# LEVELS OF PBDEs, TBBPA, TBPs, PCDDs/DFs, PXDDs/DFs AND PBDDs/DFs IN HUMAN MILK OF NURSING WOMEN AND DAIRY MILK PRODUCTS IN JAPAN

Souichi Ohta[1], Takashi Okumura[1], Hajime Nishimura[1], Teruyuki Nakao[1], Yoshiko Shimizu[2], Fumie Ochiai[2], Osamu Aozasa[1], Hideaki Miyata[1]

[1]Setsunan University, Hirakata
[2]Shizuoka Prefecture institute of University, Shizuoka

**Introduction**

Many consumer products in modern life contain brominated flame-retardant (BFR) chemicals for the purpose of fire protection. Polybrominated diphenyl ethers (PBDEs), Tetrabromobisphenol A (TBBPA) and Tribromophenol (TBPs) are used in large quantities for many applications such as television sets, computers, paints, and textiles etc. In Japan, the annual consumption of flame-retardants in 2002 was 164,000 tons, and the rate of BFRs for total consumption was 39 % (31,000 tons/yr). Further, that of DecaBDE,TBBPA and TBPs in 2002 was 2,200, 31,000 and 3800 tons, respectively. As a result, there is growing evidence that the large amounts of such BFRs in the environment are due to released during the manufacturing of these chemicals or consumer products containing these chemicals[1-3)]. In addition, there is sufficient evidence that the incineration of consumer products or manufacturing process of plastic materials containing such flame-retardant chemicals results in the formation of polybrominated/chlorinated dibenzo-p-dioxins (PXDDs) and –furans (PXDFs), polybrominated dibenzo-p-dioxins (PBDDs) and –furans (PBDFs)[4,5)]. These chemicals, as well as the BFRs, have been found to occur throughout the environment. And the intake of these contaminants from food, air and water is suspected to be the primary route of human. In humans, it was reported in 1990 on high concentration of PBDEs in adipose tissue[6)]. Since that time, several studies in Sweden, Canada and the USA have reported human contamination in adipose tissue, serum blood lipid, and breast milk by PBDEs[7,8)]. Therefore, because PBDEs are extremely lipophilic and stable substances in vivo, there has been much concern about future adverse effects on human health in these countries and elsewhere.

At present, little is known about human pollution of the above brominated compounds in Japan[9,10)]. In this study, in order to generally evaluate Japanese body burden and future adverse effects of newborn infant by PBDEs, TBBPA, TBPs, PCDDs/DFs, PXDDs/DFs and PBDDs/DFs, we investigated the pollution levels of above contaminants in Japanese Brest milk. As similar purpose, it was also investigated the contamination levels in some dairy milk products like cow's milk and powdered milk.

**Materials and Methods**

**1) Samples**

The samples of mother's milk were collected from sixteen primiparae and twenty multiparae at one month after delivery in 2002. Each 50 g of mother's milk from four primiparae and four multiparae individual was gathered as one sample, Finally, 200g of mother's milk (four group of primiparae: age; 21-31 years old, sample A ~ D, five group of multiparae: age; 22~33 years old, sample a ~ e) was used for this study. After health conditions, clinical history, dietary and smoking habit etc. among the women were ascertained using the brief questionnaire method, and then selected the above thirty-six healthy women at the beginning of this investigation. Samples of cow's milk and powdered milk for newborn baby were purchased from four markets in Hirakata and Osaka city of Osaka prefecture of Japan in 2004.

**2) Analytical method**

For the analysis of PBDEs, the quantification of PBDE congeners was performed by the method of relative calibration curves using seven different $^{13}C_{12}$-labelled BDE isomers (#28, 47, 99, 154, 183, 209) and thirty-four unlabelled native standards (TriBDEs; #17, 25, 28, 30, 32, 33, 35, 37, TeBDEs; #47, 49, 66, 71, 75, 77, PeBDEs; #85, 99, 100, 105, 116, 119, 126, HxBDEs; #138, 140, 153, 154, 155, 166, HpBDEs; #181, 183, 190, OcBDEs; #196, #203, NoBDEs; #206, DeBDE; #209) purchased from Cambridge Isotope Laboratories (MA, USA) and Wellington Laboratories (Canada). $^{13}C_{12}$-labelled BDE isomer for the analysis of native OcBDE and NoBDE congeners was substitute for that of HpBDEs and DeBDE isomer, respectively. Other analytical conditions were performed according to our previous paper[9]. Next, after addition of $^{13}C_{12}$-labelled TBBPA and TBPs, and then the lipids was similarly extracted. TBBPA and TBPs (2,4,5- and 2,4,6-TBP) in the extracts were treated with diethyl sulphate. Then, TBBPA and TBPs derivatives were performed to purify by florisil column chromatography, with an eluent of 4% diethyl ether/n-hexane. With respect to the quantification of seventeen PCDDs/DFs (seven PCDD isomers and ten PBDF isomers), five PBDDs (2,3,7,8-TeBDD, 1,2,3,7,8-PeBDD, 1,2,3,4,7,8-HxBDD, 1,2,3,6,7,8-HxBDD and 1,2,3,7,8,9-HxBDD), five PBDFs (2,3,7,8-TeBDF, 1,2,3,7,8-PeBDF, 2,3,4,7,8-PeBDF, 1,2,3,4,7,8-HxBDF and 1,2,3,4,6,7,8-HpBDF), six PXDDs (2-Br-3,7,8-TriCl-DD, 2,3-DiBr-7,8-DiCl-DD, 1-Br-2,3,7,8-TeCl-DD, 2-Br-3,6,7,8,9-PeCl-DD, 1-Br-2,3,6,7,8,9-HxCl-DD and 1-Br-2,3,6,7,8,9-HxCl-DD) and two PXDFs (3-Br-2,7,8-TriCl-DF and 1-Br-2,3,7,8-TeCl-DF) congeners, the purified method was multi-layer silica-gel column chromatography, with an eluent of n-hexane and $CH_2Cl_2$:n-hexane (1:4). The eluate was concentrated and purified by an active carbon dispersed silica-gel column with eluent of n-hexane, $CH_2Cl_2$: n-hexane (1:3) and toluene. All purified sample was analyzed by the use of HP6890 GC-JEOL JMS700 MS (HRGC-HRMS) at high-resolution condition (R=10,000) in EI-SIM mode. TBBPA and TBPs was also determined by HRGC-HRMS in EI-SIM mode using $^{13}C_{12}$-labelled internal standard. As the evaluation method of toxicity or budy burden for PXDDs/DFs and PBDDs/DFs, It was assumed that the toxicity of same congener of PBDDs/DFs or PXDDs/DFs is nearly equal to that of PCDDs/DFs. On the basis of this assumption, the contribution ratio to total TEQ by PCDDs/DFs, PBDDs/DFs and PXDDs/DFs was calculated by using 2,3,7,8-TCDD equivalent factors (WHO-TEF).

**Results and Discussion**

At first, lipid concentration of each mixed brest milk sample was compared, their lipid concentration ranged between 2.96 and 4.21 % (average concn, 3.39%). The big difference of lipid concentration was not recognized among the nine mixed samples. As shown in Figure 1, the levels of PCDDs/DFs and PXDDs/DFs in the mixed brest milk of multiparae and primiparae were investigated. Actual concentrations of Σ PCDDs/DFs in both samples (except sample b) was approximately equal, but, TEQ concentration(average concn.; 17.8 pg TEQ/g lipid) in the samples from primiparae was over two-fold against that (average concn.; 8.4 pg TEQ/g lipid) from multiparae. On the other hand, an extremely interesting phenomenon was observed; actual concentrations of PXDDs/DFs was very low, compared to that of PCDDs/DFs, however, it was clear that tha ratio of Σ PXDDs/DF for Σ PCDDs/DFs calculated as TEQ concentartion ranged between 4 and 46 %. This data suggested that both dioxin analogues were arised from same contamination sources like municipal and industrial incinerators.

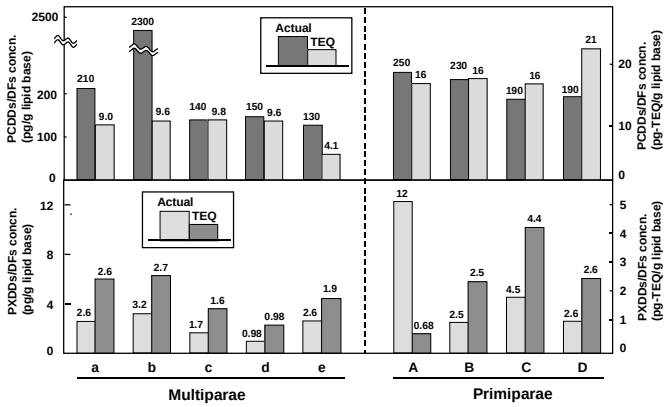


**Fig. 1: Levels of PCDDs/DFs and PXDDs/DFs in the mixed brest milk of multiparae and primiparae**

The concentations of PBDDs/DFs and PBDEs in the mixed brest milk of multiparae and primiparae are presented in Figure 2. Actual Σ PBDDs/DFs concentration (average concn.;269 pg/g lipid) in the brest milk of primiparae was over six-fold against that (average concn.;41.4 pg/g lipid) of multiparae. Futher, TeBDDs was dominant (50 ~ 90%), especially observing as 1,3,6,8- and 1,3,7,9-TeBDD isomer (data not shown). From this observation, it was estimated that there are a large amount of TeBDDs in human bodies, which derived from the pyrolysis or photoysis of BFRs like bromophenol in the environment. The average contribution ratio of PBDDs/DFs for Total TEQ concentation in the sample from primiparae and multiparae was 1.2 and 3.2%, respectively, and it was basically neglectable level. With respect to PBDEs contamination, its concentartion ranged between 2,000 and 13,000 pg/g lipid. In addition, A remarkable contamination in the mixed

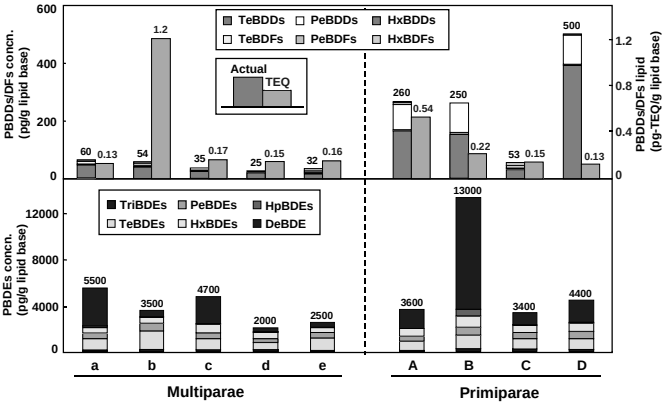


**Fig. 2: Levels of PBDDs/DFs and PBDEs in the mixed brest milk of multiparae and primiparae**

sample B was notified, observing at 13,000 pg/g lipid. Therefore, it was suggested that sample B contains the brest milk polluted by extreme high concentration of PBDEs.

Figure 3 shows the comparison of the levels of TBBPA and TBPs in the mixed samples of brest milk from multiparae and primiparae. The annual demand of TBBPA was about 10 folds against that of TBPs, however, the range of TBBPA concentration in multiparae and primiparae was between 180 to 450 pg/g lipid and 270 to 940 pg/g lipid, respectively. In contrast, that of TBPs concentration in the multiparae and primiparae was between 810 to 3,900 pg/g lipid and 1,800 to 110,000 pg/g lipid, respectively. Similar to the case of PBDEs as described above, TBBPA and TBPs concentration in the sample B was also most high among all samples analyzed, Thus, among four testee of sample B, it was estimated that there was a primipara contaminated by extreme high concentration of the above BFRs. This estimation was also supported from the average lipid content (3.12%) in the sample B.

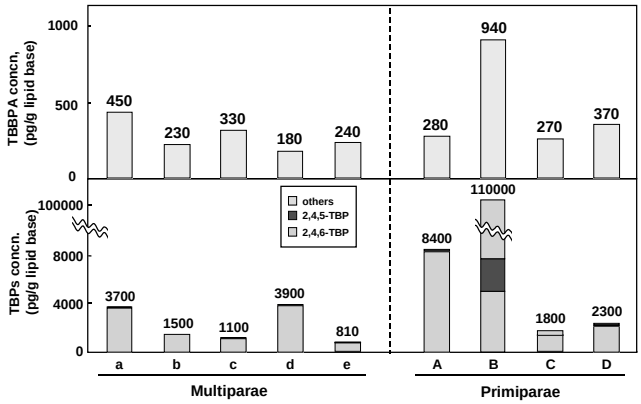


**Fig. 3: Levels of TBBPA and TBPs in the mixed brest milk of multiparae and primiparae**

Figure 4 compares the congener ratio for chlorine/bromine substituted dioxins/furans and 2,3,7,8- bromine substituted dioxins/ furans in both samples. With respect to the ratio of PBDDs/DFs, the abundant isomers were 2,3,7,8-TeBDF and 2,3,4,7,8-PeBDF, other 2,3,7,8-bromine substituted isomers were not detected, except low level of 1,2,3,7,8-PeBDF in the samples of primiparae. However, comparative high levels of 1,3,7,8- and 1,3,7,9-TeBDD could be detected in all samples (data not shown). In the case of the ratio of PXDDs/DFs, it was observed that the level of other congeners except 2,3,7,8-chlorine/bromine substituted compounds was extreamly low, and abundant isomers were 2,3-Br-7,8-Cl-DD (over 60%), followed by 3-Br-2,7,8-Cl-DD and 2-Br-3,7,8-Cl-DD. As there are theoretical five thousands and twenty kinds of congeners in the group of PXDDs/DFs, it needs further detail survay of PXDDs/DFs contamination in the brest milk.

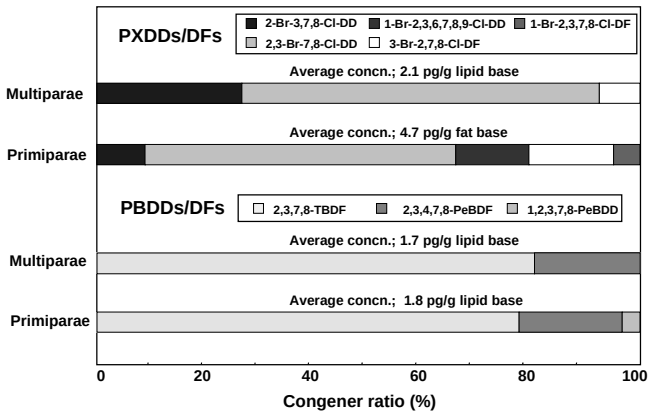


**Fig. 4: Congener ratio for Σ 2,3,7,8-PXDDs/DFs and Σ 2,3,7,8-PBDDs/DFs**

As shown in Table 1, total body burdens for new-born babies by Σ PCDDs/DFs, Σ PXDDs/DFs and Σ PBDDs/DFs were tried to calculate by using the toxic equivalent quantity of 2,3,7,8-TCDD. The average body burden (99.4 pg TEQ/g lipid) of newborn babies from primiparae is approximately two-fold for that (55.8 pg TEQ/g lipid) from multiparae. In addition, the contribution ratio for total body burdens by each congener was in order, Σ PCDDs/DFs > Σ PXDDs/DFs > Σ PBDDs/DFs. Especially, average ratio of Σ PXDDs/DFs in the samples of multiparae and primiparae was 17.9 and 12.6%, respectively. The impact of health for newborn babies is not known, but it was over our expectation results.

Figure 5 shows the concentrations of Σ PBDEs in the samples of mixed mother's milk and dairy milk products (cow's milk and powdered milk for new-born baby). The concentrations of PBDE in the cow's milk and powdered milk were ranged between 11,000 to 40,000 and 2100 to 130,000 pg/g lipid, respectively. Both levels with PBDEs contamination were higher than that of mother's milk. A marked contamination was observed in two samples (II and IV) of powdered milk, showing incredible concentration by highly brominated congeners like HpBDEs. The reason of contamination is presently unclear, from raw milk materials or manufacturing process.

Further study is warranted to evaluate whether PBDEs exposures to nursing infants pose a health risk. Additional investigations of PBDEs in dairy milk products, fish and other foods are warranted to better understand the nature and extent of PBDE contamination of Japanese food supply.

**Table 1: Estimation of total body burdens for new-born babies by Σ PCDDs/DFs, Σ PXDDs/DFs and Σ PBDDs/DFs in brest milk**

| | Multiparae (pg-TEQ/kg/day) | | | | | | Primiparae (pg-TEQ/kg/day) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | d | e | Average | A | B | C | D | Average |
| PCDDs/DFs | 45.6 | 44.4 | 45.9 | 60.5 | 22.6 | 43.8 | 69.8 | 47.9 | 70.0 | 74.4 | 65.5 |
| Ratio (%) | 76.8 | 70.4 | 85.0 | 89.4 | 66.7 | 78.8 | 92.7 | 78.9 | 77.4 | 82.0 | 82.7 |
| PXDDs/DFs | 13.1 | 12.9 | 7.26 | 6.21 | 10.4 | 9.97 | 3.04 | 11.8 | 19.7 | 15.5 | 12.5 |
| Ratio (%) | 22.1 | 20.4 | 13.5 | 9.17 | 30.7 | 17.9 | 4.07 | 19.4 | 21.8 | 17.1 | 15.8 |
| PBDDs/DFs | 0.68 | 5.84 | 0.79 | 0.96 | 0.85 | 1.82 | 2.41 | 1.03 | 0.66 | 0.77 | 1.22 |
| Ratio (%) | 1.10 | 9.20 | 1.50 | 1.43 | 2.60 | 3.30 | 3.23 | 1.70 | 0.80 | 0.90 | 1.50 |
| Total | 59.4 | 63.1 | 54.0 | 67.7 | 33.9 | 55.6 | 75.3 | 60.7 | 90.4 | 90.7 | 79.2 |

* Total body burden for newborn baby was calculated, assuming that baby drink 150 mL of mother's milk per kg weight for one day.

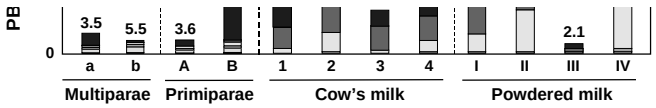


**Fig. 5: Comparison of PBDEs concentrations in the samples of mixed mother's milk and dairy milk products**

**Ackowlegements**

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