process, consumption of 1,3-DAG would result in relatively low postprandial triacylglycerolaemia compared with the consumption of TAG.

There is scientific evidence supporting the above hypothesis as reported by the applicant both in humans (Hara *et al.*, 1993; Katsuragi *et al.*, 1999; Taguchi *et al.*, 2000) and animals (Murata *et al.*, 1997). Besides, the effects of the intake of DAG on lipid and glucose metabolism have been recently reviewed (Tada, 2004). The main reported effect of the ingestion of DAG is a reduction in the levels of serum triglycerides, a modest decrease in body weight in higher body weight individuals and in some cases a moderate reduction in

body fat. Reacylation to TAG in small intestinal cells was, in animal models, found to be slower with DAG feeding than TAG feeding. Expression of mRNA of β -oxidative and uncoupling proteins 2 was also increased in liver and/or intestinal cells on feeding DAG compared with TAG (Tada, 2004). It was suggested that the stimulation of enzyme activities responsible for β -oxidation and regulation of lipid metabolism-related gene expression in the small intestine may contribute to reduced postprandial hyperlipidaemia as well as to increased energy expenditure (Tada, 2004). In conclusion, these differences between DAG and TAG did not represent adverse effects.

Various human trials comparing DAG *versus* TAG intake have been published (Nagao *et al.*, 2000; Taguchi *et al.*, 2000; Tada *et al.*, 2001; Watanabe *et al.*, 2001b; Maki *et al.*, 2002; Kamphuis *et al.*, 2003; Yasunaga *et al.*, 2004). Other human trials are unpublished reports described in the dossier (Watanabe *et al.*, 1996; Watanabe *et al.*, 1997a, b and c; Katsuragi, 1999; Hasegawa, 2000; Matsuyama, 2000; Kobayashi, 2001). Studies include single-dose and repeat dose, short and long duration studies (1 to 12 month) which examined the human tolerance and nutritional effects of DAG oil, in healthy men, women and children and conditions such as obesity, diabetes and patients undergoing haemodialysis. In these studies, DAG and TAG oils that had a similar fatty acid composition were compared. DAG oil was shown to be well tolerated and not to cause any toxicologically or nutritionally relevant adverse effect (up to 0.78 g/kg body weight). The main effect of the ingestion of DAG is a reduction on the levels of serum triglycerides.

The Panel was aware of two published studies in rats by the group of Sugimoto *et al.* (2003 a and b) that were not reported in the dossier. Normal and genetically obese Wistar rats received diets containing 10% (by weight) DAG or TAG oil for up to 12 weeks. The animals showed increased plasma free fatty acid and glucose concentrations in the portal vein and inferior vena cava by dietary DAG versus TAG oil. Glucose intolerance was observed particularly in genetically obese and older rats. In the studies on subacute and chronic toxicity in rats, provided by the applicant (section XIII), however, DAG-related changes in blood glucose levels were not observed.

In humans, however, several of the clinical studies measured blood levels of glucose, insulin or glycosylated haemoglobin and did not show DAG-related adverse effects.

Yamamoto *et al.* (2001) have specifically assessed the long-term nutritional effects of a DAG oil diet in patients with type II diabetes mellitus (16 diabetic patients for a period of 3 months). A double-blind parallel study design compared the use of DAG oil for ordinary cooking with respect to the customary cooking oil. Metabolic alterations related to DAG oil consumption (13 g/day) were not observed. On the contrary, serum triglycerides levels were decreased from 222 ± 66 to 135 ± 25 mg/dL.

Bioavailability of vitamins

Data on the absorption of fat-soluble vitamins during DAG intake come from a 12-week study on a small number (n=12) of male volunteers consuming 20 g DAG oil/person/day compared with those consuming 20 g TAG oil (n=15)(Watanabe *et al.*, 2001b). At 4, 8 and 12 weeks, fasting blood samples were drawn and the serum concentrations of vitamins A (retinol), E (α , β and δ -tocopherols) and D [25(OH), 1-25(OH)₂ and 24,25(OH)₂] were measured. However, according to two-way repeated measures of the analysis of variance, the authors concluded

that the results indicate that DAG does not affect the absorption of fat soluble vitamins in diets. There was no reference to carotenoids.

The data are considered insufficient to the Panel to make a complete assessment. However it is not expected from the mode of action of Enova oil that bioavailability of fat-soluble vitamins would be affected.

Nutrient composition

A comparison between compositions of Enova oil and traditional edible plant oils currently consumed in the EU has not been performed by the applicant. For instance, limited information is provided on tocopherol composition. Also, information on phenolic compounds has not been provided.

With the exception of *trans* fatty acids (TFA), no adverse effects are expected from the compositional information provided (section I). The main fatty acid components in the NF ingredient are oleic acid (20-65%) and the essential fatty acids linoleic (15-65%) and linolenic (about 15%). Thus, DAG oil will not limit the dietary provisions of these nutrients. However, the margin of variation in the composition of these fatty acids is quite wide and will limit the use of the NF for precise nutritional recommendations.

The levels of TFA in Enova oil (currently 2.8% of fatty acids) may suppose an additional intake of about 0.5 g/day (by considering a daily intake of 20 g oil) to the Europeans intake of TFA, a contribution that is significant when considering the estimated 0.5-1.5% current contribution of TFA to daily energy intake (NDA, 2004). A similar or greater impact is deduced (about 0.8 g/day) if it is considered that DAG oil will replace up to 100% of the oil contribution to the diet and that oil consumption is about 30% of the total fat consumption from added sources (Leatherhead, 1999). In contrast to Enova oil, vegetable oils and liquid margarines currently consumed in the EU have a low proportion of TFA, usually below 1% (NDA, 2004). As recently stated by the Panel, TFA raise LDL cholesterol levels in the blood and reduce blood levels of HDL cholesterol, thereby increasing the risk of coronary heart disease (see NDA, 2004).

XII. Microbiological information on the NF

The applicant states that Enova oil gives no reason for concern with regard to microbiological hazards due to the manufacturing processes. In view of the high temperatures experienced by the oil, 200°C for 10 minutes during a distillation step and subsequently at 230°C for approximately three hours during deodorization, microorganisms would not be expected to survive. In addition, growth of microorganisms would not occur in the oil because of the limited water content. Enova oil would be expected to be as safe as traditional oils from a microbiological perspective.

XIII. Toxicological information on the NF

Acute toxicity

In acute toxicity studies with rats using DAG (“Kao Diglyceride”) and DAG oil (“Diglyceride Healthy Oil”) which were not further characterised mortality was not observed at oral doses of 15 g/kg body weight.

Subacute toxicity

Groups of 10 male and female rats received diets with 0.2%, 1% or 5% “Kao Diglyceride” equivalent to doses of approximately 0.14, 0.72 and 3.48 g/kg body weight/day for 28 days. A total fat content of 10% in these diets was achieved by addition of the respective amounts of conventional maize oil to a fat-free feed. Control group 1 received a normal feed (fat content 4.3%) with 5.7% maize oil and control group 2 a fat-free feed with 5% maize oil and 5% rapeseed oil. According to the applicant, the test material consisted of 77% DAG, 18% TAG and 4% MAG and the fatty acid composition corresponded to that of the rapeseed control oil.

Mortality was not observed and there were no relevant treatment-related clinical signs or ocular changes. No statistically significant differences in body weight and body weight gain were noted between the groups. The only statistically significant difference found in haematology, clinical chemistry and urinalysis parameters was a lower blood urea nitrogen (BUN) level in females of all three dose groups and control group 2 compared to control group 1. This finding resulted from a relatively high BUN level in control group 1 which was considered incidental. The mean absolute mandibular salivary gland weight was increased in females of the low dose group. Females of all three dose groups showed decreased relative kidney weights and males of all three dose groups had decreased relative liver weights compared to control group 1. As these changes were small, not dose-related and the values did not differ from those in the second control group, they are not considered toxicologically relevant. Gross examination at necropsy and histopathological examination of selected organs and tissues did not reveal any specific changes in animals receiving “Kao Diglyceride” compared to the controls.

Chronic toxicity

Groups of 60 male and female rats received diets with 2.65% or 5.3% DAG oil equivalent to doses of approximately 1.0 and 2.1 g/kg body weight/day for up to 105 weeks (Soni *et al.*, 2001). The test material consisted of approximately 90% DAG, 7% TAG and 1% MAG. The total fat content in the diets was 7% resulting from a fat content of 1.7% in the basal feed and, in the case of the low dose diet, from addition of 2.65% of vegetable oil mixture with a fatty acid composition comparable to that of DAG oil. Control group 1 received 5.3% of the same vegetable oil mixture and control group 2 received 5.3% of a mixture of rapeseed and soybean oils with a slightly different fatty acid composition.

Haematology, clinical chemistry and urine examinations, organ weight determinations, macroscopic and histopathological examinations were carried out after 30 and 77 weeks with 10 animals/group. The remaining animals were sacrificed after 105 weeks but not assessed in detail.

The cumulative survival rate and occurrence of clinical signs were comparable in all groups, total mortality, however, was high in all groups, probably as a result of the high fat content of the diet. After 105 weeks only 12/18, 13/19, 16/10 and 11/11 of each 40 male/female animals were alive in control groups 1 and 2, the low and high dose group, respectively. There were no relevant differences in body weights and food consumption.

Several statistically significant differences in haematology and clinical chemistry parameters were noted in the high dose group compared to the controls (increased fibrinogen concentration in males; increased mean corpuscular haemoglobin concentration, decreased platelet count, increased activated partial thromboplastin time, increased insulin, reduced activity of lactate dehydrogenase and reduced Na concentration in females). These findings were limited to one sex and/or one time point (week 30 or 77), and generally occurred only in comparison to one of the control groups. The standard deviations in these examinations, however, were high. Male animals of the treatment groups showed a higher incidence of haematuria and slight to mild nephropathy after 77 weeks.

Changes in organ weights concerning brain, thyroid, heart, liver, kidney and spleen were found in one sex and/or at one time point (week 30 or 77) and generally only in comparison to one of the control groups. The histopathological examinations after 77 weeks revealed a low incidence of pituitary tumours in all groups and mammary tumours in all female groups. After 105 weeks there was a high incidence of epithelial mammary gland tumours (50-92%). Females of all groups showed slight to moderate necrosis of the liver and extramedullary hematopoiesis of the spleen.

Genotoxicity

The applicant made reference to genotoxicity studies in bacteria using the same test material as in the subacute feeding study. "Kao Diglyceride" dissolved in ethanol was not genotoxic in the mammalian microsome reverse mutation assay with *Salmonella enterica* var. Typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 (Ames Test) and a gene mutation assay using *Escherichia coli* strain WP2 uvrA up to the highest tested concentration of 5 mg/plate with and without metabolic activation (S-9 mix).

Human studies

A number of studies with normal subjects and some patient groups including children with hyperlipidaemia and/or obesity were conducted to examine the physiological effects of DAG, in particular the influence on plasma lipid levels (see section XI). In some of these studies a number of haematology and clinical chemistry parameters were examined. The test materials were administered to up to 131 subjects either in the form of edible oils or incorporated into foodstuffs resulting in exposures of up to 0.78 g/kg body weight/day for up to 12 months. These studies revealed no toxicologically relevant effects. In one of the studies small but statistically significant differences in parameters indicating liver and pancreas toxicities (increases in the activities of the enzymes glutamate oxaloacetic acid transaminase (GOT), glutamate pyruvate transaminase (GPT) and amylase) compared with the start of the treatment period were found. These changes are not considered relevant as the values were all within the normal ranges for these parameters.

Comment

The chronic feeding study with rats was not carried out in accordance with internationally agreed protocols. Haematology, clinical chemistry and urine examinations, organ weight determinations and histopathological studies after 105 weeks are lacking. Such examinations were carried out, however, after 30 and 77 weeks and revealed no relevant treatment-related effects.

In both the subacute and the chronic study, diets with relatively high fat contents of 10% and 7%, respectively, compared with normal rodent diets were administered. The changes observed in the control and treatment groups in the late phase of the chronic study, in particular liver effects and increased mammary tumour rates, are typical findings in studies using high calorie diets. There were no relevant differences between groups receiving different types of fat and therefore no effects which can be attributed specifically to the administration of DAG oil. A no-adverse-effect level (NOAEL), however, cannot be established.

The human short-term studies revealed no toxicologically relevant effects.

In conclusion, the toxicological data do not indicate that Enova oil induces specific effects compared with conventional oils with a similar fatty acid composition.

DISCUSSION

The Panel has considered the data that were supplied by the applicant with its original application under the Novel Foods Regulation and subsequently in response to questions and concerns expressed by the Dutch Competent Authority, other Member States and the Panel itself, in relation to the objections and concerns maintained by the Spanish Competent Authority, as requested by the Commission, as follows:

The use of genetically-modified material

This assessment has been conducted as if the fatty acids used in the esterification process were derived from non-GM crops. However the Panel is aware that for the time being the source material may be derived from GM crops as well as conventional ones. The applicant has confirmed that any GM varieties of soybean and rapeseed used as raw materials for the manufacture of Enova oil will only be those that have been authorised for food use in the European Union under appropriate EU Directives and Regulations such as (EC) 1829/2003, (EC) 258/97 and (EC) 2001/18. These will have been assessed for safety in Europe and are considered to be as safe as their conventional non-GM counterparts. It has therefore been unnecessary to review their safety again.

The production process

The applicant has provided sufficient detail on the production process to be confident that the immobilisation resin support for the lipase does not contribute any toxic material into the Enova oil. The levels of potential compounds that could have leaked or been formed from the resin were below the levels of detection in the oil. The protein content of the oil was similar to that of conventional plant oils (0.19 µg/mL). Any enzyme activity would be expected to be

lost by the severe temperature-time exposure during the refining steps. Other steps involved in the production process are conventionally used in plant oil manufacture.

Stability of the product

The data supplied indicate that DAG oils are as stable as conventional oils with comparable fatty acid composition when stored under normal household conditions and during cooking procedures such as frying.

***Trans* fatty acids**

A NF should not differ from foods or food ingredients which they are intended to replace to such an extent that their normal consumption would be nutritionally disadvantageous for the consumer (Regulation 258/97/EC). At this respect a low content of *trans* fatty acids can be considered an important parameter to guarantee a nutritional quality product for the consumer. Edible plant oils currently marketed in the EU appear to be the appropriate comparators to assess Enova oil (see general guidance in Commission Recommendation (EC) 97/618). Vegetable oils and liquid margarines have a low proportion of TFA, usually below 1% (NDA, 2004) suggesting that any NF intending to replace these oils should not contain a higher amount of TFA.

Adverse health effects on sensitive population groups

Based on the available evidence, no adverse effect on health is anticipated under the proposed conditions of use of DAG as a NF ingredient within a normal diet. However, there are no studies on health effects in particular population groups, such as infants, children, and pregnant and lactating women. The Panel notes that the company does not intend to use this ingredient as a fat source in infant formulae and follow-on formulae.

The increase in GPT, GOT and amylase in human subjects

In one human study statistically significant increases in parameters which might indicate liver and pancreas toxicities, i.e. the serum activities of the enzymes GPT, GOT and amylase, were observed. These effects are not considered clinically relevant since all values were within the normal ranges and the effects did not occur in other studies.

Absorption and bioavailability of fat soluble vitamins and micronutrients

The applicant provided data showing no significant effects of Enova oil consumption on the absorption and bioavailability of fat soluble vitamins. Although the data provided are insufficient to make a complete assessment, the Panel considers unlikely from the mode of action of Enova oil that absorption and bioavailability of fat soluble vitamins is affected.

Post-launch monitoring

Several Member States suggested that if permission were given to market Enova oil in Europe then it should be on condition that there should be post-launch monitoring. The Panel notes that the applicant has indicated that they expect to implement a monitoring programme in Europe that may include consumer complaint reporting and investigation, adverse event

reporting and investigation and continuing monitoring of the scientific literature regarding new findings or reports on the safety of diacylglycerols.

CONCLUSIONS

The Panel concludes that the product is safe for human consumption. However, in order for it not to be nutritionally disadvantageous to consumers, the *trans* fatty acid content should be reduced to the level in the conventional vegetable oils that the novel oil is intended to replace. Vegetable oils and liquid margarines have a low proportion of TFA, usually below 1% (NDA, 2004).

The Panel notes that the applicant does not intend to use this ingredient as a fat source in infant formulae and follow-on formulae.

DOCUMENTATION PROVIDED TO EFSA

Dossier on Enova oil (diacylglycerol oil) as a novel food ingredient in the European Union. Application pursuant to Regulation (EC) 258/97 submitted by ADM. September 2002.

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