HEXABROMOCYCLODODECANE IN FISH OIL SUPPLEMENTS

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Abstract

Hexabromocyclododecane (HBCD) residue in the supplement of fish oil and sea mammal oil purchased in Japan market was determined. Twenty brands of dietary supplements were analysed using liquid chromatography-tandem mass spectrometry (LC/MS/MS). The concentrations of the total HBCD isomers (α, β, and γ) ranged from <0.9 to 67 ng/g lipid weight. We found that 1 sardine oil brand and 3 shark liver oil brands extracted from fish caught in sea water around Japan contained relatively high levels of HBCD, indicating that HBCD may have contaminated both the surface and deep-sea waters around Japan.

Introduction

Oils of fish and sea mammals are sold 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 A_2 via enzyme competition. Furthermore, the metabolite of EPA such as prostaglandin I_3 shows antiplatelet aggregation and vasodilatory activity. Because of these effects, n–3 PUFA is widely recognized as an agent that decreases the risk of vascular or allergic diseases. In Japan, such fish and sea mammal oil supplements have become popular, and various products have entered the market. However, lipophilic organic chemicals can accumulate in fish oils, and polychlorinated biphenyls (PCBs), organochlorinated pesticides, and polybrominated diphenyl ethers (PBDEs) have been previously detected from such oils.[1] Recently, the organohalogen compounds mentioned above have commonly been studied with respect to marine oil contamination, whereas data on HBCD, a brominated flame retardant (BFR), are very limited—only 1 analytical result has been reported for HBCD to date.[2] HBCD is the principal BFR in polystyrene foams used as thermal insulation in building materials; 2,600 metric tons of this substance was used in Japan (The Chemical Daily Co., 2005). HBCD was detected in various marine fish and marine mammals.[3] Ueno et al. (2006) reported that the total HBCD (α, β, and γ) concentrations in the pooled muscle of skipjack tuna collected from sea water around Japan ranged from 6.5–45 ng/g lipid weight.[4] Fish oil is obtained from fishes caught from various area of the world, and the fact that oil extracted from fish caught 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.[5] Due to its bioaccumulation potential, HBCD is included as a Type I monitoring Chemical Substance for ongoing monitoring in the future in Japan.[6] 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 oils available in retail outlets in Japan.

Materials and Methods

Sample collection

The dietary oil supplements were purchased from 6 Japanese retailers between January and June 2004. All the oil supplements were in capsule form except for 1 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. The sample Nos. 5a and 5b and 7a and 7b belonged to the same brand but were from 2 different batches (different lot Nos.).

Chemicals

The nonlabeled (α, β, and γ) and ^{13}C-labeled (α and γ) HBCD standards were purchased from Wellington Laboratories (Ontario, Canada). HBCD of chemical grade and organic solvents of pesticide analysis grade used

for extraction and cleanup of samples, respectively, and sulfuric acid-impregnated silica gel of dioxin analysis grade were purchased from Wako Pure Chemicals (Osaka, Japan).

Sample extraction and cleanup

Samples (0.5 g) were spiked with ^{13}C-labeled standard mixtures (^{13}C-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 column used was CLNpakEV-G AC (guard column, 100 × 20 mm I.D.) and CLNpakEV-2000 AC (300 × 20 mm I.D.). 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 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.

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. Retention times for the α-HBCD, β-HBCD, and γ-HBCD isomers were 5.2, 5.8, and 7.7 min, respectively. A sample volume of 5 μl was typically used for each analysis.

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 and the extractor voltage, –2 V, while the cone voltage was –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 to 80.6 for ^{13}C-labeled HBCD. The m/z 640.5 to 78.9 transition was verified further. 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-labeled α-HBCD).

Results and Discussion

The concentrations of HBCD and PBDE are summarized in Table 1. HBCD was detected in 15 out of 22 samples. The concentrations of the total HBCD isomers (α, β, and γ) (ΣHBCD) were relatively high in the oil of sardine (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 (approximately 50 km off the west coast of Kyushu, Japan). These results indicate that HBCD contaminated both the surface and deep-sea waters around Japan. The liver oils obtained from shark caught in sea water around New Zealand (Nos. 9–11), which were also refined through cold process, did not contain the HBCD residue. This result suggests that the sea waters in Japan were

contaminated to a greater extent than those in New Zealand. Ueno et al. (2006) reported that residue HBCD levels in skipjack tuna collected from certain areas in the northern hemisphere were apparently higher than those from the southern hemisphere, and they believed that this tendency was noted due to the difference in the consumption level of HBCD in these regions. The concentrations of ΣHBCD were approximately 10 times higher than those of ΣPBDEs in the sardine oil from the Japanese sea waters.[7] On the other hand, the concentrations of ΣPBDEs were higher than those of ΣHBCD in cod liver oil from the North Sea. In the shark liver oils from Japan (Nos. 7a–8), the concentrations of ΣHBCD were approximately the same as those of ΣPBDEs.

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 (>70%) followed by α-HBCD and β-HBCD at room temperature.[8] HBCD is subject to thermal rearrangement at temperatures higher than 160 °C, resulting in a specific mixture of stereoisomers (α-HBCD 78%), and decomposition of HBCD begins around 230 °C.[9] 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).[10] 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 supposed to influence the amount of HBCD residue in oil. In marine fish/mammals, it is reported that α-HBCD is the dominant residue among the HBCD isomers.[3] 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.[11] Additionally, a study using rat hepatic microsomes showed that the biotransformation rate of α-HBCD was slower than that of the other isomers.[12] 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 the HBCD isomers varied according to the sample type. In the sardine oil (No. 1), lamprey oil (No. 2), shark liver oil (No. 7a–8), and sea snake oil (No. 20), the concentration of γ-HBCD was approximately the same as that of α-HBCD, whereas in the other samples, the concentration of γ-HBCD was less than half that of α-HBCD. The variety in the composition of the HBCD isomers among the samples was supposed to be partially due to the difference in the refinement process, the frequency of exposure, and species.

Table 1. Sample information and concentrations of HBCD

sample no.	sample type (sample site)	recommended dose (g/day)	conc. HBCD (ng/g lipid weight) α	β	γ	Σα,β, and γ	conc. Σ10PBDEs (ng/g lipid weight)[c]
1	sardine oil (Japan)*	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)*	1–2	3.7	0.4	1.3	5.4	32
5b	cod liver oil (the North sea)*	1–2	5.0	0.3	0.9	6.2	28
6	cod liver oil (the North sea)*	0.4–1	4.0	<0.2	<0.5	4.0	16
7a	shark liver oil (Japan)*	0.75–1.75	22	0.7	21	44	49
7b	shark liver oil (Japan)*	0.75–1.75	24	<0.2	21	45	53
8	shark liver oil (Japan)*	0.75–1.75	25	0.3	19	44	52
9	shark liver oil (New Zealand)*	1.8	<0.2	<0.2	<0.5	<0.9	0.7
10	shark liver oil (New Zealand)*	0.6–1.2	<0.2	<0.2	<0.5	<0.9	0.2
11	shark liver oil (New Zealand)*	1.5	<0.2	<0.2	<0.5	<0.9	0.6
12	shark liver oil	2.6	<0.2	<0.2	<0.5	<0.9	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	<0.9	0.3
15	shark liver oil	0.9–1.2	<0.2	<0.2	<0.5	<0.9	0.1
16	shark liver oil	1.8–2.7	<0.2	<0.2	<0.5	<0.9	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

[c]Σ10PBDEs: #28, #37, #75, #47, #66, #77, #100, #99, #85, #154, #153, #138, and #183 (Akutsu et al., 2006)
*no heating step in the refining process

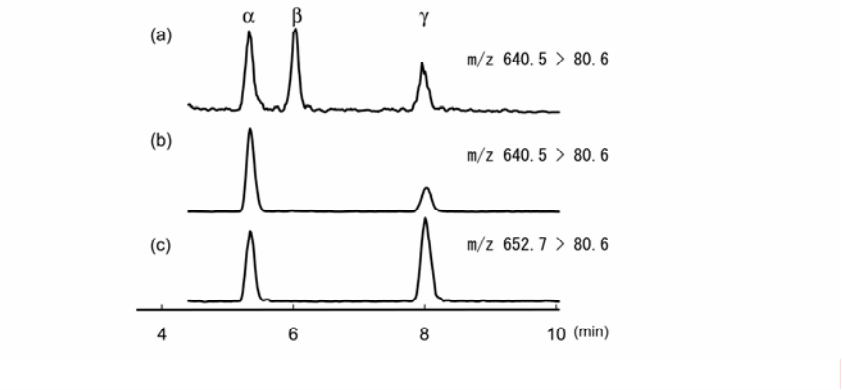

Fig. 1. Representative chromatograms. (a) 50 pg of HBCD standard mixtures, (b) fish oil sample (sample No.1), and (c) ^{13}C-labeled standard mixtures (sample No.1)

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