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Toxicologic evaluation of DHA-rich algal oil: Genotoxicity, acute and subchronic toxicity in rats

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ABSTRACT

DHA-rich algal oil ONC-T18, tested in a battery of *in vitro* and *in vivo* genotoxicity tests, did not show mutagenic or genotoxic potential. The acute oral LD50 in rats has been estimated to be greater than 5000 mg/kg of body weight. In a 90-day subchronic dietary study, administration of DHA-rich algal oil at concentrations of 0, 10,000, 25,000, and 50,000 ppm in the diet for 13 weeks did not produce any significant toxicologic manifestations. The algal oil test article was well tolerated as evidenced by the absence of major treatment-related changes in the general condition and appearance of the rats, neurobehavioral endpoints, growth, feed and water intake, ophthalmoscopic examinations, routine hematology and clinical chemistry parameters, urinalysis, or necropsy findings. The no observed adverse effect level (NOAEL) was the highest level fed of 50,000 ppm which is equivalent to 3,305 and 3,679 mg/kg bw/day, for male and female rats, respectively. The studies were conducted as part of an investigation to examine the safety of DHA-rich algal oil. The results confirm that it possesses a toxicity profile similar to other currently marketed algal oils and support the safety of DHA-rich algal oil for its proposed use in food.

1. Introduction

DHA-rich algal oil is extracted and refined from the wild-type heterotrophic micro-algae *Schizochytrium* sp. ONC-T18. This micro-algae is a member of the *Thraustochytriaceae* family which historically has been comprised of seven genera, *Japanochytrium*, *Schizochytrium*, *Ulkenia*, *Althornia*, *Diplophrys*, *Aplanochytrium*, and *Thraustochytrium* (Burja et al., 2006). It is a mixture of triglycerides containing polyunsaturated fatty acids (PUFA) in which the predominant fatty acid (>35%) is docosahexaenoic acid (DHA).

DHA is an omega-3 fatty acid that has been widely studied for its beneficial effects on human health, particularly brain, eye and heart health. Along with the omega-3 fatty acid arachidonic acid (ARA), it is commonly found in most commercial infant formula, either from algal or tuna oil sources. DHA is accumulated in the brain during gestation, and early infancy and is continually replenished from the plasma (Innis, 2005). Neurodevelopment progresses rapidly during this time and demands for DHA are high to ensure neurite outgrowth and proper brain and retina development (Marszalek and Lodish, 2005). DHA has a prominent role in the development of photoreceptors and synaptic networks (Kurlak and Stephenson, 1999), with as much as two thirds of the fatty acids in the retinal photoreceptor phospholipid membranes being 22 carbon omega-3 fatty acids (Bazan et al., 1986). DHA affects neuronal excitability and transmission by modulating ion channel function and the resulting flow of ions (Assisi et al., 2006). It may interact with membrane proteins and affect signal transduction pathways that activate transcription factors such as steroid hormones and glucocorticoids. These transcription factors can affect

Abbreviations: 2AA, 2-aminoanthracene; 2NF, 2-nitrofluorene; ALB, albumin; ALKP, alkaline phosphatase; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Ca, calcium; CFR, Code of Federal Regulations; Cl, chloride; CHOL, total cholesterol; CP, cyclophosphamide; DHA, docosahexaenoic acid; DMSO, dimethylsulfoxide; FOB, functional observation battery; GLOB, globulin; GLUC, fasting glucose; GRAS, Generally Recognized As Safe; Hct, hematocrit; Hgb, hemoglobin concentration; K, potassium; LD_{50}, median lethal dose; MA, motor activity; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; mg/kg, milligrams per kilogram; MMC, mitomycin C; Na, sodium; NAAZ, sodium azide; NOAEL, no observed adverse effect level; NCE, normochromatic erythrocytes; OECD, Organisation for Economic Co-operation and Development; ONC, Ocean Nutrition Canada; PCE, polychromatic erythrocytes; PUFA, polyunsaturated fatty acids; RBC, red blood cell count; REC, recovery group; SDH, sorbitol dehydrogenase; TOX, main test group; TP, total protein; TRIG, triglycerides; US FDA, United States Food and Drug Administration; WBC, total white blood cell count.

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the expression of a number of different genes, including those for enzymes involved in downstream signal transduction pathways (Assisi et al., 2006). Another possible role for DHA in the brain may be to enhance the activity of catalase and glutathione, thereby providing greater protection from free radicals and oxygen reactive species (Hossain et al., 1999).

Schizochytrium and *Ulkenia*-based oils are currently globally marketed as DHA-rich oils. The oil from *Schizochytrium* sp. ONC-T18 has an identical proximate composition and a closely similar lipid profile to that of the presently marketed oil from *Schizochitrium* sp. ATCC 20888 produced by Martek Biosciences (now a part of DSM). This is based on an identical proximate composition (both substances being essentially 100% fat) and a closely similar fatty acid profile (see Table 1). At the intended levels of use (60–600 mg DHA-equivalent/100 g of food), the small differences in lipid profiles will have no significance on their relative nutritional value or metabolic impact. Specifications for DHA-rich algal oil ONC-T18 include analysis for omega-3 content, heavy metal and microbial contamination, and other common oil quality measures.

DHA-rich algal oil ONC-T18 is intended for use in an identical manner and same foods as the currently marketed oil. Therefore, it will replace, rather than add to, intake from the currently marketed oils. DHA has been incorporated into a variety of food products with specific limitations including, but not limited to, breads, cereals, fats and oils, condiments, yogurt, cheese, frozen dairy, meat, egg, nut, and fish products (Kroes et al., 2003).

The potential toxicity of various algal oils rich in DHA have been previously studied and its safe use in food evaluated by numerous government agencies and regulatory authorities. Published preclinical studies include subchronic, genotoxicity, and developmental and reproductive toxicity studies (Fedorova-Dahms et al., 2011a,b; Kroes et al., 2003; Burns et al., 1999; Hempenius et al., 2000; Hammond et al., 2001a,b,c, 2002; US FDA 2004a,b, 2010). Numerous clinical trials have been conducted on DHA-containing fish and marine-based oils. The trials have included adults, children, and infants as DHA oil is used commercially in infant formula. Arterburton et al. (2007) stated that algae are the primary producers of DHA in the food chain, and algal sources of DHA are available for fortification of infant formulas and food, and for dietary supplements for adults and pregnant women. The clinical safety of DHA-rich oils has been reviewed by Kroes et al. (2003) and Arterburton et al. (2007) and found to be safe for human use.

The safety of dietary DHA and oils produced from both fish and algal sources is well established in the literature. In affirming the Generally Recognized as Safe (GRAS) status of menhaden oil (62 FR 30751; June 5, 1997), FDA concluded that the use of menhaden oil as a direct food ingredient is GRAS, provided that the combined daily intake of DHA and eicosapentaenoic acid (EPA) does not exceed 3 g/day. The proposed uses of DHA-rich algal oil ONC-T18 comply with this requirement, and when used at the intended levels, the nutritional value and metabolism of the oil from *Schizochytrium* sp. ONC-T18 is indistinguishable from that of the presently authorized and marketed oils.

DHA rich oils from micro-algal sources have been the subject of four authorization decisions and/or notifications under the EU Novel Food Regulation 258/97. The first such measure was Commission Decision 2003/427/EC in June of 2003 authorizing the use of DHA-rich oil from the thraustochytrid micro-algae *Schizochytrium* sp. in a range of foodstuffs and establishing a specification for the material. This was followed in December 2003 by a notification under Article 5 of the novel food regulation for placement on the market of a DHA-rich oil derived from a second thraustochytrid micro-algae *Ulkenia* sp. on the grounds of its substantial equivalence with the oil from *Schizochytrium* sp. In 2009 Commission Decisions 2009/777/EC and 2009/778/EC authorized extensions to the approved food uses of the oils from *Ulkenia* sp. and *Schizochytrium* sp., respectively. A third DHA-rich oil derived from the micro-algae *Crypthecodinium cohnii* was already on the EU market before the Novel Food Regulation came into effect and was therefore legally in use without the need for explicit approval. These three DHA rich oils have also been the subject of GRAS notifications to which the FDA had no objections (U.S. FDA GRN nos. 41, 137, 138, 319).

The present studies were conducted as part of an investigation to examine the safety of DHA-rich algal oil. The results reported herein demonstrate a similar toxicity profile as exists for other algal-based oils.

Table 1
Fatty acid composition of DHA-rich algal oils.

	Schizochytrium sp. ATCC 20888[1]	*Schizochytrium* sp. ONC-T18
Fatty acid	Mean[2]	Mean[3]
Laurate	0.40	1.10
Myristate	10.11	13.77
Tetradecatrienoate	Trace-0.45	ND
Palmitate	23.68	26.57
Palmitoleate	1.76	2.47
Hexadecatrienoate	Trace-0.5	ND
Stearate	0.45	0.80
Oleate	ND	0.43
Vaccenate	Trace-1.36	2.10
Linoleate	ND	0.07
Octadecatetraenoate	Trace-0.85	0.20
Dihomo-gamma Linolenate[4]	2.21	0.10
Arachidonate	0.94	0.23
Eicosatetraenoate	0.87	0.47
Eicosapentaenoate (EPA)	2.63	0.87
Docosatraenoatenoate	0.54	ND
Docosapentaenoate	13.50	7.90
DHA	35.00	40.23

[1] http://www.food.gov.uk/multimedia/pdfs/dhagolddossier.pdf.
[2] 5 lots; measured and expressed as weight%.
[3] 3 lots; measured and expressed as area%.
[4] *Schizochytrium* sp. ATCC 20888 EU novel food submission designates as co-eluting with Eicosatetraenoate 20:4n-7.

2. Materials and methods

The test material and methods employed are described for each individual study. In all studies, the stated concentrations or doses reflect the amount of algal oil administered, and appropriate control groups were employed as necessary.

2.1. Test material

ONC T-18 DHA-rich algal oil, a frozen liquid (lots no. 22630 and 22740; approximately 39–42% DHA; total omega-3 fatty acids, approximately 41–44%; storage condition, frozen, −10 to −30 °C) and the DHA fish oil (tuna) control article were produced and received from Ocean Nutrition Canada Limited, Dartmouth, Nova Scotia, Canada. The tuna oil control (lot no. 23442) had a DHA concentration of 26% and an EPA content of 7%. Ascorbyl palmitate and mixed natural tocopherols were added to both the algal oil and tuna oil to prevent oxidation.

2.2. Genotoxicity studies

All genotoxicity studies were conducted in accordance with U.S. Food and Drug Administration (21 CFR Part 58), and the OECD Principles of Good Laboratory Practice ENV/MC/CHEM (98)17).

2.2.1. Microbial reverse mutation assay

The assay design was based on OECD Guideline 471 (OECD, 1997) and ICH Guidelines S2A and S2B (ICH, 1995, 1997). *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2*uvrA* (328) were obtained through third parties from Dr. Bruce Ames, University of California, Berkeley, CA and the National Collection of Industrial and Marine Bacteria, Torrey Research Station, Scotland, UK, respectively.