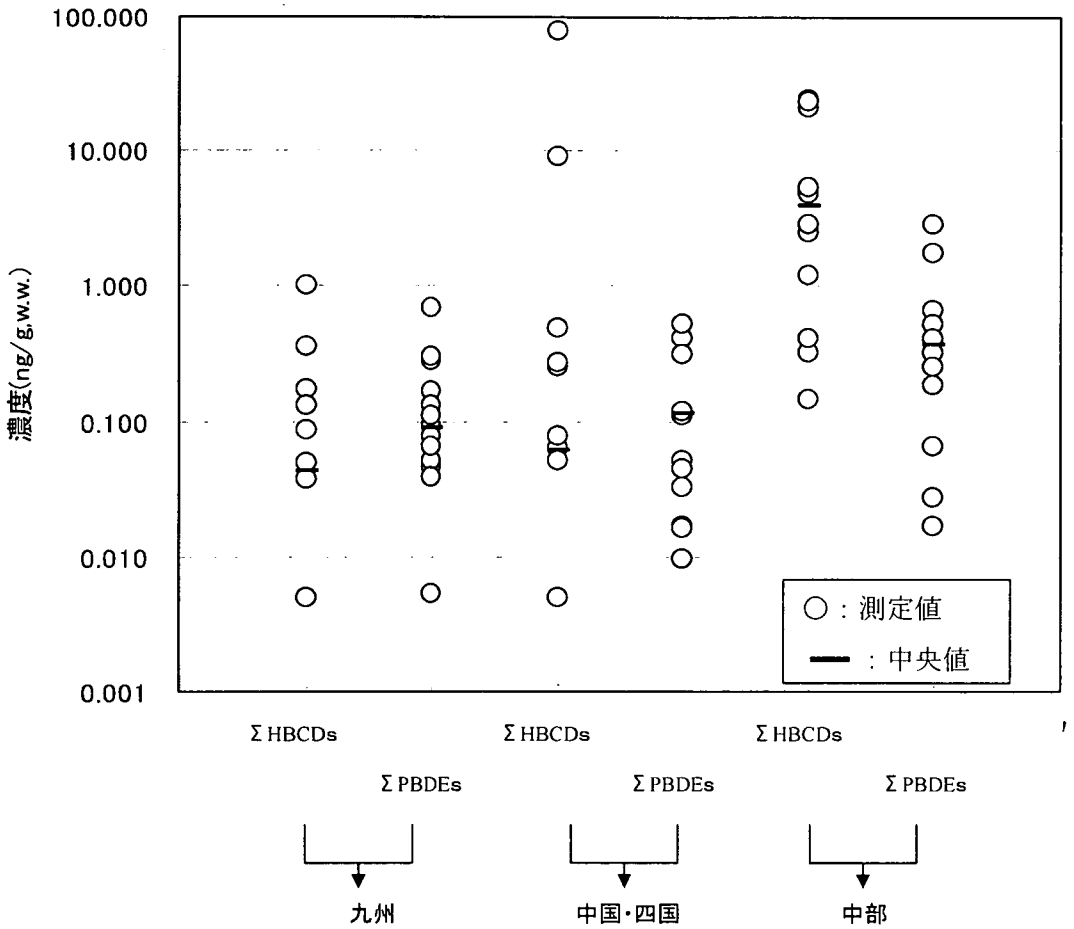


図 16　3 地域の魚介食品中のΣHBCD s 及びΣPBDE s 濃度

# Ⅲ. 研究成果の刊行に関する一覧表

## 研究成果の刊行に関する一覧表

雑誌

| | 発表者氏名 | 論文タイトル | 発表誌名 | 巻号 | ページ | 出版年 |
|---|---|---|---|---|---|---|
| 1 | Hori T, Yasutake D, Tobiishi K, Ashizuka Y, Kajiwara J, Nakagawa R, Iida T, Tsutsumi T, Sasaki K | Comparison of accelerated solvent extraction and alkaline digestion-hexane shaking extraction for determination of dioxins in animal-origin food sample | Organohalogen Compounds | 69 | 1118-1121 | 2007 |
| 2 | Murata S, Nakagawa R, Ashizuka Y,Hori T, Yasutake D, Tobiishi K, Sasaki K | Brominated flame retardants (HBCD,TBBPA and ΣPBDES) in market basket food samples of northern Kyushu district in Japan | Organohalogen Compounds | 69 | 1985-1988 | 2007 |
| 3 | Tsutsumi T, Amakura Y, Tanno K, Yanagi T, Kono Y, Sasaki K, Maitani T | Dioxins and other organohalogen compounds in fish oil supplements on the Japanese market | Organohalogen Compounds | 69 | 2371-2374 | 2007 |
| 4 | Ashizuka Y, Nakagawa R, Murata S, Yasutake D, Hori T, Horie M, Nishioka C, Takahashi T, Tamura I, Teshirogi T, Sasaki K | Daily intake of brominated dioxins and polybrominated diphenyl ethers estimated by market basket study | Organohalogen Compounds | 69 | 2769-2772 | 2007 |
| 5 | Ashizuka Y, Nakagawa R, Hori T, Yasutake D, Tobiishi K, Sasaki K | Determination of brominated flame retardants and brominated dioxins in fish collected from three regions of Japan | Mol. Nutr. Food Res. | 52 | 273-283 | 2008 |

# COMPARISON OF ACCELERATED SOLVENT EXTRACTION AND ALKALINE DIGESTION-HEXANE SHAKING EXTRACTION FOR DETERMINATION OF DIOXINS IN ANIMAL-ORIGIN FOOD SAMPLE

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**Abstract**
We studied the progressive analytical method for dioxins in animal-origin food samples such as fish, meat and dairy products. This study aimed to establish a highly sensitive and rapid analytical method using HRGC/HRMS equipped with a solvent cut large volume (SCLV) injection system and accelerated solvent extraction (ASE). When ASE was applied to extract fat from dried milk powder, high fat amounts were obtained in the case where the temperature was set to 150 °C and acetone/n-hexane (1:1, v/v) was used as the extraction solvent. A high extraction efficiency in these conditions was also found in quantitative results for 29 kinds of dioxin congeners on the identical sample. Using these conditions, a freeze-dried *tuna* homogenate was extracted by ASE and we performed a standard alkaline digestion followed by a n-hexane shaking extraction on the identical sample. The concentrations of each dioxin congener were very similar in both extraction methods. Our analysis of 20 g of various animal-origin food items according to the present method, including the ASE and SCLV injection technique, showed recovery rates for labeled congeners within the range recommended by the Japanese analytical guideline of dioxins in food (40%-120%).

**Introduction**
We previously developed a highly sensitive method for determining dioxin content in food using a solvent cut large volume (SCLV) injection system coupled to a cyanopropyl phase capillary column[1]. The SCLV injection system coupled to a 40m-length Rtx-2330 column showed sufficient separation of 2,3,7,8-chlorine-substituted isomers and had at least five-times higher sensitivity than the conventional injection technique[2]. In the conventional method, a large volume of sample (generally 100g) must be treated collectively in order to attain the desirable limit of detection (LODs) at low ppt levels, namely, 0.01pg/g for 2,3,7,8-tetraCDD/F. The SCLV injection technique method allows the reduction of a sample volume from 100g to 20g when such usual LODs are demanded and is expected to improve the efficiency of laboratory performance, especially when it is coupled to an automated extraction method such as accelerated solvent extraction (ASE). In order to examine the applicability of ASE for the determination of dioxins in food samples, it is important to verify the extraction efficiency of this method against that of the conventional technique.
We reported the applicability of an ASE for the determination of dioxins in plant food samples and compared the method's performance with that of the standard conventional shaking extraction (separatory funnel extraction) regarding recovery rates and quantitative determination[3]. The results showed that ASE could extract dioxins at high efficiency using a low-volume solvent and could provide a high level of performance for various plant matrices, especially regarding those, such as seaweed powder, from which dioxins are difficult to extract using conventional shaking extraction.
In the present study, the applicability of the combined SCLV injection and ASE methodology is evaluated for use regarding animal-origin fatty food samples. It is considered that homogeneous tissue, such as dried milk powder, is suitable for the method's quantitative validation.

**Materials and Methods**
Dried milk powder on the market was used for the examination of extraction conditions. For the comparison of quantitative determinations, about 300 g of the edible parts of *tuna* were purchased at a market in Japan. They were homogenized using a food processor, freeze dried and homogenized again. For the examination of the recovery rate, extracts were prepared from homogenates of animal-origin food samples (cow's milk, cheese, yogurt, and so on). The recovery rates for 17 kinds of $^{13}$C-labeled 2,3,7,8-substituted PCDD/Fs and 12 kinds of $^{13}$C-labeled dioxin-like PCBs were evaluated.

The analytical procedures used in this study are summarized in Table 1. In Method 1, the conventional standard method, the sample was treated with 100 ml of 1 N potassium hydroxide/ethanol for two hours with stirring at room temperature. The alkaline hydrolyzate was extracted twice with 100 ml of n-hexane using a separatory funnel for one hour each time, and then the concentrated extract was treated with 15 ml of concentrated sulfuric acid. By contrast, in Method 2, automated extraction was performed using an ASE-300 (Dionex, CA) under conditions of 1500 psi. Four individual experiments and four simultaneous blank tests were performed for each extraction method.

Dioxins were analyzed using a model 6890 gas chromatograph (Agilent Technologies, CA) coupled to a model Autospec-Ultima mass spectrometer (Micromass, UK). We employed an Rtx-2330 (0.18mm x 40m) capillary column (Restek, PA) on an SCLV injection system (SGE, Australia) in order to determine tetra- and pentaCDD/Fs, and hexaCDFs. The details of the operating conditions for the SCLV injection system are described in another paper[2]. The LOD for each congener was determined according to the provisional guidelines for analysis of dioxins in foods issued by the Ministry of Health and Welfare of Japan in 1999 ("*Guideline*"): An absolute quantity corresponding to S/N = 3 was evaluated on HRGC/HRMS chromatograms using verification standards.

Table 1 Analytical procedures for determination of dioxins in food.

| | | Method 1 | Method 2 |
|---|---|---|---|
| Extraction | | Alkaline digestion (KOH/ethanol) followed by shaking extraction*<br>Sample size: 20g<br>Time: 60 min x 2 (120 min)<br>Solvent: n-hexane<br>200 ml (100ml x 2) | Accelerated solvent extraction (ASE)<br>Sample size: 20 g<br>Time: 25 min<br>Solvent: acetone/n-hexane (1:1, v/v) 120 ml |
| Cleanup | | Sulfuric acid treatment<br>↓<br>Multi-layer silica gel column<br>↓<br>Active carbon-dispersed silica gel column | |
| HRGC/ HRMS analysis | PCDD/DFs and non-*ortho* PCBs | SCLV injection<br>Injection volume: 4 μL / 20μL<br>Pre-column:BPX-5 (0.25mm x 5m)<br>Analytical columns: a) Rtx-2330 (0.18mm x 40m)<br>b) BPX-5 (0.15mm x 30m) | |
| | Mono-*ortho* PCBs | Splitless injection<br>Injection volume: 1μL/20 μL<br>Analytical column: HT8-PCB (0.25mm x 60m) | |

* Method recommended for fat, fish and shellfish, meats, eggs, milk and dairy products in "*Guideline*".

**Results and Discussion**

Twenty grams of milk powder were extracted by ASE. After the extracts were evaporated and dried, fat contents were measured gravimetrically. Three individual experiments were performed for each extraction condition shown in Table 2. As a result, the largest fat content was obtained under the condition of 150 °C, acetone/n-hexane (1:1, v/v). This highest value agreed with that obtained from the standard fat extraction method by shaking its reconstituted aqueous solution with diethyl ether/petroleum ether (1:1, v/v). Data regarding the quantification of dioxin congeners in milk powder (pg/g whole weight basis) are shown in Table 3. Trace data showing the concentrations of the congeners under detection limits were re-evaluated and are shown in parentheses to compare concentrations between the methods. Generally, high concentrations and a large number of detected congeners were found under the condition of 150 °C, acetone/n-hexane (1:1, v/v), compared to other conditions. By contrast, there were no obvious differences among the computed data showing 29 kinds of labeled compound recoveries in each extraction condition (data not shown), all of which were adapted to the range recommended in the "*Guideline*" (40%-120%). The above results suggested that differences in quantification values between extraction conditions were due to differences in the extraction efficiency of dioxin molecules from the tissue. Hence, validation tests comparing ASE to the conventional method were carried out using the condition of "150 °C, acetone/n-hexane," which demonstrated the high extraction efficiency of the compounds and the fat content's similarity to the standard fat extraction method.

Table 2 Fat contents (%) of milk powder under various extraction conditions

| | Temperature (°C) | Solvent | Trial | Milk powder weighed (g) | Fat obtained (g) | Fat contents (%) |
|---|---|---|---|---|---|---|
| ASE | 100 | n-hexane | 1st | 20.01 | 0.20 | 1.0 |
| | | | 2nd | 20.34 | 0.31 | 1.5 |
| | | | 3rd | 20.00 | 0.30 | 1.5 |
| | | acetone/n-hexane (1:1) | 1st | 20.21 | 0.69 | 3.4 |
| | | | 2nd | 20.08 | 0.93 | 4.7 |
| | | | 3rd | 20.01 | 1.12 | 5.6 |
| | 150 | n-hexane | 1st | 20.16 | 3.02 | 15.0 |
| | | | 2nd | 20.33 | 3.22 | 15.8 |
| | | | 3rd | 20.27 | 3.13 | 15.5 |
| | | acetone/n-hexane (1:1) | 1st | 20.24 | 5.30 | 26.2 |
| | | | 2nd | 20.27 | 5.24 | 25.8 |
| | | | 3rd | 20.26 | 5.30 | 26.1 |
| Shaking extraction | | diethyl ether/petroleum ether (1:1) | 1st | 5.06 | 1.24 | 24.4 |
| | | | 2nd | 4.96 | 1.19 | 23.9 |
| | | | 3rd | 4.96 | 1.16 | 23.3 |

Table 3 Concentrations of dioxins (pg/g whole weight basis) in dried milk powder, comparison of temparature and solvent used.

| Temperature (°C) | | 100 | | | | | | 150 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Solvent | | n-hexane | | | acetone/n-hexane | | | n-hexane | | | acetone/n-hexane | | |
| Congener | LOD (pg/g) | 1st | 2nd | 3rd | 1st | 2nd | 3rd | 1st | 2nd | 3rd | 1st | 2nd | 3rd |
| 2,3,7,8-TeCDD | 0.01 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 1,2,3,7,8-PeCDD | 0.01 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 1,2,3,4,7,8-HxCDD | 0.02 | nd | nd | nd | nd | nd | nd | nd | nd | 0.021 | nd | 0.020 | (0.011) |
| 1,2,3,6,7,8-HxCDD | 0.02 | nd | nd | nd | nd | nd | nd | 0.026 | 0.034 | 0.035 | 0.046 | 0.035 | 0.026 |
| 1,2,3,7,8,9-HxCDD | 0.02 | nd | nd | nd | nd | nd | nd | nd | nd | 0.013 | (0.018) | (0.018) | 0.020 |
| 1,2,3,4,6,7,8-HpCDD | 0.02 | nd | nd | nd | (0.064) | (0.061) | 0.13 | 0.25 | 0.19 | 0.21 | 0.34 | 0.38 | 0.31 |
| OCDD | 0.05 | 0.19 | 0.25 | 0.22 | 0.51 | 0.62 | 0.99 | 2.1 | 2.2 | 2.2 | 3.3 | 3.6 | 2.9 |
| 2,3,7,8-TeCDF | 0.01 | nd | nd | nd | 0.019 | 0.016 | 0.017 | 0.055 | 0.055 | 0.049 | 0.082 | 0.080 | 0.067 |
| 1,2,3,7,8-PeCDF | 0.01 | nd | nd | nd | nd | nd | nd | 0.037 | 0.021 | 0.030 | nd | nd | nd |
| 2,3,4,7,8-PeCDF | 0.01 | nd | nd | nd | nd | nd | nd | 0.043 | 0.035 | 0.036 | nd | nd | 0.024 |
| 1,2,3,4,7,8-HxCDF | 0.02 | nd | nd | nd | nd | (0.0091) | (0.019) | 0.031 | 0.026 | 0.029 | 0.051 | 0.064 | 0.036 |
| 1,2,3,6,7,8-HxCDF | 0.02 | nd | nd | nd | nd | nd | (0.012) | 0.021 | nd | (0.012) | 0.029 | 0.030 | 0.027 |
| 1,2,3,7,8,9-HxCDF | 0.02 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.014 |
| 2,3,4,6,7,8-HxCDF | 0.02 | nd | nd | nd | nd | nd | (0.0080) | 0.022 | (0.013) | (0.016) | 0.028 | 0.032 | 0.020 |
| 1,2,3,4,6,7,8-HpCDF | 0.02 | nd | nd | nd | 0.024 | 0.020 | (0.019) | 0.056 | 0.054 | 0.085 | 0.10 | 0.10 | 0.11 |
| 1,2,3,4,7,8,9-HpCDF | 0.02 | nd | nd | nd | nd | nd | (0.010) | nd | nd | nd | nd | nd | nd |
| OCDF | 0.05 | nd | nd | nd | (0.024) | (0.014) | 0.056 | 0.14 | 0.15 | 0.16 | 0.25 | 0.19 | 0.20 |
| 3,3',4,4'-TeCB(#77) | 0.1 | (0.075) | (0.096) | (0.087) | (0.099) | (0.064) | 0.10 | 0.13 | 0.11 | 0.15 | 0.14 | 0.15 | 0.22 |
| 3,4,4',5-TeCB(#81) | 0.1 | nd | nd | nd | (0.015) | nd | nd | (0.012) | nd | (0.011) | (0.013) | nd | (0.019) |
| 3,3',4,4',5-PeCB(#126) | 0.1 | nd | nd | nd | (0.031) | (0.022) | nd | (0.082) | (0.099) | 0.11 | 0.13 | 0.11 | 0.11 |
| 3,3',4,4',5,5'-HxCB(#169) | 0.1 | nd | nd | nd | nd | (0.0083) | nd | (0.057) | (0.048) | (0.052) | (0.061) | (0.074) | (0.063) |
| 2,3,3',4,4'-PeCB(#105) | 1 | (0.23) | (0.31) | (0.32) | (0.53) | (0.55) | (0.88) | 1.7 | 1.6 | 1.8 | 2.1 | 2.1 | 2.2 |
| 2,3,4,4',5-PeCB(#114) | 1 | (0.028) | (0.029) | (0.031) | (0.046) | (0.044) | (0.075) | (0.18) | (0.18) | (0.18) | (0.22) | (0.26) | (0.23) |
| 2,3',4,4',5-PeCB(#118) | 1 | (0.81) | 1.1 | 1.2 | 1.8 | 2.0 | 3.1 | 6.9 | 6.5 | 7.1 | 8.9 | 9.9 | 9.3 |
| 2',3,4,4',5-PeCB(#123) | 1 | (0.019) | (0.032) | (0.025) | (0.042) | (0.039) | (0.066) | (0.12) | (0.087) | (0.092) | (0.13) | (0.13) | (0.17) |
| 2,3,3',4,4',5-HxCB(#156) | 1 | (0.12) | (0.11) | (0.16) | (0.33) | (0.38) | (0.56) | 1.4 | 1.4 | 1.4 | 2.0 | 2.2 | 1.9 |
| 2,3,3',4,4',5'-HxCB(#157) | 1 | (0.033) | (0.045) | (0.036) | (0.095) | (0.11) | (0.16) | (0.39) | (0.36) | (0.40) | (0.50) | (0.51) | (0.47) |
| 2,3',4,4',5,5'-HxCB(#167) | 1 | (0.054) | (0.070) | (0.064) | (0.12) | (0.14) | (0.21) | (0.50) | (0.48) | (0.47) | (0.72) | (0.75) | (0.73) |
| 2,3,3',4,4',5,5'-HpCB(#189) | 1 | (0.022) | (0.030) | (0.027) | (0.050) | (0.056) | (0.11) | (0.22) | (0.20) | (0.20) | (0.26) | (0.37) | (0.28) |

Table 4 shows the dioxin concentrations and RSD values obtained from the two extraction methods using freeze-dried *tuna* homogenates. RSD values in ASE ranged from 4% to 19%, similar to the results in alkaline digestion (1% to 27%). The concentrations of 29 kinds of dioxin congeners were close for both extraction methods other than OCDD; the ratios of estimated concentrations from ASE compared to those from the alkaline digestion-hexane shaking extraction method ranged from 0.96 to 1.4, except 2.0 for OCDD. It is considered that this result was due to ASE's high extraction efficiency compared with the shaking extraction; a tendency like this was observed in our previous examination using dried seaweed powder, in which the extraction efficiency of ASE was found to be superior to that of conventional separatory funnel extraction [3].

A recovery test in the present method, including the ASE and SCLV injection technique, was performed using 18 food items, mainly dairy products. The results showed that recovery rates for 29 kinds of labeled congeners ranged from 41% to 108 %, within the range recommended by the Japanese analytical guideline for dioxins in food (40%-120%). Our results suggest that the present method is available for rapid and sensitive determination

of dioxins in animal-origin fatty food samples of low sample size and requiring only a small volume of extraction solvent compared to the conventional extraction method. The ASE condition suited for dioxins in the animal-origin sample presented here is identical to that proposed for plant food samples[3]. Therefore, independent extraction conditions could be available for both animal- and plant-origin food samples. Moreover, fat content values obtained from the present extraction method of dioxins could be directly applied to the calculation of fat weight-based concentrations. The applicability of the combined SCLV injection and ASE methodology has been continuously verified for use regarding food mixture samples, e.g., total diet study samples.

Table 4 Concentrations of dioxins (pg/g whole weight basis) in dried *tuna* homogenates; conparison between ASE and alkaline digestion followed by hexane shaking.

| Congener | ASE ($n$=4) | | | | Alkaline digestion-hexane shaking ($n$=4) | | | | a / b |
|---|---|---|---|---|---|---|---|---|---|
| | Range | | Mean[a] | RSD(%) | Range | | Mean[b] | RSD(%) | |
| 2,3,7,8-TeCDD | 0.61 - | 0.67 | 0.64 | 4 | 0.60 - | 0.72 | 0.67 | 7 | 0.96 |
| 1,2,3,7,8-PeCDD | 0.75 - | 0.83 | 0.80 | 5 | 0.76 - | 0.80 | 0.77 | 3 | 1.0 |
| 1,2,3,4,7,8-HxCDD | 0.023 - | 0.035 | 0.028 | 19 | 0.020 - | 0.030 | 0.024 | 16 | 1.2 |
| 1,2,3,6,7,8-HxCDD | 0.20 - | 0.22 | 0.21 | 5 | 0.20 - | 0.22 | 0.21 | 4 | 0.99 |
| 1,2,3,7,8,9-HxCDD | 0.026 - | 0.037 | 0.032 | 17 | 0.022 - | 0.028 | 0.025 | 12 | 1.2 |
| 1,2,3,4,6,7,8-HpCDD | 0.058 - | 0.067 | 0.065 | 7 | 0.055 - | 0.058 | 0.057 | 2 | 1.1 |
| OCDD | 0.15 - | 0.17 | 0.16 | 6 | 0.070 - | 0.094 | 0.081 | 13 | 2.0 |
| 2,3,7,8-TeCDF | 4.4 - | 5.5 | 5.0 | 9 | 4.8 - | 5.3 | 5.1 | 5 | 0.98 |
| 1,2,3,7,8-PeCDF | 0.92 - | 1.1 | 0.99 | 6 | 0.94 - | 1.0 | 0.96 | 3 | 1.0 |
| 2,3,4,7,8-PeCDF | 2.7 - | 3.1 | 2.9 | 5 | 2.7 - | 2.8 | 2.7 | 2 | 1.1 |
| 1,2,3,4,7,8-HxCDF | 0.15 - | 0.21 | 0.19 | 14 | 0.15 - | 0.25 | 0.18 | 26 | 1.1 |
| 1,2,3,6,7,8-HxCDF | 0.14 - | 0.21 | 0.18 | 19 | 0.17 - | 0.21 | 0.18 | 11 | 1.0 |
| 1,2,3,7,8,9-HxCDF | 0.11 - | 0.15 | 0.13 | 14 | 0.12 - | 0.14 | 0.13 | 7 | 1.0 |
| 2,3,4,6,7,8-HxCDF | nd | nd | - | - | nd | nd | - | - | - |
| 1,2,3,4,6,7,8-HpCDF | 0.067 - | 0.079 | 0.072 | 7 | 0.050 - | 0.063 | 0.055 | 11 | 1.3 |
| 1,2,3,4,7,8,9-HpCDF | nd | nd | - | - | nd | nd | - | - | - |
| OCDF | nd | nd | - | - | nd | nd | - | - | - |
| 3,3',4,4'-TeCB(#77) | 220 - | 270 | 240 | 9 | 260 - | 280 | 270 | 3 | 0.91 |
| 3,4,4',5-TeCB(#81) | 17 - | 21 | 19 | 9 | 20 - | 21 | 20 | 1 | 0.94 |
| 3,3',4,4',5-PenCB(#126) | 210 - | 250 | 230 | 6 | 230 - | 240 | 230 | 1 | 0.99 |
| 33'44'55'-HxCB(#169) | 26 - | 29 | 27 | 5 | 28 - | 28 | 28 | 1 | 0.97 |
| 233'44'-PeCB(#105) | 60000 - | 72000 | 64000 | 9 | 50000 - | 68000 | 61000 | 14 | 1.1 |
| 2344'5-PeCB(#114) | 2700 - | 3400 | 3100 | 10 | 2700 - | 2900 | 2800 | 4 | 1.1 |
| 23'44'5-PeCB(#118) | 100000 - | 120000 | 110000 | 6 | 110000 - | 130000 | 120000 | 8 | 0.96 |
| 2'344'5-PeCB(#123) | 2600 - | 4100 | 3600 | 19 | 1900 - | 3700 | 2700 | 27 | 1.4 |
| 233'44'5-HxCB(#156) | 31000 - | 37000 | 36000 | 9 | 32000 - | 42000 | 39000 | 12 | 0.92 |
| 233'44'5'-HxCB(#157) | 8500 - | 10000 | 9400 | 8 | 8000 - | 11000 | 9700 | 13 | 0.97 |
| 23'44'55'-HxCB(#167) | 20000 - | 23000 | 22000 | 7 | 20000 - | 25000 | 23000 | 11 | 0.96 |
| 233'44'55'-HpCB(#189) | 4900 - | 5900 | 5600 | 8 | 4400 - | 5300 | 5000 | 9 | 1.1 |

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# BROMINATED FLAME RETARDANTS (HBCD,TBBPA AND ΣPBDES) IN MARKET BASKET FOOD SAMPLES OF NORTHERN KYUSHU DISTRICT IN JAPAN

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**Abstract**
We developed an analytical method for HBCD in food samples using gel permeation chromatography and a mini-column coupled with LC/MS/MS. In this report, to estimate the trend of human exposure to brominated flame retardants (BFRs) via food as well as to describe our validation results using this method, we analyzed two sets of market basket food samples prepared in Fukuoka prefecture in the fiscal years of 2002 and 2005. The estimated dietary intakes of HBCD, TBBPA and ΣPBDEs by an adult were 2.2, 1.1, and 2.3 ng/kg b.w./day, respectively, in F2002, and were 1.4, 0.1, and 1.4 ng/kg b.w./day, respectively, in F2005 when calculated for ND=0. The BFR intake levels by Japanese populate were considered as not so concerned.

**Introduction**
Polybrominated diphenyl ethers (PBDEs), tetrabrominated bisphenol A (TBBPA), and hexacycrododecane (HBCD) have been revealed as ubiquitous contaminants in human and wild live tissues as well as in other environmental samples. As another noteworthy point, they are closely related to the occurrence of polybrominated dioxins (PBDD/DFs)[1]. For example, the detection of PBDD/Fs in a PBDE formula or in ashes caused from the burning of plastics with PBDE as a flame retardant is well-known as a significant evidence[2]. When we estimate human exposure to these pollutants, the route via food is the most important. In our previous study, we found PBDEs in almost all fish samples collected from the Japanese near coasts and also found a tendency for PBDD/Fs to be detected in fish accompanying with highy brominated diphhenyl ethers such as nona- and deca-brominated diphenylethers. From such a viewpoint, BFR monitoring in food is important in preventing health hazardous effects not only from BFRs themselves but also from PBDD/DFs. According to a domestic trade paper, TBBPA is the mostly used BFR in Japan, with as much as 35,000 tons as a demand in F2004, followed by HBCD(2600 tons) and decabrominated diphenyl ether (2000 tons). In Japan, PBDEs without decabrominated diphenyl ether are self-controlled by the trade, and the value of the domestic demand for DBDE is only officially announced. Basically, there is only limited data on food pollution by BFRs. The best of the convenient ways to estimate the trend of human exposure to BFRs via food is to analyze market basket food samples. However, market basket food samples usually contain too many matrices that make an analysis difficult. Therefore, we have to modify the basic analytical method depending on the characteristic conditions of food—for example, whether it is a fatty food or not, or whether it is chlorophyll-rich or not. The appropriate modification is the key point in determining pollutant concentration with accuracy. In this study, we designed a pre-treatment method for the HBCD analysis for market basket samples, paying attention to the fact that HBCD is a large molecule of MW 641 and has a non-polar structure. For the method validation and the preliminary estimation of BFR intakes, the market basket samples prepared in our laboratory were used

**Materials and Methods**
Market basket food samples: Thirteen mixed food samples were prepared following the method of the Market Basket Study, alternatively termed the Total Diet Study. More than one hundred food items were chosen from 99 categories of foods that the Fukuoka populace commonly consumes, and the respective amounts of food items composing each food group (from 1 to 13) were determined by referring to the data of the latest national and prefecture survey (2002 and 2005).

Analysis of HBCD: Five grams of an homogenized sample with an addition of $^{13}C_{12}$ -α,β, and γ-HBCD were extracted twice with 20mL of dichloromethane (DCM) using a Polytron®. The extracts were dried over sodium sulfate dehydrate and concentrated. (I) Each residue of groups 1 (rice and its products), 2 (other grains, seeds, potatoes), 3 (sugar and a confectionary) and 4 (oils) was dissolved in 20mL of methanol/water (15:5, v/v) and re-extracted with n-hexane. One-half of each hexane layer was purified by liquid-liquid partition with DMSO. The purified extracts were concentrated and dissolved into 0.2mL of acetone and loaded onto a column of gel permeation chromatography (GPC). (II) Each half residue of groups 6 (fruits), 7 (colored vegetables) and 8 (other vegetables, mushroom, sea weed) was purified by multi-layered column chromatography of 22% $H_2SO_4$-silica (4g) and 44% $H_2SO_4$-silica (3g). HBCD was recovered with 10% DCM/n-hexane as an eluate. Each eluate was concentrated and dissolved into 0.2mL of acetone and subjected to GPC. (III) Each residue of the others--groups 6 (beans), 9 (drink, beverage), 10 (fish), 11 (meat and eggs), 12 (milk), and 13 (seasoning)-- was dissolved in 10% DCM/n-hexane and was treated twice with 5mL of sulfuric acid. After centrifuging at 2000 rpm, the upper hexane layer was collected and evaporated. The residue was dissolved in 0.2mL of acetone, and a half of it was subjected to GPC.
HBCD was fractionated in 12 to 14 min after large molecules such as crude fatty acids eluted in 10 to 12 min. The fraction was re-purified with a cartridge mini-column (Varian BOND ELUT-PSA, 500mg) prior to analysis by LC/MS/MS (Table 1). Detection limits of α-and γ-HBCD were 0.02 pg/g wb. That for β-HBCD was 0.01 ng/g wb.

Analysis of PBDEs: Each food group sample (fifty to one hundred grams) except for group 4 (oils) was freeze dried. After being spiked with $^{13}C_{12}$-labelled 2,3,7,8-substituted PBDD/DFs (128-500pg), $^{13}C_{12}$-1-Br-2,3,7,8-TeCDD (50pg) and $^{13}C_{12}$-labelled PBDEs (500-2500pg), it was extracted by accelerated solvent extractor(100 ℃ ,1500psi, 10% DCM/n-hexane) and with sulfuric acid and two kinds of column chromatography with silica-gel activated overnight at 130 ℃ and florisil deactivated with 1% of water. Group 4(oil) sample was directly diluted with n-hexane and then cleaned up as same as the other group. The PBDE fraction was cleaned up by liquid-liquid partition with DMSO. Prior to measurement by HRGC/HRMS (Table 2), $^{13}C_{12}$- 2,2',3,4,4',5',6 -HpBDE was added. Detection limits of tetra-to hepta-isomers were 0.1 pg/g wb.

Analysis of TBBPA: A homogenized sample (5 g) was spiked with $^{13}$C-labeled TBBPA (0.5 ng) as a clean-up standard and then extracted with methanol. The methanol extract (ca.50 mL) was defatted by liquid-liquid partition with 20 mL of hexane. Then, to the methanol layer, 120 mL of 5% sodium chloride solution was added and re-extracted with DCM. The extract was concentrated to dryness and then ethylated with diethyl sulfate under an alkaline condition. After that, TBBPA ethylate was extracted with n-hexane and was cleaned up with florisil mini-column chromatography. The purified eluate was concentrated, re-dissolved in 20 μ L of nonane with 2.5 ng of chrysene-$d_{12}$ as a syringe spike, and subjected to measurement by HRGC/HRMS (Table 2). The detection limit of TBBPA was 0.01ng/g wb.

**Results and Discussion**
HBCD are highly lipophilic chemicals and large molecules similar to PBDEs. There are three stereo isomers: α,β,and γ. So far, detecting α,β,and γ separately is difficult with GC/MS. Therefore, replacing GC/MS by LC/MS/MS is likely to be used in the analysis of HBCD. However, there is a basic problem in LC/MS/MS analysis: the suppression of ionization caused by co-eluting the matrix. Good recovery and good reproducibility are required, when developing an analytical method. Therefore, sufficient clean-up to reduce the adverse problem as much as possible is necessary. In this study, we employed GPC and an additional clean-up by mini-column chromatography. With GPC, HBCD and TBBPA eluted separately from a large molecule such as crude fatty acid of fish. In the experiment, using the group 10 sample (fish as the main food) prepared in 2007, we obtained satisfactory recoveries: a mean of 73.4% ranging from 62.2% to 81.9% for α–isomer, a mean of 83.4% ranging from 66.5% to 92.6% for β-isomer, and a mean of 73.4% ranging from 54.8% to 90% for γ –isomer, as well as satisfactory reproducibilities of 12.2%, 11.7% and 15.4% for α,β,and γ–isomers, respectively. And for the other various food group samples, the recoveries of HBCD were

42%~106% for α–isomer, 59%~130% for β-isomer and 53%~124% for γ-isomer, except for 182%~225% for group 9.

HBCD was detected in each group 10 of F 2002 and F 2005 and in group 11 of F 2002. TBBPA was detected in each group 10 of F2002 and F2005. It was also detected in the groups 3, 4, 5 and 11 of F 2002 and in the groups 3 and 11 of F 2005. On the other hand, due to the lower detection limit of each PBDE congener, PBDEs were sensitively detected in the almost samples of F 2002 and F 2005. In group 10 among the 13 food groups of each year set, PBDEs were most abundantly detected. The partition coefficient of n-octanol to water (Log $K_{ow}$) for TBBPA, HBCD and PBDEs was 4.5~5.3, 7.74 and 6.27 (as DeBDE), respectively. The very frequent detection of HBCD and PBDEs like PCBs in group 10 (fish) seems to be acceptable; in contrast, TBBPA was detected only in each group 10 of F2002 and F2005 but at a very low level, which would be due to the instability of the phenol moiety of TBBTA. TBBPA is reported to be rapidly metabolized biologically to sulfate or gluculonide conjugates in the environment[3]. On the basis of the above data, the daily intakes of HBCD, TBBPA and ΣPBDE were calculated by multiplying each pollutant's concentration by the daily food consumption amount by one person (Table 3). Assuming the average adult body weight as 50 kg, the daily intakes of HBCD, TBBPA and ΣPBDEs were 2.2, 1.1, and 2.3 ng/kg b.w./day, respectively, in F2002, and 1.4, 0.1, and 1.4 ng/kg b.w./day, respectively, in F2005, when calculated for ND=0. The daily intakes of HBCD, TBBPA and ΣPBDEs were 3.1, 1.3, and 2.3 ng/kg b.w./day, respectively, in F2002, and 2.4 ng, 0.3 ng, and 1.4 ng/kg b.w./day, respectively, in F2005 when calculated for ND=1/2xLOD. Only TBBPA varied greatly between F2002 and F2005. The dietary intakes of HBCD in F2002 and F2005 were at the same level as those of ΣPBDEs. In other reports, Swedish people's intake of BFRs was <3ng ng/kg b.w. day for HBCD[4] , and the UK people's intake of these brominated retardants in 2003 and 2004 was <5.9 ng/kg b.w./day for HBCD, <1.6 ng/kg b.w./day for TBBPA, and <5.9 ng/kg b.w./day for ΣPBDEs[5], respectively. The food standards agency of the UK that carried out the above market basket study concluded that the estimated adult dietary intake of HBCD, TBBPA and ΣPBDEs does not raise toxicological concerns. Although there is a report which simulates the increase of Br emission from TV casing waste in the near future, basically not only the available references about the intake data of BFRs, but also the fate of BFRs are too limited; the increase or decrease of emission and deposition into the environment are not completely clear. Therefore, we recommend continuing to collect more data in order to clarify the trend of food pollution by those BFRs and avoid probable human hazardous exposure.

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**Table 1 The LC/MS/MS conditions for HBCD analysis**

| |
|---|
| LC/MS/MS: Waters Quatro Micro API |
| Column:Inertsil ODS-3 (GL Sciences) 2.1mmi.d. x150m, 5μ |
| Injector volume.:5 uL |
| Column temp.:40℃ |
| Flow rate: 0.2mL/min |
| Moving phase: 10mM ammonium acetate/methanol/acetonitrile (10:55:35) |
| Monitor Ions: native-HBCD, 641 >79 (Q1) , 639 >79(Q2) |
| $^{13}C_{12}$-HBCD; 653>79(Q1), 651>79(Q2) |
| Ionization;ES negative; Ion source temp.,130℃; Capillary energy ; 2.0kV |

**Table 2 The GC/MS conditions for PBDEs and TBBPA analysis**

| |
|---|
| GC/HRMS: HP6890 (Hewlett Packard) /Autospec Ultima (MicroMass) |
| Electron energy,38eV; filament current, 750μA; ion source Temp.,270℃; resolution,10000 |
| PBDE analysis: Column: HP-5MS(Agilent) 0.25mmi.d. x15m, film thickness 0.1μm |
| Injector temp.:260℃ |
| Column temp.:120℃(2min)-20℃/min-200℃-10℃/min-300℃(7.5min) |
| TBBPA analysis: Column:DB-5 (J&W) 0.25mmi.d. x 30m, film thickness 0.25μm |
| Injector temp.:280℃ |
| Column temp.:120℃(1min)-20℃/min–300℃(8min) |

Table 3 The estimated dietary intakes of HBCD, TBBPA and ΣPBDEs

| Food group | | Food consumption (g/day) in F2002 | Daily intake in F2002 | | | | | | Food consumption (g/day) in F2005 | Daily intake in F2005 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | α-HBCD | β-HBCD | γ-HBCD | ΣHBCD | TBBPA | ΣPBDEs | | α-HBCD | β-HBCD | γ-HBCD | ΣHBCD | TBBPA | ΣPBDEs |
| I | (rice) | 459 | ND | ND | ND | ND | ND | 0.11 | 446.5 | ND | ND | ND | ND | ND | 0.19 |
| II | (other grains, potatoes,seeds) | 226.1 | ND | ND | ND | ND | ND | 0.37 | 209.9 | ND | ND | ND | ND | ND | 0.50 |
| III | (sugar, confectionary) | 36.6 | ND | ND | ND | ND | 0.33 | 0.17 | 33.7 | ND | ND | ND | ND | 0.33 | 0.67 |
| IV | (oils) | 15.2 | ND | ND | ND | ND | 0.15 | 1.85 | 11.5 | ND | ND | ND | ND | ND | 0.65 |
| V | (legume and its products) | 80.4 | ND | ND | ND | ND | 0.73 | 0.30 | 56.4 | ND | ND | ND | ND | ND | 0.13 |
| VI | (fruits) | 130.6 | ND | ND | ND | ND | ND | 0.00 | 136.0 | ND | ND | ND | ND | ND | 2.60 |
| VII | (colored vegetable) | 108.3 | ND | ND | ND | ND | ND | 0.05 | 96.6 | ND | ND | ND | ND | ND | 0.10 |
| VIII | (other vegetables) | 234.6 | ND | ND | ND | ND | ND | 0.03 | 219.0 | ND | ND | ND | ND | ND | 0.06 |
| IX | (bebariges) | 172.2 | ND | ND | ND | ND | ND | 0.02 | 504.2 | ND | ND | ND | ND | ND | 0.00 |
| X | (fish) | 100.8 | 57* | ND | 19.7* | 76.7* | 50.7* | 102* | 90.7 | 49.3* | ND | 19.7* | 49.3* | 2.72* | 57.8* |
| XI | (meat and eggs) | 157.9 | 34.5* | ND | ND | 34.5* | 4.42* | 9.17* | 133.6 | ND | ND | ND | ND | 0.67* | 4.19* |
| XII | (milk and its products) | 122.5 | ND | ND | ND | ND | ND | 1.04* | 146.8 | ND | ND | ND | ND | ND | 0.74* |
| XIII | (seasonings) | 38.1 | ND | ND | ND | ND | ND | 0.06 | 80.9 | ND | ND | ND | ND | ND | 0.65 |
| XIV | ( water) | | - | - | - | - | — | — | | - | - | - | - | — | — |
| Daily intake ng/day at ND=0 | | | 91.5 | 0 | 19.7 | 111 | 56.3 | 115 | | 49.3 | 0 | 19.7 | 68.4 | 3.7 | 68.3 |
| Daily intake ng/day at ND=1/2 xLOD | | | 108.5 | 9.4 | 37.5 | 155 | 63.9 | 116 | | 70.1 | 10.8 | 39.9 | 121 | 13.8 | 69.3 |
| Daily intake ng/kg,b.w./day at ND=0 | | | | | | 2.2 | 1.1 | 2.3 | | | | | 1.4 | 0.1 | 1.4 |
| Daily intake ng/kg,b.w./day at ND=1/2xLOD | | | | | | 3.1 | 1.3 | 2.3 | | | | | 2.4 | 0.3 | 1.4 |

LOD: 0.01ng/g for β-HBCD and TBBPA; 0.02ng/g for α-HBCD and γ-HBCD; O. 0001ng/g for each PBDE congener (from tetra- to hepta-brominated isomer)

* means an average of two food group samples prepared separately.

# DIOXINS AND OTHER ORGANOHALOGEN COMPOUNDS IN FISH OIL SUPPLEMENTS ON THE JAPANESE MARKET

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**Abstract**
Dioxin (PCDD/Fs and DL-PCBs) concentrations of 30 fish oil supplements on the Japanese market were determined to estimate the dioxin intakes resulting from their consumption. The dioxin intakes from most products were under 10% of the tolerable daily intake (TDI) of dioxins (4 pg-TEQ/kg bw/day) set in Japan. However, only a product, no. 1, had extremely high dioxin concentrations and dioxin intake of the product greatly exceeded the TDI. Four products with relatively high dioxin concentrations, including product no. 1, were further analyzed in terms of PBDD/Fs, PXDD/Fs, PCBs and PBDEs. PCBs and PBDEs were found in all samples, especially product no. 1 had much higher concentrations of PCBs and PBDEs than the other products did. By contrast, PBDD/Fs and PXDD/Fs were detected much less often in all samples.

**Introduction**
Fish oil supplements are a source of long-chain *n*-3 polyunsaturated fatty acids, such as eicosapentaenoic and docosahexaenoic acid, which are thought to have health benefits. Recently, the popularity of these supplements has increased in Japan. They are produced from various types of fish, especially from fatty tissues, and can be a major source of persistent organic pollutants such as dioxins (PCDD/Fs and DL-PCBs) and PCBs. Recently, there has been increasing concern over brominated compounds such as PBDEs, which are used as flame retardants, as well as brominated and mixed chlorinated-brominated dioxins (PBDD/Fs and PXDD/Fs, respectively). Although it is important to determine the levels of these compounds in fish oil supplements, only a few previous studies have considered them[1-4]. Here, we examined the dioxin levels in fish oil supplements on sale in Japan and estimated the dioxin intakes resulting from their consumption. Products with relatively high dioxin concentrations were further analyzed in terms of PBDD/Fs, PXDD/Fs, PCBs and PBDEs.

**Materials and Methods**
**Fish oil supplements:** In total, 30 products (29 capsule formulations and 1 bottled formulation) were purchased between 2002 and 2005 from retail outlets, or by post, in Tokyo, Japan. The analysis of encapsulated products included the capsules. All samples were stored at 4°C until they were analyzed.

**PCDD/F and DL-PCB analyses:** Dioxins were extracted, prepared and analyzed as described previously[5]. The TEQ concentrations were calculated using WHO-TEFs (1998).

**PBDD/F and PXDD/F analyses:** Samples (5–20 g) spiked with $^{13}C_{12}$-labelled internal standards were stirred with aqueous potassium hydroxide (KOH) and then kept for 16 h at room temperature (RT). The alkaline hydrolysates were extracted with *n*-hexane. The extracts were treated with concentrated sulphuric acid, and then purified on a silica gel column followed by a Florisil column (deactivated with 1% water). After washing with *n*-hexane, the elute obtained with 60% dichloromethane/*n*-hexane was loaded onto an activated carbon column. This was washed by *n*-hexane followed by 25% dichloromethane/*n*-hexane, and the fraction containing PBDD/Fs and PXDD/Fs was eluted with toluene. The fraction was spiked with $^{13}C_{12}$-labelled recovery standards, and subjected to HRGC/HRMS. The determinations of 12 2,3,7,8-substituted PBDD/Fs (2,3,7,8-TeBDD, 1,2,3,7,8-PeBDD, 1,2,3,4,7,8/1,2,3,6,7,8-HxBDD, 1,2,3,7,8,9-HxBDD, OBDD, 2,3,7,8-TeBDF, 1,2,3,7,8-PeBDF, 2,3,4,7,8-PeBDF, 1,2,3,4,7,8-HxBDF, 1,2,3,4,6,7,8-HpBDF and OBDF) and seven PXDD/Fs (2-Br-3,7,8-TrCDD, 1-Br-2,3,7,8-TeCDD, 2-Br-3,6,7,8,9-PeCDD, 1-Br-2,3,6,7,8,9-HxCDD, 1-Br-2,3,4,6,7,8,9-HpCDD, 3-Br-2,7,8-TrCDF and 1-Br-2,3,7,8-TeCDF) were performed using a DB-5HT and BP1 column. The WHO-TEFs for the chlorinated isomers were provisionally used to evaluate the toxicities of the corresponding PBDD/F and PXDD/F isomers.

**PCB analyses:** Samples (5–10 g) spiked with $^{13}C_{12}$-labelled internal standards were stirred with ethanolic KOH and then kept for 16 h at RT. The alkaline hydrolysates were added to water and extracted with *n*-hexane. The extracts were treated with concentrated sulphuric acid, and then purified on a silica gel column. The elute obtained with *n*-hexane was subjected to gel-permeation chromatography (GPC) using 5% cyclohexane/acetone. The fraction containing PCBs was concentrated and spiked with $^{13}C_{12}$-labelled recovery standards. The PCBs were quantified by HRGC/HRMS, and their determination was performed on an HT8-PCB column.

**PBDE analyses:** Samples (5–10 g) spiked with $^{13}C_{12}$-labelled internal standards were stirred with ethanolic KOH and then kept for 16 h at RT. The alkaline hydrolysates were added to water and extracted with *n*-hexane. The extracts were treated with concentrated sulphuric acid, and then purified on a silver nitrate/silica gel column. The elute obtained with *n*-hexane was subjected to GPC using acetone. The fraction containing PBDEs was concentrated and spiked with $^{13}C_{12}$-labelled recovery standards. The PBDEs were quantified by HRGC/HRMS, and their determination was performed using a DB-5HT and BP1 column.

**Results and Discussion**

The dioxin concentrations in the 30 products and the associated intakes are presented in Table 1. The dioxin concentrations varied significantly. Product no. 1, which was made from tiger shark liver (crude extract), had extremely high dioxin concentrations; however, most samples had levels below 10 pg-TEQ/g. These values were low compared with the dioxin concentrations in the source species. The fish oil purification processes could thus have effectively removed dioxins from the samples. Indeed, Hilbert and colleagues[6] reported that steam distillation, which is a refining process for fish oil, reduced the amounts of organochlorine contaminants, including PCBs.

The total dioxin intake from the most contaminated product reached 1,500 pg-TEQ/person/day, corresponding to 30 pg-TEQ/kg bw/day for an adult weighing 50 kg. This was about eight times higher than the tolerable daily intake (TDI) of dioxins (4 pg-TEQ/kg bw/day) set by the Japanese government in 1999[7]. The intakes from most products were under 10% of the TDI, although those of samples no. 2 and no. 3 corresponded to about 30 and 14%, respectively. The major contributors to the total TEQ were DL-PCBs, which accounted for more than 90% in the most contaminated sample. This was in agreement with previous reports on fish oil supplements[1,2,4].

We determined the dioxin concentrations in different batches of the same products and found no significant differences (Figure 1). Product no. 1 had the highest variation in dioxin concentrations between batches, although the ratio of the maximal/minimum dioxin concentrations was only about 2. The dioxin intakes from the four batches of product no. 1 ranged from 16 to 30 pg-TEQ/kg bw/day. Thus, if an individual consumed the product regularly over a long period, their daily dioxin intake would continuously exceed the TDI.

The four products with relatively high dioxin concentrations were also analyzed for PBDD/F, PXDD/F, PCB and PBDE (Table 2). PCBs and PBDEs were found in all samples, although their concentrations varied significantly between products. Two batches of product no. 1 had much higher concentrations of PCBs and PBDEs than the other products, with respective intakes of 32,000 to 57,000 ng/person/day and 480,000 to 670,000 pg/person/day. By contrast, PBDD/Fs and PXDD/Fs were detected much less often in all samples. Only one isomer, 2,3,7,8-TeBDF, was quantified in two products (nos. 3 and 6). The intakes of PBDD/Fs and PXCDD/Fs were calculated assuming that the levels of non-detected isomers were equal to half of their limits of detection (LODs). Overall, the intakes were much lower that those of dioxins. The dioxin-like toxicity of the PBDD/Fs and PXDD/Fs in fish oil supplements appeared to be negligible.

Thus, although rare, fish oil supplements may contain significantly high concentrations of dioxins, PCBs and PBDEs. Continuous monitoring of the levels of these compounds in fish oil supplements is therefore recommended.

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**Table 1** Dioxin concentrations in individual fish oil supplements and associated intakes

| Product no. [a] | Fish source [b] | Daily intake (g) [c] | Dioxin conc. (pg-TEQ/g) [d] | | | Dioxin intake [e] (pg-TEQ/person/day) |
|---|---|---|---|---|---|---|
| | | | PCDD/Fs | DL-PCBs | Total | |
| 1 | Tiger shark | 3.17 | 37 | 450 | 480 | 1,500 (30) |
| 2 | Sardine | 4.80 | 4.2 | 7.7 | 12 | 58 (1.2) |
| 3 | Lampern | 2.84 | 2.6 | 7.8 | 10 | 28 (0.57) |
| 4 | Cod | 2.00 | < 0.10 | 8.4 | 8.4 | 17 (0.34) |
| 5 | Gulper shark | 2.91 | 0.51 | 2.8 | 3.3 | 10 (0.19) |
| 6 | Tuna | 1.25 | < 0.10 | 6.5 | 6.6 | 8.3 (0.17) |
| 7 | Gulper shark | 3.15 | 1.3 | 0.65 | 1.9 | 6.0 (0.12) |
| 8 | Lampern etc. | 2.10 | 0.58 | 1.6 | 2.2 | 4.6 (0.092) |
| 9 | Sardine | 1.53 | 0.17 | 2.4 | 2.5 | 3.8 (0.077) |
| 10 | Gulper shark | 1.28 | 1.3 | 0.64 | 2.0 | 2.6 (0.051) |
| 11 | Herring, sardine | 2.84 | < 0.10 | 0.5 | 0.56 | 1.6 (0.032) |
| 12 | NS | 3.60 | 0.11 | 0.22 | 0.32 | 1.2 (0.023) |
| 13 | Tuna | 1.76 | < 0.10 | 0.45 | 0.46 | 0.81 (0.016) |
| 14 | Lampern etc. | 1.32 | 0.13 | 0.43 | 0.56 | 0.74 (0.015) |
| 15 | Sardine | 2.70 | < 0.10 | 0.17 | 0.25 | 0.68 (0.014) |
| 16 | NS | 2.82 | < 0.10 | 0.17 | 0.18 | 0.51 (0.010) |
| 17 | NS | 2.23 | < 0.10 | 0.12 | 0.20 | 0.45 (0.0089) |
| 18 | Sardine etc. | 2.67 | < 0.10 | 0.11 | 0.14 | 0.37 (0.0075) |
| 19 | Gulper shark | 1.52 | < 0.10 | 0.16 | 0.16 | 0.24 (0.0049) |
| 20 | Tuna, sardine | 1.80 | < 0.10 | < 0.10 | 0.10 | 0.18 (0.0036) |
| 21 | NS | 1.66 | < 0.10 | < 0.10 | < 0.10 | 0.11 (0.0023) |
| 22 | NS | 1.16 | < 0.10 | < 0.10 | < 0.10 | < 0.10 (< 0.0020) |
| 23 | NS | 1.76 | < 0.10 | < 0.10 | < 0.10 | < 0.10 (< 0.0020) |
| 24 | NS | 1.40 | < 0.10 | < 0.10 | < 0.10 | < 0.10 (< 0.0020) |
| 25 | NS | 1.28 | < 0.10 | < 0.10 | < 0.10 | < 0.10 (< 0.0020) |
| 26 | Tuna etc. | 3.87 | < 0.10 | < 0.10 | < 0.10 | < 0.10 (< 0.0020) |
| 27 | Tuna etc. | 2.10 | < 0.10 | < 0.10 | < 0.10 | < 0.10 (< 0.0020) |
| 28 | Tuna etc. | 2.19 | < 0.10 | < 0.10 | < 0.10 | < 0.10 (< 0.0020) |
| 29 | NS | 1.92 | < 0.10 | < 0.10 | < 0.10 | < 0.10 (< 0.0020) |
| 30 | Tuna etc. | 0.93 | < 0.10 | < 0.10 | < 0.10 | < 0.10 (< 0.0020) |

[a] All samples except sample 4 (bottled) were capsule formulations.

[b] Unspecified fish oil content is expressed as "NS". Nine products (nos. 3, 8, 13, 14, 26, 27, 28, 29 and 30) were a mixture of fish oils and vegetable oils.

[c] Daily intakes of each product were calculated from the maximal recommended dosages on the product labels.

[d] Dioxin concentrations are presented on a whole weight basis. For encapsulated fish oil products, the entire samples, including capsules, were analyzed. The concentrations were calculated assuming that the non-detected isomers were equal to zero.

[e] Dioxin intakes in parentheses (pg-TEQ/bw kg/day) were based on a person weighing 50 kg.

**Table 2** Summary of dioxins and other organohalogen compounds in the selected fish oil supplements

| Product no. [a] | Dioxins [b] | | PBDD/Fs+PXDD/Fs [b] | | PCBs | | PBDEs | |
|---|---|---|---|---|---|---|---|---|
| | Conc. (pg-TEQ/g) | Intake (pg-TEQ/ person/day) | Conc. (pg-TEQ/g) | Intake (pg-TEQ/ person/day) | Conc. (ng/g) | Intake (ng/ person/day) | Conc. (pg/g) | Intake (pg/ person/day) |
| 1 (B) | 510 | 1,600 | 0 | 0 | 18,000 | 57,000 | 210,000 | 670,000 |
| | (510) | (1600) | (1.3) | (4.1) | | | | |
| (D) | 250 | 800 | 0 | 0 | 10,000 | 32,000 | 150,000 | 480,000 |
| | (250) | (800) | (1.3) | (4.1) | | | | |
| 3 (D) | 9.9 | 28 | 0.13 | 0.37 | 140 | 400 | 1,800 | 5,100 |
| | (9.9) | (28) | (0.48) | (1.4) | | | | |
| 5 | 3.3 | 9.6 | 0 | 0 | 52 | 150 | 550 | 1,600 |
| | (3.3) | (9.6) | (1.3) | (3.8) | | | | |
| 6 (D) | 7.0 | 8.8 | 0.12 | 0.15 | 110 | 140 | 5,700 | 7,100 |
| | (7.0) | (8.8) | (0.47) | (0.59) | | | | |

[a] Letters in parentheses indicate batch no. in Figure 1.

[b] Dioxin concentrations are presented on a whole weight basis. For encapsulated fish oil products, the entire samples, including capsules, were analyzed. The concentrations as well as intakes were calculated assuming that the non-detected isomers were equal to zero, and also calculated in parentheses assuming that non-detected isomers were equal to half of their LODs.

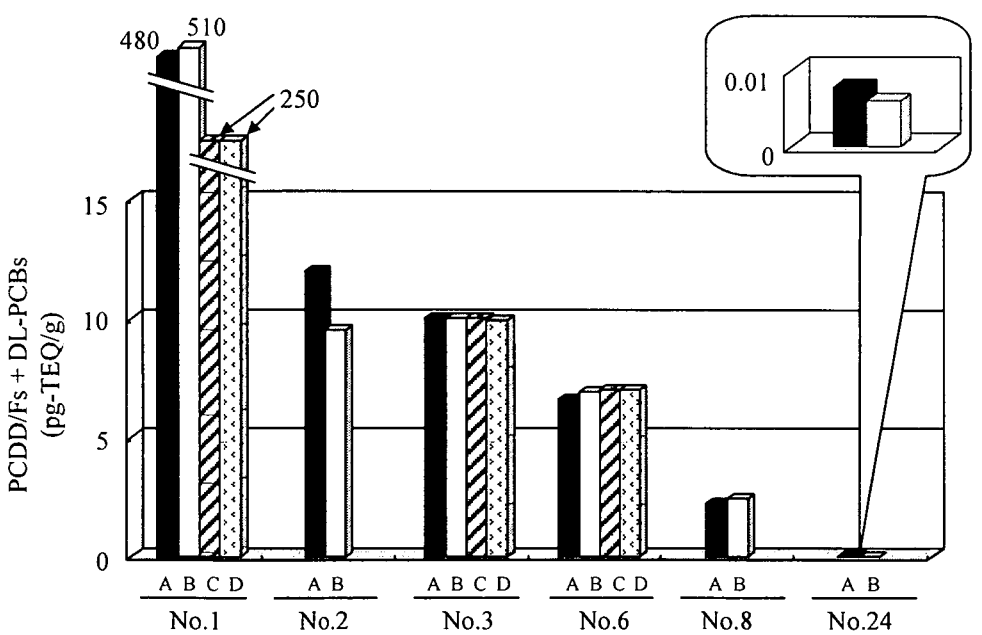


**Figure 1** Batch differences in dioxin concentrations for several fish oil supplements
The same products, two to four batches, were purchased during 2002-2005. The samples designated "A" in each product are the same samples as in Table 1.

# DAILY INTAKE OF BROMINATED DIOXINS AND POLYBROMINATED DIPHENYL ETHERS ESTIMATED BY MARKET BASKET STUDY

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**Abstract**

A market basket study of brominated dioxins and polybrominated diphenyl ethers (PBDEs) was performed to estimate daily intake levels of these compounds in Japan. We analyzed brominated dioxins and PBDEs in food mixtures from each of 13 food groups from 6 regions (Hokkaido, Tohoku, Kanto, Tyubu, Tyugoku-Shikoku and Kyushu) in Japan and calculated the daily intakes from food consumption. From the results of analyzing the brominated dioxins, only 1,2,3,4,6,7,8-HpBDF was detected in the mixture of group 4 (fats and oils) at 0.14-0.44 pg/g wb. To estimate the influence of brominated dioxins, we calculated the total TEQ per day, using TEFs of chlorinated dioxins. The mean daily intake was calculated at 0.00056 pg TEQ / kg body weight /day (assuming ND = 0). Due to the small daily consumption of fats and oils, the daily intake of brominated dioxins was at a low level. For PBDEs, the mean daily intake was calculated at 2.17ng / kg body weight /day (assuming ND = 0). Since the estimated value in this study was much less than LOAEL (1mg / kg / day), the daily intake level of PBDEs was not considered a serious problem.

**Introduction**

Brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A (TBBPA), and hexabromocyclododecane (HBCD) have been widely used in plastics and textile coatings throughout the world. For PBDEs, although the usage of low brominated PBDEs has decreased, DeBDE is currently in use. PBDEs are additives to polymers such as polystyrene and are not chemically bound to the polymer. Therefore, it is considered that they are easily released into the environment from waste products. Furthermore, polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/DFs) are pollutants generated by the manufacture of brominated flame retardants (BFRs) such as brominated diphenyl ethers (PBDEs) and are formed by combustion of substances containing BFRs. Although the toxicity of these brominated dioxins is unclear, some studies have shown that the toxicity of 2,3,7,8-TBDD is comparable to that of 2,3,7,8-TCDD [1]. In a recent report, PBDD/DFs and PBDEs have been detected in human adipose tissue in Japan [2]. Therefore, it is necessary to investigate levels of these brominated organic compounds in several foods and to estimate the influence they have on a daily intake level.

A market basket study is a useful method for estimating the average intake level in regions, based on a model of the average domestic diet. It is possible to provide information for the daily intake of food groups, such as rice, fruits, vegetables, fish and meat. In the present study, we analyzed brominated dioxins and PBDEs in food mixtures from each of 13 food group from 6 regions (Hokkaido, Tohoku, Kanto, Tyubu, Tyugoku-Shikoku and Kyushu) in Japan and estimated daily intake levels of brominated dioxins and PBDEs.

## Materials and Methods

*Sampling.*

Table 1 shows the food groups analyzed in this study and their mean daily consumption for 6 regions as calculated from the data of the Japanese Nutrition Survey carried out by the Ministry of Health, Labour and Welfare. For a market basket study, 120-200 kinds of foods were purchased from markets in each of 6 regions (Hokkaido, Tohoku, Kanto, Tyubu, Tyugoku-Shikoku and Kyushu) from 2004 to 2005. These foods were divided into 13 food groups, and weighed and cooked based on the daily consumption data of each region. Then, they were blended in a food processor. The food mixtures were prepared and analyzed for groups 10, 11 and 12 ($n$=2) and other groups ($n$=1). The food mixtures were kept below –20°C until analysis.

*Analytical Methods and Instrumentation.*

The concentrations of PBDD/DFs and PBDEs in the food mixtures were determined using high-resolution gas chromatography / high-resolution mass spectrometry (HRGC/HRMS). The analytical conditions of HRGC/HRMS are shown in Table 2. The PBDD/DFs (tetra-octa) analytical standard was purchased from Cambridge Isotope Laboratories (MA). The PBDE analytical standard was purchased from Wellington Laboratories (Ontario). Dichloromethane, $n$-hexane and toluene used for extraction and cleanup were of dioxins analysis grade (Kanto Chemicals, Tokyo). Silica gel (Wako Pure Chemical Industries, Ltd., Tokyo) was heated for 3h at 130°C. Florisil (Kanto Chemicals, Tokyo) was heated for 3h at 130°C and deactivated with 1% water. Further information about analytical methods and instrumentation is described in our previous article [3].

*Sample Preparation.*

The analytical method for the PBDD/DFs and PBDEs was as follows. Each 50g of food mixture for the market basket study was freeze dried using a model AD 2.0ES-BC (Virtis, NY) freeze dryer. Dried samples were extracted with 10% (v/v) dichloromethane / $n$-hexane by accelerated solvent extractor ASE300 (Dionex, CA). The temperature of extraction was 100°C; the time was 10 min. Extracts were treated with sulfuric acid three times and applied to a silica gel column. The mixture for group 4 was dissolved in 100ml $n$-hexane and purified by sulfuric and the silica gel column in the same way. The column was prewashed with 100ml $n$-hexane, and PBDD/DFs and PBDEs were eluted with 150ml of 10% (v/v) dichloromethane / $n$-hexane. The eluate was evaporated and dissolved in $n$-hexane. It was then loaded onto a Florisil (5 g) column. The PBDEs fraction was obtained by elution with 150 ml of n-hexane, and the successive PBDD/DFs fraction was obtained by elution with 200 ml of 60% (v/v) dichloromethane /$n$-hexane. The PBDEs fraction was treated with a DMSO /$n$-hexane partition to remove the matrix. The PBDD/DFs fraction was further loaded on an active carbon column, which in advance was washed with 50 ml of 10% (v/v) dichloromethane /$n$-hexane, eluted with 200 ml of toluene. Both fractions were concentrated to a final volume of approximately 50μl, and these samples were analyzed by HRGC/HRMS.

Table 1 Daily consumption of food (13 groups) in 6 regions of Japan

| No. | Food group | Daily consumption (g)* | Ratio (%)* |
|---|---|---|---|
| 1 | Rice and rice products | 360 (333 -382) | 17.8 (15.6-20.0) |
| 2 | Cereals seeds and potatoes | 172 (151-190) | 8.5 (7.0-9.7) |
| 3 | Sugars and confectioneries | 32.0 (27.7-36.3) | 1.6 (1.3-1.8) |
| 4 | Fats and oils | 10.8 (9.0-12.5) | 0.5 (0.4-0.6) |
| 5 | Pulses | 56.3 (43.9-64.2) | 2.8 (2.2-3.1) |
| 6 | Fruits | 135 (124-152) | 6.7 (6.3-7.1) |
| 7 | Green vegetables | 95.0 (81.4-112) | 4.7 (4.1-5.2) |
| 8 | Other vegetables and sea weeds | 203 (181-215) | 10.1 (9.2-11.2) |
| 9 | Beverages | 496 (390-587) | 24.4 (20.4-27.4) |
| 10 | Fish and shellfish | 97.7 (82.2-120) | 4.8 (4.2-6.1) |
| 11 | Meat and eggs | 110 (105-116) | 5.4 (5.1-5.7) |
| 12 | Milk and dairy products | 166 (147-194) | 8.3 (7.1-9.8) |
| 13 | Other foods (seasoning) | 88.4 (78.0-112) | 4.4 (4.1-5.2) |
| | Total | 2020 (1910-2150) | |

*Mean and range in 6 regions obtained from the data of Japanese Nutrition Survey (the Ministry of Health, Labour and Welfare of Japan).

**Results and Discussion**

We analyzed brominated dioxins and PBDEs in food mixtures from each of 13 food groups from 6 regions in Japan. In our study, the LODs (Limit of Detection) of PBDD/DFs were 0.01 pg/g wb for tetra and penta, 0.05 pg/g wb for hexa, 0.1 pg/g wb for hepta and 1 pg/g wb for octa. The LODs of PBDEs were 0.1 pg/g for tetra-hepta, 0.2 pg/g for octa, 0.5 pg/g for nona and 1 pg/g for deca.

From the results of analyzing brominated dioxins, only 1,2,3,4,6,7,8-HpBDF was detected in the mixture of group 4 (fats and oils) at 0.14-0.44 pg/g wb. MoBrPCDD/DFs congeners were not detected in any food mixtures.

Table 3 shows data for the daily intakes calculated from the concentration of brominated dioxins and PBDEs in each food group. The daily intake was estimated assuming that when a congener was below the limit of detection, the concentration was either equal to zero (ND=0) or one-half of LOD. The WHO has stated that use of the same TEF values for the PBDD/PBDF or PXDD/PXDF congeners as the chlorinated analogues appears to be justified. To estimate the influence of brominated dioxins, we calculated the total TEQ per day, using the TEFs of chlorinated dioxins. The mean daily intake was calculated as 0.00056 pg TEQ /kg body weight /day on a 50kg body weight (assuming ND = 0). Due to the small daily consumption of fats and oils, the daily intake of brominated dioxins was at a low level. In the case assuming that ND = 1/2LOD, the mean daily intake was calculated as 1.58 pg TEQ /kg body weight /day. In an investigation of chlorinated dioxin by a market basket study in Japan[4], the amount of daily intake was 1.2 pg TEQ / kg body weight /day. Even if the value of PBDD/DFs is added to the amount of chlorinated dioxin exposure, it was estimated to be within Japanese TDI (4 pg TEQ / kg body weight /day).

PBDE congeners were detected in all food mixtures. The highest PBDEs concentration was found in group 4 at 2110 pg/g wb (1190 - 3090 pg/g wb), followed by group 10 at 474 pg/g wb (237 - 840 pg/g wb). On the other hand, the concentrations of PBDEs in groups 7, 8, and 9 were at low levels. In a recent market basket study in Spain[5], the highest concentration of total PBDEs (tetra-octa) was found in oils and fats (587.7 - 569.3 ng/kg wb), followed by fish and shellfish (333.9 - 325.3 ng/kg wb), meat products (109.2 - 102.4 ng/kg wb), and eggs (64.5 - 58.3 ng/kg wb). In a market basket survey of U.S. food [6], it was reported that levels of PBDEs (tri-deca) were highest in fish (median 1725 pg/g wb), then meat (median 283 pg/g wb), and daily products such as butter and margarine (median 31.5 pg/g wb). In these reports, a high concentration of PBDEs was found in fatty food groups, such as fish, oils and fats, and meat.

For PBDE, the mean daily intake was estimated as 109 ng /day (80.2 - 140 ng /day) assuming that ND = 0. The daily intakes in other countries were reported 90.5ng / day for U.K.[7] and 81.9-97.3 ng/day for Spain[5]. Although there were some differences in analyzing congeners between studies, the daily intake in this study was close to levels of these data. The daily intake contribution of PBDEs was 45.8% (group 10), 21.1% (group 4) and 8.4% (group 11). The results suggest that the most prominent source of PBDEs is attributed to fish.

The mean daily intake was calculated as 2.17 ng / kg body weight /day (1.6 - 2.8 ng / kg body weight /day) on a 50kg body weight (assuming ND = 0). In the case assuming that ND = 1/2 LOD, the daily intake was calculated at 2.22 ng /kg body weight /day. In a recent report, the lowest observed adverse effect level (LOAEL) value suggested as reasonable for compounds or mixtures belonging to the PBDE group was 1mg / kg body weight / day [8]. Since the calculated value in this study was much less than this LOAEL value, the daily intake level of PBDEs was not considered a serious problem. However, it is important to collect more data about brominated dioxin and BFRs in food because little information is available regarding the levels of these brominated compounds.

**Acknowledgement**

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Table 2 Analytical conditions of HRGC/HRMS

| | Column | Injection temp. | Injection type Injection volume | Oven temp. | HRMS Conditions |
|---|---|---|---|---|---|
| PBDD/DFs MoBrPCDD/DFs | DB-5 (J&W Scientific, CA) 30m, 0.25mm(i.d.), 0.1μm film | 240°C | Splitless 1μl | 130° C - (20° C/min) -240 ° C - (5° C/min) 320° C(7.5min) | Electron energy 38eV Filament current 750μA |
| PBDEs | HP-5MS(Agilent Technology, CA) 15m,0.25mm(i.d.), 0.1μm film | 240°C | Splitless 1μl | 120° C (2min) - (20° C/min) -200 ° C - (10° C/min) 300° C (1min) | Ion source temp. 270° C Resolution 10,000 |

Table 3 Daily intake of brominated dioxins and PBDEs in Japan

| | Food group | Brominated dioxins pgTEQ / day | | PBDEs ng / day | |
|---|---|---|---|---|---|
| | | ND=0* | ND=1/2LOD** | ND=0* | ND=1/2LOD** |
| 1 | Rice and rice products | 0 | 15.4 (11.9-19.3) | 4.4 (1.2-8.4) | 5.0 (2.0-8.8) |
| 2 | Cereals seeds and potatoes | 0 | 9.1 (5.9-14.2) | 3.3 (2.3-4.2) | 3.6 (2.6-4.3) |
| 3 | Sugars and confectioneries | 0 | 1.4 (1.1-1.7) | 2.2 (0.7-3.8) | 2.2 (0.7-3.9) |
| 4 | Fats and oils | 0.028 (0.013-0.045) | 0.4 (0.3-0.5) | 23.3 (10.7-35.2) | 23.3 (10.7-35.2) |
| 5 | Pulses | 0 | 2.3 (1.6-3.4) | 1.5 (0.5-3.0) | 1.5 (0.5-3.1) |
| 6 | Fruits | 0 | 4.8 (4.5-5.3) | 1.5 (0.05-6.3) | 1.7 (0.3-6.4) |
| 7 | Green vegetables | 0 | 3.4 (2.7-4.2) | 0.6 (0.06-1.3) | 0.7 (0.2-1.4) |
| 8 | Other vegetables and sea weeds | 0 | 7.4 (6.6-8.4) | 0.6 (0.06-1.4) | 1.0 (0.5-1.7) |
| 9 | Beverages | 0 | 17.3 (13.6-20.5) | 1.2 (0.2-2.6) | 1.9 (1.0-3.2) |
| 10 | Fish and shellfish | 0 | 3.9 (2.9-5.3) | 50.8 (26.7-75.4) | 50.9 (26.8-75.4) |
| 11 | Meat and eggs | 0 | 4.3 (3.1-4.9) | 8.9 (5.8-13.5) | 8.9 (5.9-13.5) |
| 12 | Milk and dairy products | 0 | 5.8 (5.1-6.8) | 2.7 (0.6-4.9) | 2.9 (0.9-5.1) |
| 13 | Other foods (seasoning) | 0 | 3.8 (2.7-8.2) | 7.6 (1.6-16.5) | 7.6 (1.7-16.5) |
| | total | 0.028 (0.013-0.045) | 79.2 (66.7-95.1) | 109 (80.2-140) | 111 (82.5-142) |
| | Daily intake*** | 0.00056 (0.00026-0.0009) pgTEQ/kg/day | 1.58 (1.33-1.90) pgTEQ/kg/day | 2.17 (1.60-2.79) ng/kg/day | 2.22 (1.65-2.84) ng/kg/day |

Mean daily intakes of 6 regions are given. Values in parentheses were ranges of the daily intake in 6 regions.
*Daily intake calculated assuming that ND = zero. ** Daily intake calculated assuming that ND = 1/2LOD.
*** Daily intake calculated in the case assuming that an average body weight of a Japanese adult is 50 kg.

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Research Article

# Determination of brominated flame retardants and brominated dioxins in fish collected from three regions of Japan

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The concentrations of brominated dioxins which are polybrominated dibenzo-*p*-dioxins/polybrominated dibenzofurans (PBDD/DFs) and mono-bromo polychlorinated dibenzo-*p*-dioxins/dibenzofurans, polybrominated diphenyl ethers (PBDEs) and tetrabromobisphenol A (TBBPA) were investigated in a total of 45 fish samples collected from three regions in Japan. In the brominated dioxins, 1,2,3,4,6,7,8-heptabromodibenzofuran (HpBDF) was the most abundant congener, and it was found in seven fish samples at 0.10–25.6 pg/g wet weight (ww). The highest concentration of 1,2,3,4,6,7,8-HpBDF was found in the pike eel. Regarding other congeners, 2,3,7,8-tetrabromodibenzo-*p*-dioxin was detected in the sea bream at 0.02 pg/g ww, and 2,3,7,8-tetrabromodibenzofuran was detected in the conger eel at 0.03 pg/g ww. 3-Bromo-2,7,8-trichlorodibenzofuran was detected in the *Sardinella zunasi* and the conger eel at 0.01 pg/g ww and 0.02 pg/g ww, respectively. Using toxic equivalency factors of chlorinated dioxins, we calculated the PBDD/DFs concentrations of these fish samples at 0.001–0.256 pg TEQ/g ww. PBDEs were detected in all of the fish samples. The concentrations of total PBDEs were 0.01–2.88 ng/g ww. The seerfish and the yellowtail containd PBDEs in high concentrations. The most dominant congener in most of the fish was 2,2′,4,4′-tetrabromo diphenyl ether. TBBPA was detected in 29 fish samples at 0.01–0.11 ng/g ww. The mean level of TBBPA was about one-tenth or less of the total level of PBDEs. A good correlation was obtained between total PBDEs and fat content. On the other hand, no correlation was obtained between TBBPA and fat content. The daily intakes from fish were estimated to be 0.58 ng/kg body weight (bw)/day for total PBDEs, 0.03 ng/kg bw/day for TBBPA, and 0.01 pg TEQ/kg bw/day for brominated dioxins in the case assuming that the average bw of a Japanese adult person is 50 kg and that the average fish consumption is 82 g/day. For PBDEs, the provisionally calculated value was much less than the lowest observed adverse effect level value (1 mg/kg bw/day). For brominated dioxins, the daily intake was at a very low level compared with the Japanese daily intake of polychlorinated dioxins from fish. Even if the value of PBDD/DFs is added to the amount of chlorinated dioxin exposure, it was estimated that it is less than the tolerable daily intake (4 pg TEQ/kg bw/day) in Japan.

**Keywords:** Accelerated solvent extraction / Fish / Polybrominated dibenzo-*p*-dioxins, dibenzofurans / Polybrominated diphenyl ethers / Tetrabromobisphenol A

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## 1 Introduction

Brominated flame retardants (BFRs) have been widely used in plastics and textiles because of their low cost and superiority in flame retardation. The BFRs used most frequently in Japan are tetrabromobisphenol A (TBBPA), hexabromocyclododecane, and polybrominated diphenyl ethers

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Abbreviations: **BDE-47**, 2,2′,4,4′-tetrabromo diphenyl ether; **BFRs**, brominated flame retardants; **bw**, body weight; **DeBDE** decabromodiphenyl ether; **HpBDF**, heptabromodibenzofuran; **HR**, high resolution; **PBDD/DFs**, polybrominated dibenzo-*p*-dioxins, dibenzofurans; **PBDEs**, polybrominated diphenyl ethers; **PCB**, polychlorinated biphenyl; **TBBPA**, tetrabromobisphenol A; **TEFs**, toxicity equivalency factors; **TEQ**, toxicity equivalent quantity; **ww**, wet weight