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Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Supporting Document 2

Nutrition Risk Assessment Report – Application A1143

Food derived from DHA canola line NS-B50027-4

Executive summary

This Application seeks to amend the *Australia New Zealand Food Standards Code* (the Code) to include food derived from canola that has been genetically-modified to contain docosahexaenoic acid (DHA). As the seed oil will be available for human consumption, FSANZ has assessed whether any adverse effects can be expected from an increase in dietary intake of DHA due to consumption of DHA canola seed oil.

DHA is mainly obtained from the consumption of seafood and marine oils with a contribution due to endogenous biosynthesis from dietary α-linolenic acid (ALA). DHA plays a role in physiological functions including regulation of inflammation and immune function, lipid metabolism, and cardiovascular function.

This risk assessment includes a hazard assessment and a dietary intake assessment. The hazard assessment considers the potential adverse effects associated with DHA intake, and also includes information on the Upper Level of Intake (UL) for omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), defined as the sum of DHA, docosapentaenoic acid (DPA) and eicosapentaenoic acid (EPA).

FSANZ has previously assessed animal and *in vitro* toxicity studies conducted with DHA-rich oils from marine algae and concluded that there were no toxicity concerns. The present hazard assessment considered published randomised controlled trials (RCTs) of DHA supplementation in humans. The highest supplemental DHA dose in these studies was 6 g/day with durations of 3–15 weeks. Daily supplementation of up to 6 g DHA was associated with a number of effects that are considered beneficial, for example decreased blood total and HDL cholesterol, decreased blood triglycerides, decreased resting heart rate, and decreased systolic and diastolic blood pressure. Effects considered potentially adverse were observed in some RCTs, however none of these effects were observed consistently across studies. It is concluded that DHA intakes of up to 6 g/day do not raise safety concerns. This value is greater than the UL for n-3 LC-PUFA, namely 3 g/day. Therefore, a cautious approach was adopted and dietary intake estimates were calculated for the sum of DHA, DPA and EPA and compared to the UL.

The dietary intake estimates for all population groups in both Australia and New Zealand were below the UL of 3 g/day for n-3 LC-PUFA. It is therefore concluded that consumption of DHA canola oil will not pose a nutritional concern to the Australian and New Zealand populations.

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1 Introduction

The Application seeks to amend the *Australia New Zealand Food Standards Code* (the Code) to allow for the inclusion of food derived from canola that has been genetically-modified to contain the omega-3 (n-3) fatty acid docosahexaenoic acid (DHA). The OECD Unique Identifier for this canola line is NS-B50027-4 (herein referred to as DHA canola). As the oil from canola seed will be available for human consumption, FSANZ has assessed whether any adverse effects can be expected from an increase in dietary intake of DHA due to consumption of DHA canola seed oil.

DHA canola has been grown in Australia for field trials since 2014 under Licence DIR123 from the Office of the Gene Technology Regulator. In addition to DHA, other n-3 long-chain (≥ C20) polyunsaturated fatty acids (LC-PUFA) such as eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA) are also present but in low amounts in the seed oil of DHA canola. Trivial amounts of n-6 LC-PUFA such as arachidonic acid (ARA) are present in DHA canola.

This risk assessment includes a hazard assessment and a dietary intake assessment. The hazard assessment considers the potential adverse effects associated with DHA intake, and also includes information on the Upper Level of Intake (UL) for n-3 LC-PUFA (sum of EPA, DPA and DHA). The hazard assessment focusses on randomised controlled trials (RCTs) in humans. FSANZ has previously assessed animal toxicity studies conducted with DHA-rich oils from marine algae (FSANZ 2002, 2005, 2016).

The dietary intake assessment considers the usual intake of n-3 LC-PUFA (sum of EPA, DPA and DHA) from the current food supply (baseline intake) and two scenarios to account for potential additional intake of DHA due to the introduction of DHA canola oil.

2 Nutrition hazard assessment

The primary evidence upon which this hazard assessment is based is RCTs in subjects consuming oils containing > 90% DHA. Oils rich in, or enriched with, DHA through concentration, addition or other production technology, but with no or minimal content of other LC-PUFA, were considered as suitable interventions for the purpose of this assessment.

Primary or secondary clinical outcomes and adverse health outcomes reported in the included RCTs have been the outcomes considered in this assessment. However, in most of the studies assessed, the primary aim was investigation of the potential benefits of DHA intake and safety assessment was a secondary consideration.

2.1 Upper Level of Intake

The Upper Level of Intake (UL) is a Nutrient Reference Value (NRV) that defines the highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects increases (NHMRC and MoH 2006)[1]. In Australia and New Zealand, the UL

[1] Nutrient Reference Values, Australia and New Zealand: www.nrv.gov.au

for n-3 LC-PUFA, defined as the sum of EPA, DPA + DHA, was set at 3 g/day for children, adolescents and adults (NHMRC and NZ MoH 2006). It was stated there is some evidence to suggest that high intake of these fatty acids may impair immune response and prolong bleeding time. However it was also stated that (i) the immune function tests were performed *in vitro* and it is unclear how the results would translate to the *in vivo* situation, and (ii) prolonged bleeding times have been seen in the Inuit, but it is not known if they were caused by high consumption of n-3 LC-PUFA. The UL was based on a US Food and Drug Administration (US FDA) assessment which concluded that, when consumption of fish oils results in EPA and DHA intake of 3 g/day or less, there is no significant risk for increased bleeding time beyond the normal range (US FDA 1997). No UL has been established for DHA alone.

2.2 Biochemistry and physiology of DHA and other PUFA

Polyunsaturated fatty acids (PUFA) have a backbone of ≥18 carbon atoms with two or more unsaturated (i.e. double) bonds. Depending on the position of the first double bond, PUFA are categorised into two groups: n-3 (e.g. DHA) and n-6 (Sprecher 2000; Gawrisch et al. 2003). The fatty acid nomenclature for DHA, 22:6n-3, describes the number of carbon atoms in the backbone (22), the number of double bonds (6) and the position of the first double bond counting from the methyl end (n-3).

Linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3) are essential fatty acids that are metabolised, respectively, to n-6 and n-3 LC-PUFAs (Brenna et al. 2009; Holman 1986). Conversion of dietary ALA to EPA and DHA is limited (Alhazzaa et al. 2013; Plourde and Cunnane 2007) and the enzymatic steps in the conversion pathway vary in efficiency and are influenced by the composition of dietary fats (Goyens et al. 2006; Harnack et al. 2009).

n-3 LC-PUFA such as DHA are mainly obtained from the consumption of seafood and marine oils with a contribution due to endogenous biosynthesis from dietary ALA (Tinoco 1982; Holman 1998; Simopoulos 1991). Bioavailability of dietary n-3 LC-PUFA is influenced by factors such as their chemical form (e.g. free fatty acids or triglycerides) and other nutrients present in the food matrix, particularly the presence of other fats in a meal which appears to improve absorption of n-3 LC-PUFA (Schuchardt and Hahn 2013; Lawson and Hughes 1988).

n-3 LC-PUFA play a role in several physiological functions including regulation of inflammation and immune function, lipid metabolism, and cardiovascular function. Their multiple actions appear to involve mechanisms linking the cell membrane, the cytosol, and the nucleus (Calder 2012). Studies with DHA indicate that it can influence the physical properties of cellular and tissue membranes into which it is incorporated, thereby affecting membrane protein function and lipid raft formation resulting in the modulation of signal transduction (Chapkin et al. 2008).

Changing the n-3:n-6 PUFA ratio in the membranes of platelets and inflammatory cells can affect the production of eicosanoids, prostaglandins and cytokines that regulate inflammation and the immune response (Calder 2015; Dawczynski et al. 2011; Guarini et al. 1998).

2.3 Randomised controlled trials of DHA supplementation

RCTs that were considered particularly relevant for the hazard assessment of DHA are summarised below in order of publication year.

In a single-blind RCT of 15 weeks duration, 55 healthy men (age 23.3 ± 2.5 years) completed

a study that investigated the effect of DHA-rich algae oil intake (1.68 g/day, no EPA), compared with a fish diet (0.38 ± 0.04 g/day EPA and 0.67 ± 0.09 g/day DHA) and fish oil intake (1.33 g/day EPA and 0.95 g/day DHA) on fasting and postprandial blood lipid levels. Subjects were randomised into three intervention groups and a control group. The fish diet group (n = 13) ate 4.3 ± 0.5 fish containing meals per week and those in the fish oil (n = 14) and DHA-oil (n = 14) groups consumed 4 g oil per day. Subjects in the control group (n = 14) did not take any supplements and were not provided with fish meals. Fasting plasma triglyceride levels decreased in all test groups in 14 weeks when compared to the control group ($p < 0.05$) while the total plasma cholesterol levels did not change. The ratio of HDL cholesterol sub-fraction 2 (HDL_2) to HDL_3 increased in all test groups by over 50% ($p < 0.05$). The postprandial total and chylomicron triglyceride responses, measured as area under the response curve, were lowered in 15 weeks by the fish diet and fish oil ($p < 0.05$) but were not significantly changed in the DHA group (Agren et al. 1996).

In a double-blind RCT that recruited 26 subjects with combined hyperlipidaemia (LDL cholesterol 3.4–5.7 mmol/L and triglycerides 1.7–4.5 mmol/L), daily doses of 1.25 or 2.5 g of DHA from DHA-rich algae oil for 6 weeks were compared with a 1:1 mixture of corn and soybean oils as a control. Compared to baseline values, significant ($p < 0.01$) reductions were observed in serum triglycerides (by 21% and 18% in the 1.25 g and 2.5 g DHA groups, respectively) and an increase of approximately 6% in HDL cholesterol was observed in both DHA groups ($p < 0.02$ and 0.03 in the 1.25 g and 2.5 g DHA groups, respectively). An increase in non-HDL cholesterol was significant (5.7%, $p < 0.04$) only in the higher DHA intake group as was an increase in LDL cholesterol (13.6%, $p < 0.001$). There were no significant differences in the frequency of reported side effects between the control and treatment groups (Davidson et al. 1997).

Grimsgaard et al. (1997) evaluated the effects of dietary supplementation with highly purified EPA or DHA, derived from fish oil, on blood lipid parameters in 224 healthy men aged 44 ± 5 years in a 7-week double-blind RCT. Subjects (n = 75) received 3.8 g/day of highly purified EPA, 3.6 g/day of highly purified DHA (n = 72) or 4 g/day of corn oil as control (n = 77). HDL cholesterol increased by 0.06 mmol/L ($p = 0.0002$) in the DHA group. Compared with the control group, DHA did not affect the concentrations of LDL cholesterol, total blood cholesterol or triglycerides. Fifty-eight percent of subjects in the DHA group and 57% in the EPA group experienced belching after initiation of the dietary supplements. A taste of fish oil during the intervention was reported by 67% of subjects in the DHA group and 65% in the EPA group. No other adverse effects were reported.

A subsequent publication on the same study reported that DHA intake did not significantly affect systolic, diastolic or mean arterial blood pressure compared with the baseline or with the control treatment (corn oil). Mean heart rate decreased by 2.2 ± 4.6 beats/min in the DHA group ($p = 0.006$, compared with the control). In a pooled analysis of all subjects, the change in heart rate was inversely correlated ($r = -0.25$, $p = 0.0002$) with the change in serum phospholipid DHA. Echocardiography showed improved left ventricular diastolic filling in the DHA group compared with the corn oil group ($p = 0.02$) (Grimsgaard et al. (1998).

To assess the effect of EPA and DHA on monocyte immune response *ex vivo*, a double-blind RCT provided 58 healthy men aged 44 ± 5 years with daily supplements of 3.6 g highly purified EPA (n = 20), DHA (n = 19) or corn oil control (n = 19) for 7 weeks. Peripheral blood mononuclear leukocytes were collected prior to and after the dietary supplementation and were analysed in a crossover design. Compared with the control group, DHA supplementation did not compromise phagocytic ability and respiratory burst activity of monocytes against *Escherichia coli*. DHA supplementation improved bacterial attachment to the monocyte surface when compared with the control (Halvorsen et al. 1997).

A single-blind RCT investigated the effect of 6 g/day of DHA supplementation from highly purified algae oil for 90 days in 6 healthy males (age 33.1 ± 4.8 years) compared with a control group (4 healthy males, age 33.3 ± 6.1 years) receiving < 50 mg/day of DHA from the diet. No significant effects due to DHA supplementation were reported for blood pressure, platelet count, bleeding time or platelet aggregation. In the DHA group, triglyceride levels were reduced by 26% ($p < 0.007$) compared with the baseline. Apolipoprotein-E (Apo-E), which plays a role in the plasma clearance of triglyceride- and cholesterol-rich lipoproteins, was almost doubled in the plasma of subjects receiving 6 g/day of DHA at the end of the trial compared with day 30 ($p < 0.002$). Other Apo subclasses were unaffected. DHA supplementation did not affect plasma total and LDL cholesterol. Compared with the baseline, DHA increased HDL cholesterol by 9% ($p < 0.017$) (Nelson et al. 1997a, 1997b).

A double-blind RCT in healthy men and women (n = 19; age 31.5 ± 4.3 years) tested doses of 0.75 or 1.5 g/day DHA from highly purified algae oil for 6 weeks, compared with a corn oil control. DHA supplementation had no effect on the levels of serum lipids (total, LDL or HDL cholesterol and triglycerides), lipoprotein A, blood pressure or resting heart rate (Conquer and Holub 1998).

In a RCT of 90 days duration in healthy men, consumption of DHA supplements (6 g/day) from algae oil by 7 subjects (age 33.1 ± 1.8 years) resulted in statistically significant differences in several immune parameters when compared with the baseline or with 4 subjects (age 33.3 ± 3.1 years) in the control diet group (< 0.1 g/day DHA). It was not stated if the study was blinded. No immune parameters were affected in the control group. When compared with the baseline, DHA consumption did not affect mononuclear cell proliferation, delayed skin hypersensitivity response, the number of lymphocytes in peripheral circulation, the number of T cells producing interleukin-2 (IL-2), the ratio of helper/suppressor T cells in circulation, or serum concentrations of immunoglobulin G (IgG) or IL-2 receptor. Compared to baseline, DHA did not alter the delayed hypersensitivity skin response or the proliferation of peripheral blood mononuclear cells cultured with mitogens. DHA supplementation decreased circulating total white blood cells by 10% ($p = 0.0001$), primarily due to a decrease in granulocytes. Natural killer cell activity was reduced by 15% in the DHA group ($p = 0.004$). DHA supplementation reduced the *in vitro* secretion of IL-1β and tumour necrosis factor α by 40% ($p = 0.0004$) and 25% ($p = 0.0002$), respectively. Serum influenza antibody titres did not differ between the DHA group and control group following influenza immunization. Subjects supplemented with DHA did not show an increase in the rate of infections or any other health problems (Kelley et al. 1998; Kelley et al. 1999).

Fifty-six overweight men with mild hyperlipidaemia received 4 g/day of purified DHA for 6 weeks in a double-blind RCT. The 24-hour (systolic/diastolic: 5.8/3.3 mmHg) and awake daytime (systolic/diastolic: 3.5/2.0 mmHg) ambulatory blood pressure was significantly reduced ($p < 0.05$) by DHA supplementation. Compared with the olive oil control group, DHA reduced 24-hour heart rate by 3.5 ± 0.8 beats/min, daytime heart rate by 3.7 ± 1.2 beats/min, and night-time heart rate by 2.8 ± 1.2 beats/min (Mori et al. 1999).

In a double-blind RCT, 56 overweight, non-smoking men aged 48.8 ± 1.9 years and diagnosed with mild hyperlipidaemia with serum total cholesterol concentration > 6 mmol/L, triglyceride concentration > 1.8 mmol/L, or both, were randomly assigned to receive either 4 g EPA, 4 g DHA, or 4 g olive oil (control) daily for 6 weeks. DHA consumption did not alter total cholesterol levels, which were adjusted for baseline values, when compared to the control group or EPA. However, when compared with the baseline, DHA reduced triglyceride levels by 20% ($p = 0.003$) and increased LDL cholesterol by 8% ($p = 0.019$). DHA increased the adjusted LDL particle size by 0.25 ± 0.08 nm ($p = 0.002$), while EPA did not. Neither n-3 LC-PUFA had a significant effect on HDL levels compared to control; however, DHA produced a 29% increase ($p = 0.04$) in HDL sub-fraction 2 (HDL_2), while EPA did not affect

HDL_2. DHA had no effect on fasting glucose levels, however fasting insulin was increased by 27% (p = 0.001) (Mori et al. 2000).

Plasma lipids were measured in a 7-week double-blind RCT that included 38 subjects aged 57.8 ± 12.1 years with hyperlipidaemia; defined as having total cholesterol > 5.5 mmol/L, triglycerides > 2 mmol/L, HDL cholesterol < 1.0 or 1.2 mmol/L for men or women, respectively. Groups were randomly assigned to receive a daily dose of 3 g EPA (n = 12), 3 g DHA (n = 12) or olive oil control (n = 14). After 7 weeks of DHA supplementation, the concentrations of total and very low-density lipoprotein (VLDL) triglycerides decreased by 32% (p = 0.013) and 31% (p = 0.0019), respectively when compared with the baseline values. No significant changes were observed in the total, LDL or HDL cholesterol. The percentage concentrations of DHA in plasma at the end of the intervention correlated significantly and inversely (r = −0.63) with plasma triglyceride levels. No other statistically significant effects were reported in this study. Heart rate, blood pressure (systolic and diastolic), pulse pressure or total vascular resistance did not differ significantly between the DHA and control groups or within the DHA group over the intervention period (Nestel et al. 2002).

In a double-blind RCT in 51 hypertensive subjects with type 2 diabetes (age 61.2 ± 1.2 years) receiving 4 g/day of DHA for 6 weeks, there were no significant changes in total, LDL or HDL cholesterol when compared with the olive oil control group. There were no significant changes in the 24-h systolic blood pressure or diastolic blood pressure, during waking hours or during sleep in the DHA group. There were no significant changes in heart rate during waking hours and during sleep in the DHA group compared with control. Fasting blood glucose concentration was increased by 0.98 ± 0.29 mmol/L (p = 0.002) in the DHA group when compared with the control group. DHA did not affect fasting insulin concentrations, C-peptide, glycated haemoglobin (HbA_{1c}), insulin sensitivity or its secretion (Woodman et al. 2002). A subsequent publication on the same study reported an increase in LDL particle size (0.26 ± 0.10 nm; p = 0.02) in the DHA group compared with control (Woodman et al. 2003a). Another publication on the same study reported that DHA intake decreased platelet-derived thromboxane B2 (TXB2) production (-18.8%, p = 0.03) and collagen-stimulated platelet aggregation (-16.9%, p = 0.05) compared with control. However, there were no significant changes in platelet aggregation stimulated by platelet activating factor, fibrinolytic function, vascular function, von Willebrand factor or P-selectin in the DHA group relative to control (Woodman et al. 2003b).

A double-blind RCT was conducted on 20 children and young adults aged 9 to 19 years. Entry criteria included a clinical diagnosis of familial hypercholesterolemia characterized by LDL cholesterol levels > 130 mg/dL and a parent diagnosed with the disorder, or familial combined hyperlipidaemia characterized by elevated levels of LDL cholesterol (> 130 mg/dL) and/or triglycerides (> 150 mg/dL), and a parent with 1 of these 3 phenotypes. The study demonstrated that intake of 1.2 g/day of algal-DHA for 6 weeks shifted the lipoprotein subclass distribution toward larger and more buoyant LDL and HDL particles without significant changes in the concentrations of total cholesterol, LDL cholesterol, HDL cholesterol or triglycerides. Large, buoyant and less atherogenic lipoprotein subclasses LDL_1 and HDL_2 increased by 91% (p = 0.004) and 14% (p = 0.011), respectively compared with control (corn/soy oil mixture), while the small, dense and more atherogenic LDL_3 decreased by 48% (p = 0.002) compared with control (Engler et al. 2004, 2005).

In a double-blind RCT, postmenopausal women (n = 32; age 56.7 ± 1.5 years) receiving 2.8 g/day DHA from algae oil for 4 weeks, regardless of being on hormone replacement therapy or not, had significant (p < 0.05) changes in their blood lipids including 20% lower serum triglyceride concentrations, 8% higher HDL-cholesterol concentrations and a 28% lower overall ratio of serum triglycerides to HDL cholesterol compared with control (corn/soy

oil mixture). The DHA group showed a 7% (4.8 ± 1.3 beats/min; p = 0.004) decrease in resting heart rate compared with the baseline. DHA intake did not affect systolic or diastolic blood pressure. Changes in heart rate and the consistency in blood pressure measurements were observed regardless of the hormone replacement therapy status. DHA did not affect fasting blood glucose and insulin levels (Stark and Holub 2004).

In a double-blind RCT, 3 g/day algal DHA was provided to 17 men with hypertriglyceridaemia (age 55.0 ± 2.0 years) for 90 days. Men with hypertriglyceridaemia in the control group (n = 17, age 53.1 ± 1.0 years) received olive oil for the same duration. Compared with baseline DHA decreased the concentration of fasting triglycerides by 24% ($p < 0.05$) while LDL cholesterol increased by 12.6% ($p < 0.05$). Concentrations of large particles of VLDL and intermediate-density lipoprotein (IDL) particles were reduced ($p < 0.05$) along with the mean diameter of VLDL particles in fasting plasma compared with the initial measurements. Significant ($p < 0.05$) increases in the concentrations of small VLDL, large LDL particles and the mean diameter of LDL particles were observed in the fasting plasma, compared with the baseline, following 45 days of supplementation with DHA. These changes were sustained until the end of the intervention at approximately day 90. The area under the postprandial plasma concentration curve decreased for triglycerides, intermediate and small LDL particles, large VLDL and small HDL particles. These decreases were accompanied by increases in the area under the curve for the concentrations of large LDL and HDL particles and small VLDL particles. DHA reduced resting heart rate by 8.3% ($p < 0.05$) at day 45 but not at day 91 ($p = 0.07$) compared with the baseline. Similarly, when compared with the baseline, systolic and diastolic blood pressures were reduced by 5.6% and 4.0%, respectively ($p < 0.05$), at mid-intervention but were not significantly different at the end of the intervention (Kelley et al. 2007).

The effect of diets enriched with ALA, EPA or DHA was investigated in 48 healthy men and women aged 25.9 ± 6.8 years in a RCT. The blinding status of the study was not reported. After a 2 week wash-in period, subjects consumed meals enriched with ALA, EPA, or DHA for 3 weeks. Mean dietary intake of ALA in the ALA group was 6.0 g/day (2.5% of energy intake), mean intake of EPA in the EPA group was 2.8 g/day (1.1% of energy intake) and mean intake of DHA in the DHA group was 2.9 g/day (1.1% of energy intake). The three study diets did not lead to any significant changes in serum concentrations of total, VLDL, LDL and HDL cholesterol. DHA did not affect fasting serum concentrations of triglycerides, glucose, insulin, insulin sensitivity, HbA_{1c} or fructosamine (Egert et al. 2008).

In a double-blind RCT, 74 healthy men and women aged 25.3 ± 4.6 years consumed with their usual diet specially-formulated margarine to contain either 4.4 g/day ALA, 2.2 g/day EPA or 2.3 g/day DHA for 6 weeks during which other sources of n-3 PUFA were not permitted. Compared with baseline, DHA intake did not affect fasting serum concentrations of total and LDL cholesterol, but fasting serum triglyceride concentrations significantly decreased (median decrease of 0.30 mmol/L or 30.6%, $p < 0.001$). DHA intake increased serum HDL cholesterol by 0.21 mmol/L ($p < 0.001$) compared with the baseline (Egert et al. (2009).

In a dose escalation study, eight healthy men, mean age 58.7 years, ingested successively 0.2, 0.4, 0.8, and 1.6 g/day DHA for 2 weeks at each dose level without interruption. Blood samples were collected after overnight fasting before DHA supplementation and after each 2-week dosing period and mononuclear cells were isolated. The authors reported that the DHA supplement was well tolerated throughout the study with no significant impact on blood pressure, glucose or lipids. Compared with the baseline, expression of IL-2 mRNA started to increase after ingesting 0.4 g/day, reaching a maximum of ~2-times the baseline value at an intake of 0.8 g/day, and was positively correlated ($r^2 = 0.99$, $p < 0.003$) with DHA enrichment in cell phospholipids. Five weeks after cessation of DHA supplementation, expression of IL-2

mRNA had returned to the baseline level (Mebarek et al. 2009).

To evaluate the potential effect of maternal DHA supplementation on maternal and newborn DHA status, gestation duration, and birth weight and length, a double-blind RCT was conducted in women aged 25.1 ± 4.8 years in their second pregnancy. The control group (n = 172) took capsules of a soybean oil/ corn oil mixture. In the intervention group (n = 178) mean DHA intake, from DHA-rich algae oil, was 469 mg/day from enrolment (101 ± 26 days of gestation) until birth. Safety evaluation included recording adverse events such as hospitalisation and congenital anomalies and did not identify any safety concerns related to DHA supplementation for mothers or their newborns. Compared with the control group, DHA supplementation resulted in higher (2.6%, $p < 0.001$) maternal and cord phospholipid-DHA in red blood cells and a longer gestation period (2.9 days, $p = 0.041$). Newborns of women assigned to DHA supplementation had greater birth weight (172 g, $p = 0.004$), length (0.7 cm, $p = 0.022$), and head circumference (0.5 cm, $p = 0.012$). In addition, women who received DHA had fewer infants born before 34 weeks of gestation ($p = 0.025$) and shorter hospital stays for infants born preterm (40.8 vs. 8.9 days, $p = 0.026$) than did the control group (Carlson et al. 2013).

When taken from week 20 of gestation until delivery, supplemental DHA (600 mg/day) from algae oil increased gestational length by 4 days ($p = 0.025$) in healthy women (n = 117; age 24.6 ± 0.6 years) compared with women taking olive oil control (n =121) in a double-blind RCT. DHA did not significantly affect birth weight, birth length or head circumference. The rate of early preterm birth in women taking DHA supplements was lower compared with the control group (1.7% vs. 5.7%, $p = 0.045$) (Harris et al. 2015).

In a double-blind RCT, 0.4 g/day algal DHA was provided to 276 women aged 26.2 ± 4.9 years from their mid-pregnancy to delivery. The control group (n = 248; age 26.0 ± 4.7 years) received a mixture of corn and soy oils over the same period of pregnancy. Offspring in both groups were breastfed until age 10 months at which time blood was collected for analysis. Approximately 90% of the children were in a non-fasting state (< 8 h fasting) at the time of blood collection. DHA, compared with the control, had no effect on serum concentrations of total, LDL, HDL or non-HDL cholesterol, total-to-HDL cholesterol ratio, triglycerides, Apo B, non-fasting blood glucose, and insulin in the offspring at age 4.2 ± 0.3 years. No differences were found between the DHA and control group in the proportion of children with abnormal concentrations of lipid markers (Gutierrez-Gomez et al. 2017).

2.4 Hazard assessment discussion and conclusions

FSANZ has previously assessed Applications relating to DHA and concluded from the respective hazard assessments that there were no toxicity concerns. These hazard assessments relied on animal and *in vitro* studies that were conducted on the specific DHA-rich products for which approval was sought (FSANZ 2002; 2005; 2017).

The current hazard assessment considered published RCTs of DHA supplementation in humans. The highest supplemental DHA dose in these studies was 6 g/day and intervention durations were 3–15 weeks.

Daily supplementation of up to 6 g DHA was associated with a number of effects that are considered beneficial, for example decreased blood total and HDL cholesterol, decreased blood triglycerides, decreased resting heart rate, and decreased systolic and diastolic blood pressure.

Effects considered potentially adverse were observed in some RCTs, however none of these effects were observed consistently across studies. Increases in LDL cholesterol of 8–14%

were observed at supplementary DHA doses of 2.5–4 g/day in studies of 6–12 weeks duration (Davidson et al. 1997; Mori et al. 2000; Kelley et al. 2007). However, there was no statistically significant effect on LDL cholesterol in other studies that used DHA doses of 1.2–6 g/day for durations of 6–12 weeks (Grimsgaard et al. 1997; Nelson et al. 1997a, 1997b; Conquer and Holub 1998; Nestel et al. 2002; Woodman et al. 2002; Engler et al. 2004; Egert et al. 2009).

DHA supplementation was associated with statistically significant changes in some HDL sub-fraction parameters, for example HDL_2 and HDL_3 (Agren et al. 1996; Mori et al. 2000; Engler et al. 2004, 2005), however these parameters have been considered to have minimal value in predicting the risk of adverse cardiovascular outcomes (Superko et al. 2012).

Regarding the potential effects of DHA supplementation on glycaemic control, one study reported that fasting blood glucose concentration was increased (by approximately 1.0 mmol/L) in subjects with type 2 diabetes receiving 4 g/day of DHA for 6 weeks (Woodman et al. 2002). However, other studies showed no statistically significant effect on fasting blood glucose at DHA doses of 2.8–4 g/day for 3–6 weeks (Mori et al. 2000; Stark and Holub 2004; Egert et al. 2008). No consistent effects on other parameters relevant to glucose homeostasis were evident.

Effects on some immune system parameters were observed at DHA doses of 3.6–6 g/day (Halvorsen et al. 1997; Kelley et al. 1998, 1999), however subjects supplemented with DHA have not shown an increase in the rate of infections or any other health problems.

DHA supplementation has resulted in effects on some indicators of platelet function, however these are not considered to be adverse as DHA supplementation has not been associated with an increased risk of impaired coagulation.

It is concluded that DHA intakes of up to 6 g/day do not raise safety concerns.

3 Dietary intake assessment

3.1 Purpose

The purpose of this assessment is to estimate dietary intakes for total n-3 LC-PUFA (sum of DHA+EPA+DPA) both currently and after the introduction of DHA canola, should the application be approved. The general FSANZ methodology and approach to conducting dietary intake assessments is set out in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

The composition of DHA canola was available to carry out a full intake assessment. However, as the fatty acid composition of DHA canola was designated commercial in confidence the detailed outputs of the assessment are not included in this document.

3.2 Nutrient of interest

The assessment focusses on estimated intakes of n-3 LC-PUFA rather than DHA as there are only nutrient reference values established for n-3 LC-PUFA and none specifically for DHA.

3.3 Approach

To estimate the potential changes in n-3 LC-PUFA intake in Australia and New Zealand from the introduction of DHA canola, FSANZ added the n-3 LC-PUFA that may enter the food supply from the new canola variety (additional intake) to the usual intake of n-3 LC-PUFA reported for the current food supply (baseline intake). FSANZ used different scenarios to assess the possible changes in n-3 LC-PUFA intake.

The assessment includes the following inputs:

- current (baseline) n-3 LC-PUFA usual intakes published by the Australian Bureau of Statistics (ABS 2015a)

food consumption data from the available Australian and New Zealand national nutrition surveys (NNS) – (average (mean) amount canola oil and non-specified vegetable fat eaten eaten by consumers only, see

- Appendix 1 for details)
- market share data based on the different types of oil reported as consumed in the 2011–12 Australian National Nutrition and Physical Activity Survey (2011–12 NNPAS, (ABS 2015b)
- Population groups identified in the nutrition assessment
- Nutrient Reference Values (NRV) established UL for n-3 LC-PUFA.

3.4 Baseline intake of n-3 LC-PUFA

The baseline intakes used in the assessment of n-3 LC-PUFA for Australians aged 2 years and above were the usual intakes of n-3 LC-PUFA published by the ABS from the 2011-12 nutrition survey component of the 2011-13 Australian Health Survey (ABS 2015a).

Baseline n-3 LC-PUFA intakes for New Zealanders were not available in the 2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS) and 2008-09 New Zealand Adult Nutrition Survey (2008–09 NZ ANS). Therefore, the baseline intake for an Australian population group represented the baseline intake for the corresponding New Zealand age group. As such, this intake assessment assumes that the intake of n-3 LC-PUFA by New Zealand population groups is the same as Australia.

3.5 Food consumption data used

The food consumption data used for the dietary intake assessments were:

- 2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS): a 24-hour recall survey of 3,275 New Zealand children aged 5-14 years, with a second 24-hour recall undertaken for 15% of respondents. The assessment only used data from Day 1of the survey.
- 2008–09 New Zealand Adult Nutrition Survey (2008–09 NZ ANS): a 24-hour recall survey of 4,721 New Zealanders aged 15 years and above, with a second 24-hour recall undertaken for 25% of respondents. The assessment only used data from Day 1of the survey.
- 2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS), a component of the 2011-13 Australian Health Survey (2011-13 AHS): a 24-hour recall survey of 12,153 Australians aged 2 years and above, with a second 24-hour recall undertaken for 64% of respondents . The assessment used data from Day 1 and Day 2 of the survey (ABS 2015b).
- Dietary exposure assessments based on food consumption data from national nutrition surveys provide the best estimation of actual consumption of a food and the resulting estimated dietary intake assessment for the Australian and New Zealand populations. However, NNS data have some limitations. The design of these nutrition surveys vary the key attributes of each, including survey limitations, are set out in

Appendix 1.

3.6 Scenarios

To estimate the potential changes in n-3 LC-PUFA intake in Australia and New Zealand from the introduction of DHA canola, FSANZ used two scenarios to model potential intakes additional to baseline intake:

1. 100% DHA canola oil
2. 100% DHA canola oil plus 30% DHA non-specified vegetable oils.

The scenarios included oil consumption values for Australian and New Zealand populations combined with DHA concentrations as identified by the applicant for oil derived from DHA canola to estimate additional n-3 LC-PUFA intakes from DHA canola.

Both scenarios include estimated consumption of oil on a raw commodity basis, i.e. they included oil reported when used on its own or as an ingredient in mixed foods or dishes (e.g. in salad dressing, steak fried in oil, fried rice etc.) based on FSANZ's recipe data used in the Harvest[2] Raw Commodity model. Canola oil was the commodity used in this assessment as no consumption of other canola products (i.e. canola meal or canola seed) was reported in the nutrition surveys for Australia and New Zealand.

3.6.1 Scenario 1: 100% DHA canola oil

Scenario 1 assumed that DHA canola oil replaces all conventional canola oil currently in the food supplies of Australia and New Zealand, including canola oils used as an ingredient in mixed foods.

3.6.2 Scenario 2: 100% DHA canola oil + 30% DHA unspecified oils

Scenario 2 assumed DHA canola oil replaces all (100%) of conventional canola oil and 30% of any *non-specified oil* that consumers reported eating in the national nutrition surveys. Non-specified oil is vegetable oil reported as consumed in a nutrition survey without any specific information about its source. Any vegetable oils (other than canola) that are specifically identified (e.g. olive oil, sunflower oil) are not included in the consumption. The market share of 30% selected reflects the proportion that canola oil represents from all identified oils reported as consumed in the 2011-12 NNAPS.

3.7 Population groups assessed

The hazard assessment did not identify any population age-sex based sub-groups for which there were specific safety considerations in relation to n-3 LC-PUFA. For Australia, the population groups used for the dietary intake assessment are the same as the Nutrient Reference Value (NRV) age groups.

For children, the New Zealand survey age groups and the NRV age groups do not exactly

[2] Harvest is FSANZ's custom-built platform to calculate dietary exposures.

match because the New Zealand surveys did not sample the same age groups as the Australian survey. As the n-3 LC-PUFA usual intakes reported by the ABS for the 2011-12 NNPAS are only available for the Australian population groups, this assessment assigns the best population intake match possible to the available New Zealand population groups, based on NRV age groups.

3.8 Assumptions and limitations of the dietary intake assessment

The aim of the dietary intake assessment was to make the best estimate of dietary n-3 LC-PUFA intake. Where significant uncertainties in the data exist, FSANZ uses conservative assumptions to ensure that the estimated dietary intake is not an underestimate (for example, assuming that the population consumes the mean consumer amount of canola oil over time over-estimates potential population increases in n-3 LC-PUFA intakes).

Assumptions made in the dietary intake assessment included:

- where estimates of dietary intake to DHA were not available, estimates of n-3 LC-PUFA are sufficient to identify any associated risks to the population from increased DHA intakes from DHA canola
- n-3 LC-PUFA usual intakes published by the ABS reflect current intakes
- usual intake of n-3 LC-PUFA in New Zealand is the same as in Australia
- n-3 LC-PUFA intakes are from food only; n-3 LC-PUFA intake from complementary or other medicines (e.g. dietary supplements) is not included in the usual intakes
- The dietary intake assessments used the concentration of n-3 LC-PUFA in DHA canola provided by the Applicant. The variability and uncertainty around this concentration are unknown.
- All conventional canola oil reported as consumed is replaced by DHA canola oil (Scenario 1 – 100% DHA canola oil)
- A market share of 30% reflects the share of canola oil in non-specified oils currently in the marketplace (Scenario 2 – 100% DHA canola oil + 30% DHA unspecified oil)
- The fatty acid profile of DHA canola in the marketplace matches the Applicants data.

In addition to the specific assumptions made in relation to this dietary intake assessment, there are a number of limitations associated with the nutrition surveys per se. A discussion of these limitations is included in Section 6 of the *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

3.9 Dietary intake estimate results and conclusions

Baseline mean usual intakes of n-3 LC-PUFA for Australians aged 2 years of age and above ranged from 0.092-0.270 g/day (92-270 mg/day). The highest mean usual intake was for 31-50 year old men (ABS, 2015).

For Australians aged 2 years and above, the major contributors to baseline intakes of n-3 LC-PUFA were fish and seafood products and dishes (50%), meat, poultry and game products and dishes (23%) and cereals based products and dishes (11%). Considering the similarity in food supplies, these contributions for New Zealand are most likely very similar.

The intake estimates of n-3 LC-PUFA for Scenarios 1 and 2, all population groups and both countries were below the UL of 3 g/day.

At the highest level of estimated intake, assuming mean baseline intakes and replacement of all conventional canola oil with DHA canola oil (Scenario 1), the highest proportion of the UL reached was 25%.

Assuming that DHA canola oil replaces all (100%) of conventional canola oil and 30% of any non-specified oil that consumers reported eating in the national nutrition surveys (Scenario 2) the highest level of n-3 LC-PUFA intakes were equivalent to 55% of the UL.

The true increase of population intakes of n-3 LC-PUFA would likely be lower as both scenarios assume that all canola oil in the marketplace is from DHA canola. The intake estimates are intentionally highly protective of consumers to ensure that there is no concern associated with DHA canola increasing n-3 LC-PUFA intake in Australia and New Zealand.

4 Risk characterisation and conclusion

This risk assessment comprises a hazard assessment which considered the potential adverse effects associated with supplemental DHA intake in human studies, and a dietary intake assessment for DHA which considered two scenarios. Scenario 1 assumed that all conventional canola oil reported as consumed is replaced by DHA canola oil. Scenario 2 assumed a market share of 30% to reflect the share of canola oil in non-specified oils currently in the marketplace as well as 100% replacement of conventional canola oil with DHA canola oil (as per Scenario 1).

Based on findings from randomised controlled trials in humans, DHA intakes of up to 6 g/day do not raise safety concerns. This value is greater than the UL for n-3 LC-PUFA (defined as the sum of EPA, DPA and DHA), namely 3 g/day. Therefore, a cautious approach was adopted and dietary intake estimates were calculated for the sum of EPA, DPA and DHA and compared to the UL.

Because estimated dietary intakes were below the UL, it is concluded that consumption of DHA canola oil will not pose a nutritional concern to the Australian and New Zealand population.

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Appendix 1: Dietary Intake Assessments at FSANZ

A *dietary intake assessment* is the process of estimating how much of a nutrient (such as n-3 LC-PUFA) a population, or population sub group, consumes. Combining food consumption with food chemical concentration data allows estimates of dietary intake of food chemicals:

$$\text{Dietary Intake} = \text{Food Chemical Concentration} \times \text{Food consumption}$$

FSANZ's approach to estimating dietary intake of nutrients is based on internationally accepted procedures (FSANZ 2009). FSANZ uses different approaches depending on the assessment, the type of food chemical, the data available, and the risk assessment questions addressed. In the majority of assessments, FSANZ uses the food consumption data from each person in the national nutrition surveys to estimate their individual dietary intake. The ranked individual person's intakes from the nutrition survey form the basis of population summary statistics, such as the mean or a high percentile intake. In some cases, FSANZ will use the usual mean and high percentile intakes of nutrients published with the nutrition survey.

An overview of how FSANZ conducts dietary intake assessments and their place in the FSANZ Risk Analysis Process is available from the FSANZ website.

FSANZ has developed the custom-built platform *Harvest* to calculate dietary exposures. Harvest is a newly built program and replaces the program. Harvest is FSANZ's place for building and sharing knowledge about food consumption, dietary exposure and dietary intake. Harvest supports databased risk analysis by providing a collaborative platform for data storage, analysis, reporting and sharing.

This assessment estimates the consumption of canola and non-specified oil for Australians and New Zealanders based on the Raw Commodity model of Harvest. For further detailed information on conducting dietary intake assessments at FSANZ, refer to the *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

Food consumption data used

The food consumption data used for the dietary intake assessments were:

- 2002 NZ NCNS: a 24-hour recall survey of 3,275 NZ children aged 5-14 years, with a second 24-hour recall undertaken for 15% of respondents
- 2008–09 NZ ANS: a 24-hour recall survey of 4,721 New Zealanders aged 15 years and above, with a second 24-hour recall undertaken for 25% of respondents
- 2011-12 NNPAS, a component of the 2011-13 AHS: a 24-hour recall survey of 12,153 Australians aged 2 years and above, with a second 24-hour recall undertaken for 64% of respondents (n=7,735) (ABS 2015b)

For both New Zealand surveys, weighted mean food consumption data were from all consumers of canola oils and non-specified oils from the first 24-hour recall only.

For the 2011-12 NNPAS, mean food consumption data were from all consumers of canola oils and non-specified oils with two days of 24-hour recall data. To ensure the consumption data are representative of the Australian population the data subset of consumers has a specific set of sample weights.

The design of each of these surveys varies somewhat and key attributes of each are set out below. Further information on the National Nutrition Surveys used to conduct dietary intake assessments is available on the FSANZ website.

2011–12 Australian National Nutrition and Physical Activity Survey (2011–12 NNPAS)

The 2011–12 Australian National Nutrition and Physical Activity Survey (NNPAS) undertaken by the Australian Bureau of Statistics is the most recent food consumption data for Australia. This survey includes dietary patterns of a sample of 12,153 Australians aged 2 years and above.

The survey used a 24-hour recall method for all respondents, with 64% of respondents also completing a second 24-hour recall on a second, non-consecutive day. The collection dates of the data were May 2011 to June 2012 (with no enumeration between August and September 2011 due to the Census).

Consumption and respondent data from the *Confidentialised Unit Record File*s (CURF) data set (ABS 2015b) form part of the Harvest core data set.

2002 New Zealand National Children's Nutrition Survey (2002 NZ NCNS)

The 2002 NZ NCNS was a cross-sectional and nationally representative survey of 3,275 New Zealand children aged 5-14 years. The collection period for the data was during the school year from February to December 2002.

The survey used a 24-hour food recall and provided information on food and nutrient intakes, eating patterns, frequently eaten foods, physical activity patterns, dental health, anthropometric measures and nutrition-related clinical measures. It was also the first children's nutrition survey in New Zealand to include a second day diet recall data for about 15% of the respondents, and dietary intake from both foods (including beverages) and dietary supplements.

2008/09 New Zealand Adult Nutrition Survey (2008 NZANS)

The 2008 NZ ANS provides comprehensive information on the dietary patterns of a sample of 4,721 respondents aged 15 years and above. Collection of Data for the survey occurred on a stratified sample over a 12-month period b October 2008-October 2009.

The survey used a 24-hour recall methodology with 25% of respondents also completing a second 24-hour recall. The information collected in the 2008 NZANS included food and nutrient intakes, dietary supplement use, socio-demographics, nutrition related health, and anthropometric measures.