Exposure to Halogenated Flame Retardants from Fish Oil Supplements

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1 Introduction

Flame retardants are widely used as additives in high molecular weight organic materials such as plastics, rubbers, and fibers. Among these additives, halogenated flame retardants (HFRs), including hexabromocyclododecanes (HBCDs), polybrominated diphenyl ethers (PBDEs), and dechlorane and related compounds (DRCs), are widely used in plastic products because of their low cost and excellent flame retardant effect. However, serious concerns have been raised about such additives, particularly regarding their environmental persistence and health degradation due to long-term bioaccumulation. Among these HFRs, HBCDs, tetra- to hepta-BDEs, deca-BDE, and dechlorane are currently internationally regulated by Annex A of the Stockholm Convention on Persistent Organic Pollutants; their manufacture, use, and import/export are highly prohibited.

There have been many reports to date on HFR contents in foods.[1-3] We previously reported the estimated intake of HFRs in a total diet study based on the market basket method, finding high HFR concentrations in seafood that contributed substantially to total daily intake.[4-6]

Food supplements are currently in demand in line with growing health consciousness. Among them, fish oil supplements, which contain n-3 polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid and eicosapentaenoic acid, are popular and their consumption is increasing in Japan. There is concern about dietary exposure to HFRs through fish oil supplements due to the lipophilicity of HFRs. Thus, in the present study, we investigated the dietary intakes of HBCDs (α-, β-, and γ-HBCD), PBDEs (tri- to deca-BDEs), and DRCs (dechlorane, chlordane plus [CP], dechlorane602 [Dec602], dechlorane 603 [Dec603], dechlorane604 [Dec604], *syn*- and *anti*-Dechlorane Plus [DP]) from fish oil supplements using mass spectrometry (MS) analysis.

2 Materials and Methods

Sample collection

Thirty-seven fish oil supplements (including 5 shark liver oil supplements) were purchased from supermarkets and commercial facilities in Fukuoka, Japan between July and September 2021.

Chemicals

Non-labeled and $^{13}C_{12}$-labeled PBDE analytical standard mixtures (BFR-CVS, BFR-LCS, BFR-ISS), α-, β-, and γ-HBCDs (non-labeled and $^{13}C_{12}$-labeled), γ-HBCD-d_{18}, CP, and DP (non-labeled and $^{13}C_{10}$-labeled) standards were purchased from Wellington Laboratories. Dechlorane (non-labeled and $^{13}C_{10}$-labeled) and Dec602 ($^{13}C_{10}$-labeled) standard were obtained from the Cambridge Isotope Laboratories. Dec602, Dec603, and Dec604 were obtained from Santa Cruz Biotechnology. Each standard was mixed and diluted with appropriate amounts of acetonitrile. Dichloromethane, *n*-hexane, acetone, cyclohexane, distilled water, ethanol and acetonitrile were of mass analysis-grade (Kanto Chemical). Hydrochloric acid, sulfuric acid, 44% sulfuric acid-impregnated silica gel, sodium chloride, anhydrous sodium sulfate, 1 mol L^{-1} ammonium acetate solution of HPLC grade, and fluvalinate were purchased from Wako Pure Chemical Industries.

Preparation of the test solution

Each 1 g sample was dissolved in 5 mL of hydrochloric acid and 55 mL of distilled water. The dissolved samples were shaken and extracted with 20 mL of distilled water, 40 mL of ethanol, 60 mL of *n*-hexane, 20 mL of dichloromethane, and 20 g of sodium chloride for 5 min. Each extract (dichloromethane and *n*-hexane layer) was dehydrated and concentrated, and then re-dissolved in 20 mL dichloromethane and *n*-hexane (1:3, v/v). Next, 5 mL of each extract spiked with ^{13}C-labeled standard mixtures as surrogates ($^{13}C_{12}$-labeled α, β, and γ-HBCDs, 5 ng each; $^{13}C_{12}$-labeled PBDEs, 0.25 ng each; $^{13}C_{10}$-labeled Dechlorane, 0.25 ng; $^{13}C_{10}$-labeled Dec602, 0.25 ng; and $^{13}C_{10}$-labeled DPs, 0.25 ng each) was treated with sulfuric acid and then washed with distilled water. The extracts were concentrated and re-dissolved in 5 mL acetone and cyclohexane (3:7, v/v). Lipids were removed using gel permeation chromatography (GPC). Two mL of extract was loaded onto a column (CLNpak EV-2000 AC; Showa Denko) with a guard column (CLNpak EV-G AC; Showa Denko) and eluted with acetone and cyclohexane (3:7, v/v) at a column

temperature of 40℃ and a flow rate of 5 mL min^{-1}. The eluate was fractionated for 20 min immediately after the elution of fluvalinate, which was used as an indicator. The fraction was evaporated to dryness and redissolved in 1 mL *n*-hexane. The solution was loaded onto a mini-column (1 g of 44% sulfuric acid-impregnated silica gel) and eluted with 8 mL dichloromethane and *n*-hexane (3:7, v/v). The eluate was concentrated and re-dissolved in 0.1 mL acetonitrile containing syringe spike solution: γ-HBCD-d_{18}, 2 ng; $^{13}C_{12}$-PBDEs, 0.1 ng; and $^{13}C_{12}$-labeled PentaCB-111, 0.1 ng.

High resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) analysis

The concentrations of PBDEs and DRCs were determined using the internal standard method with HRGC/HRMS. HRGC/HRMS analysis was performed using a Thermo Scientific DFS mass spectrometer coupled to a Thermo Scientific Trace 1310 GC. The GC conditions were as follows: capillary column, Rtx-1614 (Restek, 0.25 mm i.d., ×15 m, 0.1 μm film thickness); column temperature program, 120°C (held for 1 min) to 210°C at 20°C min^{-1} and to 300°C (held for 10 min) at 10°C min^{-1}; carrier gas, helium; flow rate, 1.0 mL min^{-1}; injection mode, splitless; injector temperature, 280°C; and injection volume, 1 μL. The MS conditions were as follows: ionization mode, electron ionization mode; electron energy, 45 eV; ion source temperature, 280°C; internal mass reference, perfluorokerosene; and resolution, 10,000. The monitored ions were as previously reported.[6, 7]

Liquid chromatography (LC)-MS/MS analysis

HBCD concentrations were determined by the internal standard method using a Waters Acquity UPLC H-Class Plus Binary equipped with a Waters Xevo TQ-XS (LC-MS/MS). The LC conditions were as follows: column, Waters Aquity UPLC BEH C18 (2.1 mm, i.d., ×100 mm, 1.7 μm); column temperature, 40°C; injection volume, 2 μL; mobile phase, 2 mmol L^{-1} ammonium acetate (A) and acetonitrile (B); gradient condition, A/B (%) 45/55, which was gradually changed to 5/95 (held for 6 min) over 8 min; flow rate, 0.2 mL min^{-1}. The MS conditions were as follows: ionization mode, electron spray ionization mode (negative); capillary voltage, 2.0 kV; cone voltage, 20 V; collision energy, 20 eV; scan type, selected reaction monitoring (SRM); SRM transition, m/z 638.6 > 78.9 and 640.6 > 78.9 transition for non-labeled HBCDs, m/z 650.7 > 78.9 and 652.7 > 78.9 for $^{13}C_{12}$-labeled HBCDs, and m/z 658.7 > 78.9 for γ-HBCD-d_{18}.

Quality assurance

No interfering peak was observed in the chromatogram of any of the compounds in the fish oil food supplements. The average recovery rate of ^{13}C-labeled DRCs, HBCDs, and PBDEs added to the extracts (*n*=37) ranged from 63% to 135% with relative standard deviations between 6.7% and 23.4%. A procedural blank sample was used for each sample preparation batch. The limits of detection (LODs) calculated for the signal-to-noise ratio (S/N) of 3 were 10, 100, and 10-100 pg g^{-1} wet wt. for DRCs, HBCDs, and PBDEs, respectively.

3 Results

Concentrations of HFRs in fish oil food supplements

Figure 1 shows the box plots of HFR concentrations in fish oil supplements detected above the LODs. Out of 37 samples, HBCDs, PBDEs, and DRCs were detected in 22, 17, and 18 samples, respectively.

The total HBCD (Σ_3HBCDs) concentrations ranged from 132-1,299 pg g^{-1}. The concentrations of the isomers were as follows: α-HBCD, 132-1,198 pg g^{-1} (detected in 22 samples); β-HBCD, not detected (ND); and γ-HBCD, 101 pg g^{-1} (detected in 1 sample). Thus, in the great majority of the samples in which HBCD was detected, α-HBCD was the predominant isomer.

The total PBDE (Σ_{35}PBDEs) concentrations were 25-2,049 pg g^{-1}. The most frequently detected congeners were DecaBDE-209 (detected in 13 samples), followed by HexaBDE-154 (3 samples), TetraBDE-47 (2 samples), and PentaBDE-100 (1 sample). The detected concentrations were those of DecaBDE-209 (208-2,049 pg g^{-1}), followed by TetraBDE-47 (68 and 92 pg g^{-1}), PentaBDE-100 (79 pg g^{-1}), and HexaBDE-154 (25-89 pg g^{-1}). DecaBDE-209 was the predominant PBDE congener in most of the samples.

The total DRC (Σ_7DRCs) concentrations ranged from 10-1,229 pg g^{-1}. The concentrations of each compound were as follows: dechlorane, 10-900 pg g^{-1} (detected in 11 samples); Dec602, 114-329 pg g^{-1} (5 of 25 samples); Dec603, ND; Dec604, ND; *syn*-DP, 11-16 pg g^{-1} (4 samples); *anti*-DP, 10-26 pg g^{-1} (10 samples); CP, ND. The ratios of dechlorane concentration to total DRCs ranged from 40% to 100% in the dechlorane detected samples, and dechlorane was the predominant component in all of the samples.

The average concentrations of HFRs were higher in the order of HBCDs, PBDEs, and DRCs.

Estimated dietary intakes

Table 1 shows the dietary intakes of HFRs per day calculated based on the weight of the manufacturer's recommended daily intake. The estimated intakes assumed a value of zero when the concentration was under the detection limit (ND=0). The intakes of HBCDs, PBDEs, and DRCs ranged from 0 to 3.3 ng per day, from 0 to 6.0 ng per day, and from 0 to 3.3 ng per day, respectively.

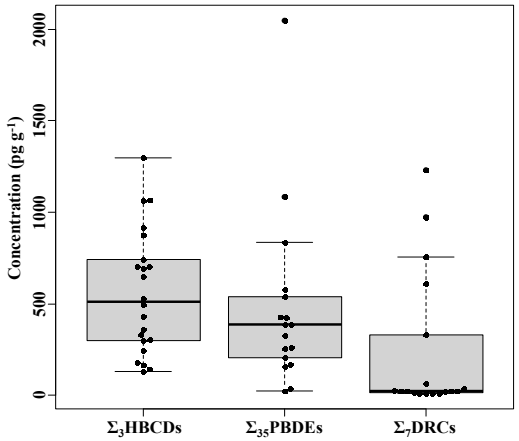

Figure 1: Overview of HFR concentrations (pg g^{-1}) in fish oil dietary supplements. The box plots show the median, 25th and 75th percentiles, range and outliers.

Table 1: Estimated dietary intakes of HFRs through fish oil food supplements.

Sample No.	Retailer	Manufacturer's recommended daily intake (mg per day)	Estimated dietary intakes (ng per day)**		
			HBCDs	PBDEs	DRCs
1	A	1320	0.9	0.0	0.0
2	A	3648	0.9	0.8	0.1
3	B	2025	1.4	1.7	0.1
4	B	3060	0.9	1.8	0.0
5	C	2020	0.3	0.0	0.0
6	C	1299	0.0	0.0	0.0
7	D	1760	0.3	0.0	0.0
8	D	2300	0.0	0.6	0.0
9	E	1610	0.7	0.0	0.0
10	F	3066	2.2	1.3	0.0
11	G	2700	1.0	2.9	0.1
12	H	2212	0.0	0.1	0.0
13	I	4080	0.0	0.0	0.0
14	J	3840	0.0	0.0	0.0
15	K	2940	3.1	6.0	0.0
16	L	4248	0.0	0.0	0.0
17	M	2170	0.7	0.0	0.0
18	N	2040	1.1	0.7	0.0
19	O	1800	0.5	0.0	0.0
20	O	3570	3.3	1.4	0.0
21	P	1200	0.9	0.5	0.0
22	P	1605	0.0	0.0	0.0
23	Q	2700	0.0	0.0	0.1
24	R	1365	1.2	0.0	0.0
25	R	2275	3.0	0.0	0.1
26	S	2200	0.4	0.0	0.0
27	T	1656	1.2	0.0	0.0
28	U	2400	0.4	0.0	0.0
29	V	2700	2.9	1.4	0.1
30	W	3150	0.0	0.1	0.0
31	X	1680	0.0	0.0	0.0
32	Y	6600	3.3	2.6	0.0
33*	D	2200	0.0	0.3	1.7
34*	F	2640	0.0	0.0	2.6
35*	G	2700	0.0	0.7	3.3
36*	Z	2736	0.0	0.0	0.9
37*	AA	1842	0.0	0.3	1.1
		Average	0.8	0.6	0.3
		Minimum	0.0	0.0	0.0
		Median	0.4	0.0	0.0
		Maximum	3.3	6.0	3.3

*Shark liver oil **Rounded to the first decimal place

4 Discussion

Alpha-HBCD was dominant in all of the samples in the present study. This finding is in accordance with those in a previous relevant study on oil fish food supplements.[8] Barontini et al. [9] also report that α-HBCD is the dominant residue among the HBCD isomers in marine fish/mammals, this report supports our results.

For PBDEs, it has been reported that relatively high concentrations of DecaBDE-209 were found in some fish and shellfish in Japan.[10] This finding is in good agreement with those in our study. Among the congeners of PBDEs, TetraBDE-47 is a known indicator of PBDE contamination in marine fish.[11] However, TetraBDE-47 was detected in only 2 samples in the present study.

DRCs have been detected at similar concentrations, as reported by Eyken et al.[12] It was characteristic that dechlorane and Dec602 were detected at relatively high concentrations in shark liver oil supplements in our study. Shark liver oil samples accounted for the top 5 samples in order of concentration, and high accumulation in large fish was observed.

The relative abundance of the *anti*-DP (f_{anti}) was calculated by dividing the concentration of *anti*-DP by the sum of the *syn*- and *anti*-DP concentrations for the 3 samples in which both *syn*-DP and *anti*-DP were detected. This yielded f_{anti} values of 0.48-0.57, which are equivalent to those previously reported, suggesting that some enrichment of the *syn*-DP occured.[12]

The obtained HFR intakes were compared with the health-based guideline values (HBGVs), which are given as follows: HBCDs, 0.051 μg kg^{-1} per day (no observed adverse effect level [NOAEL]: 10.2 mg kg^{-1} per day divided by

an uncertainty factor of 200)[13]; PBDEs, 0.05 μg kg^{-1} per day (lowest observed adverse effect level [LOAEL] of DecaBDE-209: 0.05 mg kg^{-1} per day divided by an uncertainty factor of 1000)[14]; and DRCs, 0.2 μg kg^{-1} per day (reference dose [RfD] of Dechlorane).[15] The daily intake of HBCDs, PBDEs, and DRCs from the fish oil supplements for a person weighing 50 kg ranged from 0 to 0.0001%, from 0 to 0.2%, and from 0 to 0.03% of these values, respectively.

5 Conclusions

We investigated the dietary intakes of HBCDs, PBDEs, and DRCs from 37 fish oil supplements using MS analysis. The concentrations of PBDEs and DRCs were determined using the internal standard method with HRGC/HRMS, and that of HBCDs was determined by LC-MS/MS. HBCDs, PBDEs, and DRCs were detected in 22, 17, and 18 of 37 samples, respectively. Σ_3HBCD concentrations ranged from 132 to 1,299 pg g^{-1}; Σ_{35}PBDE concentrations from 25 to 2,049 pg g^{-1}; and Σ_7DRC concentrations from 10 to 1,229 pg g^{-1}. The average concentration of HFRs was higher in the order of HBCDs, PBDEs, and DRCs. The estimated daily intake of HBCDs, PBDEs, and DRCs from the fish oil supplements ranged from 0 to 0.0001%, from 0 to 0.2%, and from 0 to 0.03% of the HBGVs, respectively. We conclude that the risk to human health due to HFR exposure from fish oil supplements is negligible.

6 Acknowledgments

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7 References

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