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Safety of 'fungal oil from *Mortierella alpina*'[1]

Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies

(Question No EFSA-Q-2007-123)

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SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies of the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the safety of 'fungal oil from *Mortierella alpina*'. The product, referred to as Fungal Oil SUN-TGA40S, is an arachidonic acid-rich oil obtained by fermentation from the fungus *Mortierella alpina* and is intended for use as a source of arachidonic acid for infant formula.

The production of long chain polyunsaturated fatty acids by micro-organisms including *Mortierella alpina* has been employed for several years. The fungus is not known as pathogenic for humans and has not been reported to produce mycotoxins. Sufficient details of the production process and comprehensive compositional data on Fungal Oil SUN-TGA40S were provided.

In two subchronic feeding studies in rats, one of them including an *in utero* exposure phase, administration of Fungal Oil SUN-TGA40S did not induce toxicologically relevant effects. Changes in haematology and clinical-chemistry parameters as well as changes in organ weights, which were observed after administration of Fungal Oil SUN-TGA40S were also seen in previous subchronic feeding studies, in which high doses of comparable oils from *Mortierella alpina* rich in arachidonic acid and docosahexaenoic acid had been fed to rats. These effects can be regarded as a physiological adaptation to the high dietary intake of fat containing high levels of polyunsaturated fatty acids. In the absence of relevant histopathological alterations, the Panel does not consider the observed changes as toxicologically relevant.

The genotoxicity studies provided did not indicate mutagenic activity.

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Clinical studies showed that preterm infants fed a modified version of a formula for prematures with arachidonic acid from fungal oil did not substantially differ in their growth rate, feeding tolerance, or morbidity (in-hospital or post discharge) compared to infants fed the premature formula without a source of arachidonic acid.

The Panel considers that SUN-TGA40S is a safe source of arachidonic acid to be used in infant formulae and follow on formulae, provided it complies with the existing legislation.

Key words:

Fungal oil, Arachidonic acid, *Mortierella alpina*, infant formula, follow-on formula

TABLE OF CONTENTS

Panel Members 1
Summary 1
Table of Contents 3
Background as provided by EC 4
Terms of reference as provided by EC 4
Acknowledgements 5
Assessment 6
I. Specification of the novel food (NF) 6
II. Effect of the production process applied to the NF 8
III. History of the organism used as the source of the NF 9
IX. Anticipated intake/extent of use of the NF 9
X. Information from previous human exposure to the NF or its source 9
XI. Nutritional information on the NF 10
XII. Microbiological information on the NF 10
XIII. Toxicological information on the NF 10
Discussion 14
Conclusions and Recommendations 14
Documentation provided to EFSA 14
References 15

BACKGROUND AS PROVIDED BY EC

In May 2000, Suntory Ltd. (represented by Abbott International Division until June 2004) submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to the Competent Authorities of the Netherlands for placing on the market `fungal oil` rich in arachidonic acid (SUN-TGA40S) as a novel food ingredient.

On 24 October 2005, the competent authorities of The Netherlands forwarded to the Commission their initial assessment report, which had reached the conclusion that `*fungal oil*` for the proposed use is acceptable.

On 17 November 2005, the Commission forwarded the initial assessment report to the other Member States. Several of these Member States submitted additional comments/objections. The issues of a scientific nature can be summarized as follows:

- A suitable specification is needed. This should include a limit for protein content, since this appears to be the basis for the allergenicity assessment.
- The legal requirements for maximum levels of contaminants according to Commission Directive 2006/141/EC on infant formulae and follow-on formulae and according to Commission Regulation (EC) No 1881/2006 have to be met.
- The assessment of mycotoxins should be included as part of the in-house control program of the applicant.
- The producer should declare the hexane content in the final product.
- Detection and determination limits of the methods used to analyse the phthalates should be provided.
- The applicant should provide a careful assessment of clinical safety related to possible effects of SUN-TGA40S fungal oil on blood coagulation.
- Information should be provided on the extent of research into the components of *Mortierella alpina* itself that might give rise to a food safety risk, such as terpenes, toxicologically significant lipid compounds, etc.
- Information on the trans fatty acid content of the oil is necessary.
- Information on the stability of the product should be provided.
- The product has to meet the restrictions set out in Commission Directive 2006/141/EC regarding the maximum limit of 1 % for arachidonic acid of total fat in infant formulae.
- A proper nutritional assessment should be carried out and a suitable monitoring programme put in place since the population group for which the product is intended is particularly vulnerable.

In consequence, a Community Decision is now required under Article 7, paragraph 1 of Regulation (EC) N° 258/97.

TERMS OF REFERENCE AS PROVIDED BY EC

In accordance with Article 29(1) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for `fungal oil` in the context of Regulation (EC) No 258/97. In particular, to assess whether the fungal oil is a safe source of arachidonic acid to be used under the provisions of Annex 1 section 5 Lipids of Commission Directive 141/2006/EEC on infant formulae and follow on formulae and replacing Commission Directive 91/321/EEC.

EFSA is asked to consider the elements of a scientific nature in the comments/objections raised by the other Member States.

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ASSESSMENT

The application relates to an arachidonic acid-rich oil obtained by fermentation from the fungus *Mortierella alpina*. In this opinion the product is referred to as ´Fungal Oil SUN-TGA40S´.

Fungal Oil SUN-TGA40S is a complex novel food ingredient derived from a micro-organisms, which has not been genetically modified. It has, therefore, been assigned to class 2, as defined in the recommendations of the Scientific Committee for Food (SCF) concerning the assessment of novel foods (European Commission, 1997). It belongs to sub-category 2.1, because the source of the novel food ingredient has a history of food use within the Community. Accordingly, information related to the structured schemes I, II, III, IX, X, XI, XII, XIII has been submitted.

I. Specification of the novel food (NF)

The specification of Fungal Oil SUN-TGA40S as proposed by the applicant is shown in Table 1.

Table 1. Specification of Fungal Oil SUN-TGA40S

Appearance	Clear yellow oil
Arachidonic acid	≥ 40 %
Peroxide value	≤ 5 meq/ kg
Acid value	≤ 0.2 mg KOH/g
Anisidine value	≤ 20
Free fatty acids	≤ 0.2 %
Unsaponifiable matter	≤ 1 %
Colour (Lovibond 50.8 mm cell) Yellow	≤ 50
Red	≤ 10
Heavy metals (as Pb)	≤ 1 mg/kg
Lead	≤ 0.1 mg/kg
Arsenic (as As_2O_3)	≤ 0.2 mg/kg
Cadmium	≤ 0.1 mg/kg
Mercury	≤ 0.1 mg/kg
Microbiological	Bacteria: ≤ 10 cells/g Coliforms: negative/g

Data on the contents of triglycerides, diglycerides and total sterols, iodine values and levels of antioxidants added to Fungal Oil SUN-TGA40S as well as data on fatty acid compositions have been provided for three lots (Tables 2 and 3).

Table 2. Compositional data on three lots of Fungal Oil SUN-TGA40S

	Lot 98041751	Lot 98071451	Lot 98100651
Triglycerides	99.4 %	97.1 %	96.1 %
Diglycerides	0.58 %	2.0 %	1.8 %
Total sterols	0.24 %	0.13 %	0.10 %
Iodine value	178.4	177.9	178.4
Antioxidants (added)	0.05 %	0.05 %	0.05 %

Table 3. Fatty acid compositions of three lots of Fungal Oil SUN-TGA40S

		Lot 98041751	Lot 98971451	Lot 98100651
Fatty acid		Area %	Area %	Area %
Myristic	C 14:0	0.35	0.35	0.40
Pentadecanoic	C15:0	0.13	0.15	0.15
Palmitic	C16:0	11.5	13.1	11.7
Palmitoleic	C16:0 (n-7)	0.07	0.10	0.10
Heptadecanoic	C17:0	0.23	0.27	0.27
Stearic	C18:0	7.02	7.88	7.23
Oleic	C18:1 (n-9)	5.41	6.38	5.65
Vaccenic	C18:1 (n-7)	0.31	0.35	0.32
Linoleic	C18:2 (n-6)	8.28	9.85	9.14
γ-Linolenic	C18:3 (n-6)	2.29	2.34	2.34
Linolenic	C18:3 (n-3)	0.22	0.34	0.35
Arachidic	C20:0	0.76	0.84	0.78
Eicosenoic	C20:1(n-9)	0.39	0.44	0.36
Eicosadienoic	C20:2(n-6)	0.66	0.84	0.66
Eicosatrienoic	C20:3(n-6)[a]	0.53	0.64	0.53
Eicosatrienoic	C20:3(n-6)	3.35	3.22	3.43
Arachidonic	C20:4(n-6)	46.2	40.3	44.0
Eicosapentaenoic	C20:5(n-3)	0.14	0.15	0.11
Behenic	C22:0	2.91	3.07	2.95
Erucic	C22:1	0.09	0.12	0.09
Docosatetraenoic	C22:4(n-6)	0.45	0.41	0.39
Lignoceric	C24:0	8.01	8.36	8.07
Nervonic	C24:1(n-9)	0.45	0.46	0.39
Hexacosanoic	C26:0	0.10	0.08	0.08
Others		0.1	0.04	0.5

[a] double bonds in position 5,11,4

According to the applicant, analytical data from ten lots of Fungal Oil SUN-TGA40S showed that the trans fatty acid concentration is below 2 % of total fats.

According to the applicant, terpenes contained in the crude oil extracted from *Mortierella alpina* are mainly sterols, and the major compound is desmosterol. According to literature, total sterols account for approximately 0.07 % (dry wt.) of the *Mortierella alpina* mycelium harvested after fermentation. The principal sterol was cholesta-5,24-dienol (desmosterol), followed by 24-methyldesmosterol (6 %), 24(28)-methylenecholesterol (3 %), lanosterol (3 %), 24ß-methyl-cholesta-5,25(27)-dienol (2 %), and other minor compounds (3 %) (Nes and Nichols, 2006). The presence of 24,25-methylenecholesterol, a sterol reported to occur in the mycelium of *Mortierella alpina* 1S-4 (Shimizu et al., 1992), has not been confirmed in later studies.

Analyses using a method with a detection limit of 2 mg/kg showed no residual hexane in the fungal oil. According to the applicant, the analytical method will be changed to comply with the EU regulation.

Di-isobutyl phthalate, di-butyl phthalate and 2-ethyl-hexyl phthalate were not detectable (detection limits: 0.1 µg/ml).

In three lots of Fungal Oil SUN-TGA40S, aflatoxins B1, B2, G1 and G2 were not detectable using methods with detection limits of 5 µg/kg. Sterigmatocystin, ochratoxin, patulin, nivalenol, zearalenone, and fumonisins B1 and B2 were not detectable using methods with detection limits of 0.05 mg/kg. The Panel emphasizes the maximum levels for aflatoxin M1 (0.025 µg/kg in infant formulae and follow-on formulae), ochratoxin A (0.50 µg/kg in baby foods for infants and young children), patulin (10 µg/kg in baby foods), deoxynivalenol (200 µg/kg in baby foods for infants and young children) in Commission Regulation (EC) 1181/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (European Commission, 2006). According to the responses of the applicant to the comments of the Member States, analyses of mycotoxins will be included in the in-house quality control programme to ensure compliance with existing legislation.

The Panel also emphasizes Annex IX (Specific maximum residue levels of pesticides or metabolites of pesticides in infant formulae and follow-on formulae) of Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. According to the responses of the applicant to the comments of the Member States, analyses of pesticides will be included in the in-house quality control programme.

The Panel draws attention to the fact that the maximum level of lead in infant formulae and follow-on formulae (0.020 mg/kg wet weight), given in the Annex, Section 3 of Commission Regulation (EC) 1181/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, is lower than the detection limit provided for lead in the specification of Fungal Oil SUN-TGA40S in Table 1.

Data on the content of polycyclic aromatic hydrocarbons were not provided. The Panel notes that Fungal Oil SUN-TGA40S should comply with the maximum levels set for polycyclic aromatic hydrocarbons in infant formulae and follow-on formulae according to Commission Regulation (EC) 1181/2006 of 19 December 2006.

In order to demonstrate its stability, the fungal oil was transferred into commercial containers after nitrogen gas exchange, and the containers were kept at 5, 25 and 40° C, respectively, for 48 months. Peroxide value, acid value and p-anisidine value were periodically analyzed and appearance, odour and fatty acid composition were assessed. All parameters were within the specification values for 48 months when the products were kept in the refrigerator (5° C).

II. Effect of the production process applied to the NF

Fungal Oil SUN-TGA40S is obtained by fermentation of the fungus *Mortierella alpina* 1S-4 using soy flour and soy oil as substrates. The process starts with two sequential propagation steps in a flask and in a stirred tank, respectively, for 2-3 days under aerobic conditions at 28° C. The subsequent main fermentation is conducted in a stirred tank for 8-14 days under aerobic conditions at 26° C. The oil is extracted from the biomass using hexane as solvent. The extract is filtered and the solvent is removed via evaporation.

For purification, the extract is degummed (using phosphoric acid) and deacidified (using sodium hydroxide). The oil slurry is washed with water, and the water is removed using vacuum evaporation (purification procedure A).

In a modified procedure the described steps of degumming, deacidification, washing and removal of water have been replaced by a mixed column treatment (purification procedure B).

A new technique described in the confidential part of the dossier is used to further purify the oil, prior to a two-step deodorization process. Mixed tocopherols are added to the oil to maintain the oxidative stability, and the oil is filled into storage containers.

Critical control points (control items and the respective specifications) for the fermentation process and for the purification procedures A and B have been provided by the applicant.

Certificates of analysis have been provided for a total of six lots of Fungal Oil SUN-TGA40S, three of them manufactured according to purification procedure A and three according to procedure B. The lots subjected to purification procedure B exhibited significantly lower anisidine values (1.0 vs. 7.6), indicating less oxidative degradation of the oil. For the other parameters listed in Table 1 as well as for the fatty acid compositions listed in Table 2, no major differences were observed between the lots manufactured according to purification procedure A and B, respectively.

According to information provided by the applicant to EFSA, purification by procedure A is not applied anymore in the production of Fungal Oil SUN-TGA40S.

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

The production of long chain polyunsaturated fatty acids and in particular of arachidonic-rich oils by micro-organisms including *Mortierella alpina* has been employed for several years (Shimidzu et al., 1990; Yamada et al., 1987). *Mortierella alpina* (ATCC 32222) is not known as pathogenic for humans and has not been reported to produce mycotoxins.

A two-week toxicity study not complying with the principles of Good Laboratory Practice (GLP) was carried out in male ICR mice. Groups of 10 animals were fed diets containing 1.6, 4 or 10 % of the Fungal Oil SUN-TGA40S-producing micro-organisms (cultured micro-organisms sterilized at 121° C for 20 min. and dried with hot air). The test material contained approximately 50 % of the production organism and 50 % of the Fungal Oil SUN-TGA40S. The actual intake in the group with 10 % of test material in the diet was 13.9 g/kg bodyweight (bw)/day. The animals were observed for two weeks and autopsied. No deaths occurred in mice throughout the experimental period. According to the study report, there were no treatment-related changes in clinical observations, body weights, food consumption, water consumption and at autopsy.

IX. Anticipated intake/extent of use of the NF

According to the applicant, arachidonic acid (via Fungal Oil SUN-TGA40S) and docosahexaenoic acid (DHA) would be added to pre-term formulae in amounts resulting in levels of these fatty acids similar to those in human breast milk. Pre-term infants consuming 120 kcal/kg/day would receive 75 mg/kg/day fungal oil and 16 mg/kg/day DHA. These levels are consistent with the calculated median arachidonic acid and DHA intakes of exclusively human milk fed European infants. The infants would receive the feedings in-hospital until their anticipated discharge weight of approximately 1800 g is reached, and then post discharge infant formula with the same source of arachidonic acid for up to 12 months of age.

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

An arachidonic acid-rich oil produced by *Mortierella alpina*, which has a similar composition as Fungal Oil SUN-TGA40S, has been approved for use in pre-term and term infant formulae.

Since the first evaluation as safe for use in pre-term and term infant formulae by the Regulatory Office of the Ministry of Health in The Netherlands in 1995, this arachidonic acid-rich oil has been assessed by various international bodies. According to the applicant, it is included in individual infant formulae available in at least 50 countries.

XI. Nutritional information on the NF

Clinical study

A prospective, randomized, double-blind, multicentre, international feeding study was conducted in premature infants. The primary objective of the study was to confirm that the addition of the long-chain polyunsaturated fatty acids arachidonic acid (AA) and docosoahexaenoic acid (DHA) in premature formulae would support normal growth (O´Connor et al., 2001).

Four hundred and seventy infants were randomized within 72 hours of first enteral feeding to one of three formula groups: (1) Control (in-hospital milk-based formula for premature infants without added AA or DHA), N=144; (2) formula with added fungal oil and a low-eicosapentaenoic (EPA) acid fish oil to provide AA and DHA at 0.40 and 0.25 g/100g total fatty acids (wt %), N=140; (3) formula with added egg-derived triglyceride (Egg-DTG) and low-EPA fish oil to provide AA and DHA in the amounts described for formula (2), N=143). Infants fed exclusively human milk served as reference (N=43). Infants were initially fed human milk and/or one of three study formulae from the time of randomisation until term corrected age (CA). At term CA, infants were transitioned to an assigned post-discharge nutrient-enriched formula with and without the same sources of AA and DHA and/or human milk to 12 months CA.

Results showed that there were no consistent differences among infants in the three study formula groups with respect to growth (weight, length and head circumference) from study day one until hospital discharge and from study day one until term CA, regardless of whether or not the analyses were controlled for human milk intake. Small, but statistically significant differences in length gain from study day one to hospital discharge were found between infants in the Control and Egg-DTG/Fish study formula groups. No statistically significant differences were found with regard to feeding tolerance, in-hospital clinical problems/morbidity, or post-discharge morbidity to term CA across study feeding groups.

It was concluded that preterm infants fed a modified version of the premature formulae with a source of AA and DHA in-hospital and until term CA did not substantially differ in their growth rate, feeding tolerance, or morbidity (in-hospital or post discharge) compared to infants fed the premature formulae without a source of AA and DHA.

From a meta-analysis of 14 trials (1846 infants), it was concluded that supplementation of infant formulae with long-chain polyunsaturated fatty acids does not influence the growth of term infants in either a positive or a negative way (Makrides et al., 2005).

XII. Microbiological information on the NF

Certificates of analysis provided for six lots of SUN-TGA40S demonstrated that the microbiological limits given in the specification (Table 1) were met.

XIII. Toxicological information on the NF

Acute toxicity

In a study on acute oral toxicity not complying with the principles of Good Laboratory Practice (GLP), Fungal Oil SUN-TGA40S was administered to five male ICR mice in a single dose of 2 g/kg bw. Animals were observed for 14 days following administration and autopsied. No deaths occurred in mice throughout the experiment and no signs of toxicity were observed.

Subchronic toxicity

In a 90-day toxicity study complying with GLP, groups of 15 male and 15 female Sprague-Dawley rats received diets containing 0.5 %, 1 % or 2 % Fungal Oil SUN-TGA40S, corresponding to doses of approximately 312, 625 and 1250 mg/kg bw/day (average intakes for males and females combined). In order to obtain diets with a consistent content of 2 % added oil, the diets of the low and intermediate dose groups were supplemented with soybean oil. A fat-equivalent control group received a diet with 2 % soybean oil and a further control group consisting of 10 male and 10 female rats was fed a standard rodent diet. An additional group received 1 % fish oil and 1 % Fungal Oil SUN-TGA40S, which was equivalent to 625 mg/kg bw/day Fungal Oil SUN-TGA40S.

There were no clinical signs and no relevant effects on body weight and food consumption. Haematology and clinical-chemistry analyses revealed several statistically significant differences in animals receiving diets with Fungal Oil SUN-TGA40S compared with the control group receiving a diet with 2 % soybean oil. Reduced plasma albumin levels and albumin/globulin ratios were observed in males and females, and the mean corpuscular haemoglobin concentration (MCHC) was increased in females. When compared with the historical control data for the same strain of rats, all values were within the normal range for the respective parameters. Males showed a prolonged prothrombin time and activated partial thromboplastin time (APTT). This effect also occurred in males receiving diets containing 1 % soybean plus 1 % fish oil. Organ weight determinations carried out at necropsy showed statistically significant differences in absolute liver weights in males, relative brain, lung and adrenal weights in females as well as absolute and relative spleen weights in females. Some of the effects were not dose-related and all values were within the normal ranges for the historical controls. In the absence of relevant histopathological changes in the respective organs, the observed differences are not considered toxicologically relevant.

On a request from the Competent Authorities of the Netherlands, a subchronic (13-week) oral toxicity study, preceded by an *in utero* exposure phase, in rats was provided, also published by Lina et al. (2006).

Fungal Oil SUN-TGA40S was administered to Wistar outbred rats at dietary levels of 0.5 %, 1.5 % or 5 %, adjusted with corn oil to 5.76 % added fat. In addition, Fungal Oil SUN-TGA40S was administered at a level of 3.65 % in combination with 2.11 % High DHA Tuna oil, containing the n-3 poly-unsaturated fatty acid docosahexaenoic acid (thus providing a ratio of arachidonic acid to docosahaxaenoic acid of 2.7 : 1). There were two control groups, one high-fat control group (5.76 % corn oil) and one low-fat control group without added oil.

The study comprised an *in utero* exposure phase in which parental (F_0) animals (24 females and 12 males per group) were fed the test and control diets starting 4 weeks prior to mating, throughout mating, gestation and lactation until weaning of the offspring (F_1 rats). Subsequently, a sub-chronic study was conducted with F_1 rats (10 rats/sex/group), in which the administration of the diets was continued for 13 weeks.

According to the study report, the overall intakes of Fungal Oil SUN-TGA40S of the F_0-animals during the premating and the gestation period were approximately 0.3, 0.9 and 3.0 g/kg bw/day in the low-, mid- and high-dose group, respectively. In the Fungal Oil SUN-TGA40S/DHA group, the overall intake during these periods was approximately 2.2 g Fungal Oil SUN-

Oil SUN-TGA40S + 1.3 g High DHA Tuna oil/kg bw/day. In the lactation period, the overall intakes of the F_0-females doubled (0.7, 2.0 and 6.7 g Fungal Oil SUN-TGA40S/kg bw/day in the low-, mid- and high-dose group, and 4.6 g Fungal Oil SUN-TGA40S + 2.7 g High DHA Tuna oil/kg bw/day in the Fungal Oil SUN-TGA40S/DHA group).

In the sub-chronic study with the F_1-animals, the overall intakes of Fungal Oil SUN-TGA40S were 0.3 (males and females), 0.8 (males) – 0.9 (females) and 2.8 (males) – 2.9 (females) g/kg bw/day in the low-, mid- and high-dose group, respectively. The intakes in the Fungal Oil SUN-TGA40S/DHA group were 2.0 (males) – 2.2 (females) g Fungal Oil SUN-TGA40S + 1.2 g High DHA Tuna oil/kg bw/day.

Results obtained in parental (F_0) rats and pups:

General condition and behaviour of F_0 rats were not adversely affected by the test substances and none of the parental rats died untimely. Gross examination of the F_0-animals at sacrifice did not reveal any effect of the test substances on maternal organs and tissues.

Body weights of F_0 females in the premating period, the gestation period and the lactation period were comparable among the test and the control groups. Food intake in the low-fat control group was higher than in all high-fat groups during the premating and gestation period. There were no differences in food intake between the treatment groups and the corn oil control group. During the lactation period, food consumption was not affected in any group.

There were no treatment-related differences in fertility and reproductive performance among the groups. There were no relevant differences among the groups in general condition and body weights of the pups, or in viability, sex ratio or number of pups per litter.

Results obtained in the sub-chronic study (F_1 rats):

General condition of F_1 rats was not adversely affected by the treatment and none of the rats died during the study. Neurobehavioural observations and motor activity assessment did not indicate any neurotoxic potential of the test substance. Ophthalmoscopic examination did not reveal tretament-related changes.

Body weights of F1 rats were comparable among the test and control groups. Food intake was higher and food conversion efficiency tended to be lower in males and females of the low-fat control group compared to all high-fat groups. There were no relevant differences in food intake or food conversion efficiency between the test groups and the corn oil control group.

In males of the 5 % Fungal Oil SUN-TGA40S and Fungal Oil SUN-TGA40S/DHA group haemoglobin concentration, red blood cell count, packed cell volume and/or MCV were decreased when compared to the corn oil control group and/or the low-fat control group, whereas reticulocytes were slightly increased. MCHC was slightly increased in these groups in both sexes and in males of the 1.5 % Fungal Oil SUN-TGA40S. There were no treatment-related changes in total or differential white blood cell counts.

Prothrombin time was increased in males of the low-fat control group as compared to all high-fat groups, including the corn oil controls. The applicant ascribed this effect to the high fat content of the test diets and the corn-oil control diet. Thus it is considered to be a physiological response to the intake of very high levels of polyunsaturated fatty acids, which are beyond the levels intended for use in infant formulae.

Cholesterol concentration in plasma was increased in the corn oil control group and in the 0.5 % and 1.5 % Fungal Oil SUN-TGA40S in both sexes, compared to the low-fat controls. At

the higher Fungal Oil SUN-TGA40S levels (5 % Fungal Oil SUN-TGA40S and Fungal Oil SUN-TGA40S/DHA groups) cholesterol levels were lower than in the corn oil controls and were comparable to those in the low-fat controls. Triglycerides were statistically significantly decreased in the 1.5 % Fungal Oil SUN-TGA40S, the 5 % Fungal Oil SUN-TGA40S and the Fungal Oil SUN-TGA40S/DHA groups in both sexes, compared to the low-fat controls and/or the corn oil controls. Phospholipids were decreased in the 5 % Fungal Oil SUN-TGA40S and the Fungal Oil SUN-TGA40S/DHA group in both sexes, compared to the low-fat controls and/or the corn oil controls. Bilirubin concentration was decreased in females of the 5 % Fungal Oil SUN-TGA40S group as compared to the low-fat controls only. Alkaline phosphatase activity was increased in several high-fat groups, including the corn-oil controls, when compared to the low-fat controls, and the test groups.

Urinary volume and density, semi-quantitative urinary observations and microscopy of the urinary sediment did not reveal relevant changes.

The absolute and the relative weight of the spleen were increased in males of the 5 % Fungal Oil SUN-TGA40S and the Fungal Oil SUN-TGA40S/DHA group both compared to the low-fat controls and the corn oil controls. Macroscopic examination at necroscopy and microscopic examination of organs and tissues did not reveal any treatment-related findings.

Oestrogen receptor-binding activities

Oestrogen receptor-binding activities were evaluated for Fungal Oil SUN-TGA40S, for the total unsaponifiable fraction of the oil and for 24,25-methylenecholesterol. Using a microsomal fraction from calf uterus as receptor, Fungal Oil SUN-TGA40S itself showed no binding activity, the unsaponifiable fraction and the sterol showed weak binding activities only at high concentrations of 10 µg/ml. The binding activities were the same or lower than those observed for other oils, such as soybean, fish and rapeseed. Using yeast expressing the human estrogen receptor (hER) and estrogen-sensitive reporter as test system, neither Fungal Oil SUN-TGA40S nor the unsaponifiable fraction exhibited oestrogenic or anti-oestrogenic activity.

Potential Allergenicity

Mortierella alpina is not known to have an allergenic potential (Streekstra, 1997).

Soy flour and soybean oil are used as fermentation materials. In the responses to the comments of the Member States, the applicant reported that soy trypsin inhibitor, a soy allergen, was analysed in the *Mortierella alpina* biomass before the oil extraction using an ELISA method (detection limit 0.1 mg/kg). The analyte was not detectable in several lots of the biomass.

According to the applicant, the purification steps applied to oil extracted from the *Mortierella alpina* biomass correspond to refinement processes currently employed to food grade oils. Analyses employing a fluorescence spectrophotometer (Fukuzawa et al., 2004) confirmed that the protein contents in three representative batches of Fungal Oil SUN-TGA40S purified by a mixed column treatment (purification procedure B) were below the detection limit of this technique (1 mg/kg).

Genotoxicity

In bacterial gene mutation tests (Ames test) using *Salmonella enterica* Typhimurium strains TA97, TA98, TA100 and TA102, Fungal Oil SUN-TGA40S was not genotoxic up to the highest dose of 5 mg/plate with or without metabolic activation (S9-mix).

Fungal Oil SUN-TGA40S was not clastogenic in tests on chromosome aberrations using Chinese hamster lung fibroblasts up to the highest dose of 1 mg/ml, with or without S9-mix.

The results of these studies do not indicate genotoxic activity of Fungal Oil SUN-TGA40S.

DISCUSSION

Genotoxicity studies did not indicate mutagenic activity. Taking into account the nature of the product and the outcome of previous studies using comparable fungal oils rich in arachidonic acid (Arterburn et al., 2000), no further genotoxicity studies are considered necessary.

In two subchronic feeding studies, one of them including an *in utero* exposure phase, administration of Fungal Oil SUN-TGA40S did not induce toxicologically relevant effects. The changes in haematology and clinical-chemistry parameters as well as the changes in organ weights, which were observed after administration of Fungal Oil SUN-TGA40S and also Fungal Oil SUN-TGA40S in combination with the DHA-rich oil, were also seen in previous subchronic feeding studies, in which high doses of comparable oils from *Mortierella alpina* rich in arachidonic acid and docosahexaenoic acid were fed to rats (Burns et al., 1999; Hempenius et al., 2000). These effects can be regarded as a physiological adaptation to the high dietary intake of fat containing high levels of polyunsaturated fatty acids. There is no reason to assume that Fungal Oil SUN-TGA40S is less safe than comparable oils containing high amounts of arachidonic acid.

CONCLUSIONS AND RECOMMENDATIONS

The Panel considers that SUN-TGA40S is a safe source of arachidonic acid to be used in infant formulae and follow on formulae, provided it complies with the existing legislation.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission to the Chairman of the European Food Safety Authority with the request for an opinion on the safety of 'fungal oil'. SANCO E4/AK/bs(2007) D/540391.
2. Initial assessment report by the Bureau Nieuwe Voedingsmiddelen (NL) concerning the assessment of Arachidonic acid rich oil SUNTGA40S (fungal oil from *Mortierella alpina*).
3. Letters from Member States with comments on the initial assessment report on 'fungal oil from *Mortierella alpina*' from Bureau Nieuwe Voedingsmiddelen (NL)
4. Response to Member States comments on the Netherland Opinion for fungal oil from *Mortierella alpina* as a novel food ingredient.
5. Application under regulation No 258-97 for the use of fungal oil from *Mortierella alpina* as a novel food ingredient.

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