Anthropogenic and Naturally Occurring Organobrominated Compounds in Fish Oil Dietary Supplements

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Fish oil dietary supplements (FODS) are recommended to increase the intake of polyunsaturated fatty acids (PUFAs), renowned for their beneficial effects on human health. However, FODS also contain anthropogenic contaminants, such as polychlorinated biphenyls and polybrominated diphenyl ethers (PBDEs). Sixty-nine ($n = 69$) PUFA-enriched FODS from 37 producers were collected in 2006 and then analyzed for their levels of organobrominated compounds. Levels of the sum of tri- to hepta-BDEs (BDEs 28, 47, 49, 66, 85, 99, 100, 153, 154, and 183) were typically below 5 ng/g oil, while only a few had higher values of up to 44 ng/g oil. Several peaks in the chromatograms were identified as methoxylated PBDEs (MeO−PBDEs) and polybrominated hexahydroxanthene derivatives (PBHDs). These two groups of compounds have been suggested to be produced by marine organisms (e.g., algae and sponges) and have also been reported in marine samples, such as fish and marine mammals. Median concentrations of MeO−PBDEs and PBHDs (6.2 and 5.3 ng/g oil, respectively) were higher than median concentrations of PBDEs (0.6 ng/g oil), and their maximum values were 1670 and 200 ng/g oil, respectively. FODS are intended to be consumed on a daily basis, and the median daily intakes of MeO−PBDEs and PBHDs from FODS were 3 and 6 times higher than the median intake of PBDEs (3 ng/day). Consumption of FODS does not appear to substantially increase the total dietary intake of PBDEs since the median daily intake from FODS was 8 and 16 times lower than the intake from either fish consumption alone or from total diet. These findings indicate that FODS might be a suitable alternative to fish consumption for certain segments of the population for which fish consumption advices have been issued. The present study also strongly supports the need for not only the inclusion of new anthropogenic contaminants (e.g., PBDEs) but also of naturally occurring compounds in monitoring schemes of marine products destined for human consumption.

Introduction

Both marine fish and fish-derived products (e.g., fish oils) contain essential long chain polyunsaturated fatty acids (PUFAs), such as 5,8,11,14,17-eicosapentaenoic acid (EPA) and 4,7,10,13,16,19-docosahexaenoic acid (DHA), which are essential in the human diet. They are needed for many metabolic functions including growth, structural maintenance, repair of nervous tissue, cellular membrane phospholipid structure, or regulation of lipid metabolism (*1*−*3*). Moreover, the intake of high amounts of PUFAs has been suggested to have several beneficial effects on human health, including decreasing the incidence and progression of vascular diseases, as well as reducing the symptoms of multiple sclerosis and/or osteoporosis (*2*, *3*).

Recently, it has been shown that consuming fatty fish (e.g., salmon, herring, etc.) may result in increased exposure to a variety of persistent contaminants, such as dioxins, polychlorinated biphenyls (PCBs), or polybrominated diphenyl ethers (PBDEs), resulting in a potential increase in health risks that could counteract the beneficial effects of PUFAs (*4*, *5*). A wide range of toxicological and hormonal effects, including endocrine disruption, reproductive, neurobehavioral, and developmental disturbances, are caused by these environmental contaminants (*3*, *6*). Several environmental and health agencies have already issued consumption recommendations, which range between 0.5 and 2 meals of fatty fish per month (*4*). The general public is given seemingly conflicting reports about the risks and benefits of fish intake, resulting in controversy and confusion over fish and fish-derived products and their role with regard to a healthy diet (*7*).

In recent years, fish oil dietary supplements (FODS) have been increasingly promoted as an alternative to fish consumption. Indeed, FODS contain high and balanced custom-made amounts of DHA and EPA (*1*). However, FODS are also a potential source of toxic contaminants, especially when the fish oil produced originates from fish caught in contaminated waters or from farmed fish fed with contaminated feed. Fish oil produced from these sources may contain markedly higher amounts of contaminants than fish originating from less polluted sites (*8*−*10*). Since FODS are recommended to be taken on a daily basis, it is therefore important to closely monitor the levels of contaminants that might be present in PUFA-enriched FODS.

Several recent studies have described the presence in the marine environment of naturally occurring halogenated compounds, such as methoxy-PBDEs (MeO−PBDEs), including fish species used for the preparation of FODS (*11*−*13*). Also, recently, a new class of brominated compounds of natural origin, polybrominated hexahydroxanthenes (PBHDs), has been discovered in marine samples (*14*, *15*). Both classes of naturally produced compounds (Figure 1) have been measured in concentrations higher than contaminants

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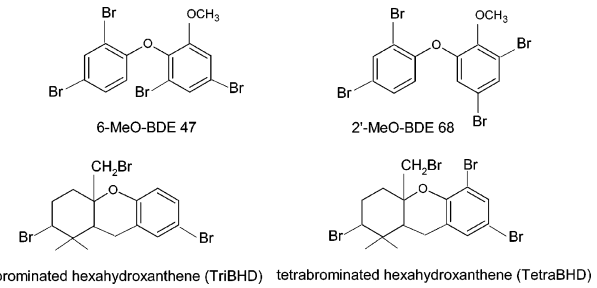

FIGURE 1. Chemical structures of the two tetrabrominated methoxy-PBDEs (6-MeO-BDE 47 and 2′-MeO-BDE 68) and two brominated hexahydroxanthenes (tri-BHD and tetra-BHD).

usually targeted in monitoring schemes, but not much is known about their occurrence, their dietary intake from fish and fish-derived products, or the potential toxicological effects of these compounds.

Since there are little data available in the public domain about FODS, this study reports the levels of brominated compounds of both anthropogenic and natural biogenic origins in PUFA-rich FODS obtained through pharmacies and retail outlets in various countries. Furthermore, we have estimated the daily intake of these brominated compounds through FODS and compared it with the intake from fish consumption alone or in a total diet, respectively.

Materials and Methods

Sample Description. PUFA-rich FODS were collected between January and March 2006. Although this is not a comprehensive survey of all brands available, samples were chosen to cover the diversity of products available for sale on the Belgian market. Additionally, FODS bought in The Netherlands, Ireland, U.K., and South Africa were also analyzed. In total, 69 ($n = 69$) FODS from 37 producers were collected in duplicate. Product expiry dates were checked to ensure the validity of the product shelf life during the measurement period. Information on recommended dosage as provided on the product labels together with the EPA and DHA composition were also recorded.

Materials. All solvents used for the analysis (*n*-hexane, dichloromethane(DCM), isooctane) were of SupraSolv grade (Merck, Darmstadt, Germany). Individual reference standards of PBDEs and MeO−PBDEs were purchased from Wellington Laboratories (Guelph, ON, Canada), while MeO-PBDE standard were a gift from Accustandard (New Haven, CT). 2,7-dibromo-4a-bromomethyl-1,1-dimethyl-2,3,4,4a,9,9a-hexahydro-1*H*-xanthene (tri-BHD) and 2,5,7-tribromo-4a-bromomethyl-1,1-dimethyl-2,3,4,4a,9,9a-hexahydro-1*H*-xanthen e (tetra-BHD), synthesized as described by Melcher et al. (*15*), were obtained at a concentration of 1.6 ng/μL in isooctane from W. Vetter (University of Hohenheim, Hohenheim, Germany). Silica (0.063−0.200 mm, Merck) was pre-washed with *n*-hexane before use.

Sample Preparation. Prior to analysis, samples were stored in their original containers at room temperature. For samples sold as capsules, the contents of 10 capsules were pooled. Initially, samples were analyzed for 10 PBDE congeners (nos. 28, 47, 49, 66, 85, 99, 100, 153, 154, and 183). Following reports of biologically produced MeO−PBDEs in marine samples (*11*), two tetrabrominated MeO−PBDEs (2′-MeO-BDE 68 and 6-MeO-BDE 47) were also included in the analysis. Analytical procedures have been previously described in detail (*9, 16*), and a brief summary is presented next. An aliquot of 200−250 mg of oil was solubilized in 3 mL of *n*-hexane, internal standards (1 ng of BDE 77 and BDE 128) were added, and the mixture was mixed via vortex for 30 s. The extract was applied to an *n*-hexane pre-washed cartridge filled with 8 g of acidified silica (45%, w/w) and was eluted with 15 mL of *n*-hexane and 10 mL of DCM. The final eluate was concentrated with a rotary evaporator and then dried under a nitrogen stream. The dried extract was redissolved in 100 μL of isooctane.

For the identification and confirmation of MeO−PBDEs and PBHDs, some extracts were further fractionated on silica cartridges (500 mg, 3 mL, BondElut, Varian), and full scan mass spectra were acquired in electron ionization (EI), positive chemical ionization (PCI), and electron capture negative ionization (ECNI) modes. After loading the previously obtained extract, two fractions were collected. The first fraction was eluted with *n*-hexane, mainly containing PCBs and less-polar pesticides, while the second fraction, containing all investigated brominated compounds, was eluted with 3 mL of DCM.

Analysis. A Hewlett-Packard 6890 gas chromatograph (GC) coupled to a HP 5973 mass spectrometer (MS) was operated in ECNI mode. The system was equipped with a 14 m × 0.18 mm × 0.20 μm AT-5 capillary column (Alltech, Lokeren, Belgium). The ion source, quadrupole, and interface temperatures were 250, 150, and 300 °C, respectively. Helium was used as a carrier gas at constant flow (0.8 mL/min). The electron multiplier voltage was set at 2200 V. One μL of the extract was injected in solvent vent mode (initial injector temperature 90 °C, held for 0.03 min, then heated at 700 °C/min to 300 °C, vent time 0.03 min, vent flow 100 mL/min). The splitless time was 1.50 min. The temperature of the AT-5 column was programmed from 90 °C (1.50 min) to 200 °C at a rate of 25 °C/min and then to 300 °C at a rate of 5 °C/min and held for 10 min. Bromine ions ($m/z = 79$ and 81) were acquired in selected ion monitoring (SIM) mode. Dwell times were set at 50 ms. For the additional confirmatory acquisition of ECNI and PCI full scan spectra (m/z range 70−650 amu), the GC was operated under the same chromatographic conditions.

For confirmation of MeO−PBDEs and PBHDs, the second fraction, obtained from silica fractionation, was injected into a GC/MS operated in electron ionization (EI) mode and equipped with a 25 m × 0.22 mm × 0.25 μm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole, and interface temperatures were set at 230, 150, and 300 °C, respectively. The mass spectrometer was used in full scan (m/z range 70−650). One μL of the fractionated extract or standard was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min), then to 300 °C at 700 °C/min, pressure pulse 25 psi, pulse time 1.50 min). The splitless time was 1.50 min. Helium was used as a carrier gas at constant flow (1 mL/min). The temperature of the HT-8 column was kept at 90 °C for 1.50 min, then increased to 180 °C at a rate of 15 °C/min (kept for 2.0 min), further increased to 280 °C at a rate of 5 °C/min, and finally raised to 300 °C at a rate of 40 °C/min and kept for 15 min.

Quality Assurance and Quality Control. Quality control was performed by regular analysis of procedural blanks and replicate samples (for which relative standard deviations (RSD) were <5 %). A fish tissue from the BROC study (*17*) was used as laboratory reference material. Recoveries of analytes and internal standards were between 81 and 105% (RSD <7%) as measured by spiking experiments ($n = 3$) at a concentration of 5 ng/g oil for each individual compound. Additionally, the method performance was assessed through successful participation to Quasimeme interlaboratory studies (PBDEs in fish and fish oil) (*18*). Procedural blanks of PBDEs were consistent (RSD <20%), and therefore, the mean value of each analyte in the procedural blanks was used for subtraction. MeO−BDEs and PBHDs were not present in the procedural blanks. After blank subtraction, the limit of quantification (LOQ) was set at 3 × SD of the value obtained in the procedural blanks, ensuring >99% certainty that the

TABLE 1. Median Concentrations and Range (ng/g of Oil) of Brominated Contaminants in Fish Oil Dietary Supplements in Relation to Their Country of Production[a]

	all samples	Belgium	The Netherlands	U.K.	other countries[b]
n	69	28	18	12	11
BDE 47	0.42	0.41	0.55	0.11	0.22
BDE 99	<0.1	<0.1	<0.1	<0.1	<0.1
BDE 100	<0.1	<0.1	<0.1	<0.1	<0.1
sum PBDEs	0.59 (<0.2−44)	0.56 (<0.2−44)	0.73 (<0.2−16.9)	0.33 (<0.2−2.3)	0.47 (<0.2−7.5)
2-MeO-BDE 68	2.6	1.9	2.6	6.0	1.3
6-MeO-BDE 47	3.8	2.2	7.0	7.6	2.8
sum MeO-PBDEs	6.2 (<0.2−1670)	4.6 (<0.2−1670)	9.2 (<0.2−180)	14 (<0.2−320)	4.1 (<0.2−230)
tri-BHD	3.4	3.2	8.0	1.6	7.1
tetra-BHD	2.0	0.6	11.6	1.1	7.1
sum PBHDs	5.3 (<1.3−200)	3.8 (<1.3−98)	23 (<1.3−160)	2.8 (<1.3−160)	14.2 (<1.3−200)

[a] For samples for which individual measurements were below LOQ, a value equal to $f \times$ LOQ (f: fraction of samples with values above LOQ) was used. Only congeners with a frequency of detection higher than 25% were reported. No significant differences in the sum PBDEs, sum MeO-PBDEs, or sum PBHDs were found for FODS produced by different countries (Kruskal−Wallis test, $p > 0.05$). [b] Denmark, South Africa, U.S., France, and Sweden.

reported value is originating from the sample. Method LOQs ranged from 0.1 to 0.2 ng/g oil for individual PBDE and MeO-PBDE congeners and were 1 ng/g oil for each PBHD. In agreement with previous reports (*14*, *15*), the response factors of PBHDs were 6−8 times lower than those of PBDE congeners with the same number of bromine atoms.

Statistical Analysis. For calculation of statistical parameters, samples with levels below the LOQ were assigned a value of $f \times$ LOQ, where f is the fraction of measurements above the LOQ (*16*). Concentrations of contaminants in the samples were not normally distributed (Shapiro−Willks test), and therefore, nonparametric statistics were used. Spearman-rank correlations were calculated between concentrations of PBDEs, MeO−PBDEs, and PBHDs in the samples. A nonparametric Kruskal−Wallis test was used to check for differences among concentrations of contaminants as a function of their country of production. All statistical analyses were performed using SPSS v.11 for Windows.

To estimate the contaminant intake, daily recommended consumption of the supplements, as provided on the product labels, was multiplied by the corresponding concentrations. Intakes (ng/day) were calculated using lower bound (LB) and upper bound (UB) methods, where nondetects were replaced with a value equal to zero or LOQ, respectively.

Results and Discussion

PBDEs. Only BDEs 47, 99, and 100 had a detection frequency >25% and are presented in Table 1. BDE 183 was not detected in any sample, while BDEs 66, 85, 153, and 154 had a detection frequency <5%. BDEs 28 and 49 were detected at frequencies between 5 and 15%. For congeners with values below LOQ, a value of $f \times$ LOQ was used for the calculation of sum PBDEs, which here is referred to as the sum of tri- to hepta-BDEs (Table 1). With a few exceptions for which PBDE levels were up to 44 ng/g oil, the PBDE levels were generally very low, with the median concentration of all samples <1 ng/g oil (Table 1). This is probably due to an improved selection of fish used for the FODS preparation and/or to the final purification methods used by different producers. Although BDE 209 was not analyzed in the present study, it is expected to be found at very low concentrations or not detected in marine biota (*5*). Interestingly, several FODS with an elevated PBDE content also had higher DHA contents. It is not clear whether this is due to the fish sources used for the preparation of FODS with a high DHA content (e.g., tuna) or to the purification processes specific for DHA-enriched FODS. In general, PBDE levels measured in the present study are similar to or lower than those found in recent reports on PBDEs in FODS (*9*, *19*) and are lower than those found in fish oil used in the aquaculture industry, which in most cases is unpurified (*8*, *20*, *21*). In accordance with previously reported PBDE data in FODS (*9*, *19*), the congener profile was dominated by BDE 47 (53%), followed by BDE 100 (13%) and BDE 99 (11%) (Figure SI-1).

MeO−PBDEs. In addition to PBDEs, two tetrabrominated MeO−BDEs (2′-MeO-BDE 68 and 6-MeO-BDE 47) were identified in the GC/ECNI-MS chromatograms based on their retention times (Figure 2). Both findings were confirmed by EI-MS spectra (Figure SI-2). These two compounds were detected in 90% of the analyzed FODS. Concentrations of tetrabrominated MeO−BDEs were, in most cases, higher than the PBDE concentrations (Table 1), with values up to 1670 ng/g oil in some samples. This is not unusual, however, since such high concentrations (up to 1900 ng/g lipid) have been previously reported in marine mammals (*11*). In all the tested samples, the median ratio between concentrations of 2′-MeO-BDE 68 and 6-MeO-BDE 47 was 0.67, with a range from as low as 0.24 up to 3.76.

MeO−PBDEs are produced by algae, bacteria, or sponges (*13*) and have previously been found in various marine organisms, including fish and marine mammals (*11*, *12*). The presence of elevated concentrations of these compounds found in the higher levels of the marine food chain demonstrates their bioaccumulative properties. However, little is known of their potential toxicological effects.

Another interesting finding in this study is that there was no significant correlation between the levels of BDE 47 and 6-MeO-BDE 47 (Spearman rank $R_s = 0.32$, $p > 0.05$), suggesting that 6-MeO-BDE 47 is not only a possible metabolic product of BDE 47 in fish but could also come from other marine sources. Since the levels of 6-MeO-BDE 47 were highly correlated with the levels of 2′-MeO-BDE 68 ($R_s = 0.93$, $p < 0.01$), it is highly plausible that these compounds have both accumulated from similar sources. Furthermore, the presence of a methoxy group in the ortho position of both compounds supports their natural biogenic origin (*12*, *22*). HO−PBDEs with the HO group in the meta and para positions were the predominant metabolites observed after exposure of rats to a cocktail of PBDE congeners (*22*). In that study, 6-HO-BDE 47 was observed only in traces, while MeO−PBDEs could not be evidenced.

Interestingly, some FODS showing high levels of MeO−PBDEs were obtained from fish of the Pacific and southern Atlantic oceans. This observation agrees with reports on high concentrations (up to 1900 ng/g lipid weight) of MeO−PBDEs measured in marine mammals from the Southern hemisphere (*11*).

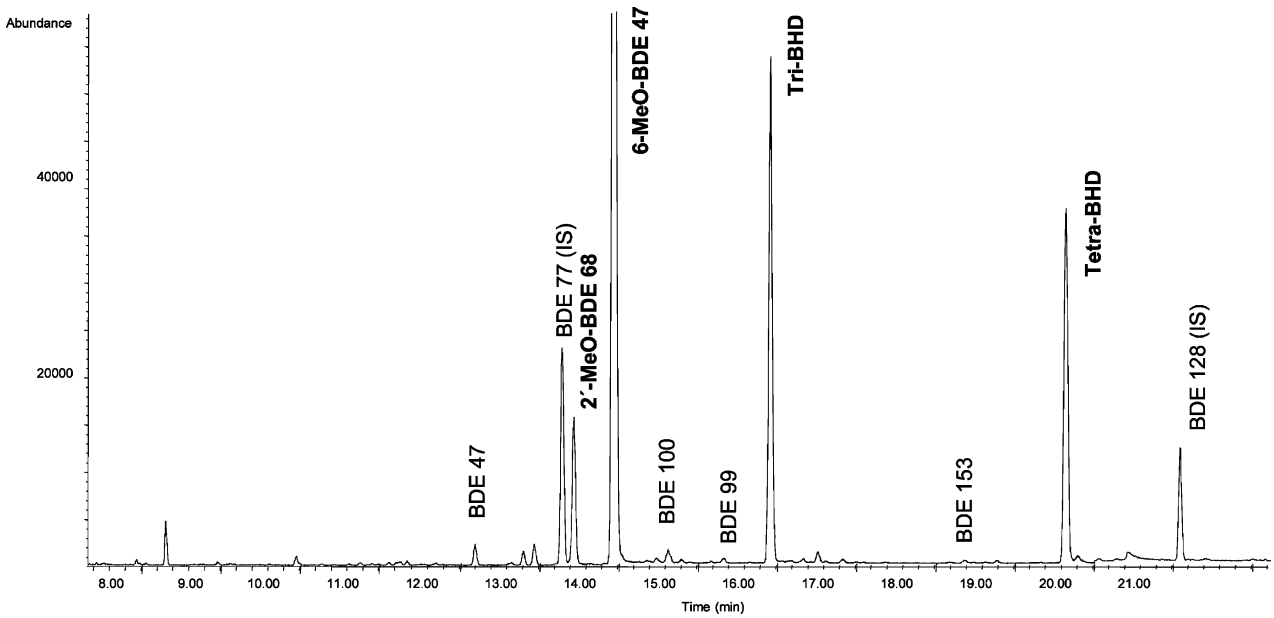

FIGURE 2. GC/ECNI-MS chromatogram (TIC of *m/z* = 79 and 81) of a fish oil extract on an AT-5 capillary column. Several PBDE and MeO-PBDE congeners together with two newly identified naturally produced tri- and tetrabrominated hexahydroxanthene derivatives (tri-BHD and tetra-BHD) are indicated.

PBHDs. In several chromatograms obtained by ECNI-MS, the retention times of two peaks that eluted later than the tetrabrominated MeO−PBDEs did not match the retention times of available penta- or hexabrominated MeO−PBDEs. Recently, Hiebl et al. (*14*) have reported the identification of two PBHDs in both fish and shellfish. By comparing their retention times (Figure 2) and mass spectra of the peaks in the samples (Figures 3 and 4) with reference standards, it could be established that these two compounds were indeed PBHDs. The synthesis of both compounds was previously described by Hiebl et al. (*14*).

The ECNI-MS full scan spectra of PBHD standards were identical with the compounds detected in samples and matched the data provided by Hiebl et al. (*14*). The ions at $m/z = 79$ and 81 in the ECNI-MS full scan of tri-BHD and tetra-BHD proved the presence of bromine substituents (Figure 3). The molecular ion at $m/z = 468$ $[M]^-$ in the ECNI spectra of tri-BHD had a very low abundance, while the fragment at $m/z = 387$ $[M - Br]^-$ was also weak and indicated a fragment containing two bromine atoms. The EI-MS spectra of tri-BHD also showed an intense fragment ion at $m/z = 385$ $[M - Br]^+$, while the isotope pattern for the fragment at $m/z = 464$ $[M]^+$ corresponded to three bromine substituents. The base peak at $m/z = 121$ $[C_9H_{13}]^+$ originated from a non-brominated fragment ion (Figure 3). The fragments obtained in the PCI−MS spectra had a much lower abundance than the corresponding fragments from ECNI-MS and EI-MS spectra (Figure 3). The ionization pattern was similar to that observed in EI-MS. In contrast to tri-BHD, tetra-BHD showed a relatively abundant molecular ion in ECNI-MS at $m/z = 542$ $[M]^-$ (Figure 4), while fragment ions were found at $m/z = 466$ $[M - Br]^-$. Likewise, the molecular ion of tetra-BHD ($m/z = 546$ $[M]^+$) was detected in EI-MS mode, while the fragment ions at $m/z = 384$ $[M - Br]^+$ (three bromine atoms) and $m/z = 264$ $[M - HBr_2]^+$ (two bromine atoms) corresponded with the fragments at $m/z = 306$ $[M - Br]^+$ and $m/z = 185$ $[M - HBr_2]^+$ in the EI-MS spectra of tri-BHD (Figure 3). Unfortunately, the acquisition of PCI−MS spectra for tetra-BHD was not possible due to both its low response factor and the low concentration of the standard (1.6 ng/μL).

Concentrations of PBHDs were similar to concentrations of MeO−PBDEs (Table 1), and in the vast case majority, higher than PBDEs. Tri-BHD and tetra-BHD had a detection frequency of 65 and 58%, respectively. The median of sum PBHDs across all samples was 5.3 ng/g oil, while the highest levels reached 200 ng/g oil (Table 1). Such high concentrations of PBHDs were already determined in farmed fish from the Mediterranean Sea (*14*, *15*) and in farmed mussels from New Zealand (*14*, *15*), but also in bird eggs from Norway (*23*), indicating the transfer of these compounds throughout the marine food web. In the latter article, tetra-BHD was one of the major brominated compounds detected in shag eggs.

The median ratio between concentrations of tri-BHD and tetra-BHD across all samples was 1.17 (mean 1.82) and ranged from 0.51 to 8.51. Since the levels of tri-BHD were highly correlated with the levels of tetra-BHD ($R_s = 0.89$, $p < 0.01$), it is plausible that these compounds both accumulate from similar sources. However, levels of sum PBHDs did not correlate with sum MeO−PBDEs ($R_s = 0.24$, $p > 0.01$), suggesting separate (natural) sources for these two groups of compounds. Indeed, sponges of the *Cacospongia* genus, reported to occur in Australia, but also in the Mediterranean Sea, have been suggested as potential natural producers of PBHDs (*14*, *15*), while cyanobacteria and red algae (*Ceramium tenuicorne*) have been associated with the production of MeO−PBDEs (*13*).

Dietary Intake. Since FODS are intended to be consumed on a daily basis, we have also calculated the daily intake of brominated compounds from FODS. The investigated FODS contain 200−800 mg/g EPA and DHA, and the recommended dosing for human consumption ranges between 1 and 3 g/day (*1*−*3*). Because of the low contamination levels (Table 1), FODS do not appear to increase substantially the dietary intake of PBDEs: the median daily intake was 8 and 16 times lower than the intake from fish consumption alone or from a total diet, respectively (Figure 5). Although fish consumption is an important contributor to the total dietary intake of PBDEs (Figure 5), the low intake of PBDEs from FODS suggests that purification processes were present during the preparation of the vast majority of the investigated FODS. Since the PBDE intake from FODS covers a wide range of values (Figure 5), some FODS brands are either produced from contaminated fish or are insufficiently purified. Various purification procedures involving supercritical fluid extrac-

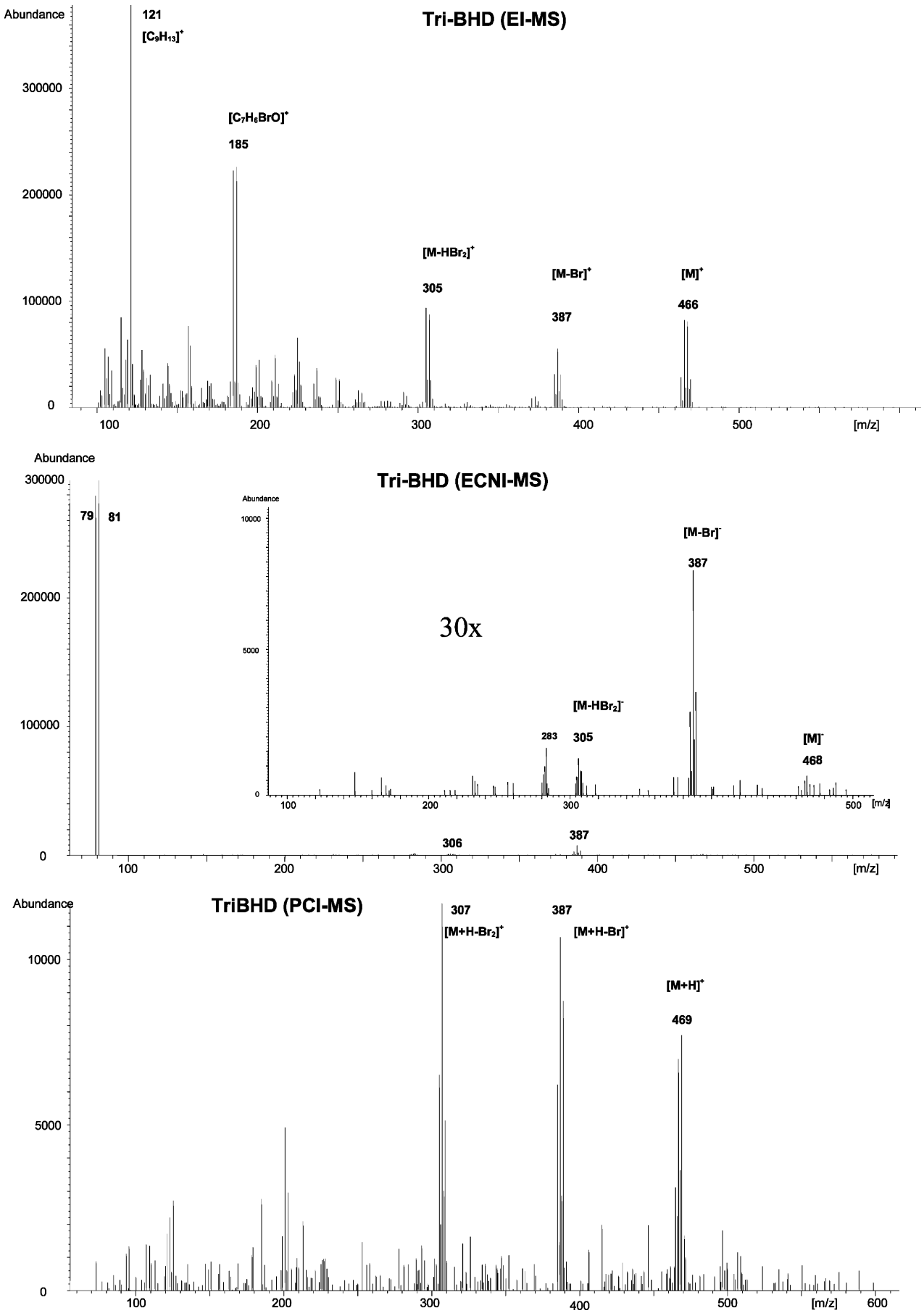

FIGURE 3. EI-MS (top), ECNI-MS (middle), and PCI-MS (bottom) mass spectra of 2,7-dibromo-4a-bromomethyl-1,l-dimethyl-2,3,4,4a,9,9a-hexahydro-1*H*-xanthene (tri-BHD).

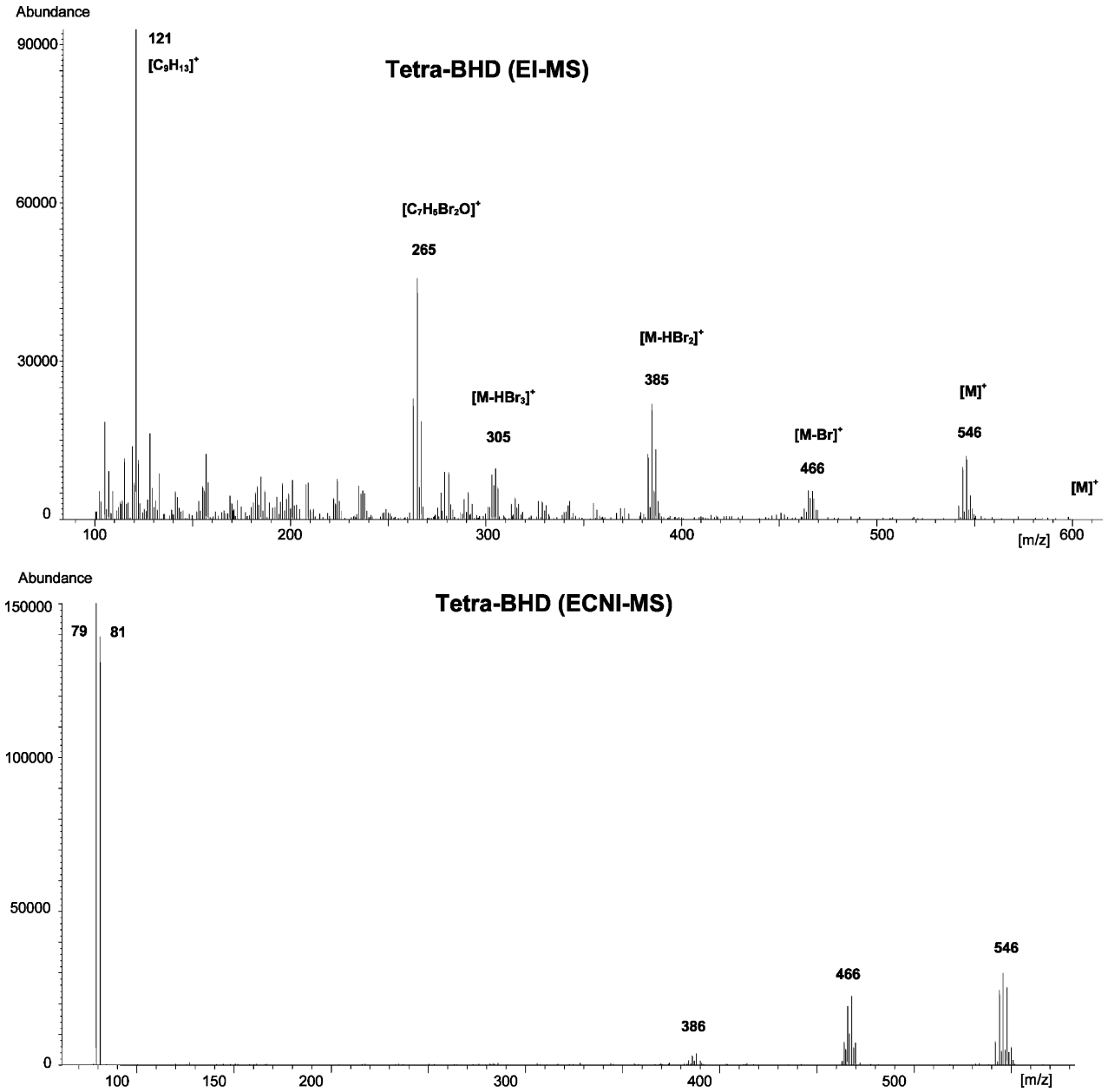

FIGURE 4. EI-MS (top) and ECNI-MS (bottom) mass spectra of 2,5,7-tribromo-4a-bromomethyl-1,l-dimethyl-2,3,4,4a,9,9a-hexahydro-1*H*-xanthene (tetra-BHD).

TABLE 2. Median and 10−90th Percentiles of Daily Intake (ng/day) for Organobrominated Contaminants in Fish Oil Dietary Supplements ($n = 69$)[a]

	adults ($n = 59$) (ng/day)		children ($n = 15$) (ng/day)	
	LB	UB	LB	UB
sum PBDEs	1.0 (0.0−6.9)	3.0 (1.4−7.8)	0.1 (0.0−2.1)	1.7 (0.5−3.0)
sum MeO-PBDEs	10 (0.0−140)	10 (0.4−140)	6.7 (0.7−230)	6.7 (0.7−230)
sum PBHDs	19 (0.0−130)	19 (2.0−130)	1.7 (0.0−11)	2.4 (1.0−11.)

[a] Five samples were destined for both adults and children, while the other samples were destined for only one group (54 of just adults and 10 of just children). Values of individual compounds below LOQ were replaced by 0 × LOQ (lower bound (LB)) or LOQ (upper bound (UB)).

tion of carbon chromatography (*37*) are described in the literature for the removal of dioxins and PCBs, but until now, the reduction in PBDE levels of fish oils after purification has not yet been described.

The low intake of PBDEs through FODS therefore makes these supplements a suitable alternative for populations with a low consumption of PUFA-rich food or for which fish consumption recommendations have been issued (e.g., pregnant women). FODS also prove to be a powerful source of EPA and DHA as compared with PUFA-containing vegetables oils, such as soybean and rapeseed oil, which have approximately 10 times less EPA and do not appear to provide an effective metabolic source of DHA for the average consumer (*9*). This renders FODS an efficient, relatively clean, and low caloric source of PUFAs.

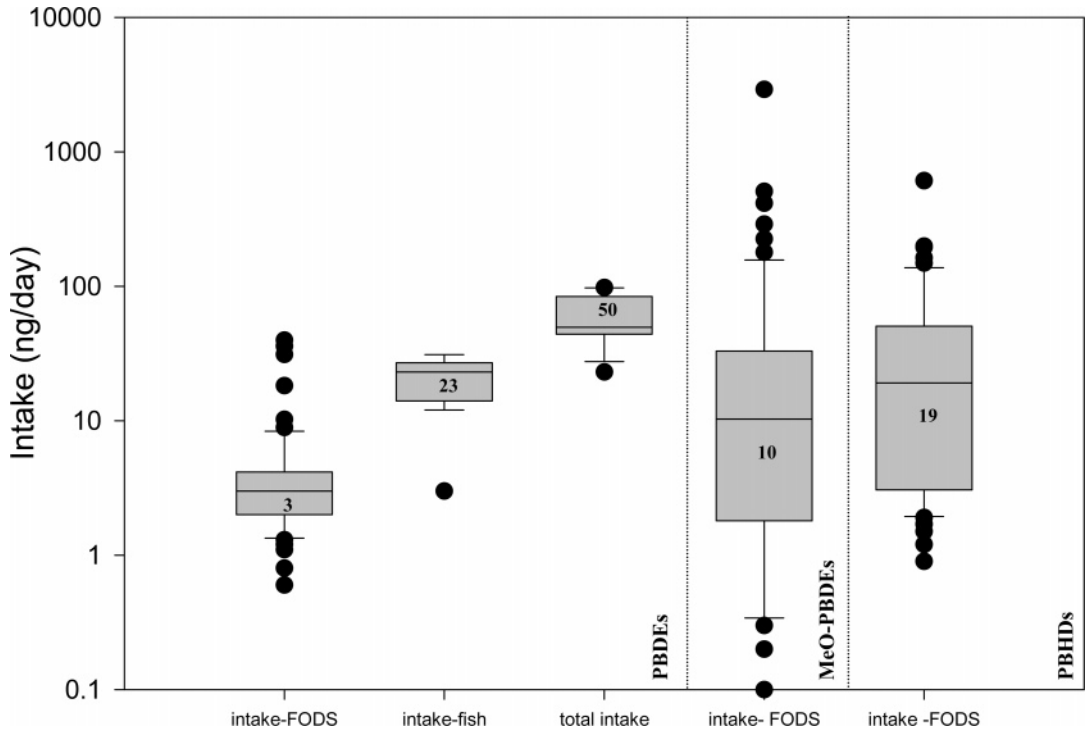

FIGURE 5. Dietary intake (ng/day) of anthropogenic (PBDEs) and naturally produced (MeO-PBDEs and PBHDs) organobromine compounds from fish oil dietary supplements (present data), fish consumption, and total diet (literature data). The box plots show the median and 25th and 75th percentiles, the lines give the 10th and 90th percentiles, while the dots represent the outliers. Values in the box plots represent the medians for each category. Literature data used for the daily intake of PBDEs from fish consumption and total diet are taken from refs *24*−*36*.

The median daily intakes of MeO−PBDEs and PBHDs from FODS were, respectively, 3 and 6 times higher than the median intake of PBDEs (3 ng/day), respectively (Figure 5). For some brands, the daily intake of MeO−PBDEs and PBHDs from FODS was even higher than the total dietary intake of PBDEs. Since BDE 209 levels in marine biota are very low (*38*), it is not expected that the PBDE intake would substantially increase with the inclusion in the calculations of BDE 209. This emphasizes that the presence of these scarcely investigated compounds should not be overlooked. Likewise, MeO−PBDEs and PBHDs show a large variation of intake estimates, encompassing the range of values obtained for PBDEs.

An estimation of the dietary intake of brominated FODS compounds for children has also been determined. For each group of compounds, the intake was lower for children than for adults (Table 2), due to a combination of lower amounts needed to be ingested daily and lower concentrations of brominated compounds in the FODS used for children.

The dietary intake from fish and seafood has also been reported for other natural compounds, halogenated dimethyl bipyrroles (DBPs). The daily intake estimate for sum DBPs was <3.5 ng/day (*39*), a lower result as compared to the naturally produced compounds investigated in the present study. Unfortunately, DBPs degrade in the presence of sulfuric acid-impregnated silica used during the sample cleanup and, thus, could not be measured in the samples.

The present study strongly supports the need for the inclusion of new anthropogenic contaminants (e.g., PBDEs), but also naturally produced halogenated compounds, in monitoring schemes of marine products destined for human consumption. This study also supports the need for appropriate monitoring and legislation for FODS.

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Supporting Information Available

Levels of PBDEs, MeO-PBDEs, and PBHDs in individual samples (Table SI-1). PBDE congener distribution in the investigated FODS and mass spectra of 6-MeO-BDE 47 (Figures SI-1 and SI-2, respectively). This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Sidhu, K. S. Health benefits and potential risks related to consumption of fish or fish oil. *Regul. Toxicol. Pharm.* **2003**, *38*, 336−344.

(2) Simopoulos, A. Essential fatty acids in health and chronic disease. *Am. J. Clin. Nutr.* **1999**, *70*, 560−569S.

(3) Mozaffarrian, D.; Rimm, E. B. Fish intake, contaminants, and human health. Evaluating the risks and the benefits. *JAMA* **2006**, *296*, 1885−1899.

(4) Hites, R. A.; Foran, J. A.; Carpenter, D. O.; Hamilton, M. C.; Knuth, B. A.; Schwager, S. J. Global assessment of organic contaminants in farmed salmon. *Science* **2004**, *303* (5655), 226−229.

(5) Hites, R. A.; Foran, J. A.; Schwager, S. J.; Knuth, B. A.; Hamilton, M. C.; Carpenter, D. O. Global assessment of polybrominated diphenyl ethers in farmed and wild salmon. *Environ. Sci. Technol.* **2004**, *38*, 4945−4949.

(6) Birnbaum, L. S.; Staskal, D. F. Brominated flame retardants: Cause for concern? *Environ. Health Perspect.* **2004**, *112*, 9−17.
(7) Verbeke, W.; Sioen, I.; Pieniak, Z.; Van Camp, J.; De Henauw, S. Consumer perception versus scientific evidence about health benefits and safety risks from fish consumption. *Publ. Health Nutr.* **2005**, *8*, 422−429.
(8) Jacobs, M.; Covaci, A.; Schepens, P. Investigation of selected persistent organic pollutants in farmed Atlantic salmon (*Salmo salar*), salmon aquaculture feed, and fish oil components of the feed. *Environ. Sci. Technol.* **2002**, *36*, 2797−2805.
(9) Jacobs, M. N.; Covaci, A.; Gheorghe, A.; Schepens, P. Time trend investigation of PCBs, OCPs, and PBDEs in *n*-3 polyunsaturated fatty acid-rich dietary fish oil and vegetable oil supplements; Nutritional relevance for human essential *n*-3 fatty acid requirements. *J. Agric. Food Chem.* **2004**, *52*, 1780−1788.
(10) Storelli, M. M.; Storelli, A.; Marcotrigiano, G. O. Polychlorinated biphenyls, hexachlorobenzene, hexachlorocyclohexane isomers, and pesticide organochlorine residues in cod liver oil dietary supplements. *J. Food Protect.* **2004**, *67*, 1787−1791.
(11) Melcher, J.; Olbrich, D.; Marsh, G.; Nikiforov, V.; Gaus, C.; Gaul, S.; Vetter, W. Tetra- and tribromophenoxyanisoles in marine samples from oceania. *Environ. Sci. Technol.* **2005**, *39*, 7784−7789.
(12) Teuten, E. L.; Xu, L.; Reddy, C. M. Two abundant bioaccumulated halogenated compounds are natural products. *Science* **2005**, *307*, 917−920.
(13) Malmvärn, A.; Marsh, G.; Kautsky, L.; Athanasiadou, M.; Bergman, Å.; Asplund, L. Hydroxylated and methoxylated brominated diphenyl ethers in red algae (*Ceramium tenuicorne*) and blue mussels from the Baltic Sea. *Environ. Sci. Technol.* **2005**, *39*, 2990−2997.
(14) Hiebl, J.; Melcher, J.; Gundersen, H.; Schlabach, M.; Vetter, W. Identification and quantification of polybrominated hexahydroxanthene derivatives and other halogenated natural products in commercial fish and other marine samples. *J. Agric. Food. Chem.* **2006**, *54*, 2652−2657.
(15) Melcher, J.; Janussen, D.; Garson, M. J.; Hiebl, J.; Vetter, W. Polybrominated hexahydroxanthene derivatives (PBHDs) and other halogenated natural products from the Mediterranean sponge *Scalarispongia* in marine biota. *Arch. Environ. Contam. Toxicol.* **2007**, *52*, 512−518.
(16) Voorspoels, S.; Covaci, A.; Schepens, P. Polybrominated diphenyl ethers in marine species from the Belgian North Sea and the Western Scheldt Estuary: Levels, profiles, and distribution. *Environ. Sci. Technol.* **2003**, *37*, 4348−4357.
(17) van Leeuwen, S. P. J.; Van Cleuvenbergen, R.; Abalos, M.; Pasini, A. L.; Eriksson, U.; Cleemann, M.; Hajslova, J.; de Boer, J. New certified and candidate certified reference materials for the analysis of PCBs, PCDD/Fs, OCPs, and BFRs in the environment and food. *Trends Anal. Chem.* **2006**, *25*, 397−409.
(18) de Boer, J.; Wells, D. E. Pitfalls in the analysis of brominated flame retardants in environmental, human, and food samples—Including results of three international interlaboratory studies. *Trends Anal. Chem.* **2006**, *25*, 364−372.
(19) Zennegg, M.; Schmid, P. PCDD/F, PCB, dioxin-like PCB, and PBDE in fish oil used as a dietary supplement in Switzerland. *Organohalogen Compd.* **2006**, *68*, 1967−1971.
(20) Herrmann, T.; Lohmann, N.; Paepke, O. Polybrominated diphenyl ethers (PBDEs) in fish, fish oil, fish meal, and fish feed samples of various origins. *Organohalogen Compd.* **2005**, *67*, 640−643.
(21) Bethune, C.; Nielsen, J.; Lundebye, A. K.; Julshamn, K. Current levels (2003−2004) of brominated flame retardants in feed and selected Norwegian seafood. *Organohalogen Compd.* **2005**, *67*, 619−621.
(22) Malmberg, T.; Athanasiadou, M.; Marsh, G.; Brandt, I.; Bergman, Å. Identification of hydroxylated polybrominated diphenyl ether metabolites in blood plasma from polybrominated diphenyl ether exposed rats. *Environ. Sci. Technol.* **2005**, *39*, 5342−5348.
(23) Vetter, W.; von der Recke, R.; Herzke, D.; Nygard, T. Natural and man-made organobromine compounds in marine biota from Central Norway. *Environ. Int.* **2006**, *33*, 17−26.
(24) Bakker, M. I.; De Winter-Sorkina, R.; De Mul, A.; Boon, P. E.; Van Donkersgoed, G.; Van Klaveren, J. D.; Baumann, B. A.; Hijman, W. C.; Van Leeuwen, S. P. J.; de Boer, J.; Zeilmaker, M. J. Dietary intake of polybrominated diphenyl ethers in The Netherlands. *Organohalogen Compd.* **2006**, *68*, 387−390.
(25) Bocio, A.; Llobet, J. M.; Domingo, J. L.; Corbella, J.; Teixido, A.; Casas, C. Polybrominated diphenyl ethers (PBDEs) in foodstuffs: Human exposure through the diet. *J. Agric. Food Chem.* **2003**, *51*, 3191−3195.
(26) Darnerud, P. O.; Atuma, S.; Aune, M.; Bjerselius, R.; Glynn, A.; Petersson Grawé, K.; Becker, W. Dietary intake estimations of organohalogen contaminants (dioxins, PCB, PBDE, and chlorinated pesticides, e.g., DDT) based on Swedish market basket data. *Food Chem. Toxicol.* **2006**, *44*, 1597−1606.
(27) Darnerud, P. O.; Eriksen, G. S.; Johannesson, T.; Larsen, P. B.; Viluksela, M. Polybrominated diphenyl ethers: Occurrence, dietary exposure, and toxicology. *Environ. Health Perspect.* **2001**, *109* (Suppl. 1), 49−68.
(28) Domingo, J. L. Human exposure to polybrominated diphenyl ethers through the diet. *J. Chromatogr., A* **2004**, *1054*, 321−326.
(29) Harrad, S.; Wijesekera, R.; Hunter, S.; Halliwell, C.; Baker, R. Preliminary assessment of U.K. human dietary and inhalation exposure to polybrominated diphenyl ethers. *Environ. Sci. Technol.* **2004**, *38*, 2345−2350.
(30) Kiviranta, H.; Ovaskainen, M. L.; Vartiainen, T. Market basket study on dietary intake of PCDD/Fs, PCBs, and PBDEs in Finland. *Environ. Int.* **2004**, *30*, 923−932.
(31) Lind, Y.; Aune, M.; Atuma, S.; Becker, W.; Bjerselius, R.; Glynn, A.; Darnerud, P. O. Food intake of the polybrominated flame retardants PBDEs and HBCD in Sweden. *Organohalogen Compd.* **2002**, *58*, 181−184.
(32) Nakagawa, R.; Ashizuka, Y.; Hori, T.; Tobiishi, K.; Yasutake, D.; Sasaki, K. Determination of brominated flame retardants in fish and market basket food samples of Japan. *Organohalogen Compd.* **2005**, *67*, 498−501.
(33) Ryan, J. J.; Patry, B. Body burdens and food exposure in Canada for polybrominated diphenyl ethers (PBDEs). *Organohalogen Compd.* **2001**, *51*, 226−229.
(34) Schecter, A.; Päpke, O.; Harris, T. R.; Tung, K. C.; Musumba, A.; Olson, J.; Birnbaum, L. Polybrominated diphenyl ether (PBDE) levels in an expanded market basket survey of U.S. food and estimated PBDE dietary intake by age and sex. *Environ. Health Perspect.* **2006**, *114*, 1515−1520.
(35) Voorspoels, S.; Covaci, A.; Neels, H. Dietary PBDE intake: A market basket study in Belgium. *Environ. Int.* **2007**, *33*, 93−97.
(36) Zennegg, M.; Kohler, M.; Gerecke, A. C.; Schmid, P. Polybrominated diphenyl ethers in whitefish from Swiss lakes and farmed rainbow trout. *Chemosphere* **2003**, *51*, 545−553.
(37) Kawashima, A.; Iwakiri, R.; Honda, K. Experimental study on the removal of dioxins and coplanar PCBs from fish oil. *J. Agric Food Chem.* **2006**, *54*, 10294−10299.
(38) Law, R. J.; Allchin, C. R.; de Boer, J.; Covaci, A.; Herzke, D.; Lepom, P.; Morris, S.; Tronczynski, J.; de Wit, C. A. Levels and trends of brominated flame retardants in the European environment. *Chemosphere* **2006**, *64*, 187−208.
(39) Tittlemier, S. A. Dietary exposure to a group of naturally produced organohalogens (halogenated dimethyl bipyrroles) via consumption of fish and seafood. *J. Agric. Food Chem.* **2004**, *52*, 2010−2015.

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Supporting Information

Anthropogenic and naturally-occurring organobromine compounds in fish oil dietary supplements

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1 Table + 2 Figures

Levels of PBDEs, MeO-PBDEs and PBHDs in individual samples, together with information about fish oil supplements are given in Table SI-1. PBDE congener distribution in the investigated FODS and mass spectra of 6-MeO-BDE 47 are given in Figures SI-1 and SI-2, respectively.

Figure SI-1. PBDE congener distribution (mean percentage ± 2 SE) in fish oil dietary supplements (n = 69). For congeners with values below LOQ, a value of f * LOQ was used for the calculation of Sum PBDEs and subsequently of PBDE distribution. Herein f is the fraction of the samples above LOQ.

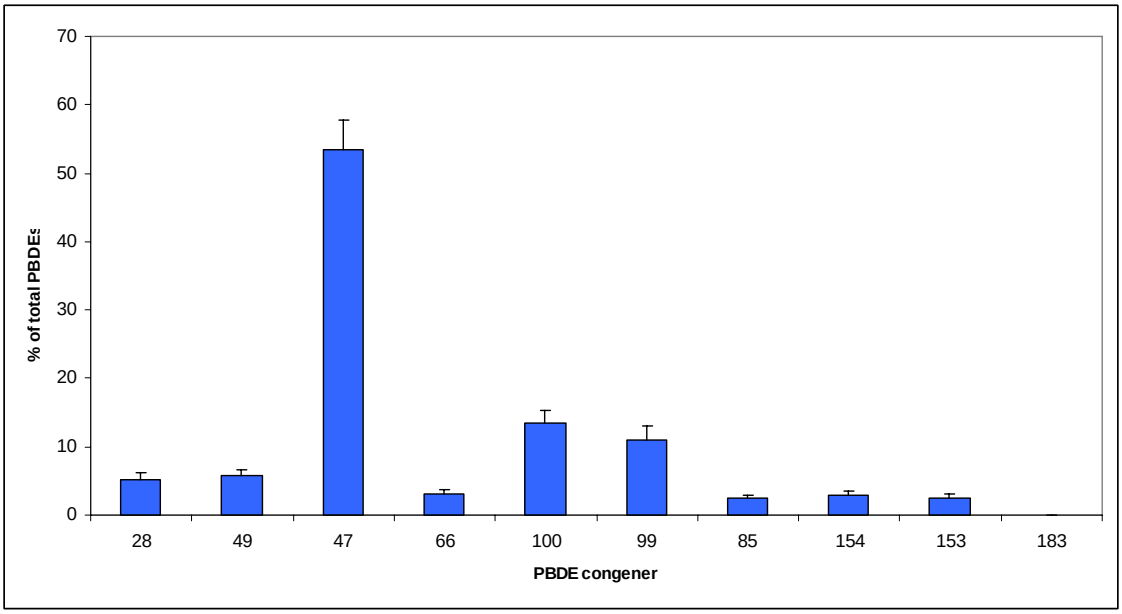

Figure SI-2. Mass spectra of 6-MeO-BDE 47 holding the methoxy group in the *ortho* position. The characteristic fragment ions for *ortho*-MeO-PBDEs ($[M\text{-}Br\text{-}CH_3]^+$) are present in the EI-MS spectra (top), while ions $[Br]^-$ ions are dominant in the ECNI-MS spectra (bottom). Note the low intensity of ions $[M]^-$ and $[M\text{-}HBr]^-$ in the ECNI-MS spectra, a characteristic of aromatic brominated compounds.

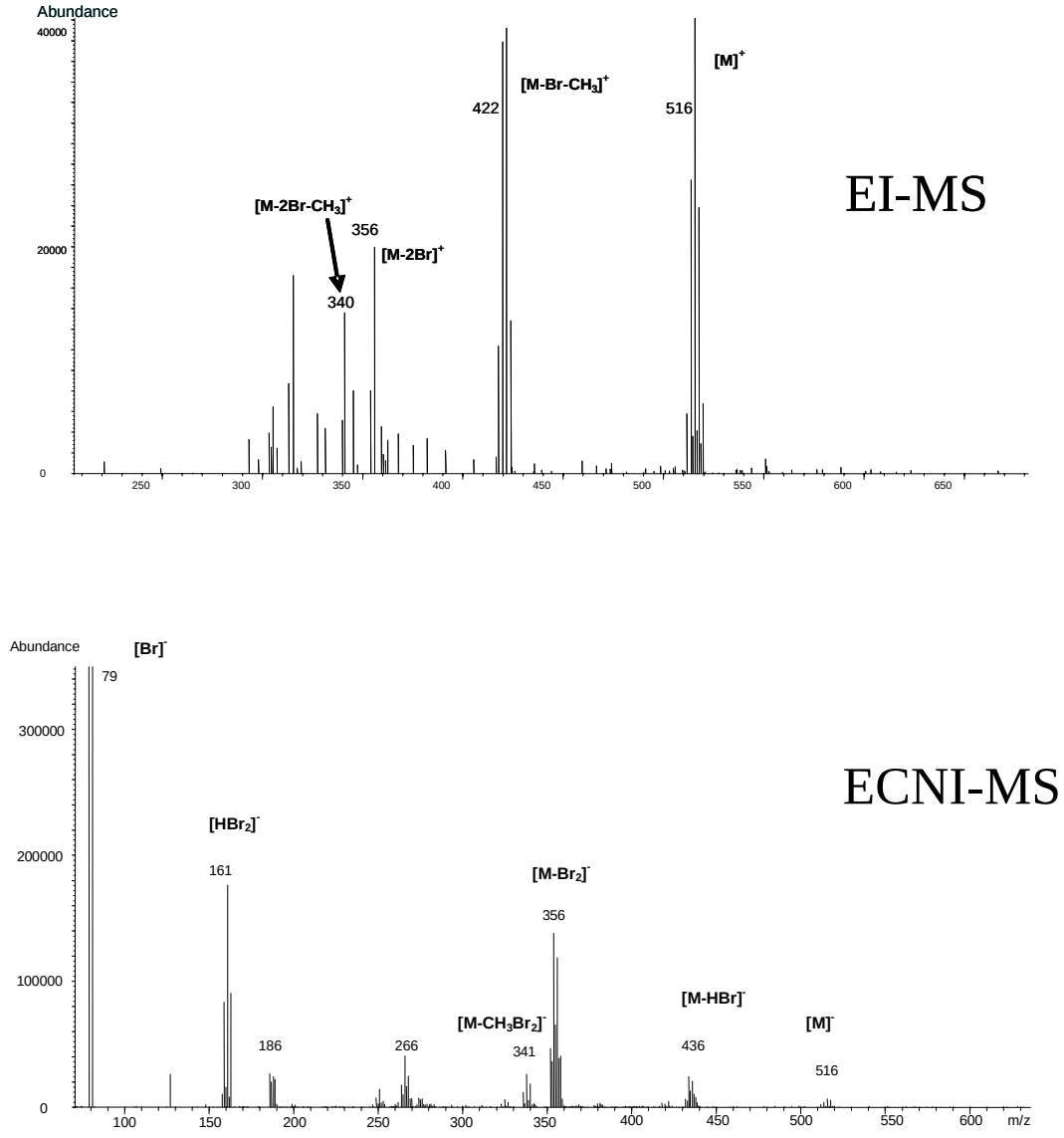

Table SI-1. PBDEs, MeO-PBDEs and PBHDs (ng/g oil) in individual fish oil dietary supplements.

Producer	Country of origin	BDE 47	BDE 100	BDE 99	2-MeO-BDE68	6-MeO-BDE47	Tri-BHD	Tetra-BHD	Content capsule (mg)	EPA (mg) per capsule	DHA (mg) per capsule	Capsules per day-adults	Capsules per day-children
LOQ		0.10	0.10	0.10	0.1	0.1	1	1					
P1	Belgium	0.70	0.10	0.07	2.1	4.7	10.9	11.0	1000	180	120	2.0	
P1	Belgium	0.57	< LOQ	< LOQ	2.3	3.8	9.9	8.9	1000	180	120	2.0	
P1	Belgium	0.20	< LOQ	< LOQ	1.5	2.1	3.3	< LOQ	425				3.0
P2	France	< LOQ	< LOQ	< LOQ	0.3	0.2	< LOQ	< LOQ	180	72	50	2.5	
P3	Belgium	0.84	0.43	0.31	7.7	26.2	60.9	27.6	560	125	60	4.0	2.0
P3	Belgium	1.21	0.59	< LOQ	13.9	33.9	62.1	35.4	500	155	110	4.0	
P3	Belgium	0.50	< LOQ	< LOQ	3.6	4.3	3.4	< LOQ	500	250	0	4.0	
P4	Belgium	0.17	< LOQ	< LOQ	3.2	1.9	< LOQ	1.1	295	260	40		2.0
P4	Belgium	0.79	0.34	0.70	280.5	113.6	3.8	4.6	291	30	230		1.0
P4	Belgium	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	555	500	-	1.5	
P4	Belgium	0.13	< LOQ	< LOQ	4.1	2.1	< LOQ	< LOQ	1340	766 (together)		1.0	
P4	Belgium	< LOQ	< LOQ	< LOQ	0.1	0.2	< LOQ	< LOQ	1340	767 (together)		1.0	
P4	Belgium	0.21	< LOQ	< LOQ	0.4	0.4	< LOQ	< LOQ	1000	580	80	1.0	
P4	Belgium	15.39	5.73	10.43	14.3	39.1	18.8	5.1	800	65	465	1.0	
P4	Belgium	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	850	630	190	1.0	
P5	United Kingdom	< LOQ	< LOQ	< LOQ	0.4	0.4	< LOQ	< LOQ	500	300	-	3.0	1.0
P6	United Kingdom	< LOQ	< LOQ	< LOQ	27.7	14.7	2.6	1.7	200	15	100		2.0
P7	United Kingdom	< LOQ	< LOQ	0.19	7.8	9.0	2.7	1.9	200	15	100		2.0
P7	United Kingdom	0.78	0.14	0.07	4.1	6.2	15.3	11.1	500	110 (together)		2.0	
P7	United Kingdom	< LOQ	< LOQ	< LOQ	21.3	14.3	3.4	1.9	200	14	100		2.0
P8	United Kingdom	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	380	280	-	3.0	1.0
P9	United Kingdom	< LOQ	< LOQ	< LOQ	0.3	0.3	< LOQ	< LOQ	1000	290	190	1.0	
P10	USA	0.39	0.23	< LOQ	7.9	33.4	129.3	73.6	1200	385	260	2.5	
P10	USA	1.18	0.13	0.20	5.9	8.3	18.5	13.4	1200	180	120	1.0	
P11	The Netherlands	0.59	0.14	< LOQ	3.9	13.3	41.2	33.1	500	90	30	4.0	
P11	The Netherlands	0.62	0.18	< LOQ	4.1	12.6	34.4	25.9	500	90	30	4.0	
P11	United Kingdom	0.12	< LOQ	< LOQ	0.7	2.3	6.0	5.4	4450	186	58	1.0	
P11	The Netherlands	0.49	< LOQ	< LOQ	2.6	7.2	24.2	18.2	500	90	30	4.0	
P12	South Africa	1.94	0.31	1.27	1.2	2.1	3.2	2.0	1000	45	30	2.0	
P13	The Netherlands	6.36	0.43	2.43	7.7	14.4	35.6	12.4	930	300	200	2.5	
P13	The Netherlands	9.42	0.64	2.00	9.86	8.17	2.7	< LOQ	930	300	200	2.5	
P14	The Netherlands	0.63	< LOQ	< LOQ	4.6	9.6	29.9	20.9	1000	180	120	2.0	
P15	The Netherlands	0.83	< LOQ	0.17	2.5	3.5	6.8	4.9	1200	180	120	1.0	

P16	The Netherlands	0.68	< LOQ	0.07	1.5	3.9	14.7	10.8	790	90	60	2.0	
P16	The Netherlands	0.52	< LOQ	< LOQ	1.23	2.10	< LOQ	< LOQ	500	90	60	3.0	
P17	The Netherlands	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	300	120	80	2.0	
P18	The Netherlands	3.64	0.62	0.31	69.6	110.2	141.6	16.6	500	175	125	2.5	
P18	Denmark	0.22	< LOQ	< LOQ	1.3	2.7	9.4	11.7	1000	170	115	1.5	
P18	Denmark	4.97	1.19	0.34	88.39	143.67	66.7	10.0	500	175	125	2.5	
P19	United Kingdom	0.43	< LOQ	< LOQ	0.3	0.6	< LOQ	< LOQ	930	80	20	1.0	
P20	United Kingdom	1.47	0.18	0.37	250.0	66.5	< LOQ	< LOQ	400	15	65	4.0	3.0
P20	United Kingdom	1.09	< LOQ	0.35	188.3	70.7	< LOQ	< LOQ	400	15	65	4.0	3.0
P21	Belgium	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	500	350	50	3.0	
P21	Belgium	< LOQ	< LOQ	< LOQ	0.5	0.5	< LOQ	< LOQ	425	170	40		3.0
P21	Belgium	5.62	0.83	1.53	1294.6	377.1	14.6	8.5	580	120	350	3.0	
P21	Belgium	< LOQ	< LOQ	< LOQ	1.5	2.6	3.4	< LOQ	425	170	40		3.0
P22	The Netherlands	0.31	< LOQ	< LOQ	1.0	2.0	5.7	5.1	1200	90	60	2.0	
P22	The Netherlands	< LOQ	< LOQ	< LOQ	0.94	2.86	2.4	4.5	1200	90	60	2.0	
P23	Belgium	< LOQ	< LOQ	< LOQ	0.3	0.3	< LOQ	< LOQ	1000	270	170	3.0	
P23	Belgium	0.75	0.13	0.13	7.2	3.3	< LOQ	< LOQ	1000	60	430	1.5	
P23	Belgium	0.86	0.26	0.35	53.0	46.7	15.0	3.7	530	93	240		1.5
P24	Belgium	0.52	0.16	< LOQ	2.7	9.7	26.8	27.7	500	90	60	4.0	
P25	Belgium	2.20	0.20	< LOQ	1.7	0.7	< LOQ	< LOQ	750	530	60	1.0	
P25	Belgium	1.10	< LOQ	< LOQ	1.64	0.67	< LOQ	< LOQ	360	250	30		2.0
P26	Belgium	0.44	< LOQ	< LOQ	1.2	2.4	3.0	< LOQ	580	390	-	3.5	
P27	Belgium	< LOQ	< LOQ	< LOQ	0.9	2.1	8.6	12.1	265	-	-	4.0	
P28	Belgium	0.37	< LOQ	< LOQ	0.9	1.5	1.9	< LOQ	560	330	80	2.0	
P28	France	0.73	< LOQ	0.12	49.7	21.7	7.1	7.1	1000	200 (together)		2.5	
P29	Belgium	0.16	0.16	< LOQ	5.2	16.3	44.9	32.8	500	165	110	3.0	
P30	France	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	555	-	50	4.0	
P31	Sweden	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	450	80	55	4.5	
P31	Sweden	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	4450	750	500	1.0	
P32	France	0.20	0.16	< LOQ	5.0	19.4	40.3	35.0	530	175	115	1.5	
P33	The Netherlands	0.42	< LOQ	< LOQ	5.51	10.49	8.6	15.4	1000	180	120	2.0	
P34	The Netherlands	0.41	< LOQ	< LOQ	2.55	1.51	< LOQ	< LOQ	1000	180	120	2.5	
P35	The Netherlands	0.30	< LOQ	< LOQ	2.57	5.00	5.7	9.8	660			3.0	
P36	The Netherlands	0.40	< LOQ	< LOQ	1.99	6.74	7.4	14.3	1000	180	120	1.0	
P37	The Netherlands	0.58	< LOQ	< LOQ	3.82	11.02	12.7	19.8	1000	767 (together)		1.5	