

# Scientific Advisory Committee on Nutrition

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**Paper for Information: Initial ACNFP opinion on DHA Gold<sup>TM</sup> under the EC Novel Food Regulation**

**Agenda item 9**

Please see attached paper for information.

**The Committee is asked to note the ACNFP opinion.**

# Scientific Advisory Committee on Nutrition

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## Initial ACNFP opinion on DHA Gold™ under the EC Novel Food Regulation

1. The UK received an application from Omegatech for the approval of DHA Gold™ for use as a nutritional ingredient. DHA Gold™ was identified as a “novel food from a non GM source” and that as “the source of the novel food has no history of use in the community”, and therefore required clearance under EC Novel Food Regulation.
2. Under the EC Novel Food regulation, applications for approval of novel foods are made to one Member State, which then has 90 days to consider the dossier and submit an initial opinion. The UK received an application from Omegatech for the approval of DHA Gold™, DHA-rich oil obtained from marine microalgae - *Schizochytrium* species. The ingredient has been previously assessed in the United States and was given clearance as a Generally Recognised as Safe (GRAS) ingredient with a recommended maximum daily intake of 1.5g/day of DHA from this source. Prior to this, the microalgae had been given GRAS status for use in chicken feed suggesting that components of the oil may already be present in the human food chain.
3. Omegatech are intending to market DHA Gold™ only as an ingredient to food manufacturers for incorporation into a range of foods - dairy products, fine bakery wares, confectionery, sauces, breakfast cereals and cereal bars, spreads, potato crisps and pasta. The ingredient will not be sold directly to consumers. The level of incorporation of DHA Gold™ will depend on the existing background levels of intake. It is estimated that UK adults on average consume 107mg/day of DHA, and 401mg/day at the 97.5<sup>th</sup> percentile. It is anticipated that the combined background and incorporated level of DHA would equal a mean intake in adults of 550mg/day.
4. The company provided extensive data to support the application, which has been placed on FSA’s website. The data included animal and human clinical studies. The ACNFP considered the application and formulated an initial opinion that has been forwarded to other Member States which will further consider the application.
5. ACNFP is satisfied by the evidence provided and concluded that DHA Gold™ is safe for use as a nutritional food ingredient on the following basis:
  - Oil consists only of lipid components already present in other existing dietary forms
  - Low level of residual protein and carbohydrate in the final refined oil indicates that the oil is likely to elicit only a low risk of allergenicity.
  - Production process is well controlled and with appropriate in-process monitoring steps to ensure a safe and consistent product.
  - The parent organism has no history of toxin production

- The increased need for vitamin E arising from increasing intake of DHA should be met by supplementing the oil with vitamin E (The Company has agreed to do this)
  - Final products containing DHA Gold™ would have to be labelled to inform consumers of recommended levels of intake from DHA Gold™.
  - Final products will need to be labelled with the ingredient name and prescribed nutritional labelling and comply with the criteria for making nutrient content and health claims.
6. The ACNFP concluded that it was satisfied with the evidence provided by Omegatech that DHA Gold™ is safe for use as a nutritional ingredient, for types of uses described above and subject to the labelling requirements described above.

**The Committee is asked to note the ACNFP opinion.**

**ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES****UK/2002/001****Opinion on an application under the Novel Food Regulation from OmegaTech for clearance of DHA Gold<sup>®</sup>, a DHA rich oil.**

**Applicant:** OmegaTech

**Responsible person:** Mr Nigel Baldwin

**Novel Food:** DHA Gold<sup>®</sup>

**EC Classification:** 2

**Introduction**

1. An application was submitted by OmegaTech to the UK Competent Authority on 13<sup>th</sup> February 2001 for approval of DHA Gold<sup>™</sup>, a DHA - rich oil. The full version of this dossier was placed on the UK competent authority website on 14<sup>th</sup> February 2001. During the course of the evaluation the UK Competent Authority sought further information to clarify certain aspects of the dossier.
2. DHA (docosahexaenoic acid) - rich oil is produced via an algal fermentation process using microalgae from the genus *Schizochytrium*, a member of the kingdom Chromista, which includes the golden algae.
3. *Schizochytrium* sp. has previously been assessed in the United States and was given GRAS (Generally Recognised As Safe) clearance as a nutritional food ingredient. In the United States, a daily intake of up to 1.5g of DHA was recommended (DHA-rich oil contains 35-45% DHA). Prior to this, the microalgae achieved GRAS status to be used in chicken feed at levels of incorporation of up to 2.8% for broilers and 4.7% in layers). There is evidence to suggest that each of the components of the oil is already present to a significant degree in the human food chain.
4. The production strain of microalgae used for DHA Gold<sup>™</sup> has been developed using conventional improvement techniques of the wild type strain and no recombinant DNA technology was used.
5. The application was prepared according to the European Commission's guidelines. DHA-Gold<sup>™</sup> was identified as belonging to class 2.2 ("complex novel food from a non-GM source", "the source of the novel food has no history of use in the community"). The Committee's consideration of the data provided is presented according to these requirements.

## I. Specification of the Novel Food

*Information on this aspect is provided in section 1 of the application dossier. Supplementary information was supplied in March 2001.*

6. DHA is a long chain polyunsaturated fatty acid, derived from heterotrophically grown microalgae. DHA Gold™, DHA-rich oil is described as a yellow to light orange-coloured oil derived from the heterotrophically grown marine microalga, *Schizochytrium* sp., intended for use as a nutritional food ingredient.
7. Quality control tests indicate that the production process is both reliable and reproducible and there is evidence to demonstrate that controls are in place to ensure individual batches meet manufacturing specifications.
8. Further information was sought regarding the compositional analysis of the oil. The Committee was concerned about the presence of components, particularly protein and carbohydrate, that may elicit an allergenic response.
9. Information was provided to demonstrate that the extraction process would not disproportionately concentrate any potentially toxic components.

### Discussion

*The Committee was satisfied that the oil consists only of lipid components already present in other existing dietary forms. The data provided (Appendix A of original application) on a number of batches show that a consistent and reliable end product is produced.*

*The producers were able to demonstrate that there is a very low level of residual protein (less than 0.1%) and carbohydrate in the final refined oil. This indicates that the oil is likely to elicit only a low risk of allergenicity.*

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

*Information on this aspect is provided in section 2 of the application dossier.*

10. The improvement of the *Schizochytrium* sp. was carried out using a classical mutagenesis/screening programme, which employs standard techniques commonly used in industrial microbial strain improvement (see section 2 of the application dossier). The production method is well defined, and a number of in-process monitoring steps are included in the manufacturing procedure to ensure the safety and quality of the oil is maintained. DHA-rich oil is manufactured under the general guidelines of the food chemical Good Manufacturing Practices (Food Chemical Codex pp xxvii, 4<sup>th</sup> edition).

### Discussion

*The Committee was satisfied that the production process is well controlled and that the in- process monitoring steps are appropriate to ensure a safe and consistent product.*

### **III. History of source organism**

*Information on this aspect is provided in section 3 of the application dossier.*

11. This class of microalga is primarily saprotrophic and is found throughout the world in estuarine and marine habitats. *Schizochytrium* sp. has a widespread distribution, and is consumed by a wide range of filter feeders. Although there are no reports of human consumption of *Schizochytrium*, the filter feeders (clams and mussels) that feed on this organism are part of the normal human diet.
12. *Schizochytrium* sp. belongs to the kingdom Chromista. This is not the same as the kingdom to which the bluegreen or dinoflagellate microalgae belong. This is significant since these two constitute the major known toxin producing microalgae, and most allergic responses to algal micro-organisms have been limited to exposure to these. Only two genera in the Kingdom Chromista are known to produce toxins, neither of which is in the same class as *Schizochytrium* sp. There have been no reports of toxins being found in this class.
13. The improved strain of *Schizochytrium* was developed from a patented wild-type parent strain, by using the standard chemical mutagen, NTG (1-methyl-3-nitro-1-nitrosoguanidine). Modified strains derived using this procedure sometimes acquire undesirable traits. Therefore, tests were conducted to characterise phenotypically the modified strain and its parent. The results indicate that the new strain performed equivalently to its parent and no adverse traits were observed due to the mutagenesis.
14. In addition, comparative compositional data of the oil from the parent (wildtype) and modified daughter strains demonstrated expected alterations in the balance in fatty acids, with the oil from the daughter strain having an increase in DHA content and a reduced level of palmitate. No unexpected fatty acids were found in the oil from the modified strain.
15. The sterol components of the parent and daughter strain oils were confirmed to be qualitatively constant when analysed. No unexpected sterols were identified in the daughter strain oil.

## Discussion

*The Committee was satisfied that the parent organism has no history of toxin production. The Committee was also content that no unexpected phenotypical changes had been introduced, and that the composition of the oil obtained from the daughter strain was similar to that from the parent apart from the desired increase in DHA content, and a compensatory decrease in palmitate levels.*

### IX. Anticipated intake/ extent of use.

*Information on this aspect is in section 9 of the application dossier.*

16. In 1994, the UK Committee on Medical Aspects on Food Nutrition Policy (COMA) recommended that individuals should increase their intake of omega-3 fatty acids, including DHA, since raised intakes are associated with a reduced risk of coronary heart disease. It must be shown, however, that by increasing levels, no detrimental effects are introduced, either from the DHA or from the other components of the oil.
17. OmegaTech are intending to market DHA Gold™ only as an ingredient to food manufacturers, it will not be sold directly to consumers. The DHA-rich oil is, however, suitable for use in a wide range of food products. These include dairy products, fine bakery wares, confectionery, sauces, breakfast cereals and cereal bars, spreads, potato crisps and pasta. However, the company has agreed to include labelling in relation to the recommended intake of DHA, so that consumers can select products as appropriate. See paragraph below.
18. The level of incorporation of DHA Gold™ into these products is dependent on the existing background DHA levels. It is estimated that UK adults currently consume 107mg/day of DHA, with the 97.5th percentile consuming 401mg/day. However, the aim would be that the combined background and incorporated level of DHA would equal a daily mean intake of 550mg.
19. Under article 2 of Directive 90/496/EEC, it will be compulsory for food manufacturers to define the quantity of DHA per serving or per 100g in the final food. OmegaTech will recommend to their customers in Europe, the food manufacturers, that daily consumption should not exceed 1.5g of DHA, as was stipulated in the US GRAS approval. Many EU Member states have their own recommendations. For example, AFFSA (France), The Health Council of the Netherlands, and COMA (UK), and so usage levels would need to be determined for particular food products and may require adjustments for different markets in various Member States. The Company has agreed to ensure that labelling meets different national requirements.

## Discussion

*The Committee was content with the information provided by the applicant. However, it was considered that the producers of the oil should provide information concerning “recommended intake” to manufacturers intending to use DHA-Gold<sup>®</sup> as a food ingredient. This information should then be included on the labelling, which would accompany the final food product, and so passed on to consumers.*

### **X. Information on previous human exposure.**

*Information on this aspect can be found in section 10 of the application dossier. Supplementary information was supplied in March 2001.*

20. DHA-rich oil contains a range of fatty acids, including eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA) as well as DHA, and traces of phytosterols. EPA and DPA both occur naturally in plant and animal products, and so estimates can be made to indicate current background levels of intake.
21. The principal sources of EPA and DHA in the diet are from oily fish. Only 35% of adults in the UK regularly consume oily fish, however. The absence of fatty fish in the diet greatly reduces the levels of DHA and EPA in the diet. The levels of fatty acid intake vary across Europe. This is probably due to the differences in trends of oily fish consumption. Most dietary DPA comes from offal.
22. Further information on previous human consumption was provided. The producers were able to give details of DHA being supplied to a customer who then sold the oil as a dietary supplement. The product has been on sale in the United States for over two years, and the information provided is in the form of a list of calls received from the after sales care helpline. Over 400 calls have been received by this helpline, none of which have recorded any adverse effects relating to this product.

## Discussion

*The Committee accepted the data provided on the background levels of consumption of the fatty acid and phytosterol components of DHA – rich oil already in the human diet. Although conscious that the information provided from the helpline call centre in the USA was anecdotal and not a formal structured study, the Committee noted that there have been no reported adverse effects in the two years that the product had been on sale.*

### **XI. Nutritional information**

*Information provided on this aspect is in section XI of the application dossier.*

23. DHA is considered to play an important role in maintaining a healthy heart. Several markers of the cardiovascular system are directly influenced by dietary DHA. These include triglyceride levels, platelet aggregation (may

lower the risk of heart attack or stroke), cardiac rhythmicity and haemodynamics.

24. DHA is also considered to be vital for the development and function of brain and eyes. DHA oil is supplemented with Vitamin E for nutritional purposes.

### **Discussion**

*The Committee accepted that nutritional advice is to increase intake of omega-3 fatty acids and was aware that this oil could improve the nutritional properties of foods to which it is added. The Committee agreed that foods containing DHA - Gold<sup>®</sup> oil would have to be labelled to inform consumers of recommended intake levels. There is an increased nutritional need for vitamin E when increasing the intake of polyunsaturated fatty acids, and we note that the oil supplemented with vitamin E to address this point.*

### **XII. Microbiological information**

*Information provided on this aspect is in section 12 of the application dossier.*

25. DHA Gold<sup>™</sup> is manufactured under the general guidelines of food chemical Good Manufacturing practices. A combination of heat treatment, environmental conditions of oil extraction and processing and the extremely low water activity of the finished oil, contributes to the inhibition of typical food-borne microbes.

### **Discussion**

*The Committee was content with the information provided by the applicant and considered the production process, the quality control measures and the nature of the final product to be sufficient to ensure no unintentional microbiological contamination of the oil.*

### **XIII. Toxicological information**

*Information on this aspect is provided in the application dossier in section 13. Additional data requested by the Committee was supplied in February 2002.*

26. A range of safety studies has been conducted with dried microalgae of the genus *Schizochytrium*. These studies were conducted in accordance to the 1982 FDA Redbook Guidelines and in compliance with the FDA Good Laboratory Practice (GLP) regulations to support the GRAS petition in the US.

27. The studies included a) a subchronic feeding study where dried DHA-rich microalga was fed to rats for at least 13 weeks, b) developmental toxicity evaluation in rats and rabbits, c) a single generation rat reproduction study and d) a mutagenicity study. Also an acute gavage study was conducted with extracted and refined DHA-rich oil, and a laying hen and a chicken broiler study were also conducted. The actual material tested in each of

the studies is shown in the table attached below. The Company also presented the findings from a swine toxicity study carried out on the algal biomass, but the Committee did not feel this contributed any further information for the safety assessment of the oil for human consumption.

Summary of the test material used in the toxicology studies.

Trial	Test Article	Strain
<u>Acute Feeding</u> Mouse	oil	N230D (Daughter, production strain)
<u>Sub-chronic Feeding</u> Rat - 90 day <sup>a</sup>	algae	ATCC 20888 (Parental, wildtype strain)
Laying Hen -112 day <sup>d</sup>	algae	N230D
Broiler Chicken – whole life	algae	N230D
<u>Developmental &amp; Reproductive Toxicity</u> Rat – developmental toxicity <sup>b</sup>		
maternal	algae	ATCC 20888
offspring	algae	ATCC 20888
Rat – single generation reproduction <sup>c</sup>		
males	algae	ATCC 20888
females	algae	ATCC 20888
Rabbit – developmental toxicity <sup>b</sup>		
maternal	algae	ATCC 20888
offspring	algae	ATCC 20888

<sup>a</sup> Hammond et al. (2001b); <sup>b</sup> Hammond et al. (2001a); <sup>c</sup> Hammond et al. (2001c); <sup>d</sup> Abril et al. (2000);

Summary of the genetic toxicity studies performed using dried algae of the genus *Schizochytrium* and oil containing DPA(n-6) and DHA.

Trial	Test Article	Strain
Ames	oil (DHALIP-NS) <sup>NB</sup>	N230D
Ames <sup>a</sup>	intact algae	ATCC 20888
In Vitro Human Lymphocytes <sup>a</sup>	intact algae	ATCC 20888
Mouse Lymphoma <sup>a</sup>	intact algae	ATCC 20888
Ames <sup>a</sup>	lysed algae	ATCC 20888
AS52/XPRT Gene Locus <sup>a</sup>	lysed algae	ATCC 20888
Mouse Micronucleus <sup>a</sup>	lysed algae	ATCC 20888

<sup>a</sup> Hammond et al. (2001d)

NB. DHALIP-NS refers to the Company's internal product code for commercial article.

28. A human clinical study on the DHA-Gold™ oil was supplied in 2002.
29. It was noted that the toxicology studies were carried out on either the parental (ATCC 20888) or the production daughter (N230D) strains. Evidence was provided to demonstrate that the oil from the two strains were comparable, with the exception of the expected increases in DHA content. For further information, see section III, The History of the Source Organism.
30. Each of the studies is summarised below.

**Safety Assessment of DHA-Rich Microalgae of the genus *Schizochytrium*:**

**Part I: Sub-chronic Rat Feeding Study.**

31. The purpose of this study was to determine the effects of DHA (docosahexaenoic acid) -rich microalgae (DRM) of the genus *Schizochytrium*, administered in the diet of rats for 13 weeks.
32. A dried preparation of DRM was administered in the diet to groups of 20 male and 20 female Sprague-Dawley rats to provide an intake of 0, 400, 1500 and 4000 mg/kg/day for at least 13 weeks. Untreated controls received basal diet only. An additional group of 20 males and 20 females received rodent diet mixed with fish oil to provide a target dose of 1628 mg/kg bw/day. In view of DRM's high fat content (41%, mainly unsaturated fatty acids), vitamin E acetate had been added (during manufacture of DRM) to all test substance and fish oil-treated groups to provide supplementary dietary antioxidant.
33. The stability and homogeneity of the diets were checked regularly during treatment periods. Animals were observed twice daily for mortality and clinical signs. Ophthalmoscopic examinations were carried out prior to start of study and prior to killing the controls, fish oil treated rats and the high-dose DRM treated animals. Blood collected under halothane anaesthesia was collected from 10 rats/sex/group during week 6-7 and prior to killing; urine was also collected during the latter periods from the same rats. Haematological measurements were carried out on 13 relevant parameters (including thromboplastin-clotting time). Serum clinical chemistry measurements were carried out on 24 parameters. Urinalysis was carried out on at least 13 end points. At the end of the study, the animals were anaesthetized by sodium pentobarbital and killed by exsanguination. Organ weight data were collected for the liver, spleen, heart, thymus, ovaries, testes, kidneys, adrenals, pituitary and brain. Complete sets of tissues were also collected for histopathology. All tissue slides from control, fish oil, and high dose DRM groups were examined microscopically. In addition, heart, kidneys and pituitary for males in all groups and liver from females in all groups were also examined microscopically.

34. Based on the results from a previous 13-week rat feeding study (CTBR study) with DRM, a Pathology Working Group was formed to review the heart slides from this study and CTBR study to resolve differences in terminology and severity scores between the two studies. The review also assessed the accuracy and consistency of the initial histopathological examinations of the hearts of male and female rats.
35. All animals survived during the study. There were no treatment-related clinical and ophthalmologic signs of toxicity. There were no treatment-related effects on body weight or food consumption compared with controls.
36. While there were a few significant intergroup differences in haematological parameters, these were not considered treatment-related, as there was no dose-response. The main clinical chemistry finding involved a drop in cholesterol and HDL levels in both sexes of the fish oil and the high-dose groups. A lowering in the latter two parameters was also noted in males receiving DRM at 1500 mg/kg bw/day.
37. At necropsy, no treatment-related effects on gross lesions and terminal body weights and absolute and relative organ weights were noted. Microscopic changes were mainly confined to the liver, kidneys and heart. In the liver, the incidence of periportal hepatocellular vacuolation was significantly increased in the female fish oil group (18/20) and all female treatment DRM groups (low dose, 16/20, mid-dose, 18/20 and high dose 19/20) when compared with the female untreated control group (8/20); there were no treatment-related differences in the severity of this observation. There was a significant increase in pelvic dilation of the kidneys in high dose DRM males (5/20) compared to control (0/20) and fish oil groups (2/20).
38. The nature of the histological changes affecting the heart (slight increases in the incidence and/or severity of inflammation and degenerative changes in the male rats) of animals from this study was similar to that reported in a previous study, although in this study, diets were not supplemented with vitamin E. A slight increase in the incidence, but not severity, of cardiomyopathy was observed in the 4000 mg/kg bw/day DRM dosed males in this study. The changes were characterised by small foci of mononuclear inflammatory cells and degeneration of myofibres, sometimes accompanied by fibrosis of the myocardium. The changes were reported to be identical to spontaneous "cardiomyopathy" associated with ageing. The incidence and severity of cardiomyopathy in this study were greater in male rats than females.

**Safety Assessment of DHA-Rich Microalgae of the genus *Schizochytrium***  
**Part II: Developmental Toxicity Evaluation in Rats and Rabbits**

39. The developmental toxicity of DRM (supplemented with vitamin E during manufacture) was assessed in Sprague-Dawley rats (25/group, provided

with dried DRM in the diet at 0, 0.6, 6, and 30% on gestation days (GD) 6-15) and in New Zealand White Rabbits, 22/group, dosed with DRM at levels of 180, 600, and 1800 mg/kg bw/day by gavage (in 2 equal daily doses, 6 hrs apart for 13 consecutive days on GD 6-19). An additional group of 22 rabbits dosed with fish oil (also supplemented with vitamin E) was used as a negative control to provide an equivalent amount of fat to that received by the high-dose DRM rabbits. Control animals (22 per group) received the vehicle (0.5% carboxymethyl cellulose and 0.1% polysorbate 80).

40. Sperm-positive female animals were weighed and food consumption determined on GD 0, 6, 9, 12, 16, 18, and 20 (rats) and GD 0 through to GD 29 (rabbits). Animals were observed twice daily for clinical signs and mortality. All rats were killed on GD 20 by asphyxiation with carbon dioxide. Rabbits were killed on GD 29 by a lethal injection of sodium pentobarbital. A complete gross necropsy was conducted on all rats and rabbits. At necropsy, the uterus and ovaries were excised from each animal and the number of corpora lutea recorded. The uteri were examined for the location of foetus, resorptions and implantation sites. Live foetuses were dissected from their uterus, weighed and examined for morphologic and visceral abnormalities. All foetal carcasses were eviscerated and stained and examined for skeletal malformations.

#### Rats

41. No rats died during the course of the study. There were no treatment-related clinical signs. Animals in the 30% DRM group exhibited a reduction in weight gain from GD 16 to GD 18. Food consumption was also reduced in the latter group during GD 6 to GD 9 and between GD 16 and GD 18. Examination of the uteri confirmed that 88%, 88%, 92% and 80% of the mated animals in the control through to the high-dose DRM groups were pregnant and produced foetuses by GD 20. There were no treatment-related effects on corpora lutea, implantations, live foetuses, or in percent resorptions or late deaths. Statistical increases in the number of male foetuses and in the male sex ratio were noted in low- and mid-dose DRM groups (mainly due to a low percentage (39.1%) of male foetuses/litter in the control group). The incidence of foetuses with ossification centres in the first lumbar vertebrae (2%) was significantly lower in the high-dose-DRM group but was within the historical control range (1.5-15%). A statistically higher incidence of foetuses (but not litters) with reduced ossification of the ribs was seen in the mid- and high-dose DRM groups. This resulted from a single litter with a number of affected pups (mid-dose, 8 foetuses, high-dose, 5 foetuses). Treatment with DRM did not result in other skeletal and visceral anomalies in rats.
42. Since at the highest dietary concentration of DRM (30% - which equates to 22 g/kg/day) no treatment related clinical signs were observed, the authors proposed a NOEL to be the highest dose, 22 g/kg bw/day.

## Rabbits

43. One animal in the 600 mg/kg bw/day DRM group died during GD 10 and a second was killed by an intubation error on GD 10 in the 1800 mg/kg/day DRM group. One female in the fish oil control group aborted on GD 23, and two females in the high-dose group aborted on GD days 25 and 26. No treatment-related clinical signs were reported in the DRM dosed groups. Reductions in body weight gain and food consumption were noted in the animals in the high-dose DRM group during GD 12-19 and when the entire treatment period was evaluated (there was reversal of this effect during the first half of the post-treatment period, GD 24-29). A similar loss in weight gain was noted in the fish oil-treated group.
44. Uteri examinations confirmed that 77%, 81%, 77%, 81%, and 89% of the artificially inseminated females in the control, fish oil and low through to high-dose DRM groups were pregnant when killed.
45. There were no significant differences between the DRM or fish oil treated groups and the control in mean number of corpora lutea, implantation sites, litter size, post implantation loss, and foetal body weight. Treatment with DRM did not result in skeletal and visceral anomalies in rabbits. The authors proposed a NOEL in rabbits of 600 mg/kg/ day of DRM for maternal toxicity and 1800 mg/kg bw /day of DRM for developmental toxicity.

## **Safety Assessment of DHA-Rich Microalgae of the genus *Schizochytrium***

### **Part III: Single generation rat reproduction study**

46. The reproductive toxicity of dried DRM was examined in Sprague-Dawley rats (30/sex/group) in the diet at concentrations of 0, 0.6, 6 and 30% (equivalent to a dose of 400, 3900, and 17800 mg/kg bw/day for F0 males and 480, 4600, and 20700 mg/kg bw/day for F0 females respectively). Treatment in males continued throughout mating and until termination. Females were treated throughout gestation and through lactation day 21. Females were killed after raising their offspring to weaning at 21 days of age.
47. Animals were observed twice daily for clinical signs and mortality. Body weight and food consumption data were recorded weekly. For each F1 litter, the number of live and dead pups, gross abnormalities and individual weight of live pups were recorded at birth and on days 4, 7, 14 and 21 of lactation. Culled F1 animals (4/sex/litter) were subjected to gross necropsy. F0 animals were terminated (males, 3 weeks after the end of mating period and females on days 21, 22 or 23 *post partum*) by carbon dioxide asphyxiation and gross necropsies performed. The left testes from F0 males were used to assess sperm count, motility and morphology. Uteri and ovaries from F0 females were used for assessment of implantation sites and implantation loss. In addition, histopathological examinations

were conducted on epididymis, liver, ovaries, prostate, seminal vesicles, testis (right), uterus, vagina and tissues showing gross pathology.

#### F0 generation

48. Three male rats died during the study (a high-dose male died during week 5 and a control and two mid-dose DRM animals died, one during week 13 and one during week 14). These deaths were not related to treatment with DRM. Statistically non-significant increases in body weight in high dose males were noted from week 10 through to week 16. The body weight in female high-dose DRM group was increased significantly during pre-mating weeks 1 and 2 and throughout both gestation and lactation. Food consumption in the male DRM groups was reduced (only statistically significant in the high dose group during weeks 2-16). Food consumption was also reduced in high-dose DRM females during gestation. There were no treatment-related adverse effects in reproductive performance, or in the duration of gestation, mean litter size, mean pup weight, number of litters with dead pups, or post implantation loss. Apart from an increased hepatocellular vacuolation in the mid- and high-dose DRM female groups, there were no treatment related histopathological changes noted. Dietary DRM treatment had no effect on epididymal weights, sperm counts, percent motility, sperm morphology or spermatogenic cycle.

#### F1 generation

49. There were no treatment-related clinical signs apparent in the F1 pups. Pup viability and survival was similar in control and treated animals. DRM treatment had no effect on F1 body weights recorded on lactation days 0, 4, 7, 14, or 21. No treatment-related internal or external gross abnormalities were noted in pups that were born dead or in those that were subjected to necropsy at the termination of the trial.

#### **An Evaluation Of The Mutagenic Potential Of DHALIP-NS In the Ames Salmonella/ Microsome Assay (EX 4709).**

50. A precipitate was observed at doses of 500 and 1000 µg/plate. The plates dosed at 5000 µg/plate were not countable due to this precipitate. There was no toxicity observed at any test article concentrations. There appeared to be a two-fold increase in mean revertant colony numbers over that of the vehicle control (dimethyl sulfoxide) with strain TA98 at 500 and 1000 µg/plate without metabolic activation. This increase was neither dose-related nor reproducible in a repeat assay. There were no compound-related increases in the number of revertant colonies over the controls for the other 4 tester strains. The increases in the number of revertants as a result of treatment with the positive control compounds demonstrated the capability of the system to detect mutagens in this assay. It was concluded that DHALIP-NS is negative in the Ames test.

## **Exploratory Acute Oral Limit Study of DHALIP-NS in Mice**

51. This (non-GLP) study was performed to assess the acute oral toxicity of DHALIP-NS (yellowish oil) in mice when administered as a single oral gavage dose. The compound (suspended in 0.5% carboxymethyl cellulose and 0.1% Tween 80 in distilled water) was dosed to 5 male and 5 female CD mice at 2000 mg/kg bw/day. The animals were observed for clinical signs at approximately 1, 2.5 and 4 hours after dosing and daily thereafter. Body weights were recorded before and after fasting on Day 0 and on post-dosing day 7. The animals were killed on day 7, post dosing.
52. There were no treatment-related deaths or clinical signs, or significant effects on body weight and gross necropsy related to administration of the test substance at a dose of 2000 mg/kg bw/day.

## **Laying Hen Study**

53. A target animal safety trial with laying hens was conducted using dried DHA-rich microalgae of the genus *Schizochytrium* at three different dose levels: 82, 240, and 408 mg DHA/bird per day. Each treatment consisted of 64 laying hens divided into eight replicates per group with a total of 320 animals. Body weights, food conversion, egg production, egg weight, shell thickness and interior quality were measures at the end of the month for each of the four dosing periods. Eggs were also collected and analysed at the end of months 2 and 4 for their weight, shell thickness, interior egg quality and fatty acid profile.
54. At the end of the four-month period, two randomly selected hens from each dose level and replicate were killed and evaluated for haematological and histopathological changes. Blood clotting time was also determined, since it is known that fatty acids lead to a decrease in platelet reactivity. Gross necropsy was completed on all layers that died during the study or killed for scheduled evaluation. Weights were determined for most of the major organs and a range of tissues were studied for fatty acid content. The results of the experimental diets were determined via statistical analysis.
55. The results showed that there were no significant differences in any of the organ weights measured. No alterations were noted in the histopathological study, and there were no significant differences between treatments for any of the haematological analyses.
56. It was concluded, based on this study, that dried DHA-rich microalgae of the genus *Schizochytrium* is safe as a feed ingredient for laying hens at 3,040 mg/kg body weight/day dried microalgae delivering 532mg DHA/kg body weight/day.

## **Broiler Chicken Study**

57. A similar target animal safety trial with broiler chickens was carried out using 2240 birds. They were sexed and randomly assigned to one of four dietary treatments, including three different levels of DHA-rich microalgae and one control group. 560 broilers were included in each group, and then divided into eight replicates, with 70 birds in each, 35 each of both sexes.
58. The same studies that were carried out with the laying hens were also used to analyse the diet affect on the broiler chickens.
59. The results indicated that there was no effect of treatment level on any of the evaluated broiler growth performance measures. No significant difference regarding weight gain, feed intake or feed conversion between treatment levels were noted. No histopathological or haematological differences were observed between the treatment groups.
60. Based on these results, it was concluded that dried DHA-rich microalga is safe as a feed ingredient for broiler chickens at 2331 mg/bird/day delivering approximately 408 mg DHA/bird/day.

### **Human Clinical Study**

Following consideration of the animal data, the Committee requested further confirmatory information in humans, and this was subsequently provided in January 2002.

61. The aim of the study was to evaluate the effects of consuming 1.5g/day of DHA oil from DHA-Gold, considered to be in excess of estimated usage, on plasma lipids, haematology, biochemical markers of liver and cardiac functions, and certain haemostatic risk factors, which include plasma fibrinogen concentration, factor VII coagulant activity, C-reactive protein concentration, plasminogen activator inhibitor type 1 (PAL-1) activity and von Willebrand factor antigen concentrations (vWF). Platelet counts were also included in the study, since studies with fish oils have reported a fall in platelet count.
62. Seventy-nine individuals were divided randomly between two groups, the test group and the control, each containing approximately equal numbers of male and female subjects. Individuals in the test group each consumed 4 capsules containing 1000mg of oil per day, providing 1.5g DHA from DHA Gold. The control group received an olive oil placebo, which has been shown to be inert.
63. Responses were assessed by measurements made on entry to the trial, day 1 and on days 28 and 29 of each treatment. Measurements included seated blood pressure and body weight. Fasting blood samples were collected on test days 1 and 28/29, and were used to determine blood counts, erythrocyte fatty acid composition, serum lipids, glucose, creatine kinase activity and liver function tests. Citrated samples were collected to determine PAL-1 activity, fibrinogen and factor VII coagulant activity. Further blood samples were also obtained for lipid analysis on these days.

64. Subjects recorded signs of illness, medication used, menstrual phase and deviations from the protocol in a diary. At the end of the study, subjects completed a questionnaire about their appreciation of the treatment, and any side effects experienced.

65. This study demonstrated that a consumption of 1.5g DHA daily as DHA Gold resulted in the expected changes in serum lipids within the normal ranges. A rise in plasma concentration of LDL cholesterol was noted, but this was accompanied by an increase in HDL cholesterol, and no overall net change in the LDL/HDL ratio was observed. DHA Gold was well tolerated among the test group, with no adverse effects on liver function, cardiac enzymes, glucose metabolism, and haematology markers of inflammation or haemostatic function being recorded, apart from the statistically significant increase in Factor VIIc. However, an increase is also seen with fish oils and is a result of the compensatory increase in clotting in response to the known effects of DHA on platelet vessel wall interactions. Therefore, it is of no toxicological concern.

## 66. Discussion and Conclusion

*The safety of fatty acids present in DHA-rich oil is based on four factors:*

- i) Extensive knowledge of fatty acid metabolism.*
- ii) Extensive previous exposure due to their high level in background human diet and the small quantities expected to be present in foods using DHA-rich oils.*
- iii) Published literature on the safety of fatty acid components and comparable oils.*
- iv) Confirmatory safety studies.*

*The safety of phytosterols found in DHA-rich oil is based on five factors. These factors allow a conclusion that an intake of phytosterols present in DHA-rich oil is safe:*

- i) Experience of use due to their natural abundance in food and low levels expected to be used.*
- ii) Extensive knowledge of the absorption, distribution, metabolism and excretion of phytosterols in mammalian species.*
- iii) Extensive safety information as the result of testing these and similar phytosterols.*
- iv) Easy identification of at risk populations.*
- v) Confirmatory safety studies.*

*A number of confirmatory toxicological studies have been conducted with the DHA-rich microalgae, including 90-day rat feeding studies, teratology studies in the rat and rabbit and a single generation reproduction study in the rat. The NOEL for DHA in these animal studies ranged from 153 - 1,868 mg/kg/day, although in some cases these levels were the top tested and the actual NOEL could be higher.*

*The periportal vacuolation seen in the 90-day rat study is likely to be the result of the high fat level in the diet and therefore does not represent an adverse effect as such. There is, however, a slight increase in the incidence, but not in the severity, of cardiomyopathy in the high dose male rats in this study, which may be a result of treatment. However, it was not within the test protocol to histologically examine the heart in the single-generation rat reproduction study. The lesion seen was similar to that seen spontaneously in ageing rats of this strain. An expert panel in the United States reviewed the cardiomyopathy data. They concluded that the treatment related findings of the 13-week study had little relevance to the safety assessment of the use of DHA as a nutritional ingredient for humans. The ACNFP sought expert advice from an animal pathologist from the Committee on Toxicity. The same conclusion was drawn that the presence of heart lesions in the rat was of no significance in the safety evaluation of DHA for use in humans.*

*In the USA, DHA Gold<sup>®</sup> has GRAS status at an intake level of 1.5g a day. This figure was based on the presence of DHA in human breast milk, although the safety data indicated the oil was safe at higher intake levels. The level of 1.5g a day is considered to be an adequate upper level of intake when considering individual Member States recommendations to achieve the intended effects, which include key cardiovascular and immune system health benefits.*

*The results of the 90 day rat feeding study indicates that the NOEL for DRM in the rat 90 day feeding study is at least 4000mg/kg/day (the highest dose tested), which is equivalent to an intake of 340mg/kg/day of DHA. No significant adverse effects were seen in either of the teratology studies or the single-generation reproduction study in the rat, other than slight reductions in food intake and body weight gain at high levels of incorporation of DRM in the diet. If the recommended dose for DHA were accepted to be 1500mg/person/day, this would equate to 25 mg/kg/day DHA for a 60-kg person, which would give a safety factor of 13.5 in relation to the rat study.*

*Published and unpublished scientific data relating to Schizochytrium sp., the species from which DHA-rich oil is derived, have not shown any adverse effects that are relevant to the safety of this oil for humans. There have been no reports of toxic compounds being produced by Schizochytrium sp., it occurs widely in the marine environment and is an indirect component of the human food chain.*

*The Committee agreed that a formal study in humans to confirm the safety of the oil was needed. The study provided by the Company was able to demonstrate that the inclusion of DHA Gold in the diet of human volunteers, at a level considered to be in excess of expected usage, had no effect on target parameters.*

*The study tested the Null Hypothesis that the mean values did not differ between treatments, since the aim of the safety testing is to demonstrate toxicity rather than to demonstrate equivalence. The study was designed to detect 1SD unit change in all the variables of interest, since a value of less*

*than 1 unit is considered unlikely to be of clinical significance. A sample size of 32 subjects has the power to detect a 0.7 SD unit change, and so the study recruited 40 individuals to allow for dropouts. This Committee was satisfied with the statistical power of the study.*

## **OVERALL DISCUSSION**

67. The application dossier contains good product specification data and a detailed description of the production process. The process is well monitored, with quality control and safety measures in place. The product is manufactured using a standard method, which has been shown to be both reliable and reproducible.

68. No nutritional concerns have been raised, since the entire product components are already present to some degree, as background, in human diets, and there is a recommendation generally for an increase in the level of DHA in the diet.

69. The product has undergone toxicological testing, and there are data from animal and other studies to support the safety of the derived oil. The inclusion of a human clinical trial provided further reassurance as to the safety of the DHA-Gold™ ingredient.

70. If this product is approved, the Applicant Company will make recommendations to food manufacturers regarding appropriate intake levels for DHA and appropriate labelling for the final food product. The Applicant Company has agreed to make recommendations to food manufacturers as described in the application dossier. Final products will need to be labelled with the ingredient name and the prescribed nutritional labelling. Since the oil is to be used as a nutritional ingredient, any claims made on the food due to the inclusion of the oil must comply with the general criteria for making nutrient content claims. Also, any health claims made will have to comply with the appropriate legislation in this area, regardless of any nutritional claim made.

## **Conclusion**

The Advisory Committee on Novel Foods and Processes is satisfied by the evidence provided by Omegatech that DHA Gold™ is safe for use as a nutritional food ingredient, for the types of uses as described in the application dossier, subject to the labelling requirements described above.