ANSES Opinion
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French agency for food, environmental and occupational health & safety

The Director General

Maisons-Alfort, 14 February 2012

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES)

on an application for authorisation to market a novel food ingredient: DHA-EPA-rich oil from the micro-algae *Schizochytrium* sp.

ANSES undertakes independent and pluralistic scientific expertise work.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.
It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).
Its opinions are made public.

On 20 December 2011, the *Direction générale de la concurrence, de la consommation et de la répression des fraudes* (DGCCRF – Directorate-General for Competition, Consumer Affairs and Fraud Prevention) requested that ANSES provide an expert assessment on the application for authorisation to place on the market the novel food ingredient: DHA-EPA-rich oil from the micro-algae *Schizochytrium* sp.

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1. BACKGROUND AND PURPOSE OF THE REQUEST

This request falls within the scope of Regulation (EC) No 258/97 concerning novel foods and novel food ingredients (NI). The product applied for belongs to class 2.1, i.e. a complex NI from non-genetically modified sources which has a history of food use in the Community.
According to Table II of Recommendation 97/618/EC, the information required for NIs belonging to class 2.1 is as follows:

I. Specification of the NI
II. Effect of the production process applied to the NI
III. History of the organism used as the source of the NI
IX. Anticipated intake/extent of use of the NI
X. Information from previous human exposure to the NI or its source

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XI. Nutritional information on the NI
XII. Microbiological information on the NI
XIII. Toxicological information on the NI

As part of the novel foods evaluation procedure, the applicant has already submitted two dossiers for an oil from the microalgae *Schizochytrium* s.p.: the first in 2001 for the placing on the market of an oil rich in DHA (docohexaenoic acid)[1] (hereinafter: DHA-S), and the second in 2008 for an extension the use of this oil[2]. The oil for which authorisation is requested in this case differs from the oil previously authorised, since it has both a high EPA (eicosapentaenoic acid, C20: 5 n-3) and DHA content. The applicant proposes that it be incorporated in the same vector foods as DHA-S, with a slight alteration of the incorporation levels in line with the EFSA recommendations for EPA and DHA intakes (EFSA, 2010).

2. ORGANISATION OF THE EXPERTISE WORK

The expertise work was prepared in compliance with standard NF X 50-110 'Quality in expertise work – general competency requirements for expertise' (May 2003).
The joint expertise work was prepared by the Expert Committees (CES) on 'Human Nutrition' (NUT) (CES pilot) and on 'Additives, Aromas and Processing Aids' (AAAT) on the basis of four initial reports drafted by four rapporteurs.
The Biological risk evaluation unit conducted an internal expert assessment of the parts of the dossier falling within its sphere of competence.
The work carried out was discussed and then the opinion was validated by correspondence by the CES NUT and the CES AAAT owing to the short response times imposed.

3. ANALYSIS ANS CONCLUSIONS OF THE CES

3.1 Specification of the NI

The applicant proposes a specification for the following components: acid value, peroxide value (PV), moisture and volatiles, unsaponifiables, *trans*-fatty acids, DHA and EPA. *Trans*-fatty acids must not account for more than 1% of the NI, while DHA and EPA should account for not less than 22.5% and 10% of the NI respectively. The applicant also describes the test results for three production batches of the NI, which were compliant with the specifications. In these three batches the percentage represented by DHA and EPA in the total fatty acids is stable and well above the specification (DHA accounts for 40.6% of fatty acids on average for the three batches and EPA accounts for 19.3%). The applicant points out that this enables the NI to be mixed with vegetable oils so that the mix obtained resembles fish oils, thereby allowing fish oils to be directly substituted with the NI in recipes.
As regards the profile of the fatty acids, the percentage of palmitic acid (20.6%) is similar to that of EPA. A comparison with DHA-S oil reveals that the NI has a different fatty acid

[1] Comission Decision of 5 June 2003 authorising the marketing of oil rich in DHA (docohexaenoic acid) from the microalgae *Schizochytrium* sp. as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (2003/427/EC).
[2] Commission Decision of 22 October 2009 concerning the extension of uses of algal oil from the micro-algae *Schizochytrium sp*. as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council.

profile although the DHA content remains the same. The most significant variations are increases in EPA (x19) and oleic acid (x2), and a decrease in myristic acid (x4). Total sterols (cholesterol, cholestanol, phytosterols and phytostanols) represent 0.77% by weight of the NI. The applicant notes that the fatty acids and sterols in the NI are commonly found in plant and animal-based foods.
For the three batches, the applicant also provides results of analyses of residual solvents, certain heavy metals (copper, iron, mercury and lead), arsenic, protein content, fatty acids and sterols profiles. It also provides results of contaminant analyses: dioxins (dioxins and dioxin-type PCB), polycyclic aromatic hydrocarbons, pesticide residues and acrylamide, stressing that their content is lower than the statutory limits (except in the case of acrylamide, for which no such limit has been stipulated). The applicant stresses that the methods used are standardised and/or published methods.
The Advisory Committee on Novel Foods and Processes (ACNFP) of Britain's Food Standards Agency (FSA) considers that the composition of the NI does not give rise to any food safety concerns.

The CES NUT and the CES AAAT agree with the ACNFP's view that the NI's composition is satisfactory.
In its opinion on DHA-S oil (AFSSA, 2008), AFSSA stressed that 'this oil does not contain EPA and therefore alone could not replace fish oils which contain the two fatty acids of nutritional interest, DHA and EPA'. The NI, with its fatty acid profile similar to that of fish oils may be considered an alternative in order to promote DHA and EPA intakes close to the recommended nutrient intake (RNI).
The CES AAAT and the CES NUT consider that a lipid oxidation measure should be added, such as the anisidin value or volatile components (propanal), in order to ensure that the oil is not significantly oxidised during refining or storage. A low peroxide value does not rule out the possibility of significant oxidation of the oil during these phases. It therefore considers that the peroxidation level of the food enriched with the NI should be checked regularly. Moreover, the nature and quantity of the antioxidants in the final product must be specified.
They also point out that French legislation provides that edible oils such as vegetable oils 'must not contain polar compounds or triglyceride polymers in concentrations higher than 25% and 14% respectively. Oils which fail to meet this requirement are considered unfit for human consumption[3]**.**

3.2 Effect of the production process applied to the NI

The NI is produced via an *in vitro* fermentation process following a protocol identical to that for DHA-S oil. The oil is recovered from a culture or from dried algae following rehydration. The oil recovered in this way is dried and purified using a method similar to that used by vegetable oil producers. First antioxidants are added, followed by heating and pH adjustment prior to homogenisation to release the oil. The applicant must take care to apply HACCP procedures at every production stage, monitoring critical points and recording the quality controls, thereby ensuring that the batches of the NI comply with the stated specifications.

[3] Decree No 2009-184 of 26 February 2008 applying the Consumer Code (*Code de la Consommation*) in respect of fats and edible oils (OJ 28 February 2008).

The ACNFP notes that the production process is very similar to that used for the production of DHA-S from Schizochytrium sp., except in the oil recovery phase, in which hexane is replaced by isopropylic alcohol. The committee considers that this point does not give rise to any cause for concern.

The CES NUT has no particular comment to make on this point.
The CES AAAT considers that the fact that the procedures followed are those commonly used in the oil manufacturing industry and are similar to those used for obtaining DHA-S oil (except for the replacement of hexane with isopropylic alcohol), and that an HACCP procedure is implemented in the company are positive factors. It therefore shares the opinion of the ACNFP.

3.3 History of use of the organism used as the source of the NI

The microalga used in the production of the NI belongs to the genus *Schizochytrium* and was selected by the applicant for its ability to produce EPA. The production strain has not been genetically modified and further improvements in EPA production were obtained by optimisation of the fermentation process.
The applicant presents a detailed overview of algal toxin production, based on published and unpublished studies, indicating that there have been no reports of toxic compounds or links to toxic compounds produced by the Thraustochytrid group, to which *Schizochytrium* belongs. The applicant stresses that most toxic compounds produced by microalgae are produced by blue-green algae and dinoflagellates, which belong to a different kingdom. Two toxic compounds, domoic acid and prymnesin, are known to be produced in the Chromista kingdom, to which *Schizochytrium* belongs. However, these toxins are mainly produced by two genera (*Pseudonitzschia* and *Prymnesium*) which belong to a class (*Prymnesiophyta*) and phylum different from those to which the Thraustochytrids belong. Additional tests carried out by the applicant confirm that *Schizochytrium* sp. produces neither domoic acid nor prymnesin.
The ACNFP considers that although Schizochytrium had previously been used to produce DHA-rich oils and although the NI was produced from a newly characterised member of the genus, this does not give cause for concern, since there were no reports of toxins being produced by any members of the Class to which the genus Schizochytrium belongs. The Committee also considers that the results of the tests carried out by the applicant confirming the absence of domoic acid and prymnesin offer additional reassurance in this regard.

The Biological food risk assessment unit has no particular comment to make on this point.

3.4 Anticipated intake/extent of use of the NI and information based on previous human exposure to the NI or its source

The applicant proposes that its NI be added to the same vectors as those authorised for DHA-S oil, as well as to biscuits (200 mg/100 g), cooking oils (360 mg/100 g) and food supplements for pregnant and lactating women (450 mg/per day). These vectors are listed in Table 1. However, the applicant proposes some changes to the intake quantities. It

indicates that these changes are relatively minor and take into account a recent EFSA opinion on reference values for lipids. That opinion concludes that there is a link between the intake of n-3 polyunsaturated fatty acids PUFAs (EPA and DHA) of 250 mg/day and cardiovascular health (EFSA, 2010). The applicant points out that its proposal to incorporate the NI in food supplements for pregnant and lactating women is in line with the recommendations of a number of national health agencies and of the European authority (EFSA).

Table 1: Food vectors and incorporation levels of DHA-S oil and the NI

Food use	DHA-S oil	NI
Dairy products except milk based drinks	200 mg/100 mg; 600 mg/100 g for cheese	Unchanged
Dairy analogues except drinks	200 mg/100 mg; 600 mg/100 g for cheese analogues	Unchanged
Spreadable fat and dressings	600 g/100 g	Unchanged
Breakfast cereals	500 mg/100 g	Unchanged
Foods for particular medical purposes, but excluding infant and follow-on formulae	In accordance with the particular nutritional requirements of the persons for whom the products are intended	Unchanged
Foods intended for use in energy restricted diets for weight reduction	200 mg/meal replacement	250 mg/meal replacement
Bakery products (bread and rolls)	200 mg/100 g	Unchanged
Biscuits (cookies)	Not included	200 mg/100 g
Cooking oils	Not included	360 mg/100 g
Nutrition bars	500 mg/100 g	Unchanged
Non-alcoholic beverages (including milk-based drinks)	60 mg/100 g	80 mg/100 g
Food supplements	200 mg/100 g	250 mg/100 g
Food supplements for pregnant and lactating women	Not included	450 mg/day

3.5 Estimated intakes

The applicant estimated the impact that enriching foods with its NI would have on DHA and EPA intakes based on British consumption data provided by the FSA. Estimates were made for the entire population as well as for consumers of at least one of the products likely to be enriched with the NI (hereinafter: 'consumers'). The applicant considers that this method assumes the highest possible consumption, as all products within a category contain the maximum permissible level of the NI.

The result of this analysis indicates that adolescent 'consumers' have the highest intakes, with a 0.88 g/day mean intake and an intake of 1.72 g/day of EPA+DHA for the 97.5th percentile. For adult male 'consumers', the mean intake is 0.77 g/day and the intake for the 97.5th percentile is 1.65 g/day. In general, the intake levels of 'consumers' are no different from those of the general population, since 94 to 99% of individuals, depending on their age group, consume at least one of the products likely to be enriched with the NI.
The applicant considers that food supplements are consumed as an alternative to enriched foods and are therefore unlikely to have a significant impact on the above-mentioned estimated intake levels. They are therefore not taken into account in this estimation. The FSA's ACNFP takes the view that the changes to use levels proposed by the applicant will not lead to a significant increase in the level of consumption amongst the general population. It observes that the high dose supplements recommended for pregnant and lactating women is in line with the recent EFSA opinion on the claims concerning n-3 PUFAs.

The CES NUT considers that the maximum estimated intake is lower than that observed in certain segments of the population which consume large amounts of fish (2.8 g/day) (Bemrah et al. 2008). However, it regrets that these estimated intakes do not take into account the basal exposure level and the consumption of food supplements rich in EPA/DHA, which varies greatly depending on the level of seafood consumption in particular. In a population which consumes large quantities of fish, consumption of enriched products could lead to very high EPA/DHA intake levels, the long-term effects of which are not yet known. As indicated by AFSSA in its opinion on DHA-S oil (request 2008-SA-0316), very high intakes of DHA could be harmful as regards peroxidation phenomena which may occur within foods. These phenomena vary in intensity, depending in particular on the composition of the food matrix, and the cooking or storage methods (AFSSA, 2003). Thus n-3 PUFA oxidation compounds were identified in different foods and food supplements (Surh et al., 2007; Michalski et al., 2010; Halvorsen et Blomhoff, 2011). Questions remain concerning the risks linked to ingesting products of n-3 PUFA oxidation (Turner et al., 2006; Eritsland, 2000; Mesa et al. 2004), aldehydes from lipid oxidation, 4-hydroxy-hexenal (4-HHE) and 4-oxo-2-hexenal in particular, with various biological effects (Kasai et Kasai, 2008; Long and Picklo, 2010; Pillon et al, 2011). In healthy adult males, heavy DHA consumption (1.6 g/day) leads to increased 4-HHE plasma levels (Calzada et al. 2010). The CES NUT highlights that there is no mention of a study on the stability of the NI in the different food matrices proposed, particularly in those which undergo heat treatment, such as biscuits or cooking oils.
It considers that the concerns relating to high intake levels of long-chain n-3 PUFAs are greater for children, since there are fewer studies for this age group.
However, the CES NUT takes the view that this problem is not unique to the NI. Rather it applies to all n-3 PUFA-rich ingredients, and appropriate labelling of the NI on enriched foods should prevent populations which consume large quantities of seafood from consuming EPA- and DHA-enriched foods.
The CES NUT considers that the dose proposed for food supplements for pregnant and lactating women is similar to the RNI for this population category, which is 500 mg/day EPA+DHA, just like the RNIs for the general population (ANSES, 2010). It points out that it is likely that the sum of the EPA and DHA intakes from a normal diet and from food supplements for pregnant and lactating women is higher than the RNIs and stresses that, in general, there is no benefit in exceeding the RNIs.

3.6 Nutritional information on the NI

The applicant refers again to the rationale for the changes in enrichment levels in the food vectors and to a novel food marketing authorisation[4] for a DHA+EPA rich oil from Antarctic Krill (*Euphausia superba*). The latter is authorised in a list of vectors similar to that authorised for DHA-S oil. The applicant also compares the fatty acids profile of the NI with a range of oils such as krill oil, salmon oil and cod liver oil.

The ACNFP considers that the nutritional information provided by the applicant is adequate and the non-fat nutritional profile of a product containing the NI would not be significantly different when compared with an equivalent product enriched with fish oil. The committee also notes that the fatty acid profile of the product is broadly comparable to that of fish oils, and does not therefore give rise to any safety concerns. It finally points out that the applicant does not state the calorific load of the NI, but since it is almost entirely composed of triglycerides, a caloric value of 9 kcal/g can be used on nutritional labels.

The CES NUT has no particular comment to make on this point.

3.7 Microbiological information on the NI

The applicant notes that the NI is a lipid with little water activity and would not support the growth of microorganisms, whether they come from the source organisms or an external contamination. Moreover, the applicant provides a specification for the presence of microorganisms (yeasts, moulds, coliformes, Escherichia coli, Staphylococcus coagulase+, Salmonella) and the test results for three individual batches, which are all lower than the specifications.

The ACNFP accepts the data provided by the applicant although it had considered that the possibility of contamination by cyanobacteria could not be discounted. This concern has been allayed by quality control and by the applicant's confirmation that the manufacturing process was carried out in the absence of light, under axenic conditions (pure culture of a single microorganism). The Committee accepts that these measures are sufficient to ensure that any risk of cyanobacterial contamination was no greater than for any other fermentation process used in food production.

The Biological risk evaluation unit has no particular comment to make on this point.

3.8 Toxicological information on the NI

The applicant points out that the traditional counterpart of the NI, fish oil, is widely used in both food supplements and in enriched foods in the EU without restriction. It highlights the absence of algal toxins and the broad similarity between the NI and microalgal oil rich

[4] Commission Decision of 12 October 2009 authorising the placing on the market of a lipid extract from Antarctic Krill *Euphausia superba* as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (2009/752/EC).

exclusively in DHA (DHA-S), which was previously placed on the market by the applicant, meaning that the studies on the latter may be relevant to analysis of the toxicity of the NI.

14-day range finding study

Administration of the test product (oil from Schizochytrium sp.) in the diet (stability during the period tested).

The test was carried out in 2010 in accordance with OECD Guideline 407 and US Red Book 2000 and 2003.

100 Sprague Dawley (Hsd: SD) rats, 50 males and 50 females, aged between seven and eight weeks, were divided into five groups of ten males and ten females, including two control groups. Group 1: basic diet, group 2: fish oil, group 3: 1% NI (corresponding to 833 mg NI/kg bodyweight (bw)/day, group 4: 3% NI (2 500 mg NI/kg bw/day) and group 5: 6% NI (5 000 mg NI/kg bw/day). The total fat content of the diet was adjusted to 10.2% for groups 2, 3, 4 and 5, with maize oil added for groups 3 and 4. The traditional parameters were monitored: mortality, consumption, weight changes, biochemistry, haematology, coagulation, exploratory anatomopathology, etc. The results do not reveal any effects linked to treatment, except for an increase in the absolute and relative liver weights compared with group 1 (basic diet control rats). This effect is non-specific and is also observed in group 2 animals (fish oil). This increase was predictable and can be explained by the high fat content of the diet and the specific composition of fatty acids in algal oil.

The conclusion is that the rats can tolerate a dose of 5 000 mg NI/kg bw/day (6%) without any adverse effects.

The CES AAAT considers this study acceptable. The number of animals is relevant and the correlation between the concentrations of oil in the diet and the dose per kg of bodyweight has been verified.

90-day sub-chronic toxicity study

Administration of the test product (oil from Schizochytrium sp.) in the diet (stability during the period of testing, as well as the stability of DHA and EPA).

The test was carried out in 2010 in accordance with OECD Guideline 408 and the US Red Book 2000 and 2003, and in line with quality assurance procedures.

100 Sprague Dawley (Hsd: SD) rats, 50 males and 50 females, aged seven to eight weeks, were divided into five groups of 10 males and 10 females, including two control groups, group 1: basic diet, group 2: fish oil. Three additional animal groups were exposed to 0.5% (group 3), 1.5% (group 4) and 5% (group 5) of the NI incorporated consistently in the diet. The doses correspond to 357, 1 071 and 3 571 mg NI/kg bw/day. The total fat content of the diet was adjusted to 9.2% for groups 2, 3, 4 and 5, with maize oil added for groups 3 and 4.

The traditional parameters were monitored: mortality, food consumption level, weight changes, biochemistry, haematology, coagulation, macroscopic anatomopathological examination of all animals and histological examinations of a number of organs for groups 1 and 5, as well as an ophthalmological test (day 90 vs. day 0) and a neurological examination in week 13. The latter consisted of a functional observational battery (FOB) and motor activity (MA). A toxicokinetic approach was carried out in the plasma, liver and brain of all the animals.

The results do not reveal any increased mortality, nor any abnormalities linked to the administration of the product (clinical observations, biochemistry, haematology, coagulation, ophthalmology, neurology). Significant decreases in bodyweight and weight gain in both males and females were observed in the last part of the study, as well as reduced consumption by males and females in group three, but these effects were

attributed by the authors of the study to high concentrations of n-3 PUFAs. Moreover, these effects were also observed in the control group 2 (fish oil) and are comparable to those in group 5 (maximum exposure to the NI). The results of toxicokinetic testing revealed a dose-dependent increase (groups 3, 4, 5) in plasma concentrations of DHA and EPA, and also in the liver and brain of the female animals in particular.
Anatomopathological examination did not reveal any effect linked to the NI. The increase observed in the absolute and relative weights of the liver in males and females is statistically significant only in groups 2 and 5. The weight increase in females, without clinical effects, is attributed to the high fat content of the diet. No histological changes in these organs associated with the levels of the NI in the diet were observed.
The conclusion of the study is that a NOAEL of 3 149 mg NI/kg bw/day can be attributed to the NI for males and 3 343 mg NI/kg bw/day for females, equivalent to the highest exposure through the diet containing 5% NI.
The CES AAAT considers this study acceptable even though, in the light of the OECD guidelines, it would have been preferable to have more animals in each group. The CES agrees with the NOAEL determined in this study.

Three genotoxicity studies (two *in vitro*, one *in vivo*) are reported in the toxicological dossier.

In vitro: Detection of gene mutations through a bacterial reverse mutation test
The study was carried out in 2009 in accordance with good laboratory practice, quality control and internationally recognised guidelines (OECD 471, German Guidelines EPA, GLP) to examine the mutagenic potential of the NI on four strains of *Salmonella typhimurium* (TA 98 and TA 1537 for frameshift mutations, and TA 100 and TA 1535 for base pair substitutions and the strain Escherichia coli VVP2 uvra for base substitutions). Two separate experimental series using six different concentrations were tested in triplicate with and without activation (microsomal fraction S9 mix). The maximum concentration tested for the two series was 5 000 µg/plate. No cytotoxicity was observed at this dose. The test encompassed negative and positive controls (2 AA, MMS, NaN3).
The results for the five strains and for all tests, with and without activation, do not reveal any significant increase in the number of revertant colonies. The validity of the test was proved by means of the positive controls.
The study concludes that under the test conditions applied, the NI tested is considered to have no mutagenic effect.
The CES AAAT considers that this traditional Ames test is acceptable provided that the strains are carefully selected. The NI tested can be considered to have no mutagenic effects.

In vitro: Chromosomal aberration test in cultured mammalian cells
The study was carried out in 2009-2010 in accordance with good laboratory practice, quality assurance procedures and internationally recognised guidelines (OECD 473, German EPA GLP guidelines) to examine the potential mutagenic effect of the NI on human lymphocytes (peripheral blood): diploid cell cultures treated with a mitogen. Two separate experimental series were performed with six different concentrations for each series, with the maximum concentration tested for each series (5 µL/mL). No cytotoxicity was observed. The treatment consisted of two test series:
- Series I: with and without (S9) activation, 4 hrs of treatment, metaphase preparation (fixation) 24 hrs after treatment;

- Series II:
-With activation, 4 hrs of treatment, metaphase preparation 24 hrs after treatment;
-Without activation, 24 hrs of treatment, metaphase preparation 24 hrs after treatment.
In each case, 100 metaphases per culture were examined. The test also encompassed negative and positive controls (ethyl methane sulfonate, cyclophosphamide).
The results of the two series of tests, with and without metabolic activation, do not reveal any significant increase in the rate of chromosomal aberrations (breaking of chromosomes), nor in the frequency of polyploid cells. The chromosomal aberration rates observed are within the limits of the historical values for the negative controls. The validity of the test was proven by means of the positive controls.
The study concludes that under the test conditions used, the NI does not cause an increase in chromosomal aberrations in human lymphocytes and can be considered to have no clastogenic effect.
The CES AAAT considers this test acceptable and that the NI tested can be deemed to have no clastogenic effect.

In vivo: Erythrocyte micronucleus test in mice
The study was carried out in 2009-2010 in accordance with good laboratory practice, quality assurance procedures and internationally recognised guidelines (OECD 474, German GPL) to examine *in vivo* the potential mutagenic and chromosomal effect of the NI, i.e., the capacity to cause the formation of micronuclei on the polychromatophilic erythrocytes (PCEs) in the bone marrow of mice.
The authors, rather than carrying out a prior test to determine the doses, immediately carried out a limit test at 2 000 mg/kg bw: a single dose, considered here as the maximum tolerated dose (largest dose which can be administered without toxic effect). The substance was administered by gavage in a single dose (gavage, 10 mL/kg bw). Samples were taken from the peripheral blood after 44 and 68 hrs of treatment. Fifty NMRI mice, in batches of five males and five females divided into negative and positive controls, were tested. For each batch, at least 10 000 cells were examined for the presence of micronucleated polychromatophilic erythrocytes (PCEs). The controls were treated with cyclophosphamide (40 mg/kg bw, administered intraperitoneally). The statistical study was carried out using the Mann-Whitney non-parametric test.
The results reveal that the treatment with 2 000 mg/kg bw of the NI does not lead to an increase in the number of micronucleated polychromatophilic erythrocytes: the percentage of young (immature) micronucleated red blood cells compared to the total PCEs is comparable to that obtained for the negative controls.
The conclusions of the study are that under the test conditions used, the NI can be considered to have no clastogenic properties.
The CES AAAT observes that the test could have used a less traditional methodology (range-finding test, several doses, possibly with fractionated administration). However, the* in vivo *micronucleus test for examining potential chromosomal mutations – the result of which is always of toxicological significance – was negative. The CES AAAT therefore believes that the NI can be considered not to have any clastogenic effects.

General conclusions of the FSA and the CES AAAT concerning the toxicological data
The ACNFP concludes that the toxicological studies carried out by the applicant are adequate to ensure the safety of the product at the proposed levels of use. It notes that the concerns relating to post-date births were not addressed by the applicant's response. It

disagrees with the applicant's conclusions regarding reviews by Makrides et al. in 2006 and 2010, noting that the latter paper reveals an increase in the number of post-date births linked to high intakes of n-3 PUFAs. However, the FSA considers that longer gestation periods are a general problem which has already been considered by EFSA and the UK's Scientific Advisory Committee on Nutrition when preparing their recommendations on n-3 PUFA intakes in pregnant and lactating women. However, it does suggest that the possibility of longer gestation should be taken into account when considering the levels at which the NI is used, and the adverse effects following its introduction into the diet (if it is possible to monitor such effects).

The CES AAAT considers that the repeated-dose 90-day study in rats does not reveal any harmful effects associated with the administration of the NI. The only modifications observed can be attributed to a nutritional imbalance caused by the high fat intake, and these effects are not therefore specific to the NI. A NOAEL of 3 149 mg/kg bw/day may be determined from this study.

Much effort has been put into investigating potential genotoxicity, with two* in vitro *tests and one* in vivo *test revealing consistently negative results. The CES AAAT therefore considers that these test results demonstrate that the NI is non-genotoxic.

Moreover, the CES AAAT observes that, according to the applicant's exposure calculations, the highest intakes are in adolescent 'consumers' and are around 0.88 g/day for the mean intakes of EPA+DHA from consumption of the NI (18 mg/kg bw/day for an average weight of 50 kg) and 1.72 g/day (34 mg/kg bw/day) for intakes at the 97.5th percentile. In adult male consumers, the mean intake is 0.77 g/day (11 mg/kg bw/day for an average weight of 70 kg) and the intake at the 97.5th percentile is 1.65 g/day (23 mg/kg bw/day). These intakes are 92 to 286 times lower than the highest dose, revealing no adverse effects in the repeated-dose 90-day study in rats. These margins are therefore considered adequate from a toxicological point of view.

3.9 Allergenicity and labeling

The level of residual protein in the NI is lower than 0.02%. The applicant points out that its previous DHA-S oil from *Schizochytrium* is produced under similar conditions from a related microalgal strain which also contains low levels of protein (<0.1%), and its consumption has not been associated with any serious adverse events. The applicant also notes that reports of respiratory and dermatologic responses (including allergy) to microalgae have been restricted to human exposure to blue-green algae.

The applicant does not make any proposals for the labelling of its NI. The authorisation for DHA-S oil specifies that it must be labelled as 'oil rich in DHA from the micro-algae *Schizochytrium sp*.'.

The FSA's ACNFP considers that the NI does not present an allergenic risk and that labelling similar to that of DHA-S oil adequately describes the product.

The CES NUT agrees with the FSA's labelling proposal.

3.10 Conclusions

■ **Conclusions of the British authorities**

The FSA's ACNFP concludes that the applicant has provided sufficient scientific data to ensure that the proposed additional uses of the NI do not give rise to any specific concerns about its safety when consumed at the proposed levels of use. The FSA highlights that current UK policy is to encourage the intake of long-chain n-3 PUFAs and that this product may help consumers with low intakes to increase their intake of these fatty acids.
Concerns have been raised during a previous assessment of a novel n-3 PUFA-rich algal oil about the impact that long-term, high-level consumption of these products on health. The FSA notes that this should be kept under review and intakes of DHA should be monitored at national and/or European level. However, the FSA's ACNFP reiterates its view that this uncertainty is not solely related to the extension of the use of the NI and that any studies examining the impact of consumption of foods enriched with n-3 PUFAs should take into account all dietary sources and different age groups, particularly children.
Lastly, the ACNFP considers that the data provided by the applicant are adequate and that the proposed levels of use of the NI are acceptable.

Conclusions of the CES NUT and the CES AAAT

The CES NUT and the CES AAAT agree with the FSA that the NI does not give rise to any particular safety concern when consumed at the proposed levels.

The CES NUT considers that the estimated maximum intake level for EPA and DHA – in spite of the fact that the basal intake level for these fatty acids was not taken into account – is acceptable as regards the consumption levels potentially reached by heavy seafood consumers. However, it shares the view of the FSA concerning the long-term effects of very high doses of long-chain n-3 PUFAs and on the need to monitor consumption, taking into account all dietary sources and different age groups, particularly children. It also agrees that this problem is not specific to the NI but results from the cumulative intake owing to the fact that several ingredients are rich in EPA and DHA.

The CES NUT also considers that the formation of compounds from the oxidation of n-3 PUFAs during storage of the NI and during processing and preparation of foods enriched with the NI (in particular when this involves heat treatment, such as cooking (cookies) or frying) should be regularly checked.

The CES NUT and the CES AAAT point out as regards adding the NI to cooking oils that the criteria for polar compounds and triglyceride polymers (as defined by Decree 2008-1845[5]) must be observed.

The CES AAAT considers that from the toxicological point of view, consumption of the NI at the levels proposed would not present a health risk.

The repeated-dose 90-day study in rats does not reveal any harmful effect linked to administration of the NI. The only changes observed can be linked to a nutritional imbalance caused by a high fat intake. They are therefore not specific to the NI. A NOAEL of 3 149 mg/kg bw/day may be identified from this study. The results of the examination of

[5] Decree No 2009-184 of 26 February 2008 applying the Consumer Code (*Code de la Consommation*) in respect of fats and edible oils (OJ 28 February 2008).

potential genotoxicity are negative and the NI can therefore be considered as having no genotoxic potential.

Moreover, the NI intakes calculated by the applicant are 92 to 286 times lower than the highest dose with no adverse effects in the repeated-dose 90-day study in rats. These margins are therefore considered adequate from a toxicological point of view.

4. CONCLUSIONS ET RECOMMANDATIONS DE L'AGENCE

The National Agency for Food, Environmental and Occupational Health & Safety adopts the conclusions of the CES NUT and the CES AAAT as regards the absence of any concerns about the safe consumption of the NI at the levels proposed. However, it highlights the risk linked to a cumulative intake of long-chain n-3 PUFAs (EPA and DHA), given their presence in several vectors. It also considers it necessary to monitor the formation of compounds resulting from the oxidation of n-3 PUFAs, in particular when the NI is incorporated into foods which undergo heat treatment.

By the Director General

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KEYWORDS

Long-chain polyunsaturated fatty acids, Omega 3, novel food, food supplement, enrichment.

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