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Toxicologic evaluations of DHA-rich algal oil in rats: Developmental toxicity study and 3-month dietary toxicity study with an *in utero* exposure phase

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ABSTRACT

DHA-rich algal oil ONC-T18, tested for subchronic, reproductive, and developmental toxicity in the rat, did not produce any significant toxicologic manifestations. Based on the absence of maternal or developmental toxicity at any dosage level, a dosage level of 2000 mg/kg/day was considered to be the no observed adverse-effect level (NOAEL) for maternal toxicity and embryo/fetal development when DHA-rich algal oil was administered orally by gavage to pregnant Crl:CD(SD) rats during gestation days 6–19. In a dietary combined one-generation/90-day reproductive toxicity study in rats, the NOAEL for F0 male and female and F1 male systemic toxicity was considered to be 50,000 ppm (highest concentration administered) and 25,000 ppm for F1 female systemic toxicity (higher mean body weight, body weight gain, and food consumption). F0 reproductive performance values, estrous cycle length, gestation length, or the process of parturition, and the numbers of former implantation sites and unaccounted-for sites were unaffected by algal oil exposure. Postnatal survival and developmental parameters in the F1 generation were unaffected by algal oil exposure at all dietary concentrations. There were no neurotoxic effects noted at any algal oil exposure level. The results 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

Abbreviations: 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; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; F_0, parenteral generation; F_1, first generation; FOB, functional observation battery; GGT, gamma glutamyltransferase; GLOB, globulin; GLUC, glucose; GRAS, Generally Recognized As Safe; Hct, hematocrit; Hgb, hemoglobin concentration; HDW, red cell distribution width; K, potassium; MA, motor activity; LinTrt*Time, linear dose by time interaction; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; mg/kg, milligrams per kilogram; MPV, mean platelet volume; Na, sodium; NOAEL, no observed adverse effect level; OECD, Organisation for Economic Co-operation and Development; ONC, Ocean Nutrition Canada; PND, postnatal day; ppm, parts per million; PUFA, polyunsaturated fatty acids; RBC, red blood cell count; RRV, rat respiratory virus; SDH, sorbitol dehydrogenase; SOP, standard operating procedure; TIME, time interval; TP, total protein; TRIG, triglycerides; TRT, treatment group; TRT*TIME, time interval and treatment group; US FDA, United States Food and Drug Administration; WBC, total white blood cell count.

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interact with membrane proteins and affect signal transduction pathways that activate transcription factors such as steroid hormones and glucocorticoids. These transcription factors can affect 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. The nutritional value and metabolism of the oil under study is virtually indistinguishable from the previously authorized and marketed oil from *Schizochytrium* 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. 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 (see Table 1).

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 (Kroes et al., 2003; Burns et al., 1999; Hempenius et al., 2000; Hammond et al., 2001a, 2001b, 2001c, 2002; U.S. FDA, 2001, 2004a, 2004b, 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. Atterburton 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 Atterburton et al. (2007) and found to be safe for human use.

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

	Schizochytrium sp. ATCC 20888[a]	*Schizochytrium* sp. ONC-T18
Fatty acid	Mean[b]	Mean[c]
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[d]	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

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

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 the fish-based 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 grams/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 as described below.

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 EC Commission Decision (2003) 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 EC Commission Decision, 2009a,b 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, 319).

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

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. Prenatal developmental toxicity study

2.1.1. Test material and animals

ONC T-18 DHA-rich algal oil, a frozen liquid (lot no. 22630; approximately 42% DHA; total omega-3 fatty acids, 44%; storage condition, frozen, −10 to −30 °C) was obtained from Ocean Nutrition Canada Limited, Dartmouth, Nova Scotia, Canada.

Sexually mature, virgin female Sprague Dawley [Crl:CD(SD)] rats were used as the test system on this study (Charles River Laboratories, Inc., Raleigh, NC). This species and strain of animal is recognized as appropriate for developmental toxicity studies. Upon arrival and until pairing, all rats were individually housed in clean, stainless steel wire-mesh cages suspended above cage-board. The rats were paired for mating in the home cage of the male. Following positive evidence of mating, the females were returned to individual suspended wire-mesh cages; nesting material was not required as the females were euthanized prior to the date of expected parturition. Animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011). Test animals were provided continuous access to tap water and PMI Nutrition International, LLC Certified Rodent LabDiet® 5002.