Measurement of Environmental Contaminants in a Globally-Representative Sample of Fish Oil Supplements

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Abstract

Fish oil supplementation can increase blood concentrations of n-3 fatty acids, thought to be largely responsible for the beneficial health effects of fish. Environmental contaminants, however, tend to bio-accumulate in fatty fish tissues, which may result in the unintentional intake of toxins. To investigate, a total of 1894 fish oil samples were analyzed against GOED Voluntary Monograph tolerances. Although cadmium, lead, and mercury were detected at the mean levels of 0.0006 mg/kg, 0.0019 mg/kg, and mercury 0.0005 mg/kg, respectively, all values were below the 0.1 mg/kg GOED allowance. The mean total arsenic concentration (0.1200 mg/kg) was slightly higher than this allowance, but it is difficult to ascertain form contributions. Mean PCB, dioxin and furans, and dioxin-like PCB levels (24.54 ppb, 0.55 ppt WHO-TEQ, and 0.834 ppt WHO-TEQ, respectively) were all within respective GOED specifications (i.e. ≤ 90 ppb, 2 ppt WHO-TEQ, and 3 ppt WHO-TEQ). Less concentrated fish oil samples (≤ 50% EPA+DHA) were more polluted (with mercury and PCBs) than their higher concentrate counterparts, albeit within tolerance. Overall, results demonstrate that the samples analyzed are well within accepted standards for contaminant allowances. Moreover, this globally-representative assessment supports industry efforts to meet consumer expectations of safe, high-quality fish oil products.

Keywords: fish oil, n-3 fatty acids, EPA, DHA, environmental contaminants, heavy metals, PCBs, PCDDs, PCDFs, dioxin-like PCBs, GOED, WHO-TEQ

Introduction

In addition to the well-documented cardio-protective benefits of fish oil supplementation,[1-5] recent evidence suggests that fish oil may play a role in cancer prevention, cognitive function decline and treatment of depression, diabetes and rheumatoid arthritis.[6-11] As such, fish oil supplementation is popular among consumers and has experienced widespread success in the natural health product/dietary supplement industry.

Fish oils obtained from the liver or tissue of cold-water fish species are readily available for human consumption in capsule or liquid form. They are a rich source of the omega-3 (n-3) polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are increasingly being listed on nutritional supplement labels as fish oil constituents.[12] EPA and DHA serve essential biological roles in the human body, including prostaglandin production and central nervous system development, and are thought to be largely responsible for the wide range of health benefits associated with fish oil consumption.[12] As the science in support of EPA and DHA grows, so too does the recognition from government bodies and scientific organizations. For example, Health Canada and the United States Food and Drug Administration (US FDA) consider a daily dose of fish oil containing 100 to 3000 mg of EPA + DHA to be safe for most adults.[13,14] Further to this, the Dietitians of Canada recommend consuming at least 2 servings of fish per week, providing approximately 300 to 450 mg of EPA + DHA per serving.[15] In 2010, the Food and Agriculture Organization of the United Nations

(FAO) released recommendations for adult males and non-pregnant/non-lactating females to consume 250 mg of EPA + DHA per day.[16]

Concerns have been raised, however, that meeting these dietary recommendations may pose a risk to human health, due to the potential for inadvertent consumption of environmental contaminants (i.e. chemicals that enter the environment as a result of human industrial activity).[17] Because these lipophilic contaminants enter the marine food chain and bioaccumulate in the fatty tissues of fish, crude fish oil derived from fatty tissues may contain high concentrations of environmental contaminants.[12] However, the precise levels of contaminants present in fish depend on several factors, including the following: the species of fish; the age of fish; the type of tissue from which the oil was derived (i.e. oil extracted from the liver typically contains more contaminants than that taken from muscle tissue); the geographical location where the fish were collected; and the use of refinement during oil preparation.[12,18] The number of environmental contaminants detected in fish range from heavy metals (i.e. arsenic, cadmium, lead and mercury) to persistent organic pollutants (i.e. polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated diobenzofurans (PCDFs) and dioxin-like PCBs), named accordingly for their ability to endure the environment without breaking down.[17,19] All present a risk to human health, and, at levels exceeding threshold limits, have the potential to negate the inherent cardiovascular benefits of fish oils.[20] For instance, mercury may promote

atherosclerosis,[21] whereas PCBs and related compounds exhibit adverse dermatologic, reproductive, developmental, endocrine, hepatic and immunologic effects.[22-24]

To mitigate possible health risks associated with contamination, the fish oil industry utilizes several oil refining techniques, including steam stripping, cold filtration, distillation and activated charcoal filtration.[18] These processes however, may also remove the health promoting constituents EPA and DHA found in fish oil.[12] In an effort to monitor the equilibrium between contaminant reduction and fatty acid maintenance, the industry developed what is now known as the Global Organization for EPA and DHA Omega-3 (GOED) Voluntary Monograph. This quality standard incorporates the strictest global regulatory considerations, theoretically ensuring the safe consumption of any oil manufactured according to the Monograph's specifications. The World Health Organization and the European Pharmacopeia have not established heavy metal limits,[25] and while California's Proposition 65 (Safe Harbour) has set <15 ppm, <10 ppm, and <4.1 ppm limits for lead, arsenic, and cadmium, respectively,[26] these limits are well over the <0.1 ppm established by GOED. GOED's Monograph is updated as necessary to reflect the ever-changing international regulatory landscape.[27]

The objective of this study was to determine the n-3 fatty acid, heavy metal, PCB, PCDD and PCDF, and dioxin-like PCB concentrations in a large sample of dietary fish oil supplements readily available in the global marketplace. Determination of the latter contaminant

concentrations facilitated a comparison of respective GOED Voluntary Monograph tolerances and an overall evaluation of consumer safety of fish oil supplementation.

Materials and Methods

Sample Description. Between November 2005 and December 2010, data from finished product fish oil samples were submitted for analysis by manufacturers to Nutrasource Diagnostics Inc., a third party contract research organization located in Guelph, Ontario, Canada and were used in the construction of the dataset. Any data corresponding to samples that were for research purposes (e.g. research and development, raw material and stability studies, etc.), were not from a fish source or blended with non-marine oils, or were not analyzed for fatty acids, heavy metals, or persistent organic pollutants were removed, leaving a remainder of 1894 samples representing approximately 44 brands from 8 countries. As this study was retrospective in nature, pinpointing the source and/or type of fish oil for each sample was not feasible. Having said this, a wide representation of fish species, from the key global raw material marine oil suppliers, was used. As a result, the samples analyzed include species of *Clupeidae* (herring, menhaden, and sardine), *Engraulidae* (anchovy), *Salmonidae* (salmon), and *Scombridae* (tuna) oils at various concentrations of EPA and DHA. Crude oils were extracted and purified using urea crystallization and molecular distillation, both of which concentrate the n-3 fatty acids in fish oil.[28,29] Cis-trans isomerisation during oil processing would have been an indication of a chemical change, resulting from severe conditions (i.e. high temperature), however, no such data was available.[30]

Samples were grouped according to combined concentrations of EPA + DHA. A concentration of ≤ 50% EPA + DHA or > 50% EPA + DHA was selected as a surrogate for the type of fish oil products sampled (i.e. non-concentrated vs. concentrated, respectively). Note that while those samples allocated to the > 50% EPA + DHA category may not have been 'true' concentrates (i.e. the samples may have been several fish oils blended to reach a desired final concentration of EPA + DHA), these concentration categories are representative of blends available in the marketplace.

Analytical methods. Fatty acid analysis was conducted by an American Oil Chemists' Society (AOCS) Approved Chemist for its AOCS/GOED n-3 oils testing. The AOCS' Laboratory Proficiency Program verifies quality control practices so that EPA and DHA are consistently and accurately measured. Samples were analyzed by a Modified AOCS Official Method Ce 1b-89. This method used a gas chromatograph with capillary injection and flame-ionization to detect fatty acids in marine oils (and oil esters). The chromatograph consisted of a flexible fused silica capillary column, 25 m (or more) in length and 0.20-0.35 mm in (internal) diameter, with a liquid phase of bonded Carbowax-20M (or equivalent polyglycol). Samples were analyzed after isooctane extraction using $C_{23:0}$ methyl ester internal standard metal concentrations with an injection volume of 1-2 μl. Total fatty acid composition was determined in relative (area %) values, while EPA + DHA in absolute (mg/g) values according to the calculations provided in the method.

Heavy metal analysis was performed in a facility accredited by the Standards Council of Canada (i.e. CAN-P-1585, CAN-P-1587, and CAN-P-4E) and the Quality Management Systems ISO 9001:2008 and ISO/IEC 17025:2005. Success in obtaining these certifications is contingent on the laboratory's consistent fulfillment of customer needs and applicable statutory, quality control and regulatory requirements. Sample preparation for arsenic, cadmium, lead and mercury was completed by US EPA 3051 (Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils), where a representative sample up to 0.5 g was digested in 10 mL of concentrated nitric acid for 10 minutes in a fluorocarbon microwave vessel. After digestion, the sample was filtered, centrifuged or allowed to settle in preparation for analysis. Arsenic, cadmium and lead concentrations were measured using US EPA 200.8 (Methods for the Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma – Mass Spectrometry). Sample material in solution was exposed to radiofrequency plasma causing atomization and ionization of the sample. The ions were then extracted from the plasma and separated according to mass-to-charge ratio by a quadrupole mass spectrometer with minimum resolution of 1 amu peak width at 5% peak height. For determination of mercury concentration, US EPA 245.6 (Determination of Mercury in Tissues by Cold Vapor Atomic Absorption Spectrometry) was used. After digestion of the sample, quantification of mercury occurred via reduction of the sample with 5 mL of stannous chloride solution to elemental mercury, which was measured on the spectro-photometer.

Testing of PCBs, dioxins and furans, and dioxin-like PCBs was carried out in a facility certified to the Quality Management Systems ISO 9001:2008, ISO/IEC 17025:2005 (by the Canadian Association for Laboratory Accreditation Inc.) and ISO Guide 34:2009 (by ACLASS ANSI-ASQ National Accreditation Board), ensuring the highest level of quality testing. PCB concentrations were quantified by Method PCB US EPA 1668 Revision A. This analytical technique utilized isotope dilution high resolution gas chromatography/high resolution mass spectrometry for congener-specific determination of PCBs. For sample preparation, a 20 g aliquot of sample was homogenized and a 10 g aliquot was spiked with the labeled compounds. The sample was mixed with anhydrous sodium sulfate, allowed to dry for 12-24 hours, and then extracted for 18-24 hours using methylene chloride in a Soxhlet extractor. This extract was then evaporated and the lipid content determined. A labelled cleanup standard was spiked into the extract and back-extracted with sulfuric acid (and/or base, and gel permeation), silica gel, or Florisil chromatography. The extract was concentrated to 20 μl, labelled internal standards added, and injected into the gas chromatograph where the analytes were separated for detection by the high-resolution mass spectrometer. By applying this method, total PCB concentration was determined for all 209 PCB congeners, including the seven indicator International Union of Pure and Applied Chemistry (IUPAC) congeners 28, 52, 101, 118, 138, 153, and 180.

Dioxins and furans (i.e. PCDDs and PCDFs) and dioxin-like PCBs vary in dioxin-like toxicity, thus the use of the World Health Organization (WHO) Toxic Equivalency Factors

(TEFs) were required.[31] TEFs allow less hazardous dioxins to be expressed as an overall equivalent concentration of the most toxic dioxin, 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (2,3,7,8-TCDD).[25] All toxicologically significant concentrations were summed and shown as a single quantifiable Toxic Equivalent, or WHO-TEQ.[31] To determine the WHO-TEQ values of dioxins and furans in the fish oil samples, US EPA 1613 Revision B was used. Sample preparation and extraction were relatively similar to that of PCBs (described above), less the use of methylene chloride:hexane in place of methylene chloride. The resulting extract was concentrated to near dryness, internal standards added, and an aliquot injected into the gas chromatograph where separation and detection by the high-resolution mass spectrometer occurred.

Due to the high number of samples processed (i.e. 1800+), providing recovery information for surrogate standards, reference material or results from duplicate analysis, limits of detection, laboratory blanks, injection volumes, and/or clean up procedures was not feasible for each sample analyzed. However, all laboratories used in this investigation were controlled scientifically to respective quality assurance standards (see above).

Statistical methods. Overall means and standard errors were calculated with the SAS 9.2™ program using the PROC MEANS procedure for the following parameters: EPA; DHA; EPA + DHA; heavy metals; PCBs; PCDDs and PCDFs; and dioxin-like PCBs. Means were calculated by year and by % EPA + DHA category (i.e. non-concentrated ≤ 50% vs. concentrated > 50%). WHO-TEQ values with detection limits were calculated using a positive value, if

detected. However, if a non-detected/no detectable residue values was found, the detection limit was used in calculation for that particular compound.

Results

As this analysis was retrospectively based on manufacturer-specific requisitions, not all 1894 samples were analyzed for all parameters of interest, leading to different numbers of observations throughout the dataset. Results from the n-3 fatty acid, heavy metal, PCB, PCDD and PCDF, and dioxin-like PCB analyses are summarized in **Tables 1** through **9**, respectively, below.

Table 1. Mean Fatty Acid Content, By Year and Overall

Year	Fatty Acid	Number of Samples (N)	Mean Concentration (%)	Standard Error
2005	EPA	100	25.60	1.393
	DHA	100	20.11	0.7384
	EPA + DHA	110	41.55	2.121
2006	EPA	92	34.80	1.841
	DHA	92	23.80	1.421
	EPA + DHA	148	36.42	2.624
2007	EPA	70	27.50	2.238
	DHA	70	20.25	1.779
	EPA + DHA	127	26.32	2.703
2008	EPA	204	32.15	1.075
	DHA	204	22.66	0.9115
	EPA + DHA	255	43.85	1.753
2009	EPA	377	25.54	0.7452
	DHA	377	17.96	0.5525
	EPA + DHA	444	36.94	1.140
2010	EPA	658	27.56	0.6545
	DHA	658	20.80	0.4459
	EPA + DHA	810	39.28	0.9577
Overall (2005-2010)	EPA	1501	27.99	0.4185
	DHA	1501	20.45	0.3022
	EPA + DHA	1894	38.39	0.6261

In combination, the mean concentration of EPA and DHA was 38.39% across all samples (as per **Table 1**). Individually, these fish oil constituents showed anticipated lower mean values of 27.99% EPA (N = 1501) and 20.45% DHA (N = 1501). As representative by these mean values, the EPA content of the fish oil samples was consistently higher than that of DHA. While combined EPA and DHA content was relatively stable over the 5 year term, 2007 witnessed a significant drop to 26.32% - a stark contrast to the following year which showed the highest concentration at 43.85%.

Table 2. Mean Heavy Mental Content By Year

Year	Heavy Metal	Number of Samples (N)	Mean Concentration (mg/kg)	Standard Error	GOED Specification (mg/kg)[32]
2005	As [A]	48	0	0	< 0.1
	Cd	45	0	0	< 0.1
	Pb	50	0	0	< 0.1
	Hg	50	0	0	< 0.1
2006	As [A]	40	0	0	< 0.1
	Cd	43	0	0	< 0.1
	Pb	72	0	0	< 0.1
	Hg	76	0	0	< 0.1
2007	As [A]	39	0	0	< 0.1
	Cd	39	0	0	< 0.1
	Pb	73	0.0004	0.0003	< 0.1
	Hg	74	0	0	< 0.1
2008	As [A]	86	0.0244	0.0157	< 0.1
	Cd	86	0.0037	0.0037	< 0.1
	Pb	129	0.0036	0.0016	< 0.1
	Hg	128	0.0003	0.0002	< 0.1
2009	As [A]	141	0.3624	0.1281	< 0.1
	Cd	139	0.0003	0.0002	< 0.1
	Pb	158	0.0034	0.0020	< 0.1
	Hg	159	0.0025	0.0015	< 0.1
2010	As [A]	310	0.0854	0.0382	< 0.1
	Cd	311	0.0001	0.0001	< 0.1
	Pb	306	0.0016	0.0008	< 0.1
	Hg	305	0	0	< 0.1
Overall (2005-2010)	As [A]	664	0.1200	0.0329	< 0.1
	Cd	663	0.0006	0.0005	< 0.1
	Pb	788	0.0019	0.0006	< 0.1
	Hg	792	0.0005	0.0003	< 0.1

A. Although Total Arsenic was measured, the GOED Monograph specifies limits for Inorganic Arsenic only.

Table 3. Percentage of Samples Above and Below GOED Heavy Metal Specifications		
Heavy Metal Component	% of samples Containing <0.1 mg/kg	% of samples Containing ≥ 0.1 mg/kg
Total As (N=664)	95.8%	4.2%
Cd (N=663)	99.85%	0.15%
Pb (N=788)	99.1%	0.90%
Hg (N=792)	99.75%	0.25%

Although cadmium (0.0006 mg/kg), lead (0.0019 mg/kg), and mercury (0.0005 mg/kg) were measured and detected in 663, 788, and 792 of the total fish oil samples, respectively, all (mean) levels were below corresponding GOED allowances (as per **Table 2**). Total arsenic (organic and inorganic) was measured in 664 samples; however, GOED has not defined tolerance limits for total concentrations of this heavy metal, only inorganic levels. The total mean arsenic concentration (0.1200 mg/kg) was considerably higher than those of the other heavy metals, but as no discriminatory testing was performed, it is possible that the majority of arsenic detected was in the relatively non-toxic organic form.[33] Also, as illustrated in **Table 3**, 95.8% of the 664 samples in which arsenic was detected fell below the 0.1 mg/kg GOED tolerance limit, suggesting that perhaps the outliers of 2009 (0.3624 mg/kg; see **Table 2**) may have been responsible for the mean concentration exceeding the GOED tolerance limit for arsenic.

Table 4. Mean PCB Content, By Year, Congener Value and Overall

Year	Number of Samples (N)	Mean Concentration (ppb)	Standard Error	GOED Specification (ppb)[32]
2005	64	28.95	4.772	≤ 90
2006	53	26.24	3.056	
2007	37	21.44	4.111	
2008	80	44.94	16.25	
2009	133	26.71	4.554	
2010	316	17.65	1.682	
209 Congeners	366	30.94	5.11	
IUPAC (7 Congeners)	366	13.04	1.7	
Overall (2005-2010)	683	24.54	2.319	

Table 5. Frequency of Samples at Varying PCB Cut-off Levels

PCB Cut-off (ppb)	Number of Samples (N)	Percent of Samples (%)	Cumulative Percent of Samples (%)
≤ 30	533	78.04	78.04
≤ 45	73	10.69	88.73
≤ 90[A]	55	8.05	96.78
> 90	22	3.22	100.0

A. ≤ 90 ppb = GOED specification limit for PCBs

PCBs (209 congeners) were measured in a total of 683 samples; however, annual and overall mean levels were below the specification limits outlined by the GOED Voluntary Monograph (as per **Table 4**). Of the 683 samples measured, 661 (96.78%) samples were at or below GOED specifications for PCBs (as per **Table 5**). With respect to the more stringent PCB cut-offs of ≤ 45 and ≤ 30 ppb, 73 (10.69%) and 533 (78.04%) samples measured, respectively, fell at or below these limits. Of the 366 samples in which both total and the seven indicator PCB congeners (IUPAC numbers 28, 52, 101, 118, 138, 153, and 180) were measured, mean concentrations (30.94 ppb and 13.04 ppb, respectively) were also within GOED PCB cut-off levels (see **Table 4**).

Table 6. Mean PCDD and PCDF Content (2005-2010)

Contaminant Component	Number of Samples (N)	Mean Concentration (ppt)	Standard Error	GOED Specification (ppt WHO-TEQ)[32]
PCDDs and PCDFs [A]	484	0.55	0.028	≤ 2

A. WHO-TEQ with detection limits

Table 7. Mean Dioxin-like PCB Content (2005-2010)

Contaminant Component	Number of Samples (N)	Mean Concentration (ppt)	Standard Error	GOED Specification (ppt)[32]
Dioxin-like PCBs [A]	297	0.834	0.096	≤ 3

A. WHO-TEQ with detection limits

As previously mentioned, dioxin and furan limits include the sum of PCDDs and PCDFs, expressed as a single WHO-TEQ value.[32] PCDDs and PCDFs were measured in 484 samples at a mean concentration of 0.55 ppt WHO-TEQ, substantially lower than the 2 ppt WHO-TEQ cut-off defined by GOED (as per **Table 6**). Similarly, the mean concentration of dioxin-like PCBs (0.834 ppt WHO-TEQ) was considerably below the 3 ppt WHO-TEQ GOED limit in the 297 fish oil samples measured (as per **Table 7**).

Table 8. Mean Mercury Content Considering Fatty Acid Concentration

Fish Oil Supplement Category	Number of Samples (N)	Mean Concentration of Hg (mg/kg)	Standard Error	GOED Specification (mg/kg)[32]
Overall	792	0.0005	0.0003	< 0.1
≤ 50% EPA + DHA	266	0.0009	0.0006	
> 50% EPA + DHA	526	0.0003	0.0003	

Table 9. Mean PCB Content Considering Fatty Acid Concentration, By Year

Year	Number of Samples (N) ≤ 50% EPA + DHA	Mean Concentration (ppb)	Standard Error	Number of Samples (N) > 50% EPA + DHA	Mean Concentration (ppb)	Standard Error	GOED Specification (ppb)[32]
2005	2	14.37	9.109	62	29.42	4.910	≤ 90
2006	22	26.46	5.025	31	26.09	3.891	
2007	14	19.33	6.989	23	22.72	5.169	
2008	2	934.3	61.51	78	22.13	2.537	
2009	31	32.75	14.11	102	25.87	4.151	
2010	96	24.84	4.805	220	14.51	1.151	
Overall (2005-2010)	167	36.82	8.614	516	20.57	1.249	

To account for the differences in the types of fish oils evaluated in this sample, results were divided according to two categories of fish oil supplements: supplements containing > 50% EPA + DHA concentration and supplements containing ≤ 50% EPA + DHA concentration. The mean mercury levels from the two categories of fish oil supplements analyzed within the 5 year testing period are illustrated in **Table 8**, and the mean PCB content, overall and by year, are reported in **Table 9**. Less concentrated fatty acid samples (≤ 50% EPA + DHA) showed greater mean mercury and PCB levels (0.0009 mg/kg and 36.82 ppb, respectively), while higher

concentrates (> 50% EPA + DHA) were less contaminated (i.e. 0.0003 mg/kg and 20.57 ppb, respectively).

Discussion

Unlike many previous studies which examined n-3 fatty acid and/or contaminant concentrations in a limited number of fish oil products, the data presented above represents a vast sample size, collected globally over a 5-year period, analyzed and compared against rigorous GOED contaminant allowances for heavy metals and PCBs.

Mercury. As discussed previously, fish consumption can lead to mercury exposure due to potential for environmental contamination. It has been shown that larger carnivorous fish (i.e. shark and swordfish) have the highest concentration of mercury in their tissues at a value of 1 μg/g. Conversely, smaller fish such as bass, pike, trout, and tuna have tissue mercury concentrations ranging between 0.1 and 0.5 μg/g, followed by invertebrates (i.e. shellfish) with the lowest concentrations.[20,34,35] Consequently, the type and amount of fish consumed can affect mercury status. For example, a 70 kg American adult has a mean daily mercury intake of 3.5 μg, whereas an adult in Finland, an area of high fish consumption, has an elevated mean mercury intake of 7.6 μg per day.[34] Thus, the need arises for research assessing mercury levels in fish and/or fish oils in relation to human health.

Previous work reporting heavy metal levels in cod liver oil showed that regular use of the oil led to a weekly mercury intake of 8 μg, substantially lower than that of fish meals at 125 μg.[36] A study examining mercury levels in several popular fish oil supplement products (i.e. CVS, Kirkland, Nordic Ultimate, Omega Brita, and Sundown) found negligible amounts of mercury,

suggesting even mega-dosing with concentrated fish oil products would not lead to mercury toxicity.[21] These results are consistent with other research articles which found negligible amounts of heavy metals in fish oil supplements.[37-39] Of the 792 samples tested in this study, a mean concentration of 0.0005 mg/kg is a level well below the GOED Voluntary Monograph mercury allowance of 0.1 mg/kg. When samples were grouped according to fatty acid content, a lower mean level of mercury (0.0003 mg/kg) was observed in the more concentrated (i.e. > 50% EPA + DHA) samples, as compared to a mean mercury level of 0.0009 mg/kg in the less concentrated sample fragment. Nonetheless, both mean mercury concentration values were within GOED specification limits.

PCBs. Although the use of PCBs was banned in North America in 1977, they can still be found in transformers and capacitors made before this time.[40] Coupled with their inability to degrade, PCBs tend to endure in the environment and food chain.[41] For example, bottom-feeding fish consume PCBs, causing the contaminant to concentrate higher up in the food chain, at levels ranging from 600 to as high as 20,000 ppb in such fish.[23,42-44] Studies such as that conducted by Bourdon et al. substantiate such findings, as n-3 PUFA products derived from salmon and seal oil yielded the highest PCB concentrations, suggesting that supplements made from small, cold-water, fatty fish may be safer for human consumption.[45] Similarly, of the fish and seal oil dietary supplements analyzed by Rawn et al., a shark oil sample contained the highest mean ∑PCB concentration (10,400 ng/g), whereas supplements derived from mixed fish oil (i.e. anchovy,

mackerel, and sardine) contained the lowest levels (0.711 ng/g).[46] Two brands of Japanese deep-sea shark oil were also found to contain high levels of PCBs at 290 and 340 ng/g oil weight, respectively.[47] Jacobs et al. reported levels of PCBs in 44 fish oil products, the results of which showed a salmon oil sample from a retail outlet in the United Kingdom to contain the highest total concentration (1,132 μg PCB/L).[48] Cod liver oil and concentrated fish oils were among the samples high in PCBs, yet no value exceeded the 2.0 ppm US FDA limit for total PCBs in foodstuffs.[48] Results from Jacobs et al. showed PCB concentrations in fish oil samples tested were generally of the same order of magnitude[49], but not as high as those previously reported.[48] In a similar fashion to the trial conducted by Foran et al. assessing mercury contamination in fish oil products[21], Melanson et al. analyzed the same five popular brands for PCBs, but could not detect the compounds in any sample.[41]

Despite controls surrounding its use, results from several aforementioned studies illustrate that considerable quantities of PCBs can still be found in fish oil supplements, confirming the persistent nature of this contaminant. Therefore, a large, global, current assessment of PCB contamination in fish oil supplements was undertaken. In the 683 samples measured in this study for PCB contamination, the mean concentration of 24.54 ppb was acceptable by GOED specifications (i.e. ≤ 90 ppb). Further to this, the majority of the samples (i.e. 77.92%; **Table 5**) met even more stringent limits of ≤ 30 ppb, consistent with findings from Melanson et al., in which PCBs were not detected in any of the popular fish oil brands tested.[41]

On the whole, 96.64% of PCB measurements were below the GOED Voluntary Monograph limit, suggesting either that majority of samples were sourced from fish that naturally contain less PCBs, such as smaller, cold-water, fatty fish, or that the refining techniques employed by industry (such as steam stripping, cold filtration, distillation and activated charcoal filtration) are effective. Although Fernandes et al. previously hypothesized that these refining methods remove beneficial fatty acids,[12] this was not the case in the current analysis (see **Tables 8** and **9**), as higher fish oil concentrations (>50% EPA + DHA) were less polluted with mercury and PCBs than their lower concentrate counterparts.

Dioxins and Furans and Dioxin-like PCBs. Unlike PCBs, PCDDs and PCDFs were not made deliberately and serve no discernible purpose; rather, they are a result of industrial and combustion activities.[50] Nonetheless, human exposure of PCDD and PCDF occurs in part from dietary intake, including that of fish and fish-related products.[51] In an analysis of fish oil products by Fernandes et al., WHO-TEQ values ranged from 0.18 to 8.4 ng/kg for ∑PCDD and PCDF and from ∑1.1 to 41 ng/kg for dioxin-like PCBs, which in comparison to previous work, suggests dioxin and furan levels in fish oil supplements are decreasing (i.e. 0.3 to 10 ng/kg for ∑PCDD and PCDF observed in 1996).[12]

As PCDDs and PCDFs separate to organic matter, they collect in marine life and bio-magnify up the food chain, through ongoing consumption of contaminated prey.[52] For example, previous research analyzed 30 n-3 PUFA-rich fish oil-containing supplement samples, of which

shark samples had the highest median ∑PCDD and PCDF levels (24 pg TEQ/g), followed by seal (1.4 pg TEQ/g), salmon (1.0 pg TEQ/g), menhaden (0.47 pg TEQ/g), mixed fish oils with (0.08 pg TEQ/g) and without salmon (0.07 pg TEQ/g), etc.[53] These results fall in line with the PCB analysis conducted by Rawn et al., in which shark oils were found to contain higher contaminant levels than mixed fish oils,[46] further substantiating the biomagnifying effect of persistent organic pollutants. In a similar initiative which analyzed 15 n-3 PUFA rich supplements, concentrations of PCDDs and PCDFs and dioxin-like PCBs ranged from 0.04-2.4 pg TEQ/g and 0.01-12.1 pg TEQ/g, respectively.[54] Vegetable and mineral oil derived supplements showed expectedly lower contaminant concentrations than fish oil products, of which, cod liver oil had the highest persistent organic pollutant levels, characteristic of oil extracted from the liver.[12]

In the present investigation, PCDDs and PCDFs were measured in 484 samples at a mean concentration of 0.55 ppt WHO-TEQ, substantially lower than the 2 ppt WHO-TEQ cut-off defined by GOED. Similarly, the mean concentration of dioxin-like PCBs (0.834 ppt WHO-TEQ) was considerably below the 3 ppt WHO-TEQ GOED limit in the 297 fish oil samples measured, telling of a global reduction in dioxin and dioxin-like PCBs among dietary fish oil products. Further to this, samples may have been derived from small, young fish with little pollutant accumulation, mixed fish oils, or less contaminant-concentrated muscle tissues.

Overall, the large sample size of fish oil products evaluated herein for n-3 fatty acid, heavy metal, PCB, PCDD and PCDF, and dioxin-like PCB concentrations is representative of fish harvested from a range of geographic locations, during a variety of seasons, from an assortment of supply chains, over a 5-year period. When collectively analyzed, results demonstrate that these samples meet or exceed industry standards for contaminant allowances. The breadth and depth of this analysis supports the efforts the fish oil industry has made to ensure that the consumer has access to safe, high-quality products.

Abbreviations: Omega-3, n-3; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; US FDA, United States Food and Drug Administration; FAO, Food and Agriculture Organization of the United Nations; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzo-p-dioxins; PCDFs, polychlorinated diobenzofurans; GOED, Global Organization for EPA and DHA Omega-3; WHO-TEFs, World Health Organization Toxic Equivalency Factors (TEFs); 2,3,7,8-TCDD, 2,3,7,8-tetrachloro-dibenzo-p-dioxin; WHO-TEQ, single quantifiable Toxic Equivalent.

Acknowledgements: Maggie Laidlaw, Ph.D., Clinical Trials Director at NDI is acknowledged for editing the manuscript. Jennifer Andrews, M.Sc., Corporate Branding Manager at NDI is

acknowledged for editing, as well as Table of Contents Art photography. Preparation of this manuscript was financially supported by GOED.

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