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Simultaneous Quantitation of Multiple Classes of Organohalogen Compounds in Fish Oils with Direct Sample Introduction Comprehensive Two-Dimensional Gas Chromatography and Time-of-Flight Mass Spectrometry

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We successfully optimized an analytical method using gel permeation chromatography followed by direct sample introduction comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry to quantify multiple groups of targeted persistent organic pollutants and halogenated natural products (HNPs) simultaneously in fish oil samples. This new method has a wider analytical scope than the traditional approach to use multiple methods to cover each class of compounds. Our analysis revealed that the relatively more volatile and lighter organic compounds, such as polychlorinated biphenyls (PCBs), organochlorine pesticides, and other smaller organohalogen compounds, were still present in two brands of "PCB-free" cod liver oils, albeit at much lower levels than in an untreated commercial sample. Moreover, the less volatile organic compounds, such as polybrominated diphenyl ethers and brominated HNPs, were detected at similar levels in all three cod liver oils. This suggests that the commercial molecular distillation treatment used for removal of organic/inorganic toxic contaminants is only effective for the lighter organic contaminants.

KEYWORDS: Direct sample introduction; comprehensive two-dimensional gas chromatography; time-of-flight mass spectrometry; persistent organic pollutants; halogenated natural products; dietary supplements; fish oil

INTRODUCTION

Anthropogenic persistent organic pollutants (POPs), such as polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs), are ubiquitous in the environment. They are persistent and bioaccumulative and have thus entered both terrestrial and marine food webs. Human exposure to PCDD/Fs and PCBs is mainly through food; specifically, the consumption of fish is a major exposure pathway for these toxic pollutants in humans (*1, 2*).

In most cases, human and wildlife exposure studies have been limited to the POPs (PCDD/Fs, PCBs, OCPs, and PBDEs), and specific analytical methods have been devised for their analysis. Recently, multiple groups have reported the presence of lipophilic and bioaccumulative halogenated natural products (HNPs) in marine organisms (*3*−*6*) (see **Figure 1** for HNPs chemical structures). In addition, Pangallo and Reddy showed the possibility of biomagnification of halogenated 1′-methyl-1,2′-bipyrroles (MBPs) (*4*). However, most studies cover a single or a few groups of POPs or HNPs using a targeted analytical approach, which includes laborious sample preparation followed by multiple injections in gas chromatography−mass spectrometry (GC/MS) (*7*−*10*). A better risk analysis of POPs to humans and the environment requires data on multiple groups of POPs in several matrices. Hence, the development and usage of a

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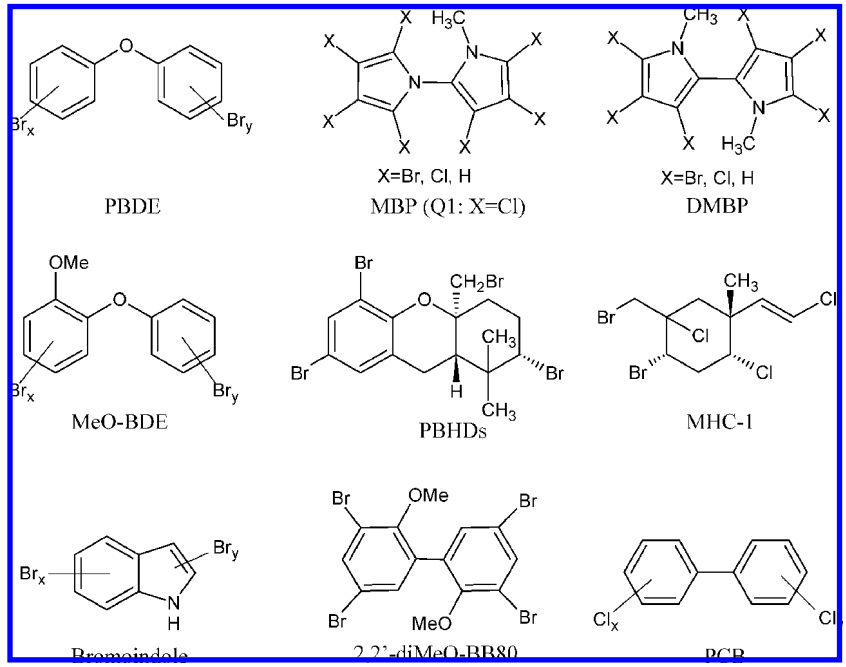

Figure 1. Chemical structures of PBDEs, PCBs, and HNPs.

single analytical method would provide the most efficient and cost-effective way to collect such data.

In a previous study, we reported the qualitative capabilities of an analytical approach using direct sample introduction comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (DSI-GC×GC/TOF-MS) and gel permeation chromatography (GPC) cleanup, which allowed identification of targeted POPs as well as untargeted organic chemicals in fish oil samples (*11*). This combined analytical approach efficiently increases the scope of organic chemicals monitored in the fish oils: GPC cleanup removes the bulk of the oil matrix, DSI enables large volume injection in a rugged manner, GC×GC provides greater separation and sensitivity than GC alone, and TOF-MS collects full mass spectra. The greater selectivity of GC×GC affords cleaner mass spectra in complex extracts (fewer interferences), which helps in quantitation and qualitative identifications of targeted and nontargeted chemicals. Using DSI-GC×GC/TOF-MS, we found anthropogenic POPs (PCBs, OCPs, and PBDEs) in a dietary supplement of cod liver oil that was labeled "PCB\heavy metal free" (*11*). In the sample, we also identified and confirmed several groups of HNPs [MBPs, 1,1′-dimethyl-2,2′-bipyrroles (DMBPs), methoxylated PBDEs (MeO-PBDEs), polybrominated hexahydroxanthene derivatives (PBHDs), polybromoindoles, and a halogenated monoterpene MHC-1] in addition to other organic contaminants (oxybenzone and octachlorostyrene) (*11*).

In this study, our objectives were to demonstrate the feasibility and applicability of the new analytical approach in a simultaneous and robust quantification of POPs, HNPs, and other organic contaminants in commercially available dietary fish oil supplements with or without treatment for PCB removal.

EXPERIMENTAL PROCEDURES

Samples. Alaskan sockeye salmon fish oil supplement in capsules was purchased from an Internet retailer. Two dietary cod liver oil supplements (liquid oil) were also purchased from an Internet retailer; one bottle had a label indicating "PCB/heavy metal free", and the other did not. A third bottle of dietary cod liver oil supplement (liquid oil) was obtained from a local supermarket in Maryland; the bottle label indicated that the cod liver oil was molecularly distilled, and heavy metals, PCDDs, and PCBs were undetected.

Materials. PCB standard mixtures (PCB-W22), OCP mixture (M-680P), PBDE standard mixture (BDE-CM, PBDE congeners of primary interest calibration mix), individual PCB standards (CB65, 155, and 204), individual PBDE standards (BDE77 and 166), octachlorostyrene, and two MeO-PBDE mixtures (2′-MeO-BDE68 and 6-MeO-BDE47) were all purchased from AccuStandard (New Haven, CT). $^{13}C_{12}$-Labeled PCB standard mixture (CB 77, 81, 126, and 169) and $^{13}C_{12}$-CB189 were purchased from Wellington Laboratories (Guelph, Canada). 5H-Bromoindole, 2,4,6-tribromoanisole, oxybenzone, and 1,4-dimethoxy-2,5-dimethoxybenzene were purchased individually from Sigma-Aldrich (Milwaukee, WI), and 4,6-dibromoindole was purchased from SiNova (Bethesda, MD).

Many HNPs, for which reference standards were not commercially available, were made in laboratories. The molecular structures and their acronyms are shown in **Figure 1**, and their purities and sources are as follows: Standards of halogenated MBPs (MBP-HBr_5Cl, MBP-HBr_6, MBP-HBr_6Cl, and MBP-Br_7) were extracted from the blubber of a common dolphin and isolated by GPC and silica column followed by preparatory capillary GC (purity >95%) (*12*). Q1 (MBP-Cl_7) was synthesized and recrystallized (purity >98%) (*13*); MHC-1 (purity >90%) was calibrated with *trans*-chlordane (*14*); and two congeners of PBHDs were isolated from Mediterranean sponge (purity >95%) (*15*). 2,2′-Dimethoxy-3,3′,5,5′-tetrabromobiphenyl (2,2′-diMeO-BB 80) was synthesized following the procedure described by March et al. (*16*) (purity >95%). Standards of halogenated DMBPs (two isomers of DMBP-Br_3Cl_3, DMBP-Br_4Cl_2, DMBP-Br_5Cl, and DMBP-Br_6, purity >99%) were donated by Sheryl Tittlemier (Health Canada, Ontario, Canada).

All solvents used in this study were high-performance liquid chromatography grade; cyclohexane and toluene were purchased from Sigma-Aldrich, and ethyl acetate (EtOAc) and isooctane were purchased from J. T. Baker (Phillipsburg, NJ). Septa for the autosampler vial caps were heated at 200 °C overnight, and all glassware and glass wool were heated at 450 °C for 6 h in a muffle furnace prior to use.

Sample Preparation. For lipid removal from cod liver oil extracts, an automated GPC instrument (J2 Scientific, Columbia, MO) was employed. The glass GPC column was 2 cm i.d. and 22.5 cm length packed with 24 g of BioBeads S-X3 in 1:1 EtOAc:cyclohexane (v:v) and was purchased from J2 Scientific. Fish oil (0.5 g) was dissolved into the mobile phase 1:1 cyclohexane:EtOAc solvent with spiked known amounts of internal standards (CB65, 155, and 204 and BDE77 and 166) and brought to 10 mL of volume. Half (5 mL = 0.25 g) of each sample was injected into the GPC at 5 mL/min, and elution was between 12.5 and 22.5 min and reduced finally to 100 μL with final

addition of recovery standards ($^{13}C_{12}$-labeled CB77, 81, 126, 169, and 189) in isooctane. For more sensitive analysis of heavier HNPs, a larger sample size of 1 g instead of 0.25 g was used but with two GPC cleanup steps.

Instrumental Parameters. A Pegasus 4D (Leco, St. Joseph, MI) GC×GC/TOF-MS was used in this study with the following column configuration: a Restek (Bellefonte, PA) Siltek deactivated column (5 m, 0.25 mm i.d.) as a guard column, a Restek Rtx-5Sil-MS (15 m, 0.25 mm i.d., 0.25 μm film thickness) as the ^{1}D column, and a J&W Scientific (Folsom, CA) DB-17MS (2 m, 0.18 mm, 0.18 μm thickness) as the ^{2}D column. Ultrapurity He (Airgas, Radnor, PA) was used as the carrier gas.

The instrument conditions were as follows: The primary oven temperature was set at 60 °C for 7.5 min, ramped at 10 °C/min to 300 °C, and held for 20 min; the secondary oven temperature was programmed to be 20 °C higher than the primary oven; for GC×GC, the modulation period was set as 3.5 s with 0.9 s hot pulse duration and 35 °C modulator temperature offset vs the primary oven temperature; MS transfer line, 270 °C; ion source temperature, 250 °C; electron energy, −80 eV; detector voltage, 1850 V; and data acquisition rate, 100 spectra/s for the *m*/*z* range of 50−800.

Injection was conducted by a Combi-PAL autosampler (Leap Technologies; Carrboro, NC) with the automated DSI accessory (Linex) in combination with an Optic 3 programmable temperature vaporizer (Atas-GL International; Veldhoven, The Netherlands). The injection volume was 10 μL. The optimized conditions for DSI of final extracts in isooctane were as follows: initial injector temperature at 70 °C for 8 min with 50:1 split ratio (to vent the evaporated solvent), ramped to 320 °C at the maximum rate (12 °C/s) with a splitless period of 7.5 min, then 50:1 split ratio for 16.5 min, at which point the split flow was reduced to 25:1 and the injector temperature was cooled to 250 °C. The carrier gas flow rate was held at 1 mL/min for 8 min, ramped to 2.5 mL/min as a pressure pulse during the 7.5 min splitless period, then reduced to 1 mL/min until 33 min, and ramped to 1.5 mL/min until the end of the analysis.

Data Processing and Quality Control. Data analysis was conducted with the Leco ChromaTOF (version 3.25) software. Quantitation of the analytes for which reference standards were available used five levels of calibration standards. Manual review was made of all integrations and identifications to ensure accuracy of the results. The average recovery of the six internal standards was 91% with an overall range of 64−122% among the sample analyses and quality control spikes. The PCB analysis was not fully congener-specific, which is a very complicated undertaking due to coelutions of PCB congeners (*17*). Instead, to simplify the analysis without affecting overall PCB quantitation, we used 15 PCB congeners including seven PCB indicators (CB28, 52, 101, 118, 138, 153, and 180). Some PBDEs, oxybenzone, and a dibromoindole were also detected in the "blank" samples, so only those results more than three times higher than the blank levels are reported. A few other analytes were detected in the blank samples but at such low levels that there was no need to correct for their concentrations in the samples.

RESULTS AND DISCUSSION

Method Optimization for Analysis of PCBs, OCPs, and PBDEs in Fish Oil. The analytical approach using DSI-GC×GC/TOF-MS only requires GPC cleanup for fish oils based on the previous sample cleanup comparison study (*11*). Fankhauser-Noti and Grob developed a GC procedure using backflushing in the inlet for injection of diluted edible oils (*18*), but their approach was not demonstrated to be effective for relatively nonvolatile components such as PBDEs. The equivalent amount of sample injected in their method was 200 μg, which is too little to achieve very low detection limits for organic contaminants. Through the initial use of GPC in our study, we injected 25 mg of equivalent sample. Without the use of GPC, the presence of too much oil in the removable microvial reduces transfer efficiency of lipophilic analytes into the GC column, and quantitation suffers.

Figure 2A shows comparison of responses of selected PCBs and PBDEs in cod liver oil of different sample sizes (0.1, 0.25, 0.5, and 1 g) after GPC cleanup by injection using microvials in DSI. The peak responses of the more volatile, smaller compounds increased as the amount of cod liver oil sample increased, as would be expected with increasing analyte concentration in the final extracts. However, the peak responses of the less volatile, larger compounds remained fairly constant regardless of the sample size because more lipids remained in the final extracts as sample size in GPC increased, which caused worse transfer efficiency of the heavier compounds from the DSI microvial to the GC column.

Therefore, use of an appropriate amount of injected sample or a larger GPC column for larger samples was an option to better remove the lipids. Because the GPC column was fixed in this study, we chose a smaller sample size to reduce the amount of lipid coextractives and improve transfer efficiency for less volatile analytes in DSI. On the basis of the result in **Figure 2A**, 0.25 g was chosen as an optimal injected amount of oil. Additionally, a higher GC injection temperature improved the transfer efficiency of the heavier analytes. Also, the use of several internal standards with different molecular weights corrected for the different analyte transfer efficiencies on account of the lipids in the final extracts (see **Figure 2B**). In a final optimized method, we added a heavier internal standard, hexa-BDE (BDE166), to better correct for the lower transfer efficiency of PBDEs with five or more bromines ($\geq Br_5$).

Limits of detection (LODs) calculated based on signal/noise of 3 using the instrument software were 0.44 pg/g for BDE77 and 0.63 pg/g for ^{13}C-CB189. We quantified selected PCBs, PBDEs, and OCPs in three samples from each of the three dietary cod liver oils with the optimized analytical method, and results are summarized in **Table 1**. The quantified concentrations of all analytes were consistent with a relative standard deviation (RSD) of 11% on average, and 95% of the total data had RSDs between 1.0 and 25%.

PCBs, OCPs, PBDEs, Lighter Halogenated Compounds, and Oxybenzone. Table 1 summarizes the concentrations of selected POPs (OCPs, PBDEs, and PCBs including seven indicators: CB28, 52, 101, 118, 138, 153, and 180), the relatively more volatile halogenated compounds, and oxybenzone that were previously confirmed and identified in the cod liver oil, sample 2 (*11*). The three cod liver oils are named as samples 1, 2, and 3 as indicated in **Table 1**. Two cod liver oil supplements, samples 1 and 2, were from the same company, and their source was Norwegian cod liver oil, but only sample 2 had a label of PCB/heavy metal free with the written claim, "This product has been processed and tested to be free of PCB and heavy metals". The third cod liver oil sample was from a different company, but its source was from Arctic cod liver oil (similar to the other two samples), and the bottle was labeled "molecularly distilled" and "undetected levels of heavy metals, dioxins, and PCBs".

When contacted, the vendor of the "PCB/heavy metal free" bottle also stated that molecular distillation was conducted by the supplier in Norway. This process involved cooking and pressing of the cod fish at <95 °C, removal of protein, separation of the crude oil, addition of NaOH followed by neutralization at 95 °C for removal of free fatty acids as soap, and use of a "proprietary blend of absorbents" at 110 °C for "removal of organic contaminants, heavy metals, phospholipids, and peroxides". Then, winterization was conducted at 0−10 °C for 3 h for "removal of saturated fats (stearin)", followed by "molecular distillation and deodorization" for "removal of organic con-

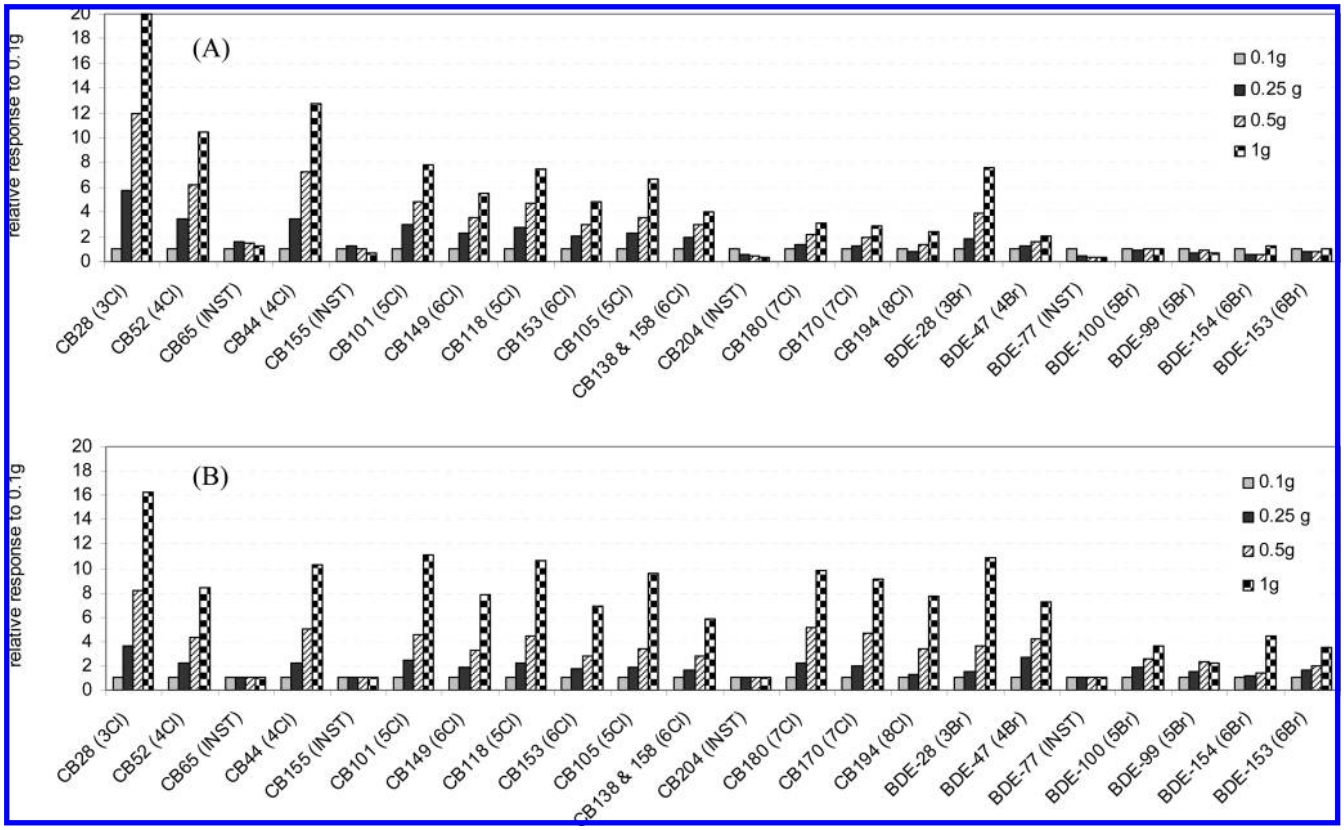

Figure 2. Comparison of responses of PCB and PBDE congeners in cod liver oil of different sample sizes (0.1, 0.25, 0.5, and 1 g) cleaned up once by GPC and injected using microvials in DSI. (**A**) The responses are normalized to the 0.1 g results. (**B**) The peak responses are normalized to internal standards (INST) in each sample and then are normalized to the 0.1 g results. For direct proportionality, the individual bars should reach 1, 2.5, 5, and 10 on the *y*-axis for the 0.1, 0.25, 0.5, and 1 g responses, respectively.

taminants (PCBs, dioxins, volatile organic materials)". Drumming was conducted under nitrogen, and antioxidants were added to produce the "refined, purified, deodorized cod liver oil", which was analyzed by the manufacturer to verify quality.

Although the three cod liver oils have a similar source, only samples 2 and 3 were treated for PCBs and heavy metals. Thus, we were able to compare the analyte concentrations among the three cod liver oils and assess the effect of the process used to remove PCBs and heavy metals. The relative amounts in samples 2 and 3 vs sample 1 are summarized in **Figure 3**. Interestingly, the relative amounts of POPs and HNPs in samples 2 and 3 increased as the molecular weights of the analytes increased except for oxybenzone in sample 2, which will be discussed later. The levels of PBDEs ($\geq Br_4$) were similar among the three samples, while PCBs and OCPs were much lower in samples 2 and 3. Moreover, the relative levels of the compounds ($\leq$300 g/mol) in samples 2 and 3 were $\leq$20% of the levels in sample 1. In addition, **Figure 4** displays the GC$\times$GC contour maps of the selected ions in samples 1 and 2, which clearly shows that the smaller molecular weight compounds such as 2,4,6-tribromoanisole, mono-dibromoindoles, and hexachlorobenzene almost disappeared in sample 2. This suggests that sample 1 was not treated to remove PCBs and that the manufacturer treatments of samples 2 and 3 removed lighter POPs more efficiently than heavier POPs. This general POP profile with respect to volatility is consistent with molecular distillation using a vacuum system as the oil-refining process (*19*).

Apparently, the analytical method used by the manufacturer also was devised to only detect the relatively low molecular weight PCB contaminants and not to detect other organic contaminants. The ability of our method to efficiently quantify and identify a wide range of organic chemicals in the oil matrices at trace concentrations demonstrates its advantages over the limited methods often used in practice.

In wild Alaskan sockeye salmon oil, concentrations were generally higher than in samples 2 and 3. The exceptions (PBDEs, dibromoinoles, a halogenated monoterpene MHC-1, and oxybenzone) were equal to the levels in procedural laboratory blanks or simply not detected. These results suggest that the salmon oil was not treated to remove contaminants. The low concentrations of these compounds may reflect their environmental levels of where the salmon were caught. Additionally, a global survey of farmed and wild salmon showed that Alaskan sockeye wild salmon contained less total PBDEs and POPs than farmed salmon fish (*7, 20*). That may be why significantly lower levels of PBDEs were detected in the salmon oil in our study. The monoterpene MHC-1 was recently identified to be naturally produced by the red seaweed *Plocamium* sp. (*14*). While the seaweed is abundant along the coast of Norway, it is not in Alaskan waters. This may explain why we did not detect MHC-1 in the salmon oil, and yet, MHC-1 was the highest concentrated halogenated compound in sample 1.

Interestingly, the concentrations of oxybenzone were high in samples 1 (370 ng/g) and 2 (690 ng/g) but were equal to the procedural laboratory blank level ($\sim$3 ng/g) in sample 3 and the salmon oil, which is a different trend than observed for the other lighter analytes reported here as shown in **Figure 3**. Considering its relatively low molecular weight of 228 g/mol and 224$-$227 °C boiling point, we expect that oxybenzone would be removed from the cod liver oil through the treatment process if present in the raw cod liver oil. Therefore, contamination probably occurred after the treat-

Table 1. Concentrations (in ng/g) of PCBs, OCPs, PBDEs, and Small Halogenated Compounds in Three Different Cod Liver Oil and Salmon Oil Supplements[a]

		cod liver oil			
	RT (s) (^1t_R, ^2t_R)	sample 1 ($n = 3$)	sample 2 ($n = 3$)	sample 3 ($n = 3$)	salmon oil ($n = 2$)
treatment		no information	PCB and metal free	moleculary distilled	no information
origin		Norwegian cod liver oil	Norwegian cod liver oil	Arctic cod liver oil	Wild Alaskan sockeye salmon oil
made in		United States	United States	United States	United States
CB17	1266, 1.230	0.59 ± 0.0046	0.031 ± 0.0010	0.022 ± 0.0009	1.3 ± 0.056
CB 28/31	1325.5, 1.170	2.1 ± 0.020	0.12 ± 0.012	0.044 ± 0.0048	2.4 ± 0.080
CB20	1336, 1.331	0.36 ± 0.081	0.025 ± 0.0043	0.008 ± 0.0073	0.61 ± 0.014
CB52	1364, 1.166	3.0 ± 0.110	0.28 ± 0.025	0.064 ± 0.0093	4.1 ± 0.27
CB44	1388.5, 1.450	1.8 ± 0.061	0.17 ± 0.0055	0.056 ± 0.0075	2.0 ± 0.004
CB101	1465.5, 1.193	14 ± 0.60	2.3 ± 0.12	0.68 ± 0.032	14 ± 0.94
CB149	1528.5, 1.530	11 ± 0.53	2.7 ± 0.22	0.78 ± 0.047	5.7 ± 0.11
CB118	1535.5, 1.420	19 ± 1.3	4.3 ± 0.45	2.0 ± 0.19	13 ± 0.76
CB153	1556.5, 1.300	30 ± 1.9	9.0 ± 0.70	4.6 ± 0.19	12 ± 0.57
CB105	1563.5, 1.847	6.2 ± 0.46	1.7 ± 0.16	0.91 ± 0.10	3.3 ± 0.40
CB138	1588, 1.680	31 ± 1.5	11 ± 0.86	6.7 ± 0.26	11 ± 0.005
CB180	1658, 1.600	6.8 ± 0.23	4.4 ± 0.51	2.7 ± 0.072	2.4 ± 0.109
CB170	1689.5, 2.030	2.8 ± 0.12	1.8 ± 0.30	1.3 ± 0.096	0.86 ± 0.071
CB 194	1752.5, 1.950	0.50 ± 0.037	0.42 ± 0.10	0.36 ± 0.043	0.12 ± 0.016
total (ng/g)		130 ± 6.9	38 ± 3.5	20 ± 1.1	73 ± 3.4
α-HCH	1210, 1.350	0.17 ± 0.036	ND	ND	ND
heptachlor epoxide	1427, 1.460	2.3 ± 0.075	0.20 ± 0.034	0.083 ± 0.0075	1.3 ± 0.034
chlordane (γ)	1455, 1.420	3.2 ± 0.17	0.82 ± 0.18	0.43 ± 0.070	1.1 ± 0.093
chlordane (α)	1469, 1.444	7.5 ± 0.34	1.2 ± 0.057	0.37 ± 0.037	4.6 ± 0.043
trans-nonachlor	1472.5, 1.141	5.8 ± 0.27	1.1 ± 0.093	0.33 ± 0.024	3.1 ± 0.12
cis-nonachlor	1542.5, 1.720	3.1 ± 0.15	1.0 ± 0.083	0.44 ± 0.071	0.45 ± 0.008
p,p′-DDE	1497, 1.346	53 ± 2.5	11 ± 0.75	3.3 ± 0.26	57 ± 2.7
p,p′-DDD	1546, 1.741	15 ± 0.85	5.6 ± 0.83	1.7 ± 0.15	10 ± 0.55
p,p′-DDT	1584.5, 1.730	8.3 ± 1.2	4.2 ± 0.32	1.8 ± 0.79	ND
dieldrin	1500.5, 1.740	26 ± 1.8	2.6 ± 0.19	1.1 ± 0.15	8.3 ± 0.89
total (ng/g)		120 ± 7.4	28 ± 2.5	9.6 ± 0.86	86 ± 4.4
BDE28	1539, 2.140	1.0 ± 0.081	0.23 ± 0.012	0.12 ± 0.027	ND
BDE47	1665, 2.660	6.5 ± 0.19	4.0 ± 0.040	4.4 ± 0.11	1.0*
BDE100	1756, 3.053	0.84 ± 0.011	0.64 ± 0.033	0.90 ± 0.063	0.14*
BDE99	1784, 3.090	1.0 ± 0.058	0.82 ± 0.14	0.74 ± 0.26	0.30*
BDE154	1857.5, 0.080	0.87 ± 0.076	1.1 ± 0.27	1.2 ± 0.31	ND
BDE153	1892.5, 0.165	0.32 ± 0.076	0.20 ± 0.029	0.19 ± 0.10	ND
total (ng/g)		11 ± 0.49	7.0 ± 0.52	7.6 ± 0.87	ND
dibromo dimethoxy benzene	1143.5, 1.149	0.96 ± 0.046	ND	ND	0.35 ± 0.023
dibromo dimethoxy benzene	1154, 1.147	0.77 ± 0.044	0.024 ± 0.0027	ND	0.21 ± 0.029
1,4-dibromo-2,5-dimethoxybenzene	1199.5, 1.528	1.3 ± 0.038	0.023 ± 0.0005	ND	1.7 ± 0.005
dibromo dimethoxy benzene	1206.5, 1.510	0.58 ± 0.032	ND	ND	6.0 ± 0.14
dibromo dimethoxy benzene	1248.5, 1.890	1.1 ± 0.017	ND	ND	1.9 ± 0.046
2,4,6-tribromo anisole	1154, 1.166	12 ± 0.63	0.085 ± 0.0032	0.049 ± 0.0069	7.5 ± 0.46
bromoindole	1171.5, 2.190	8.0 ± 0.37	1.7 ± 0.61	1.78 ± 0.21	0.91 ± 0.23
dibromoindole	1315, 2.080	2.2 ± 0.028	0.35 ± 0.10	0.030*	0.030*
dibromoindole	1392, 3.000	59 ± 1.2	4.9 ± 0.32	1.4 ± 0.064	0.08 ± 0.022
hexachlorobenzene	1213.5, 1.010	8.6 ± 0.40	0.17 ± 0.0042	0.074 ± 0.0034	25 ± 0.28
octachlorostyrene	1416.5, 0.966	0.50 ± 0.037	0.049 ± 0.012	ND	0.46 ± 0.035
MHC-1	1465.5, 1.769	69 ± 3.7	7.5 ± 0.66	ND	ND
Q1 (MBP-Cl_7)	1472.5, 1.100	3.2 ± 0.10	0.37 ± 0.0063	ND	2.8 ± 0.21
oxybenzone	1416.5, 1.917	370 ± 40	690 ± 100	2.7*	2.7*

[a] Compounds written in italics were quantifed using their isomeric standards. ^1t_R and ^2t_R indicate ^{1}D and ^{2}D retention times, respectively; ND represents not detected; and * represents the low-level background concentrations found in laboratory procedural blanks.

ment process. Oxybenzone is a common sunscreen agent (*21*), and it is also used as an UV stabilizer in plastic surface coatings for food packaging to prevent polymer or food photodegradation (*22*). On the basis of its relatively very low level in procedural blanks, its high concentrations in samples 1 and 2 are not from laboratory contamination but probably from its usage in the container as a UV stabilizer. In fact, both containers for samples 1 and 2 were made of the same plastic material from the same company, whereas the container of sample 3 was an amber glass bottle and the salmon oil was stored in a softgel capsule made of fish gelatin, glycerin, and purified water.

The PCB concentrations in the samples were comparable with a recent survey (*23*), but the PBDE concentrations in the cod liver oils were in the higher concentration range for PBDEs in a survey of 69 fish oil supplement samples (*10*). 1,4-Dibromo-

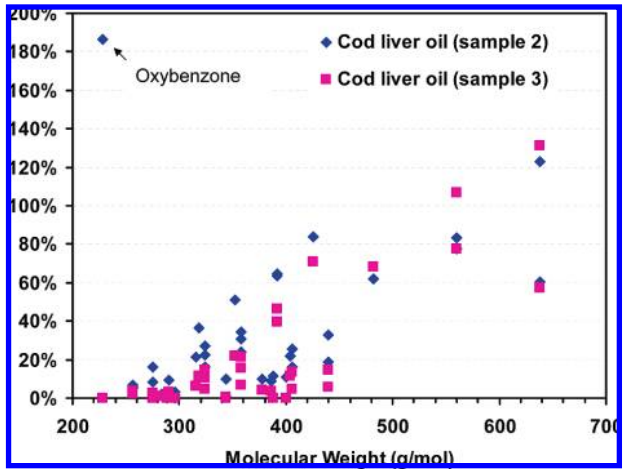

Figure 3. Relative concentrations (%) of selected PCBs, OCPs, PBDEs, and the other organohalogen compounds listed in **Table 1** in cod liver oils samples 2 and 3 (treated by molecular distillation to remove contaminants) vs sample 1 (untreated) according to their molecular weight (g/mol).

2,5-dimethoxybenzene and its potential isomers and 2,4,6-tribromoanisole were newly identified and confirmed during this study because of their high abundance in sample 1 but not in sample 2, which was used in our previous study for screening compounds qualitatively (*11*). The identification of these untargeted additional compounds shows the power of this approach for untargeted screening capacity (*11*). The source of these compounds in the cod liver oil is ambiguous, with both anthropogenic and marine natural sources as possibilities (*24*−*26*).

The concentration of a dibromoindole was as high as 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p*,*p*′-DDE) in sample 1. We found only two previous studies that measured concentrations of bromoindoles in marine sediment and water extracts from the North and Baltic Seas and the common oyster collected from coastal Georgia (*27*, *28*). Hexachlorobenzene, once used as a fungicide, was detected at a rather high level, 25 ng/g, in the salmon oil.

HNPs. We identified multiple classes of HNPs in the cod liver oil supplement (sample 2) during the qualitative evaluation study (*11*): MBPs, DMBPs, MeO-PBDEs, diMeO-BB-80, and PBHDs. Their chemical structures are similar to PBDEs and PCBs, as shown in **Figure 1**. However, the sample size, 0.25 g, was not enough to quantify these HNPs in the cod liver oils due to their heavier molecular weights (≥500 g/mol), lower concentrations in the samples, and relatively higher detection limits. Therefore, we increased the fish oil sample size to 1 g and performed the GPC cleanup procedure twice due to the small GPC column capacity. We summarized the results separately for those analytes in **Table 2**.

The concentrations of the HNPs reported in **Table 2** are comparable to the concentrations of PBDEs in each sample. The reduction of the HNPs from sample 1 to samples 2 and 3 is less than the reduction that occurred for the more volatile halogenated compounds, which is undoubtedly a function of separation by volatility in the molecular distillation process. For example, reduction of Q1 (MBP-Cl_7) from 3.2 ng/g in sample 1 to 0.37 ng/g and nondetectable in samples 2 and 3 (listed in **Table 1**) is larger than its heavier congeners (MBPs) in **Table 2** due to Q1's relatively low molecular weight of 384 g/mol.

Surprisingly, the concentrations of DMBPs in the salmon oil supplement were greater (totaling 160 ng/g) than those in the cod liver oil supplements (ND to 0.23 ng/g). We speculate that the reason for high DMBP concentrations in the salmon oil

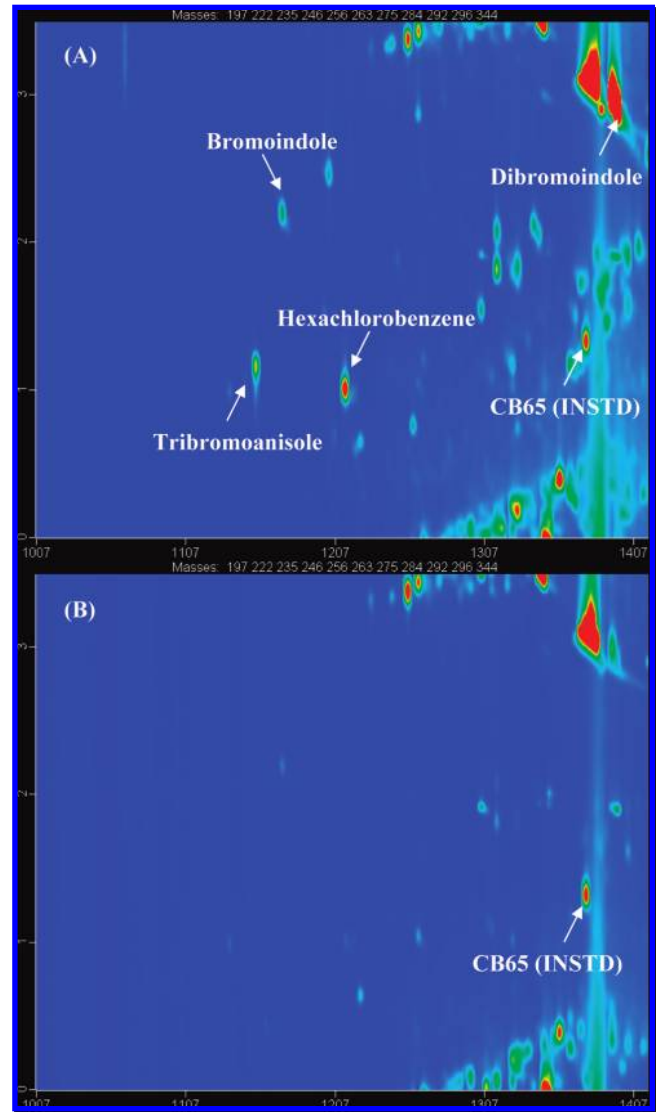

Figure 4. GC×GC contour maps of *m*/*z* 197, 222, 235, 246, 263, 275, 284, 292, 296, and 344 in cod liver oil sample 1 (**A**) and sample 2 (**B**). The peak intensities represent summation of the ions, and the shade indicates degrees of the peak intensity. The *x*-axis and *y*-axis represent ^{1}D GC and ^{2}D GC retention times (in s), respectively.

supplement relates to their higher abundance in the Pacific Ocean as compared to the Atlantic Ocean (*3*). Tittlemier also found higher DMBP concentrations in canned tuna and salmon from the Pacific Ocean than in marine fish such as cod or haddock in the northern Atlantic area (*29*).

MBPs have been detected at high levels before in marine mammals, which are top predators in the marine food web (*4*, *30*, *31*). Although the presence of Q1 (MBP-Cl_7) was previously reported in fish oil and other environmental samples (*5*), the concentrations of MBPs in cod liver oil or marine organisms at a lower trophic level have not been reported to our knowledge.

According to the literature, 2′-MeO-BDE68 and 6-MeO-BDE47 are the most abundant MeO-PBDEs present in marine organisms (*32*). These analytes were detected in all of the samples analyzed, and their levels were similar in cod liver oil sample 1 and the salmon oil supplement. Their concentrations were lower in samples 2 and 3 (again, likely due to the manufacturer's treatment process). The total concentrations of the MeO-PBDEs were almost equal to the total PBDE concentrations in the cod liver oil supplements and even exceeded the

Table 2. Concentrations (ng/g) of HNPs in the Cod Liver Oil Supplements and a Salmon Oil Supplement[a]

		cod liver oil			
name	RT (s) (^1t_R, ^2t_R)	sample 1	sample 2	sample 3	salmon
MBP-H_2Br_4Cl	1479.5, 1.740	ND	ND	ND	ND
MBP-H_2Br_4Cl	1539, 2.493	ND	ND	ND	ND
MBP-H_2Br_4Cl	1560, 2.134	ND	ND	ND	ND
MBP-H_2Br_4Cl	1623, 2.935	ND	ND	ND	ND
MBP-HBr_4Cl_2	1581, 2.082	ND	ND	ND	ND
MBP-H_2Br_5	1525, 2.083	ND	ND	ND	ND
MBP-H_2Br_5	1584.5, 2.858	ND	ND	ND	ND
MBP-H_2Br_5	1605.5, 2.470	0.13[b]	0.059[b]	ND	ND
MBP-HBr_5Cl	1623, 2.338	ND	ND	ND	ND
MBP-HBr_5Cl	1633.5, 2.450	0.058	0.023	ND	0.18
MBP-HBr_5Cl	1679, 3.221	ND	ND	ND	ND
MBP-HBr_5Cl	1714, 2.993	40	ND	ND	ND
MBP-Br_5Cl_2	1714, 2.790	ND	ND	ND	ND
MBP-HBR_6	1679, 3.221	0.057	0.031	ND	0.050
MBP-HBr_6	1668.5, 2.735	ND	ND	ND	ND
MBP-HBr_6	1675.5, 2.860	ND	ND	ND	ND
MBP-HBr_6	1728, 0.131	ND	ND	ND	ND
MBP-HBr_6	1756, 3.431	ND	ND	ND	ND
MBP-Br_6Cl	1763, 3.284	0.18	0.056	ND	0.23
MBP-Br_7	1808.5, 0.284	0.081	0.037	0.063	0.061
total MBP (except Q1)		0.51	0.21	0.063	0.52
DMBP-$H_2Br_2Cl_2$	1399, 1.302	ND	ND	ND	15[c]
DMBP-$H_2Br_2Cl_2$	1476, 1.659	ND	ND	ND	0.96[c]
DMBP-$H_2Br_2Cl_2$	1539, 2.069	ND	ND	ND	0.54[c]
DMBP-$H_2Br_3Cl_2$	1563.5, 2.100	ND	ND	ND	19[c]
DMBP-HBr_3Cl_2	1637, 2.594	ND	ND	ND	0.46[c]
DMBP-Br_3Cl_3	1668.5, 2.600	ND	ND	ND	3.5
DMBP-HBr_4Cl	1616, 2.449	ND	ND	ND	0.19[c]
DMBP-HBr_4Cl	1672, 3.290	ND	ND	ND	2.3[c]
DMBP-Br_4Cl_2	1714, 2.939	0.15	0.098	ND	110
DMBP-Br_5Cl	1766.5, 0.031	ND	ND	ND	5.1
DMBP-Br_6	1815.5, 0.541	0.075	0.027	ND	0.13
total DMBP		0.23	0.13	ND	160
6′-MeO-BDE49	1693, 2.734	0.21	0.050	ND	0.082
2′-MeO-BDE68	1710.5, 2.473	2.7	1.3	0.51	0.886
6-MeO-BDE47	1728, 2.935	9.7	4.4	1.5	7.9
2,2′-diMeO-BB-80	1714, 2.340	0.15	0.10	0.031	0.29
PBHD (3Br)	1808.5, 0.028	27	10	8.0	2.6
PBHD (4Br)	1938, 2.147	14	5.2	20	ND

[a] The compounds written in italics were quantified by their isomeric standards or congeneric standards (see also *b* and c), ^1t_R and ^2t_R indicate ^{1}D and ^{2}D retention times, respectively, and ND represents not detected. [b] The response factor of a standard compound, MBP-HBr_5Cl, was used. [c] The response factor of a standard compound, DMBP-Br_3Cl_3, was used.

PBDE concentrations in the salmon oil supplement. Because the structural similarities with PBDEs suggested an anthropogenic origin of MeO-PBDEs, these compounds have been studied more extensively than many other HNPs (*33*−*35*). Teuten et al. showed that these methoxy-BDEs in whale blubber originated from a natural source using radiocarbon analysis (*32*) and their presence in preindustrial whale oil (*36*). Furthermore, 2,2′-dimethoxy-3,3′5,5′-tetrabromobiphenyl (2,2′-diMeO-BB80) was detected in all of the samples and followed the same trend as the MeO-PBDEs. Its concentration is only available in top predator marine mammals according to the literature (*16*). The PBHDs were also detected at significant levels in the cod liver oil supplements. However, we only detected PBHD (3Br) in the salmon oil supplement. The PBHD levels in the cod liver oil supplements fall in the highest range of these compounds detected in a previous fish oil survey study in Europe (*10*).

Dietary Intake Estimate. The total daily suggested amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) range from 1 to 3 g/day for humans (*37*, *38*). The cod liver oil supplements tested in our study contained approximately 1 g EPA and DHA per serving size (5 mL) according to their labels. Therefore, 5−15 mL (1−3 serving sizes) of these cod liver oil supplements is required to meet the recommended daily dose, which corresponds with the recommendations on the bottle labels. The salmon oil contained 460 mg of EPA and DHA per its recommended daily serving size (3 softgels = 3 g) on the bottle label. The calculated total daily intake of halogenated compounds was reduced from 2.2 to 0.33−0.52 μg (for the 5 mL serving size) in the treated cod liver oil supplements (samples 2 and 3). The major reduction comes from smaller halogen compounds that are removed efficiently by the treatment processes but not from PBDEs and the heavier HNPs (MBPs, DMBPs, MeO-PBDEs, diMeO-BB-80, and PBHDs). Especially, in the case of the salmon oil, the heavy HNPs contribute almost 45% of the total daily intake of POPs and HNPs due to presence of relatively large amount of DMBPs.

Few toxicity studies of the HNPs have been conducted (*39*, *40*), and toxicity tests for multiple groups of HNPs or POPs have not been performed. Despite this lack of information, human consumption of fish oil has increased because of its reported beneficial health effects (*38*), and notably, fish oil is recommended to pregnant women for prenatal care. It may be worthwhile to analyze a greater scope of fish oil supplements and alternatives such as vegetable oil supplements to determine the presence and concentrations of POPs and HNPs together. The bioactivity of the organic contaminants in fish oil must be assessed to truly comprehend the trade-offs between the risks and the benefits of these supplements. This is especially important given the evidence presented here that current treatment processes are ineffective at removal of the heavier organic contaminants.

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