# Determination of Brominated Flame Retardants in Fish and Market Basket Food Samples of Japan

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## Introduction

Brominated flame retardants (BFRs) have been used all over the world and detected in effluents such as those from sewage treatment plants and textile plants[1]. In most cases, such effluents drain into rivers or estuaries. BFRs are suspected as sources of poly-brominated dioxins. Consequently, social concern is increasing regarding the pollution of the environment and marine products by BFRs. Polybrominated diphenyl ethers (PBDE), a well-known group of BFRs, are lipophic and easily bio-accumulated in organisms through the food web; however, the toxicities have not yet been fully clarified. Tetra-brominated bisphenol A (TBBPA) is another representative group of BFRs whose demand by Japan industries in 2001 has increased to nearly 32,000 tons[2], which is more than ten times the demand for DecaBDE (2,200 tons), the only PBDE used in Japan. Since TBBPA has a more polar structure than PBDEs and has been reported to be readily metabolized, there has been a limited number of studies on the pollution in food such as marine products, compared with studies on the pollution caused by PBDEs. In this paper, we report the pollution levels of these BFRs in marine products collected at food market stores in Japan. Additionally, we discuss the estimated daily intakes of the BFRs by analyzing food group samples prepared following the Market Basket Method.

## Materials and Methods

*Marine products*: Eighteen fish of 15 species.

*Market basket food samples*: Thirteen mixed food samples were prepared in 2002, following the method of the Market Basket Study, alternatively termed theTotal Diet Study [3]. One hundred and sixty-six food items were chosen from 85 categories of foods that the Japanese populace commonly consumes, and the respective amounts of food items composing each of 13 food groups were decided by referring to the data of the latest national and prefectural surveys.

*Preparation of samples*: A homogenized sample (5~10 g) was spiked with $^{13}$C-labeled TBBPA as a clean-up standard and then packed in a stainless steel tube and extracted with methanol. The extraction conditions are listed in Table 1. The methanol extract (ca. 30~50 mL) was defatted by liquid-liquid partition with 20 mL of hexane. Then, 120 mL of 5% sodium chloride solution was added to the methanol layer fraction and re-extracted twice with 30~50 mL of dichloromethane. The extract was concentrated to dryness and then 1 mL of 1N potassium hydroxide and 0.2 mL of diethyl sulfate(Cica-Reagent) were added to it and the mixture was kept at 25~30 ºC for 30 min. After the mixture was treated with 4 mL of 1N potassium hydroxide at 70ºC for 1 hour, 3 mL of water was added to it and the solution was re-extracted with hexane. The hexane extract was cleaned by chromatography, the first, a florisil mini-column using an elution solvent of 8 mL of 2% diethyl ether/hexane, and when necessary, the second, a sulfuric-acid-impregnated silica-gel mini-column using an elution solvent of 15 mL of dichloromethane. The final eluate was concentrated, re-dissolved in 25 mL of nonane with 5 ng of chrysene-$d_{12}$ as a syringe spike and subjected to measurement by GC/MS. The analytical conditions are listed in Table 2.

Analysis of PBDEs was described in our paper, presented at the Dioxin 2004 in Berlin[4].

## Results and Discussion

The levels of TBBPA and ΣPBDEs in 16 mixed-food samples prepared based on the nutritional classification are

shown in Table 3. In this study, the LODs were 0.1 ng/g w.w. for TBBPA and 0.0001 ng/g w.w. for each PBDE congener. TBBPA was only detected at 0.46 ng/g w.w. in one of the two X samples, constituted from fish, shellfish and processed fish products. On the other hand, PBDEs were detected ranging from 0.00012 to 1.7 ng/g w.w. in all of the mixed-food samples except VI. The total consumption of TBBPA was estimated to be 18.8 ng/day at ND=0 and 98.4 ng/day at ND=1/2LOD. The 5-fold differences in the values between the two conditions suggested dependence upon the high-level LOD, in comparison with the non-significant difference in the values of ΣPBDEs between 114 ng/day at ND=0 and 115 ng/day at ND=1/2LOD.

The levels of TBBPA and ΣPBDEs in marine products are shown in Table 4. TBBPA ranged from ND (<0. 1 ng/g) ~3.0 ng/g (saury), while ΣPBDEs ranged from ND (<0.0001 ng/g)~1.2 ng/g (yellowtail) in the raw fish. Although ΣPBDEs appeared to be correlated with the fat contents, there is no correlation for TBBPA. Considering the likelihood of it being easily metabolized by organisms to the possible metabolites such as methoxy- or glucuronized-TBBPA, the exposure to TBBPA may not be considered to continue long, even if there is temporarily highly pollution with TBBPA by sewage or sludge from plants. The exposure to ΣPBDEs is thought to occur when eating food highly contaminated by food web accumulation as same as PCBs. In this study, the medians of TBBPA and ΣPBDEs in raw fish were 0.05 ng/g and 0.22 ng/g, respectively, on the condition that ND is assumed as 1/2 LOD. Regarding all the statistical values of average, median and frequency, the latest pollution by BFRs is considered more considerable by ΣPBDEs than by TBBPA, though the demand for PBDE s (only DecaBDE is now used in Japan) was far below that of TBBPA. For the toxicities in vivo, PBDEs have been reported to pose various toxicities, such as liver toxicity, disruption of thyroid hormones, developmental neuro-toxicity and carcinogenicity on animal tests[5]. In contrast, TBBPA have shown no adverse effects in vivo[6]. However, unexpected nephro-toxicity has been recently found in newborn rats treated with TBBPA [7]. In addition, the detection of TBBPA was reported in human serum by highly sensitive analysis using LC/MS[8]. Therefore, the monitoring of those BFRs in food should be continued to prevent the further pollution that may cause and/or increase risk for the health of humans and wildlife.

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Table 1 The extraction conditions

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|---|
| Apparatus: ASE-300 (Dionex) |
| Extraction Temp.: 50°C, Extraction Pressure: 1500psi |
| Cell Capacity: 33mL, Exraction Time: 10 min |
| Extraction Cycle: 3, Flash Capacity: 90%, Purge Time: 120 sec |

Table 2 The GC/MS conditions

| |
|---|
| GC/MS: HP6890/5973MSD |
| Column: DB-5(J&W) 0.25mmi.d.x30m, film thickness: 0.25mm |
| Injection Temp: 280°C |
| Column Temp: 120°C(1min)~20°C/min~300°C |

Table 3 Average composition of total diet of average person in Japan

| Group | Foods in group | Av. Weight g/day | TBBPA (ng/g,wet weight) | ΣPBDEs (ng/g,wet weight) |
|---|---|---|---|---|
| I | Rice and rice products | 409 | <0.1 | 0.00026 |
| II | Grains, seeds and potatoes | 192.8 | <0.1 | 0.0019 |
| III | Sugar and confectionaries | 32.6 | <0.1 | 0.0053 |
| IV | Oils | 15.2 | <0.1 | 0.12 |
| V | Legume and legume products | 73.2 | <0.1 | 0.0041 |
| VI | Fruits | 113.9 | <0.1 | ND |
| VII | Carrots and green leafy vegetables | 86.9 | <0.1 | 0.00056 |
| VIII | White leafy vegetables, mushrooms, and seaweeds | 184.6 | <0.1 | 0.00016 |
| IX | Seasonings and beverages | 172.2 | <0.1 | 0.00012 |
| Xa | Fish and fish products | 82.3 | 0.46 | 0.83 |
| Xb | | 81.8 | <0.1 | 1.7 |
| XIa | Meat and eggs | 110.5 | <0.1 | 0.1 |
| XIb | | 105.3 | <0.1 | 0.07 |
| XIIa | Milk and milk products | 122.5 | <0.1 | 0.0062 |
| XIIb | | 122.5 | <0.1 | 0.011 |
| XIII | Other processed foods | 38.1 | <0.1 | 0.0016 |
| XIV | Water | — | — | — |
| Total estimated comsumption(ng/day) at ND=0 | | | 18.8* | 114* |
| Total estimated comsumption(ng/day) at ND=1/2LOD | | | 98.4 | 115 |

* caliculated using the average TBBPA(or ΣPBDEs) values of Xa and Xb
LODs of 14 PBDE congeners are all below 0.0001ng/g.

Table 4 TBBPA and ΣPBDEs Concentrations in Various Marine Products

| Raw fish and shellfish | Fat Contents (%) | TBBPA (ng/g,wet weight) | ΣPBDEs (ng/g,wet weight) |
|---|---|---|---|
| yellowtail | 15 | <0.1 | 1.2 |
| horse mackerel | 13 | 3.0 | 0.087 |
| saury | 11 | 1.8 | 0.47 |
| sea bream A | 10 | 0.14 | 0.50 |
| salmon trout | 10 | <0.1 | 0.52 |
| sardine | 9.8 | 0.19 | 0.24 |
| mackerel | 9.1 | 0.81 | 0.55 |
| grunt | 4.8 | 1.6 | 0.11 |
| sea bream B | 3.5 | <0.1 | 0.28 |
| oyster-I | 3.2 | <0.1 | 0.01 |
| amberjack | 2.5 | 1.4 | 0.29 |
| oyster-k | 1.7 | <0.1 | 0.04 |
| striped beakperch | 1.3 | <0.1 | 0.22 |
| cuttlefish | 1.3 | <0.1 | 0.26 |
| razor−shell | 0.98 | <0.1 | 0.12 |
| tuna | 0.89 | <0.1 | 0.0086 |
| sea bass | 0.54 | <0.1 | 0.045 |
| leatherfish | 0.04 | 0.67 | <0.0001 |