# Levels of polybrominated diphenyl-ethers and polybrominated dioxins in fish, total diet study food groups, and Japanese meals

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## Introduction

Since they were found in mother's milk and blood in several studies [1-3], the polybrominated diphenyl-ethers (PBDEs) and other polybrominated flame-retardants (BFRs) that are used in plastics, electrical appliances, and textiles have been recognized as ubiquitous pollutants. BFRs are precursors of polybrominated dibenzo-*p*-dioxins/ polybrominated dibenzofurans (PBDD/Fs). Recently, 2,3,7,8-TBDD/Fs and PBDEs have been detected in adipose tissue and blood in Japanese people[4]. Food is naturally suspected. However, there is very few information on food contamination with those brominated compounds in Japan . Therefore, we measured the levels of PBDEs and PBDD/Fs in various fish samples, meal samples, and total diet study (TDS) food groups and estimated Japanese people's dietary intake of PBDD/Fs and PBDEs.

## Methods and Materials

*Fish samples*: Nine fish samples (grunt, horse mackerel, thread-sail filefish, mackerel, pacific saury, razor-shell, sardine, sea bream, and young yellowtail) were purchased from grocery stores in Fukuoka during 2002~2003.

*Meal samples*: Samples weighing from 1565 to 3151 g/day were collected from six persons for 2~3 days, homogenized, and frozen before analysis.

*Total diet study* (TDS) *samples*: In Japan, total diet studies are carried out annually as a program of National Nutrition Survey. There, all foods that Japanese eat daily are classified to 14 TDS food groups as seen in Table 1. One sample per one group was prepared except for groups X, XI and XII. The preparation was as follows: several typical foods in each food group were chosen and sampled according to the amounts consumed by an average adult in Fukuoka district in the latest survey[5]. In each food group, foods were either cooked (boiled, grilled or roasted) beforehand or kept raw (such as sashimi), just as they were served for meal. Then all of the foods in each food group were mixed together. For groups X, XI, and XII, two samples (e.g. Xa and Xb) per group were prepared, using different foodstuffs from the respective groups. Group X IV (water) was not analyzed in this study.

One hundred grams of a fish sample or a TDS sample, or 500 g of a meal sample homogenate were freeze-dried and a $^{13}$C-labeled PBDD/F and PBDE mixture was added as a

clean spike. The sample was then extracted with hexane using an accerrelated solvent extractor (ASE 300,Dionex, USA) under the conditions of 100 °C, 1,500 psi. Each concentrated extract solution was treated with sulfuric acid and then cleaned using silica-gel column chromatography with 150 mL of 10% dichloromethane (DCM) in hexane as the eluate, and then using Floridil column chromatography with 150 mL of hexane (PBDE fraction) and 200 mL of 60% DCM/hexane (PBDD/F fraction). The PBDE fraction was cleaned using DMSO/hexane partitioning. The PBDD/F fraction was further cleaned using activated carbon chromatography with 50 mL of 10% DCM/hexane and 200 mL of toluene (PBDD/F fraction). The cleaned PBDD/F and PBDE fractions were concentrated and dissolved in 25 μL of nonane with $^{13}C_{12}$-OCDD or $^{13}C_{12}$-2,2',3,4,4',6-HxBDE as a syringe spike, respectively.

**Table 1 Average composition of total diet of average person in Fukuoka**

| Group | Foods in group | Av. wt, g/day | %(by wt) of total diet |
|---|---|---|---|
| □ | Rice and rice products | 409.0 | 25.6 |
| □ | Grains, seeds and potatoes | 192.8 | 12.1 |
| □ | Sugar and confectionaries | 32.6 | 2.0 |
| □ | Oils | 15.2 | 1.0 |
| □ | Legume and legume products | 73.2 | 4.6 |
| □ | Fruits | 113.9 | 7.1 |
| □ | Carrots and green leafy vegetables | 86.9 | 5.4 |
| □ | White leafy vegetables, mushrooms, and seaweeds | 184.6 | 11.5 |
| □ | Seasonings and beverages | 172.2 | 10.8 |
| □ | Fish and fish products | 82.1 | 5.1 |
| □□ | Meat and eggs | 107.9 | 6.8 |
| □□ | Milk and milk products | 122.5 | 7.7 |
| □□ | Other processed foods | 5.6 | 0.4 |
| □□ | Water | — | |
| | Total | 1598.5 | 100.0 |

*Analysis of PBDD/Fs and PBDEs by HRGC/HRMS*

GC6890 (Agilent, USA) /Autospec Ultima (Micromass, USA) was used at a resolution of >10,000 for PBDD/Fs. GC6890/MS5973 (Agilent) was used for PBDEs. The columns were a DB-5 (J&W, USA) (0.25 mm i.d. × 30 m, film thickness 0.1 μm) for the PBDD/Fs and a HP-5MS (Agilent, USA) (0.25 mm i.d. × 15 m, film thickness 0.1 μm) for the PBDEs.

**Results and Discussion**

***(1) Analysis of PBDD/Fs and PBDEs***

In this survey, no PBDD/Fs were detected in any of the fish or food samples. The limits of detection (LODs) were 0.01 (0.003 for meal samples) pg/g for tetra- and penta-BDD/F and 0.05 (0.005 for meal samples) pg/g for HexaBDD/F.

By contrast, PBDEs were found at 79~547 pg/g in eight of the nine fish samples; the exception was thread-sail filefish. The PBDE levels in mackerel, sea bream, and horse mackerel, which are favorites in the Japanese diet, were particularly high at 547, 503, and 472 pg/g, respectively. The major isomers in these samples were 2,2',4,4'-TeBDE (IUPAC No.#47)(avg. 39.3%), 2,2',4,5'-TeBDE (#49)(avg,16.3%), 2,2',4,4',6-PeBDE (#100) (avg,13.4%), and 2,2',4,4',5,6'-HxBDE (#154)(avg.13.7%)(Fig. 1).

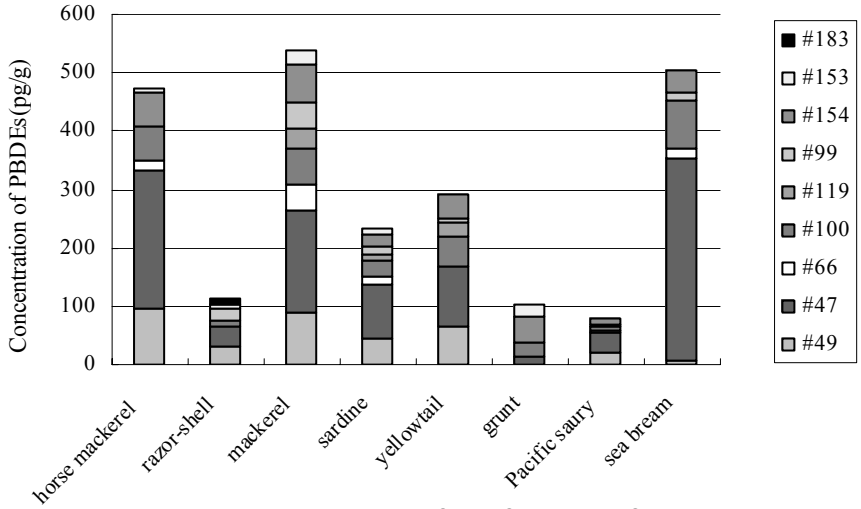


**Fig.1 Levels and congener profiles of PBDEs in fish samples**

In the meal samples collected from six individuals, PBDEs were found at 3.4~81.1 pg/g, and the major contributors were #47 (avg. 37.3%), #99 (2,2'4,4'5-PentaBDE, avg. 22.1%), #100 (avg. 11.2%), and #49 (avg. 8.7%) (Table 2, Fig.2).

On the other hand, among the 13 food group samples in the TDS, PBDEs were found in groups IV (Oil), X (Fish), XI (Meat & Eggs), and □□(Milk & milk products) at the concentrations of 122, 1259 (avg.), 64.7(avg.) and 8.6pg/g(avg.), respectively□Fig.3□. In the other groups PBDEs were below detection limit. By multiplying PBDE concentrations by consumption amounts of food groups in Table 1, daily intake of PBDEs can be calculated. In result, our data suggest that more than 90% of total PBDE intake by Japanese people derives from group X .

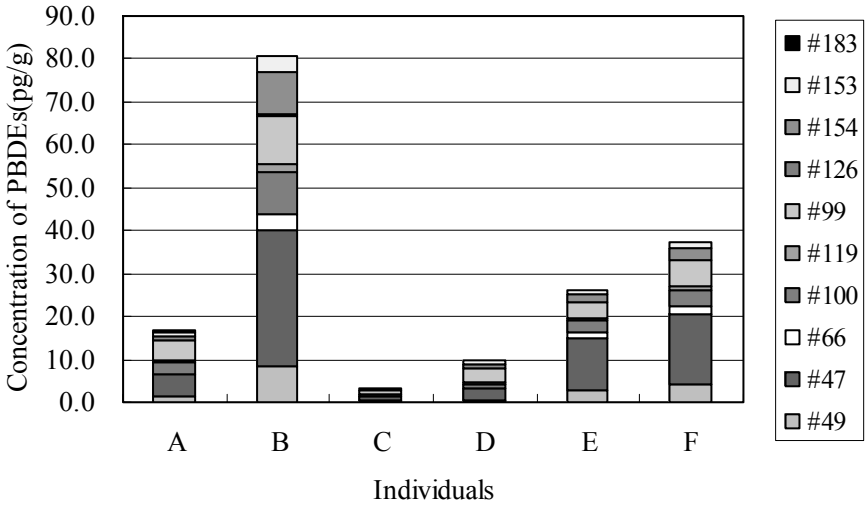


**Fig.2 Levels and congener profiles of PBDEs in the meal samples collected from 6 persons**

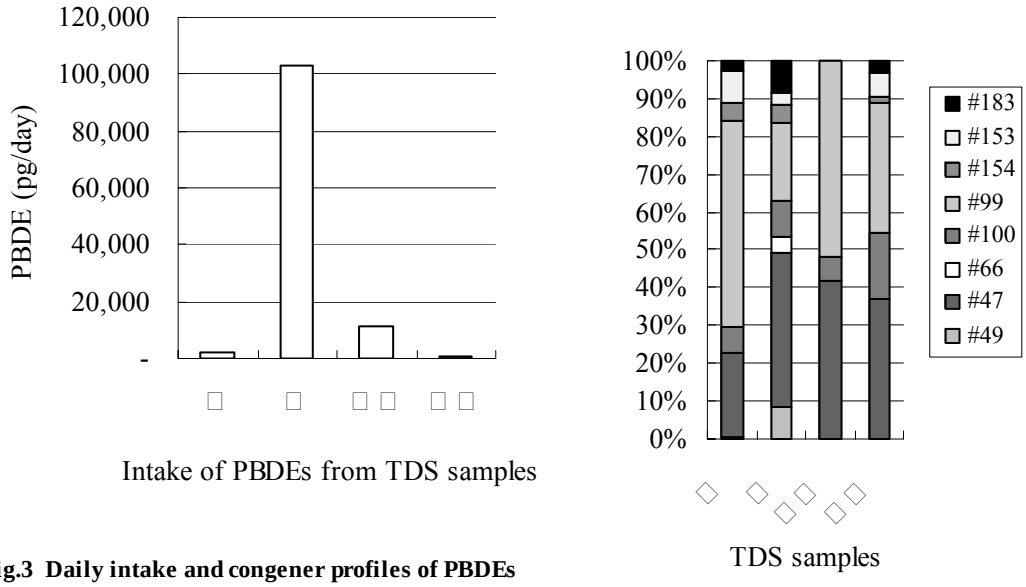


**Fig.3 Daily intake and congener profiles of PBDEs in the TDS samples**

Within the group X , congeners #47, #99, and #100 contributed an average of 44.3, 22.3, and 10.5%, respectively. Conversely, in groups IV and XI, penta-brominated congener #99 contributed more than tetra-brominated congener #47.

The concentration ratios between congener #99 and congener #47 in the meal samples and the group X are somewhat different from those in the raw fish samples. The PBDE congener profiles in the raw fish in this study are similar to those in raw Michigan salmon[6]. This is analogous to the phenomenon that lower chlorinated PCB congeners are more frequently found than higher ones in raw fish. We further speculate that not only various backgrounds of fish samples (i.e., fish age, cultured or not) but also certain cooking or processing procedures might have affected the isomer profile of PBDEs in the meal samples and the semi-cooked TDS sample (group X).

***(2) Estimation of dietary intake of PBDEs and PBDD/Fs by Japanese people***

As seen in Table 3, even after taking the intake of PCDD/Fs[7] into account, the total TEQs under the two conditions that are ND=0 and ND=1/2 × LOD were 1.24 and 1.59 pg TEQ/ kg b. w. /day, respectively. These TEQs are less than TDI (4pg TEQ /kg b. w. /day ) set in the Japanese law. The contribution of PBDEs and PBDD/Fs to the total TEQ was 5.3% at ND=0 and 19.3% at ND=1/2 × LOD. Therefore, PBDD/Fs and PBDEs have not so significant influence on the total TEQ at present. Nevertheless, given the huge volume of industrial wastes containing BFRs, the levels of these brominated contaminants in food should be continually monitored.

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**Table 2 Levels of PBDEs in the meal samples and daily intake of PBDEs by the six persons**

| PBDE congener | | A | B | C | D | E | F | Average |
|---|---|---|---|---|---|---|---|---|
| Tetra-brominated | #49 | 1.2 | 8.6 | 0.2 | 0.4 | 3.0 | 4.4 | 3.0 |
| | #71 | ND | ND | ND | ND | ND | ND | 0.0 |
| | #47 | 5.2 | 31.6 | 1.3 | 2.8 | 12.0 | 16.1 | 11.5 |
| | #66 | 0.3 | 3.8 | ND | 0.2 | 1.3 | 2.1 | 1.3 |
| | #77 | ND | 0.3 | ND | ND | ND | ND | 0.0 |
| Penta-brominated | #100 | 2.9 | 9.5 | 0.3 | 1.0 | 2.8 | 3.3 | 3.3 |
| | #119 | 0.4 | 2.0 | ND | 0.3 | 0.6 | 1.1 | 0.7 |
| | #99 | 4.5 | 11.1 | 1.1 | 3.2 | 3.6 | 6.0 | 4.9 |
| | #85 | ND | ND | ND | ND | ND | ND | 0.0 |
| | #126 | ND | 0.3 | ND | ND | ND | ND | 0.0 |
| Hexa-brominated | #154 | 0.9 | 10.1 | ND | 0.9 | 1.7 | 2.8 | 2.7 |
| | #153 | 1.2 | 3.6 | 0.3 | 0.9 | 0.9 | 1.3 | 1.4 |
| | #138 | ND | ND | ND | ND | ND | ND | 0.0 |
| Hepta-brominated | #183 | 0.5 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Total (pg/g) | | 17.0 | 81.1 | 3.4 | 9.9 | 26.2 | 37.4 | 29.2 |
| Total weight of meal (g) | | 1565 | 2623 | 3151 | 2123 | 2392 | 2016 | 2312 |
| daily intake of PBDEs(ng) | | 26.6 | 212.7 | 10.8 | 21.1 | 62.8 | 75.5 | 68.2 |
| TEQ* pg/kg b.w./day (ND=0) | | 0.0254 | 0.2438 | 0.0055 | 0.0159 | 0.0441 | 0.0562 | 0.065 |
| TEQ pg/kg b.w./day (ND=1/2xLOD**) | | 0.0419 | 0.2438 | 0.0416 | 0.0401 | 0.0704 | 0.0812 | 0.087 |

*TEQ was caliculated with the relative EROD activities (0.0032 for #77, 0.00024 for #100, 0.00035 for #119, 0.0024 for #126, 0.000048 for #153) by Chen *et al.*[8].

** LOD was 0.2pg/g for each PBDE congener.

**Table 3 Estimated average dietary intake of PBDEs, PBDD/Fs and PCDD/Fs by Japanese persons**

| | pgTEQ/kg.b.w./day | | | |
|---|---|---|---|---|
| | ND=0 | (%*) | ND=1/2 xLOD | (%*) |
| PBDE | 0.065 | 5.3 | 0.087 | (5.5) |
| PBDD/Fs** | 0.000 | (0.0) | 0.218 | (13.8) |
| PCDD/Fs | 1.170 | (94.7) | 1.280 | (80.8) |
| Total | 1.235 | | 1.585 | |

(%*):contribution

**:The TEFs[9] assigned to PCDD/Fs were tentatively used for PBDD/Fs.