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Analytical Methods

Evaluation of hexabromocyclododecane in fish and marine mammal oil supplements

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Abstract

We analyzed the dietary fish and marine mammal oil supplements purchased from Japanese markets for hexabromocyclododecane (HBCD); 20 brands were analyzed by liquid chromatography–tandem mass spectrometry (LC/MS/MS). HBCD was detected in 15 of 22 samples, and the concentrations of all the HBCD isomers (α, β, and γ) ranged from <0.9 to 67 ng/g lipid weight. α-HBCD was the dominant residue among the HBCD isomers. However, the composition of HBCD isomers varied according to the sample type. We found that 1 sardine oil brand and 2 shark liver oil brands extracted from fish captured in seawater around Japan contained relatively high levels of HBCD, indicating that both the surface and deep seawaters around Japan may have been contaminated with HBCD.
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1. Introduction

Oils of fish and marine mammals are marketed as polyunsaturated fatty acid (PUFA) supplements, particularly long-chain $n-3$ PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It is generally accepted that $n-3$ PUFA consumption suppresses the production of arachidonic acid metabolites such as thromboxane A2 (TXA2) via enzyme competition. Furthermore, prostaglandin I_3, the metabolite of EPA, shows antiplatelet aggregation and vasodilatory activity. Due to these effects, $n-3$ PUFA is widely recognized as an agent that decreases the risk of vascular or allergic diseases. In Japan, these types of fish and marine mammal oil supplements are popular, and various products have entered the market. However, lipophilic organic chemicals can accumulate in fish oils; moreover, polychlorinated biphenyls (PCBs), organochlorinated pesticides, and polybrominated diphenyl ethers (PBDEs) have been previously detected in such oils (Jacobs, Covaci, Gheorghe, & Schepens, 2004). In recent years, the organohalogen compounds mentioned above have been commonly studied with respect to marine oil contamination; on the other hand, limited data is available on hexabromocyclododecane (HBCD), a brominated flame retardant (BFR) – only one analytical result has been reported for HBCD to date (UK Food Standards Agency, 2006). HBCD is the principal BFR in polystyrene foams that are used as thermal insulation in construction materials; to date, 2600 metric tons of this substance has been used in Japan (The Chemical Daily Co., 2005). HBCD was detected in various marine fish and mammals (Covaci et al., 2006). Ueno et al. (2006) reported that the total HBCD (α, β, and γ) concentrations in the pooled muscle of skipjack tuna collected from seawater around Japan ranged from 6.5 to 45 ng/g lipid weight (Ueno et al., 2006). Fish oil is obtained from fishes captured from various parts of the world, and the fact that oil extracted from fish captured from polluted waters may be contaminated with HBCD is a cause of concern. It is suspected that HBCD is a potential endocrine disruptor and affects thyroid hormone receptor-mediated gene expression (Yamada-Okabe, Sakai, Kashima, & Yamada-Okabe, 2005). Due to its

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bioaccumulation potential, HBCD is classified as a Type I monitoring Chemical Substance for ongoing monitoring in the future in Japan (BSEF, 2007). In this study, we investigated the HBCD levels in dietary fish/mammal oil supplements purchased from the Japanese market. To our knowledge, this study represents the first assessment of HBCD levels in fish/mammal oil supplements available in retail outlets in Japan.

2. Materials and methods

2.1. Sample collection

The dietary oil supplements were purchased from six Japanese retailers between January and June 2004. All the oil supplements were in capsule form, except for one brand of bottled cod liver oil (sample nos. 5a and 5b) (Table 1). The capsule shells were removed, and the homogenized oil was regarded as a single batch sample for each product. Sample nos. 5a and 5b, and 7a and 7b belonged to the same brand but were from two different batches (different lot nos.).

2.2. Chemicals

The nonlabeled (α, β, and γ) and $^{13}C_{12}$-labeled (α and γ) HBCD standards were purchased from Wellington Laboratories (Ontario, Canada). HBCD of chemical grade and organic solvents of pesticide analysis grade were used for extraction and cleanup of samples, respectively, and sulfuric acid-impregnated silica gel of dioxin analysis grade was purchased from Wako Pure Chemicals (Osaka, Japan).

2.3. Extraction and clean up

Samples (0.5 g) were spiked with $^{13}C_{12}$-labeled standard mixtures ($^{13}C_{12}$-labeled HBCD, α and γ; 12.5 ng each). Lipid purification was then performed using a gel permeation chromatography (GPC) system equipped with a 515 HPLC pump, 717 plus Autosampler, and Fraction Collector III (Waters, MA, USA). The GPC columns used were CLNpak EV-G AC (guard column, 100 × 20 mm i.d.; Showa Denko, Tokyo, Japan) and CLNpak EV-2000 AC (300 × 20 mm i.d.; Showa Denko). The column was eluted with an acetone–cyclohexane mixture (3:7, v/v) at 40 °C. The flow rate was 5 ml/min; the first 60 ml of the eluate was discarded to remove the bulk of lipids, and the subsequent 25 ml of the eluate was collected. This fraction was evaporated to dryness and redissolved in hexane. The solution was subjected to a cleanup procedure by using a 44% sulfuric acid-impregnated silica gel column (1 g). Hexane (10 ml) was used as an eluent. After adding 20 μl of dimethyl sulfoxide (DMSO), the eluate was evaporated until hexane was completely removed. Finally, 80 μl of methanol was added to the residue for liquid chromatography–tandem mass spectrometry (LC/MS/MS) analysis.

Table 1
Sample information and concentrations of HBCDs and PBDEs (ng/g lipid weight)

Sample no.	Sample (origin of sample)	Recommended dose (g/day)	Concentration of HBCD				Concentration of Σ10PBDEs[a]
			α	β	γ	Σα, β, and γ	
1	Sardine oil (Japan)[b]	1–3	43	1.1	23	67	6.0
2	Lamprey oil	0.6–1.8	4.5	1.8	3.0	9.3	1.8
3	Lamprey oil	1.5–3	2.1	<0.2	<0.5	2.1	7.8
4	Globe fish liver oil	0.75	9.5	<0.2	1.5	11	3.6
5a	Cod liver oil (the North sea)[b]	1–2	3.7	0.4	1.3	5.4	32
5b	Cod liver oil (the North sea)[b]	1–2	5.0	0.3	0.9	6.2	28
6	Cod liver oil (the North sea)[b]	0.4–1	4.0	<0.2	<0.5	4.0	16
7a	Shark liver oil (Japan)[b]	0.75–1.75	22	0.7	21	44	49
7b	Shark liver oil (Japan)[b]	0.75–1.75	24	<0.2	21	45	53
8	Shark liver oil (Japan)[b]	0.75–1.75	25	0.3	19	44	52
9	Shark liver oil (New Zealand)[b]	1.8	<0.2	<0.2	<0.5	n.d.	0.7
10	Shark liver oil (New Zealand)[b]	0.6–1.2	<0.2	<0.2	<0.5	n.d.	0.2
11	Shark liver oil (New Zealand)[b]	1.5	<0.2	<0.2	<0.5	n.d.	0.6
12	Shark liver oil	2.6	<0.2	<0.2	<0.5	n.d.	0.1
13	Shark liver oil	0.5	4.0	1.4	1.9	7.3	15
14	Shark liver oil	0.6–1.5	<0.2	<0.2	<0.5	n.d.	0.3
15	Shark liver oil	0.9–1.2	<0.2	<0.2	<0.5	n.d.	0.1
16	Shark liver oil	1.8–2.7	<0.2	<0.2	<0.5	n.d.	0.5
17	Shark liver oil	2.2–2.8	0.4	<0.2	<0.5	0.4	0.1
18	Seal oil (Canada)	1.5	0.4	<0.2	<0.5	0.4	0.9
19	Seal oil (Canada)	0.9	0.5	<0.2	<0.5	0.5	0.8
20	Sea snake oil	0.9–1.8	2.1	1.3	1.7	5.1	4.7

[a] Σ10PBDEs: #28, #47, #49, #66, #99, #100, #153, #154, #155, and #183 (Akutsu et al., 2006).
[b] No heating step in the refining process.

2.4. Liquid chromatography

The HPLC system consisted of an Acquity binary solvent manager, Acquity sample manager, and Acquity column manager (Waters). The temperature in the sample manager was set at 8 °C. Chromatographic separation of the HBCD isomers was performed on an Acquity UPLC BEH C18 column (100 × 2.1 mm i.d., 1.7 μm, Waters) with an in-line filter (0.2 μm). The column temperature was maintained at 40 °C. Isocratic elution was then performed using 10 mM ammonium acetate in a mixture of acetonitrile, water, and methanol (65:23:12) at a flow rate of 0.2 ml/min, and the HPLC flow was directed into the MS detector between 4 and 10 min. A sample volume of 5 μl was typically used for each analysis.

2.5. Mass spectrometry

Mass spectrometric analyses were performed using a tandem quadrupole mass spectrometer (model Quattro Premier XE, Waters) equipped with an electrospray ionization (ESI) interface. The ESI source and MS/MS parameters were automatically optimized using the MassLynx automatic tuning procedure. Infusion analysis was performed as follows. The flow of standard compounds (5 μg/ml) from a syringe pump at a flow rate of 10 μl/min was mixed with the mobile phase through a T-piece. The temperature and flow rate of the desolvation gas (nitrogen) were maintained at 350 °C and 900 l/h, respectively. The flow rate of the cone gas (nitrogen) was set at 50 l/h. The ion source temperature was set at 120 °C. The instrument was operated in the negative ion mode. Collision-induced dissociation was performed using argon as the collision gas at a pressure of 6.1×10^{-3} mbar. The capillary voltage was 2800 V; the extractor voltage, −2 V; the cone voltage, −16 V; and the collision energy, 9 eV. The selected reaction monitoring (SRM) mode was used for quantitation – m/z 640.5 to 80.6 transition for nonlabeled HBCD and m/z 652.7–80.6 for $^{13}C_{12}$-labeled HBCD. The m/z 640.5–78.9 transition was further verified. MassLynx was used to control the LC/MS/MS system and to acquire and process the data. The HBCD isomers were quantified by the isotope dilution method using the corresponding ^{13}C-labeled congeners (β-HBCD was quantified against $^{13}C_{12}$-labeled α-HBCD).

2.6. Quality assurance and quality control

The recoveries of α-HBCD, β-HBCD, and γ-HBCD from spiked cod liver oil samples ($n = 5$) were 101%, 73%, and 110%, respectively, with a relative standard deviation of 8%, 18%, and 8%, respectively (spiking level: 40 ng/g lipid weight for each sample). The limits of quantification for α-HBCD, β-HBCD, and γ-HBCD were calculated using the lowest calibration level standard (10 ng/ml) and 20, 20, and 40 pg/injection, respectively (signal-to-noise ratio >10).

3. Results and discussion

Fig. 1 shows the representative chromatograms of an HBCD standard mixture and a fish oil sample. The concentrations of HBCD and PBDE are summarized in Table 1. HBCD was detected in 15 of 22 samples. The concentrations of all the HBCD isomers (α, β, and γ) (ΣHBCD) were relatively high in sardine oil (67 ng/g lipid weight) landed in Choshi (90 km to the east of Tokyo) and the shark liver oils (nos. 7a–8) (44–45 ng/g lipid weight). The samples (nos. 7a–8) were obtained from selected bathyal sharks captured from a depth of approximately 600–800 m around the Goto Islands. Goto Islands is approximately 50 km off the west coast of Kyushu, Japan, and is surrounded by Japan, Korea, and China. These results indicate that both the surface and deep seawaters around Japan were contaminated with HBCD. The UK Food Standards Agency (2006) reported that the cod liver oil supplements available in the UK market contained ΣHBCD in the range of 1.5–5.8 ng/g lipid weight; these values are approximately the same as those detected in this study. The liver oils obtained from shark captured in seawater around New Zealand (nos. 9–11), which were also refined through the cold process, did not contain the HBCD residue. This result suggests that seawaters of Japan were more contaminated than those of New Zealand. Ueno et al. (2006) reported that the residue HBCD levels in skipjack tuna collected from certain areas in the northern hemisphere were apparently higher than those from the southern hemisphere; the authors believed that this tendency was due to the difference in the consumption level of HBCD in these regions. In the shark liver oils from Japan (nos. 7a–8), the concentrations of ΣHBCD were approximately the same as those of ΣPBDEs (Akutsu, Konishi, & Tanaka, 2006). Furthermore, in the cod liver oil from the North Sea, ΣPBDEs concentrations were higher than those of ΣHBCD. On the other hand, the concentrations of ΣHBCD were approximately 10 times higher than those of ΣPBDEs in the sardine oil from Japanese seawaters. The Tone river

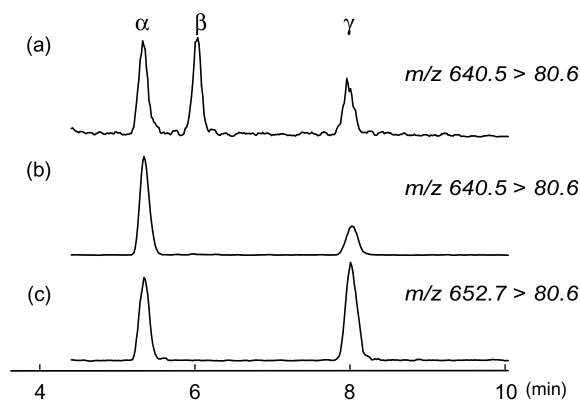

Fig. 1. Representative chromatograms: (a) 50 pg of HBCD standard mixtures, (b) fish oil sample (sample no. 1), and (c) $^{13}C_{12}$-labeled standard mixtures (sample no. 1).

basin houses a textile industry area, and Choshi is situated at the mouth of the Tone river. Thus, the above results suggest the possibility that the HBCD effluent from the textile industrial area pollutes the seawater around Choshi or the upper waters of the river.

In our study, contamination in the samples refined without heat treatment appeared to be relatively high. In the technical mixture, γ-HBCD was the most dominant isomer (>70%) at room temperature, followed by α-HBCD and β-HBCD (Tomy et al., 2004). HBCD undergoes thermal rearrangement at temperatures higher than 160 °C, resulting in a specific mixture of stereoisomers (α-HBCD, 78%), and the decomposition of HBCD starts at around 230 °C (Barontini, Cozzani, & Petarca, 2001). Fish oils are usually refined in a stepwise manner via neutralization (removal of free fatty acids, pigments, and metal traces), bleaching (removal of pigments and oxidation products), and deodorization (removal of volatile compounds) (Hilbert, Lillemark, Balchen, & Hojskov, 1998). In the deodorization process, the oil was heated at a high temperature (around 180 °C) after degassing under vacuum. For thermal decomposition of HBCD, the deodorization temperature is assumed to influence the amount of HBCD residue in oil. In marine fish/mammals, α-HBCD is reported to be the dominant residue among the HBCD isomers (Covaci et al., 2006). Our study results were in agreement with this observation. Among the HBCD isomers, α-HBCD appeared to have a relatively longer environmental and biological half-life than β-HBCD and γ-HBCD. The degradation rates of α-HBCD in freshwater aquatic sediments were slower than those of β-HBCD and γ-HBCD (Davis et al., 2006). Additionally, a study using rat hepatic microsomes showed that the biotransformation rate of α-HBCD was slower than those of the other isomers (Zegers et al., 2005). Ueno et al. (2006) reported the possibility of high atmospheric transportability and bioaccumulation potential of α-HBCD. These studies suggested that α-HBCD is the dominant isomer in top predators. In our study, the composition of HBCD isomers varied according to the sample type. In the sardine oil (no. 1), lamprey oil (no. 2), shark liver oil (nos. 7a–8), and sea snake oil (no. 20), the concentration of γ-HBCD was approximately the same as that of α-HBCD, whereas in other samples, the concentration of γ-HBCD was less than half of that of α-HBCD. The variation in the composition and the amount of HBCD isomers among the samples may partially be due to the difference in the refinement process, the frequency of exposure, the distance from the pollution source, and the difference in the metabolizing ability between species. Additionally, there is a possibility of contamination from construction materials or purification equipment during the refinement process of oils.

When the daily intake of ΣHBCD was estimated according to the daily doses proposed by the product manufacturers, the maximum intake in the present study (200 ng/day) was higher than the reported median intake of HBCD (141 ng/day) (Covaci et al., 2006). Our data indicate that the frequent consumption of oil supplements will increase dietary exposure to HBCD.

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References

Akutsu, K., Konishi, Y., & Tanaka, Y. (2006). Analysis of organohalogen pollutants in fish oil supplements and drugs. *Bulletin of Osaka Prefectural Institute of Public Health, 44*, 47–53.

Barontini, F., Cozzani, V., & Petarca, L. (2001). Thermal stability and decomposition products of HBCD. *Industrial and Engineering Chemistry Research, 40*, 3270–3280.

BSEF (2007). <http://www.bsef.com/> Accessed April 25, 2007.

Covaci, A., Gerecke, A. C., Law, R. J., Voorspoels, S., Kohler, M., Heeb, N. V., et al. (2006). Hexabromocyclododecanes (HBCDs) in the environment and humans: A review. *Environmental Science and Technology, 40*, 3679–3688.

Davis, J. W., Gonsior, S. J., Markham, D. A., Friederich, U., Hunziker, R. W., & Ariano, J. M. (2006). Biodegradation and product identification of [14C]hexabromocyclododecane in wastewater sludge and freshwater aquatic sediment. *Environmental Science and Technology, 40*, 5395–5401.

Hilbert, G., Lillemark, L., Balchen, S., & Hojskov, C. S. (1998). Reduction of organochlorine contaminants from fish oil during refining. *Chemosphere, 37*, 1241–1252.

Jacobs, M. N., Covaci, A., Gheorghe, A., & Schepens, P. (2004). Time trend investigation of PCBs, PBDEs, and organochlorine pesticides in selected $n-3$ polyunsaturated fatty acid rich dietary fish oil and vegetable oil supplements; nutritional relevance for human essential $n-3$ fatty acid requirements. *Journal of Agricultural and Food Chemistry, 52*, 1780–1788.

Tomy, G. T., Budakowski, W., Halldorson, T., Whittle, D. M., Keir, M. J., Marvin, C., et al. (2004). Biomagnification of alpha- and gamma-hexabromocyclododecane isomers in a Lake Ontario food web. *Environmental Science and Technology, 38*, 2298–2303.

Ueno, D., Alaee, M., Marvin, C., Muir, D. C., Macinnis, G., Reiner, E., et al. (2006). Distribution and transportability of hexabromocyclododecane (HBCD) in the Asia-Pacific region using skipjack tuna as a bioindicator. *Environmental Pollution, 144*, 238–247.

UK Food Standards Agency (2006). Brominated chemicals in farmed & wild fish & shellfish and fish oil dietary supplements. <http://www.food.gov.uk/multimedia/pdfs/fsis0406.pdf> Accessed April 25, 2007.

Yamada-Okabe, T., Sakai, H., Kashima, Y., & Yamada-Okabe, H. (2005). Modulation at a cellular level of the thyroid hormone receptor-mediated gene expression by 1,2,5,6,9,10-hexabromocyclododecane (HBCD), 4,4′-diiodobiphenyl (DIB), and nitrofen (NIP). *Toxicology Letters, 155*, 127–133.

Zegers, B. N., Mets, A., Van Bommel, R., Minkenberg, C., Hamers, T., Kamstra, J. H., et al. (2005). Levels of hexabromocyclododecane in harbor porpoises and common dolphins from western European seas, with evidence for stereoisomer-specific biotransformation by cytochrome p450. *Environmental Science and Technology, 39*, 2095–2100.