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Confidentiality	**None**

TITLE:
DHA HISTORY AND UTILIZATION

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ABBREVIATIONS

5-HIAA	5-hydroxyindoleacetic acid
AI	adequate intake
ALA	alpha-linolenic acid
AMDR	Acceptable Macronutrient Distribution Ranges
ANDA	Abbreviated New Drug Application
ARA	arachidonic acid
DGLA	dihomo-γ-linolenic acid
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
FA	fatty acid(s)
GLA	γ-linolenic acid
L	liter
LA	linoleic acid
LC-PUFA	long chain polyunsaturated fatty acid(s)
M	million
mg	milligram
µL	microliter
µM	micromolar
NDA	New Drug Application
No.	Number
PUFA	polyunsaturated fatty acid(s)
®	registered trademark
§	section
™	trademark
UL	upper limit
USDA	United States Department of Agriculture
VLC-PUFA	very long chain polyunsaturated fatty acid(s)
ω	omega
ω3	omega-3
ω6	omega-6

EXECUTIVE SUMMARY

The seed of NS-B5ØØ27-4 **DHA canola** is expected to contain omega-3 fatty acid (ω3 FA) not otherwise present in canola seed, such as eicosapentaenoic acid (EPA), docosapentaenoic (DPA), and particularly docosahexaenoic acid (DHA). For example, the amount of EPA, DPA, and DHA fatty acid is expected to be significant (%wt. total fatty acid) in seed oil from NS-B5ØØ27-4 **DHA canola**. This report discusses the safety and nutritional impact of ω3 FA and particularly DHA.

I. OMEGA-3 FATTY ACID

Fatty acids (FA) are carboxylic acids with long-chain hydrocarbon side groups, typically found in esterified form as the major component of lipids. Lipids and fatty acids are sources of energy, integral in cell membranes, and indispensable for processing biological and biochemical information. ω3 FA are essential FA: required for human health and obtained primarily from diet. ω3 FA are a group of polyunsaturated fatty acids (PUFA) that are important for a number of bodily functions, including muscle activity, blood clotting, digestion, fertility, cell division and growth, and reducing inflammation (Gogus & Smith 2010). ω3 PUFA play a critical role in the development and function of the central nervous system (Bourre 2004; Dyall 2015). Indeed, approximately 20% of the dry weight of the brain is made up of PUFA, and one out of every three fatty acids in the central nervous system is PUFA (Logan 2004). A number of countries (Canada, Sweden, United Kingdom, Australia, Japan) as well as the World Health Organization and North Atlantic Treaty Organisation have made formal population-based dietary recommendations for omega-3 fatty acids (Kris-Etherton et al. 2002).

The three principal ω3 FA are α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). DHA is a primary structural component of many human tissues, and is important for brain development and function (Logan 2004; Dyall 2015).

II. OMEGA-3/ OMEGA-6 FATTY ACID BALANCE

Omega-6 fatty acids (ω6 FA) are also essential fatty acids: required for human health and obtained primarily from diet. Along with omega-3 fatty acids, ω6 FA play a crucial role in brain function, and normal growth and development. As a type of PUFA, ω6 FA help stimulate skin and hair growth, maintain bone health, regulate metabolism, and maintain the reproductive system. Most ω6 FA in the diet is in vegetable oils, such as linoleic acid (LA) (not to be confused with α-linolenic acid (ALA), which is an ω3 FA). LA is converted to γ-linolenic acid (GLA) in the body. It is metabolized further to arachidonic acid (AA). GLA is found in several plant-based oils, including evening primrose oil, borage oil, and black currant seed oil. Although some ω6 FA tend to promote inflammation, GLA can be converted to dihomo-γ-linolenic acid (DGLA), which reduces inflammation. Having enough of certain nutrients in the body (including magnesium, zinc, and vitamins C, B3, and B6) helps promote the conversion of GLA to DGLA.

Importantly, a proper ratio of ω6 FA to ω3 FA avoids metabolic problems such as imbalance of membrane fluidity. Membrane fluidity affects function of enzymes such as adenylate cyclase and ion channels such as calcium, potassium, and sodium, which in turn affects receptor numbers and functioning, as well as serotonin neurotransmitter levels. The ideal dietary ratio of ω6 FA to ω3 FA has been recommended by an international panel of lipid experts to be about 2:1; or less than 4:1 (Logan 2004; Dyall 2015). However, the dietary intake of ω3 FA has dramatically declined in Western countries over the last century. Most modern societies have imbalanced diets in which at least 90% of PUFA are ω6 FA (Dyall 2015) with ω6 FA:ω3 FA ratios exceeding 20:1. It is thus evident that western diets are deficient in ω3 FA and excessive in ω6 FA; and that balancing of this ratio would confer numerous health benefits (Logan 2004; Gogus & Smith 2010). There is mounting evidence supporting the beneficial effects of an increased intake of ω3 PUFAs in a variety of neurodegenerative and neurological conditions (Dyall 2015). Seed oil from NS-B5ØØ27-4 is expected to provide a source of dietary DHA.

III. DHA STRUCTURE, SYNTHESIS, AND FUNCTION

DHA is a long chain polyunsaturated fatty acid (LC-PUFA), described structurally as a carboxylic acid (-oic acid) consisting of a 22-carbon chain (docosa- *Greek* for 22) with six (hexa-) *cis* carbon double bonds (-en) (C=C), with the first double bond located at the third carbon from the ω-end of the FA (ω3). The chemical formula of DHA is $C_{22}H_{32}O_2$. DHA's systematic name is "all-*cis*-docosa-4,7,10,13,16,19-hexa-enoic acid." In fatty acid nomenclature, DHA referred to as $22{:}6^{(4,7,10,13,16,19)}$, C22:6(n-3) or C22:6(ω3), or simply 22:6(n-3) or 22:6(ω-3). DHA's trivial name is "cervonic acid."

DHA is considered an accessory nutrient by the USDA. The term "accessory nutrient" refers to substances that are not specifically classified as vitamins or minerals but are found to promote optimal health. Accessory nutrients can be contrasted with the essential nutrients such as LA and ALA FA, which cannot be synthesized by the body (IFC Int'l 2011).

DHA is primarily obtained from diet, although it can be synthesized to some extent from ALA, an essential FA, through a series of elongations and desaturation, including the intermediates EPA (C20:5(n-3)) and DPA (C22:5(n-3)). The main sources of ALA in the U.S. diet are vegetable oils, particularly canola and soybean oils. Generally flaxseed, hemp and walnut oils also provide rich sources of ALA. Dietary ALA can be metabolized in the liver to the longer-chain EPA and DHA, but this conversion is limited in human beings: at best only 5–15% of ALA is ultimately converted to DHA (Swanson et al. 2012). DHA may also be converted from EPA that is elongated to a C24:5 ω3 FA, desaturated to C24:6 ω3 FA, and finally shortened to DHA via beta oxidation (Voss et al. 1991). Aging, illness, stress, and consuming excessive amounts of ω6-rich oils can compromise conversion of precursor FA to DHA (Logan 2004). Therefore, sources of LC-PUFA remain important in the diet. DHA can be obtained directly from maternal milk (breast milk), fish oil, or algal oil. The main sources of EPA and DHA are seafood, including fatty fish (e.g., salmon, tuna, and trout), shellfish; and algal oils provide a vegetarian source of DHA. Commonly used dietary supplements that contain ω3 FA include fish oil (which provides EPA and DHA), and flaxseed oil (which provides ALA).

As noted, DHA provides many health benefits: DHA is an essential component of cell membranes of various tissues and organelles in mammals (e.g., nerve, retina, brain, and immune cells). DHA is quantitatively the most important omega-3 PUFA in the brain, and consequently the most studied. Clinical studies have shown that DHA is essential for the growth and development of the brain in infants, and for maintenance of normal brain function in adults; has significant effects on photoreceptor function involved in the signal transduction process, rhodopsin activation, and rod and cone development; and provides some positive effects on diseases such as hypertension, arthritis, atherosclerosis, depression, thrombosis and cancers. There are high concentrations of DHA in synaptic membranes of the brain, which is critical for synaptic transmission and membrane fluidity (Logan 2004). There is mounting evidence supporting the beneficial effects of an increased intake of omega-3 PUFA in a variety of neurodegenerative and neurological conditions (Dyall 2015). Low plasma concentrations of DHA predict low concentrations of cerebrospinal fluid 5-hydroxy-indoleacetic acid (5-HIAA); and low concentrations of 5-HIAA in the brain is associated with depression and suicide (Rees et al. 2005). DHA and EPA have also been shown to protect against cancers by suppressing neoplastic growth, inhibiting angiogenesis, inducing apoptosis, suppressing omega-6 eicosanoid production, and inhibiting mammary, colon, and prostate carcinogenesis and tumor growth (Nat'l Acad. 2005; Gogus & Smith 2010). Because such fatty acids cannot be efficiently synthesized by infants, young children and senior citizens, it is particularly important for these individuals to adequately intake these fatty acids from the diet (Dyall 2015).

The current commercial source of DHA is fish oil. Marine stocks are diminishing as a result of over-fishing, and alternative sustainable sources of EPA and DHA are needed to meet increasing demand. Numerous efforts have been made to develop transgenic oilseed plants that produce VLC-PUFAs, including DHA (Dyall 2015).

IV. DHA HISTORY OF SAFE USE

Human breast milk reportedly has a fatty acid profile comprising from about 0.15% - 0.36% DHA, about 0.03% - 0.13% EPA, about 0.30% - 0.88% arachidonic acid (ARA), from about 0.22% - 0.67% DGLA, and from about 0.27% - 1.04% GLA (Das et al. 2014). Worldwide, however, the range of DHA concentrations in human milk are much broader than the ranges that have been evaluated in clinical trials (ICF Int'l 2011).

Both the USDA and the American Heart Association and American Stroke Association recommend eating fish two to three times per week, primarily for vascular health (USDA 2011; Kris-Etherton et al. 2002). The 2010 U.S. Department of Health and Human Services dietary guidelines recommend that women who are pregnant or breastfeeding should consume 8 to 12 ounces of seafood per week from a variety of seafood types (Swanson et al., 2012). According to the UK Scientific Advisory Committee on Nutrition guidelines (2004), a healthy adult should consume a minimum of two portions of fish a week to obtain the health benefit of LC-PUFA (ICF Int'l 2011).

The acceptable macronutrient distribution (AMDR) for ALA is 0.6% - 1.2% of energy; 10% of which can be consumed as EPA and/or DHA. The National Academy of Sciences *Dietary Reference Intakes* notes that the physiological potency of EPA and DHA is much greater than that for α-linolenic acid, making it impossible to estimate one AMDR for all ω3 FA. The adequate intake (AI) set for ω3 FA (as ALA) according to age and sex is shown in Table 1. The AI for ALA has no upper limit (UL). EPA and DHA contribute about 10% of the total omega-3 FA intake, and thus this percent contributes to the AI for ALA (Nat'l Acad. 2005).

Table 1. Adequate Intake for Omega-3 (ALA)

	ADEQUATE INTAKE
AGE GROUP	OMEGA-3 (AS ALA)
Infants-12 months	0.50 g/day
Children 1-3	0.7 g/day
Children 4-8	0.9 g/day
Boys 9-13	1.2 g/day
Girls 9-13	1.0 g/day
Boys 14-18	1.6 g/day
Girls 14-18	1.1 g/day
Men	1.6 g/day
Women	1.1 g/day
Pregnancy	1.4 g/day
Lactation	1.3 g/day

A large variety of fish and fish products, as well as meat, dairy, margarine, and egg products containing DHA are available in grocery stores and supermarkets. Whereas fish and fish products contain the highest amounts of DHA, eggs, milk, and margarine also provide some dietary DHA (ICF Int'l 2011). Fish oils are the most common source of DHA for enrichment of foods and supplementation and provide the highest amounts of DHA. Some fish oils were found to have higher levels of EPA than DHA, and supplementation of infant formula with these oils was apparently associated with adverse effects on infant growth (ICF Int'l 2011). Nevertheless, the FDA (ICF Int'l 2011) considers Menhaden oil, the fish oil with the highest DHA and EPA content, Generally Recognized as Safe (GRAS).

Additional sources of DHA are also GRAS. For example, DHA-rich single-cell oil (DHASCO), as a source of dietary DHA, is GRAS according to FDA GRAS Notice No. GRN 000041. The FDA began permitting use of DHASCO in infant formula in 2001. DHASCO contains approximately 40% DHA, and the use level corresponded to a maximum of 0.5% of total fat as DHA. Another oil, DHA Algal Oil as a source of DHA, is GRAS when used alone or in combination with ARA or EPA according to FDA GRAS Notices No. GRN 000041 and No. GRN 000137. DHA Algal Oil is obtained from processing of the dinoflagellate *Crypthecodinium cohnii* or the thraustochytrid *Schizochytrium* species. DHA Algal Oil from *C. cohnii* contains 35% - 45% DHA; DHA Algal Oil from *Schizochytrium* species contains about 35% DHA, about 13.5% DPA, and about 3% EPA (Int'l 2011). Additionally, GRAS Notice No. GRN 000094 relates to ARA-rich oil from *Mortierella*

alpina (ARASCO) and DHA-rich tuna oil; at a maximum level of 1.25% each of the total dietary fat and at a DHASCO:ARASCO ratio ranging from 1:1 to 1:2 (ICF Int'l 2011).

GRAS Notice No. GRN 000553 refers to algal oil derived from *Schizochytrium* sp. at a maximum use level of 1.25% dietary fat, corresponding to a maximum use level of 0.5% total fat as DHA. This algal oil was also combined with ARA, at a ratio of DHA:ARA from 1:1 to 1:2. This algal oil contains 91% -95% triglycerides, with the major FA in this algal oil including myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (OA) (18:1 n-9), LA, EPA, and n-6 DPA (22:5 n-6) -- quite different from the DHA Algal Oil from *C. cohnii*. The contemplated daily dietary exposure to this algal oil was based on the assumption that human infants consume 100 to 120 kilocalories (kcal)/kg body weight per day (bw/d), of which fat comprises about 50%: infants consume about 50 to 60 kcal of fat/kg bw/d which corresponds to approximately 5.5 g to 6.7 g of fat/kg bw/d. Based on the maximum use level for algal oil, an infant would consume approximately 75 mg algal oil/kg bw/d (ICF Int'l 2011).

DHA Algal Oil from *Schizochytrium* species is also added to soy protein bars, processed vegetable drinks, hard and soft candies, non-dairy and powdered cream substitutes, jams and jellies, non-dairy milk, imitation milk, and soymilk (Int'l 2011). In a petition to the FDA by Unilever United States, Inc., the "Future Intended Use Levels" of fish oil in milk products was 2.9% by weight. According to 21 CFR § 184.1472(a)(3), menhaden fish oil, may be added to milk at a maximum level of 5.0% to ensure that the intake of EPA and DHA does not exceed 3.0 grams per person, per day. The GRAS notices state that FDA has no objection to the use of these DHA oils under the proposed conditions of use (ICF Int'l 2011).

As noted, DHA is manufactured and sold as a dietary supplement. The DHA in dietary supplements may be obtained from fish oil or from DHA Algal Oil. Fish oil supplements are widely available in grocery and health food stores. According to a 2010 survey by ConsumerLab.com, fish oil/ω3 FA supplements are the top multivitamin among people who use multiple dietary supplements: 74% of respondents reported that they used fish oil/ω3 FA supplements (ICF Int'l 2011).

The NIH has reported that consuming ω3 FA supplements does not usually have negative side effects. When side effects do occur, they typically consist of minor gastrointestinal symptoms, such as belching, indigestion, or diarrhea. It is uncertain, however, whether people with fish or shellfish allergies can safely consume fish oil supplements. Additionally, ω3 supplements may extend bleeding time, such that people taking anticoagulants or nonsteroidal anti-inflammatory drugs should discuss the use of ω3 FA supplements with a health care provider (NIH NCCIH 2015; ICF Int'l 2011).

Genotoxicity studies with algal oil produced utilizing aqueous extraction were compared with genotoxicity studies conducted on algal oil produced utilizing isopropyl alcohol extraction (algal oil-IPA). Subchronic toxicity studies conducted in rats apparently showed that administration of algal oil and algal oil-IPA caused no adverse effects at the highest levels tested (3279 mg/kg bw/d and 4122 mg/kg bw/d, respectively) (ICF Int'l 2011).

The FDA has also licensed several prescription formulations comprising ω3 FA. For example, LOVAZA® (omega-3-acid ethyl esters), NDA #021654 (initial approval as OMACOR®, NDA #021853 approved 2004), provides a 1 g oral capsule containing at least 900 mg of fish oil-derived ω3 FA including about 465 mg EPA and about 375 mg DHA. LOVAZA® is prescribed as an adjunct to diet to treat hypertriglyceridemia (LOVAZA® Package insert). Similarly, OMTRYG™ (Omega-3-acid ethyl esters Type A), NDA 204977 (approved 2014), provides a 1.2 g oral capsule containing at least 900 mg of ω3 FA ethyl esters. Generic versions have also been approved for Omega-3-Acid Ethyl Esters (ANDAs #090973, #091018, #091028, #204940). EPANOVA® (Omega-3-carboxylic acids) capsules (NDA #205060), contain fish-oil derived source of EPA and DHA as an adjunct to diet to treat hypertriglyceridemia.

V. CONCLUSIONS: SAFETY AND NUTRITION ASSESSMENT

In conclusion, the safety of DHA in NS-B5ØØ27-4 **DHA Canola** is based on (a) its occurrence as a product of *in vivo* metabolism of ALA, (b) a long history of consumption of foods that contain DHA, (c) GRAS status of DHA oils for use in foods and dietary supplements, and (d) FDA approval of pharmaceuticals comprising DHA.

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