Determination of Co-Planar Polybrominated/chlorinated Biphenyls in Human Breast Milk from Twenty Women of JAPAN

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Introduction

As is generally known, there has been extremely homologue/isomer of numbers in so-called "dioxins", which has consisted of halogenated compounds having chemical structure of dibenzo-p-dioxins, dibenzofurans and biphenyls. In fact, there are 75 isomers as dibenzo-p-dioxins, 135 isomers as dibenzofurans in polychlorinated dioxins (PCDDs/DFs), while are 209 isomers as biphenyls in polychlorinated biphenyls (PCBs). To evaluate their toxicity, it is present situation that 29 dioxin congener are measured. On the other hand, with respect to polybrominated/chlorinated dibenzo-p-dioxins (PXDDs) and –furans (PXDFs) having the above similar bone structures, there are theoretically about 4600 homologue/isomer, and 2, 3, 7, 8- congeners exist over 900 among them.

We investigated the TEQ levels of PCDDs/DFs (17 congeners), PXDDs/DFs (8 isomer) and PBDDs/DFs (9 congeners) in thirty six samples of mother's milk[1)]. The contribution ratio of PCDDs/DFs, PXDDs/DFs (8 congeners) and PBDDs/DFs (9 congeners) for total TEQ level was 67-93, 11-31 and 0.6-3.1%, respectively. In results, it was observed that such ratio of PXDDs/DFs for human pollution was unexpectedly high, and that of PBDDs/DFs can be ignored. Similar results can refer to human pollution by coplanar polychlorinated/brominated biphenyls (Co-PXBs). From such standpoint, we have recently reported the occurrence of polybrominated chlorinated biphenyls (PXBs) in biota collected from various global regions[2)].

In this paper, comparing to the levels of Co-PCBs, we investigated that of Co-PXBs in the breast milk of Japan.

Materials and Methods

1) Samples

The samples of mother's milk were collected from twenty women (age; 22~33 years old, sample A ~ T) at one week after delivery between November 2006 and May 2007. 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 seven healthy women at the beginning of this investigation.

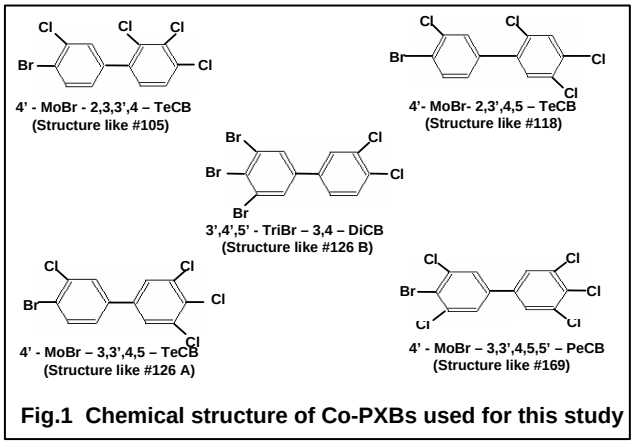


Fig.1 Chemical structure of Co-PXBs used for this study

2) Analytical method

As shown in Fig. 1, we used five $^{13}C_{12}$-labelled and five unlabelled Co-PXBs in this study; 4'-MoBr-2,3,3',4-TeCB (structure like PCB #105), 4'-MoBr-2, 3',4,5-TeCB (like PCB #118), 4'-MoBr-3,3',4,5-TeCB (#126A; like PCB #126), 3',4', 5'-TriBr-3,4-DiCB (#126B; like PCB #126) and 4'-MoBr-3,3',4,5,5'-PeCB (like PCB #169) purchased from Cambridge Isotope Laboratories (MA, USA). Each 50 g of mother's milk were used for this study. The extractions of Co-PXBs congeners in these samples were performed according to our previous paper [2- 4)]. For the analysis of Co-PXBs, the purified method was multi-layer silica-gel column chromatography, with an eluent of n-hexane. The

eluate was concentrated and purified by an active carbon mixed silica-gel column with eluent of 25% CH_2Cl_2 in n-hexane (#105, #118) and toluene(#169, #126A&B). 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. As the evaluation method of toxicity (TEQ level) for Co-PXBs and PXDDs/DFs, It was assumed that the toxicity of same congener of Co-PXBs or PXDDs/DFs is nearly equal to that of Co-PCBs and PCDDs/DFs. On the basis of this assumption, each contribution ratio to total TEQ by PCDDs/DFs, Co-PXBs, PXDDs/DFs and Co-PXBs was calculated by using 2, 3, 7, 8-TCDD equivalent factors (WHO-TEF, 2005)[5]. Other detail methods should be referred to our paper[2, 6].

Results and Discussion

From many results of the past Co-PCBs pollution in biological specimen, we selected and investigated to the above five Co-PXBs, having high frequency and high TEF. As shown in Table 1, it was compared the actual Co-PXBs concentrations in Japanese breast milk. Co-PXBs were detected in all samples, their total concentration ranged between 11.9 and 348.5 pg/g lipid wt, showing as average concentration of 57.0 pg/g lipid wt. Especially, it was the highest concentration in the Sample O. Interestingly, four congeners except #126B were observed in almost samples. Thus, we detected 3', 4', 5'-TriBr-3, 4-DiCB (#126B) detected only in almost

Table 1. Coplanar PXB Concentrations in Breast Milk from Twenty Women (Sample A - T) of Japan (all values in pg/g lipid). *** ND: not detected (S/N< 3)**

Co-PXBs / Sample	4'Br-23'45Cl-B (#118)	4'Br-233'4Cl-B (#105)	4'Br-33'45Cl-B (#126A)	4'Br-33'455'Cl-B (#169)	3'4'5'Br-34Cl-B (#126B)	Total (pg/g lipid)
A	26.5	22.7	20.7	29.8	ND*	99.7
B	27.5	4.5	5.4	3.0	ND	40.4
C	17.1	13.1	10.2	10.5	ND	50.9
D	32.2	7.5	5.4	6.2	ND	51.3
E	6.6	3.6	3.2	2.0	ND	15.4
F	6.3	4.8	7.2	6.6	ND	24.9
G	20.2	13.7	17.3	15.3	ND	66.5
H	26.5	6.7	6.5	6.4	7.5	53.6
I	23.3	9.3	5.3	5.5	ND	43.4
J	40.5	15.2	12.3	11.0	ND	79.0
K	13.8	6.8	6.2	5.8	ND	32.6
L	11.6	5.1	3.9	4.0	7.4	32.0
M	11.7	6.8	7.0	5.6	9.9	41.0
N	8.1	4.0	6.1	4.8	8.6	31.6
O	105.7	91.2	75.8	75.8	ND	348.5
P	ND	2.1	0.8	11.0	0.9	14.8
Q	7.6	18	5.6	21	2.2	54.4
R	2.4	3.2	1.6	4.5	2.2	15.3
S	3.5	5.9	1.7	18	4.1	33.2
T	ND	2.3	1.3	7.1	1.2	11.9

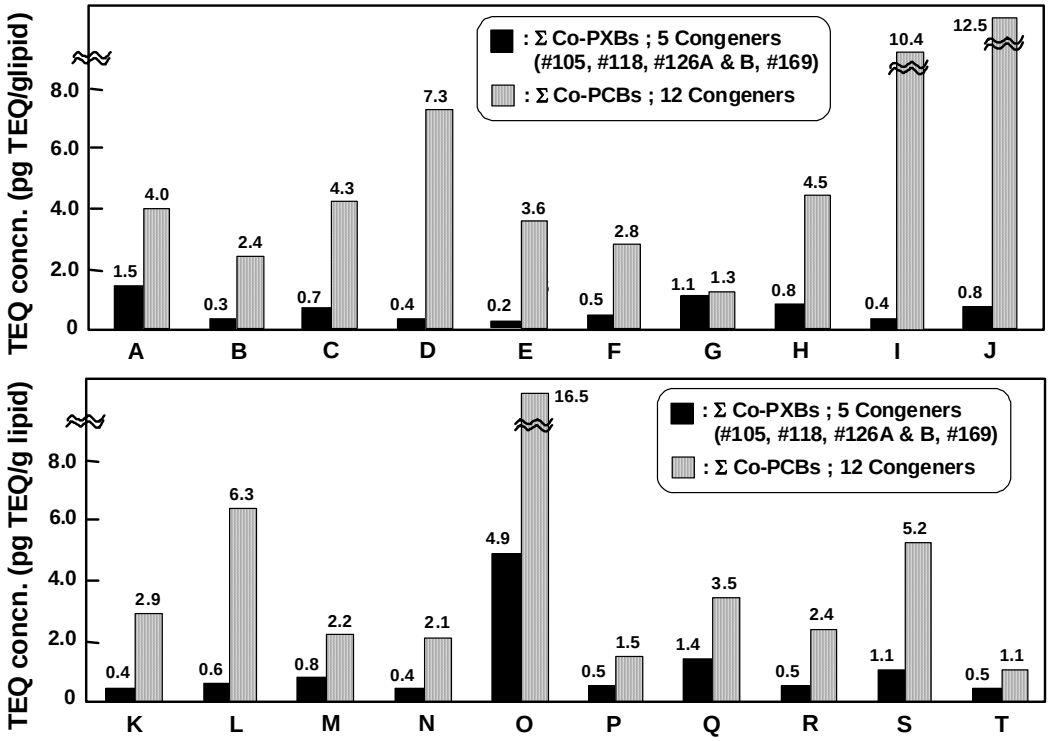
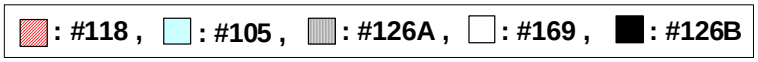
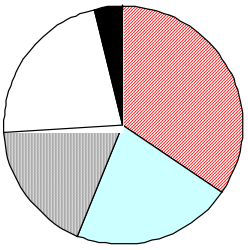


Fig.2 Comparison of TEQ levels of Co-PXBs or Co-PCBs in Breast Milk of Japan

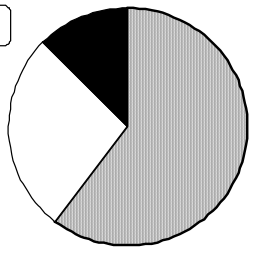


1) Actual concentration (pg/g lipid) **2) TEQ concentration (pg/g lipid)**



Data of all samples

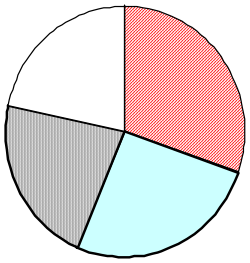
#118	19.6
#105	12.3
#126A	10.2
#169	12.7
#126B	2.2



#118	0.003
#105	0.002
#126A	0.53
#169	0.24
#126B	0.11

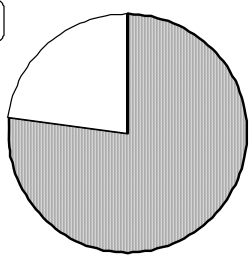
Fig.3a Average composition ratio of Co-PXBs congeners in all samples

1) Actual concentration (pg/g lipid) **2) TEQ concentration (pg/g lipid)**



Data of sample O

#118	105.6
#105	91.2
#126A	75.8
#169	75.8
#126B	ND



#118	0.002
#105	0.001
#126A	3.79
#169	1.14
#126B	ND

Fig.3b Composition ratio of Co-PXBs congeners in the highest sample O

fish samples [2)], however, only in 9 of 20 samples. Therefore, this nine women's exposure by Co-PXBs #126B may be derived from the eating of fish.

Figure 2 compares the TEQ levels by Co-PXBs and Co-PCBs in all samples. The concentration of Co-PXBs ranged between 0.2 and 4.9 pg TEQ/g lipid wt, and that of Co-PCBs ranged between 1.1 and 16.5 pg TEQ/g lipid wt., observing the highest concentration in Sample O. Further, the average concentration of Co-PXBs and Co-PCBs was 0.88 and 4.83 TEQ pg/g lipid wt, respectively. In general, the levels of Co-PXBs are lower than Co-PCBs. Consideration should be given to the fact that the number of possible PXB congeners (twelve congeners) is significantly higher than that of PCBs and that this study focuses on only five congeners.

As shown in Figure 3, the composition ratio of five Co-PXBs congeners in all samples (Fig.3a) and the highest sample O (Fig3b) were illustrated. The contribution congener for total actual concentration was in order, #118 > #169 > #105> #126A> #126B, and next, that for total TEQ concentration was #126A > #169 > #126B > #118 >#105. On the other hand, in the case of the highest sample O, high concentration of #126A was recognized, but not #126B. Therefore, it was suggested that there are two different pathway of the Co-PXBs contamination with or without #126B, depending on the amount and frequency of the eating of fish.

Further study is warranted to evaluate whether Co-PXBs exposures to nursing infants pose a health risk. Additional investigations of Co-PXBs in the daily foods are warranted to better understand the nature and extent of Co-PXBs contamination of Japanese food supply.

Acknowledgements

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