Determination of Co-Planar Polybrominated/chlorinated Biphenyls (Co-PXBs) in Thirty-eight Mother's Milk of Japan and Estimation of their Contamination Sources

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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. It has already 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). In fact, the introduction of a second halogen into the polychlorinated biphenyl moiety increases the number of possible congeners from 209 to 9180. Currently there are a limited number of PXBs commercially available and those are all co-planar.

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. Then, on the basis of our past investigation results, we compared the contribution ratio for total actual concentration by PCDDs, PCDFs, Co-PCBs and Co-PXBs, and estimated the Co-PXBs contamination sources. Further, we tried to demonstrate the formation of Co-PXBs by pyrolysis of deca-BDE as BFRs.

Materials and Methods

1) Samples

The samples of mother's milk were collected from thirty-eight women (age; 22~33 years old, sample 1 ~ 38) 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.

2) Experimental 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]. On the other hands, EROD activity was determined as followed by our previous paper[7]. Other detail methods should be referred to our paper [1, 2, 6].

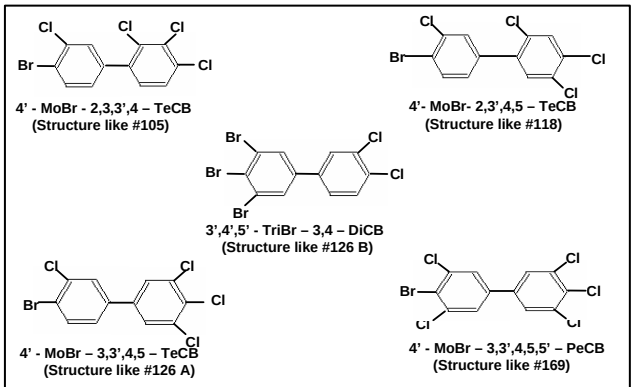

Fig.1 Chemical structure of Co-PXBs used for this study

Results and Discussion

As shown in Figure 2, it was compared the total TEQ concentration of PCDDs/DFs, Co-PCBs and Co-PXBs in breast milk (5 days after delivery) of thirty-eight women of Japan. Total TEQ concentration ranged between 2.1 and 22.4 pg/g lipid wt, showing as average concentration of 8.8 pg/g lipid wt. Co-PXBs were detected in all samples except sample #16 and #30. Figure 3 showed the average TEQ concentration of PCDDs/DFs, Co-PCBs and Co-PXBs in breast milk sample (total 76 samples) of 5days and 30days. Interestingly, it was observed no

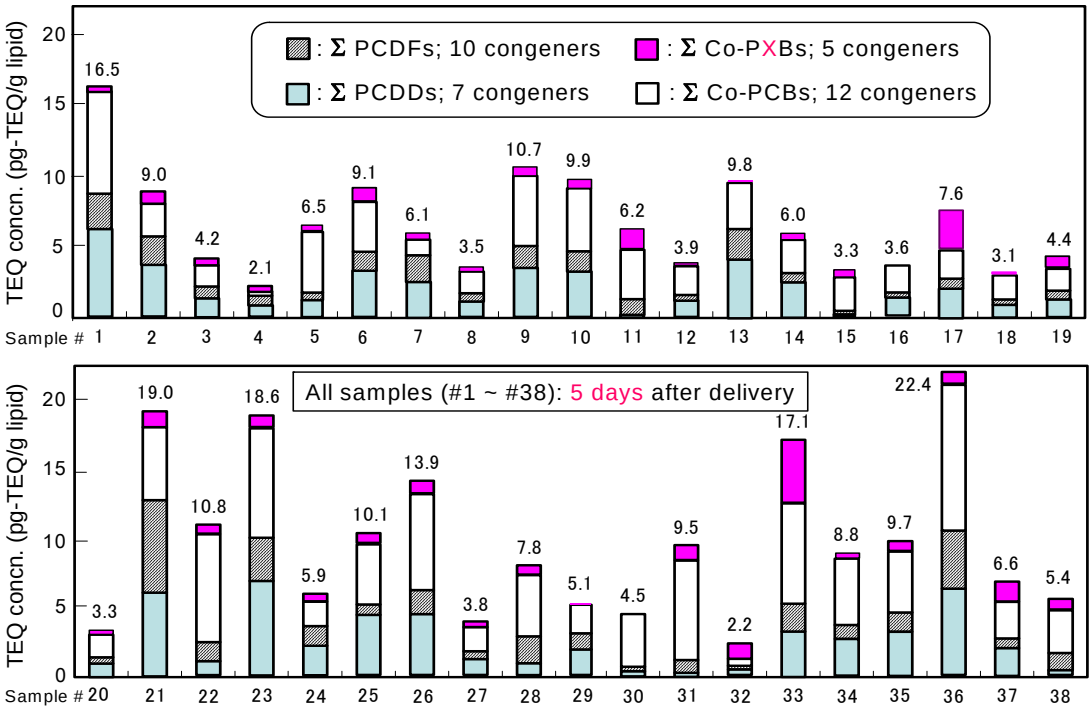

Fig.2 **Total TEQ concn. of PCDDs/DFs, Co-PCBs and Co-PXBs in 38 mother's milk of Japan**

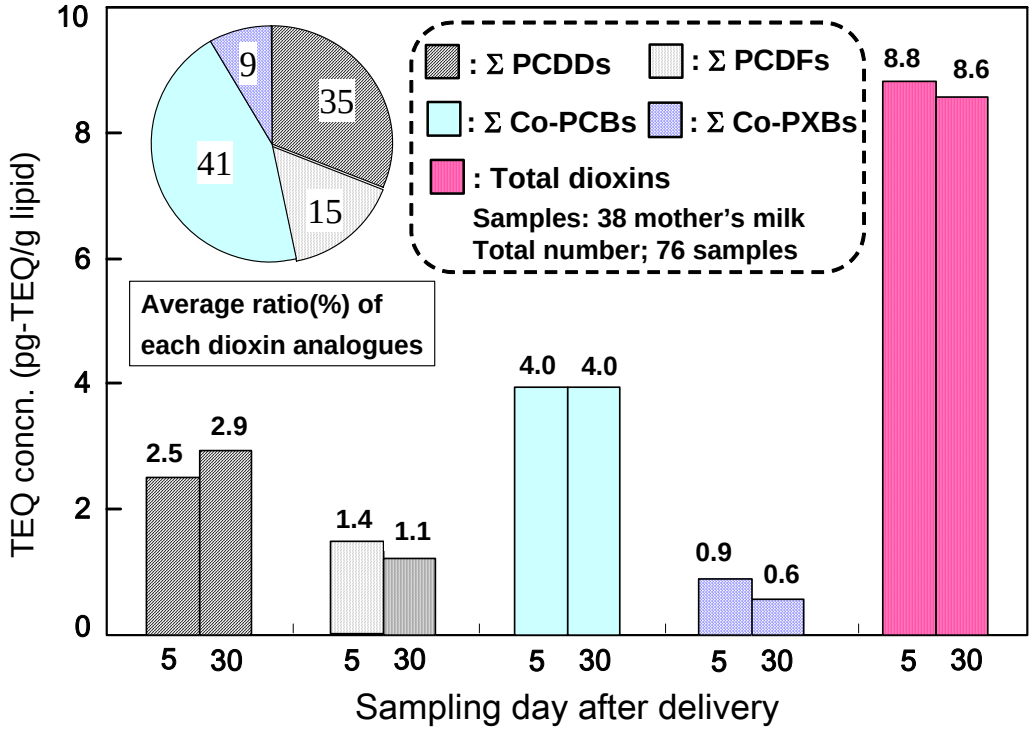

Fig.3 **Average TEQ concn. of PCDDs, PCDFs, Co-PCBs and Co-PXBs**

difference of average TEQ concentration between sample of 5 days and 30days. With respect to the average TEQ ratio of each dioxin analogues, Co-PXBs, PCDDs, PCDFs and Co-PXBs was 41, 35, 15and 9 %, respectively.

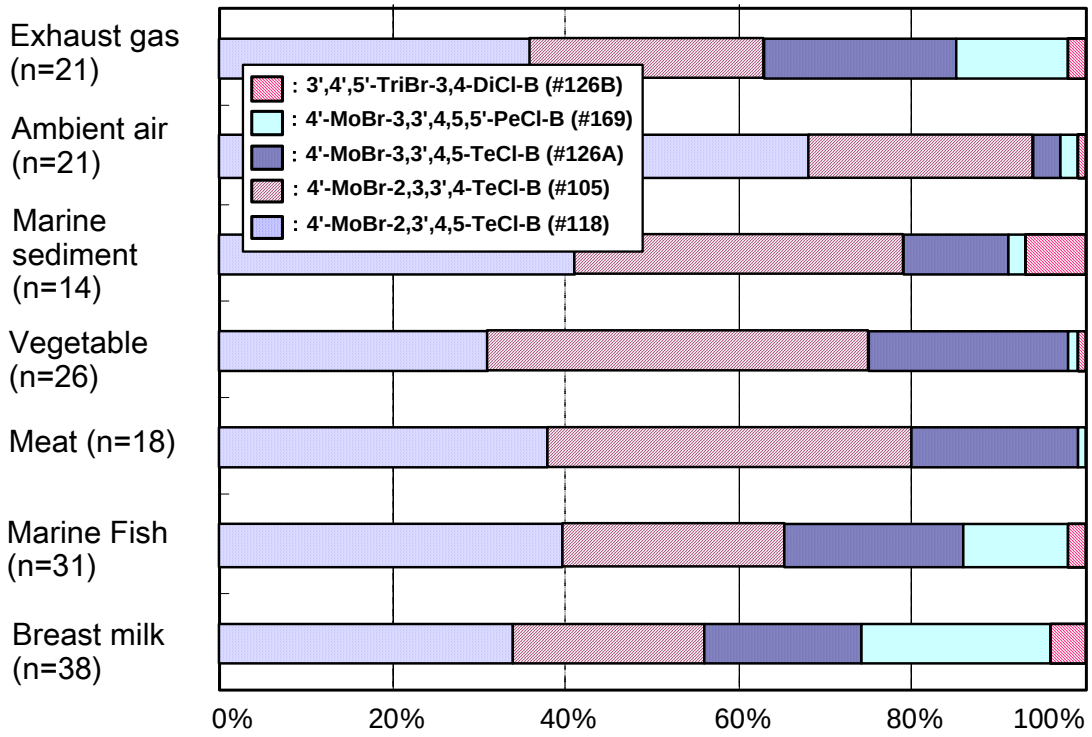

Fig. 4 Average contribution ratio for actual concentration by five Co-PXBs congeners in various samples

We have already investigated the contamination levels of Co-PXBs in various environment, food and human samples of Japan for past four years; it was determined 21 samples of exhaust (flue) gas from waste incinerators, 21 samples of ambient air and 14 samples of marine sediment as environmental samples, and 26 samples of market vegetable, 8 samples of market meat and 31 samples of market marine fish as food samples. As shown in Figure 4, in order to estimate contamination sources to human by Co-PXBs congeners, it was compared the average contribution ratio for actual concentration by five Co-PXBs congeners in the above samples. As results, Co-PXBs #118, #105 and #126A indicated highly contribution, and their pattern was comparatively similar in all samples. Therefore, the sources of human contamination were directly from food, but the food contamination is probably derived from incinerators. The slight difference of contribution ratio by Co-PXBs #169 and #126B observed in seven different samples might be due to the property of bioaccumulation and function of enzyme metabolism. As the reason why similar contribution pattern was not observed between exhaust gas and ambient air, it was estimated the existence of other contamination sources, affecting the exhaust gas from other chemical factory or indoor gas from human living house.

There are several routes of formation for PXBs. Nakano et al.[8] reported formation of co-planar PXBs (Co PXBs) during the manufacturing process of Fe_3Cl. Figure 5 showed the possible pathway of Co-PXBs and PXDFs from PBDEs in the presence of HCl, $FeCl_2$ and $CuCl_2$ under pyrolysis or photolysis. It was estimated that PBDEs decompose and become to Bromophenol etc., and also re-synthesized to PXDFs or Co-PXBs. Therefore, we tried to demonstrate this pathway by using of deca-BDEs under the above experimental conditions.

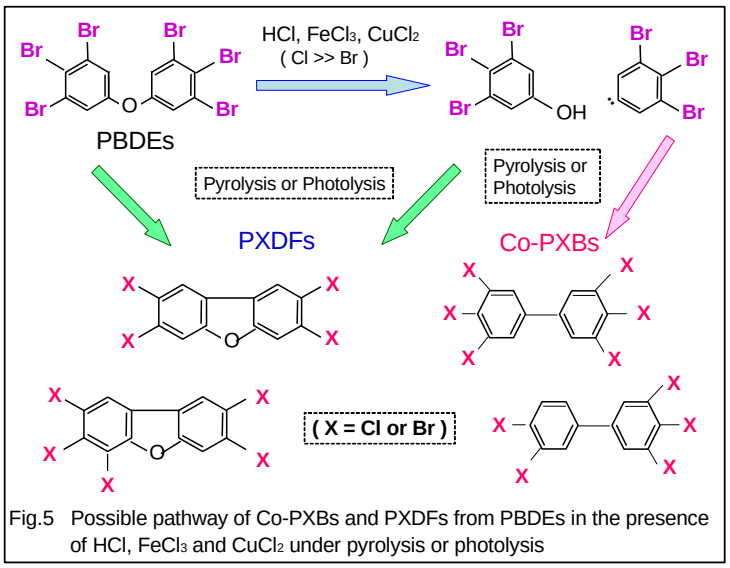

Fig.5 Possible pathway of Co-PXBs and PXDFs from PBDEs in the presence of HCl, $FeCl_3$ and $CuCl_2$ under pyrolysis or photolysis

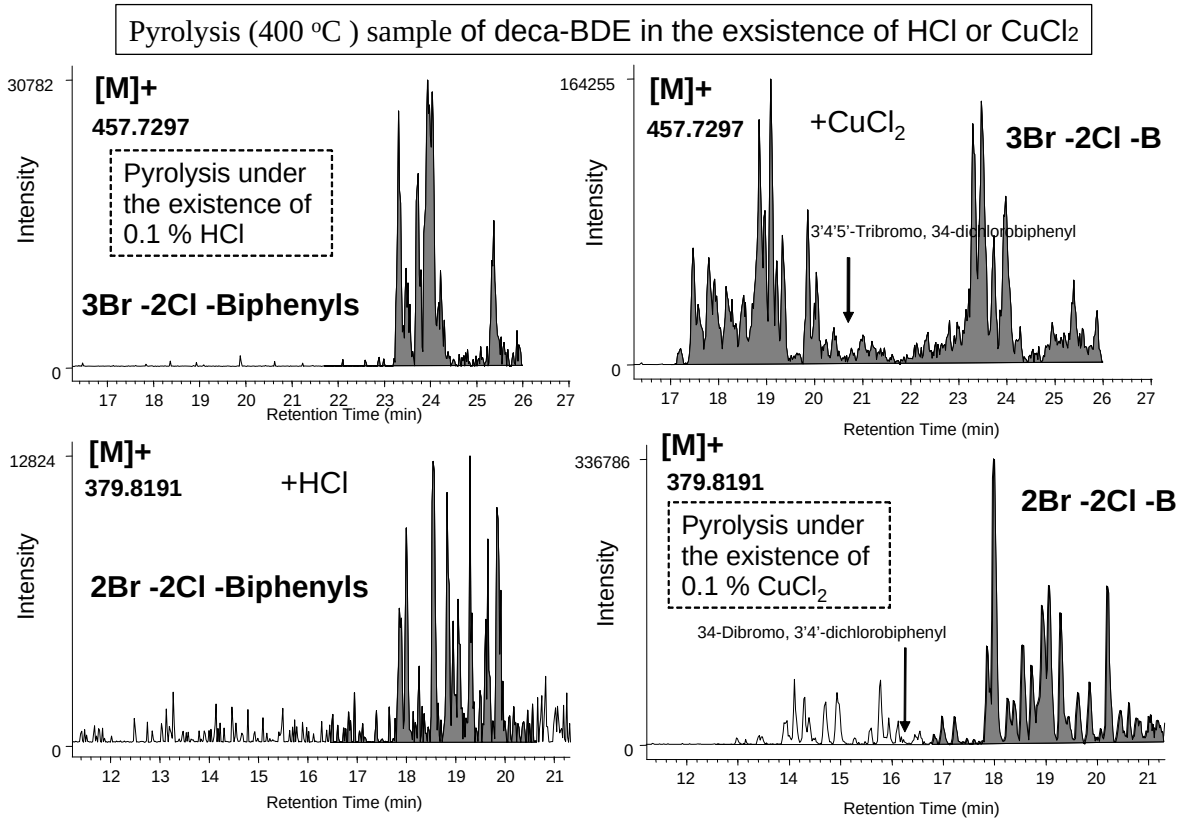

Fig. 6 EI-SIM chromatogram of unknown peaks of polybrominated/chlorinated biphenyls observed in pyrolysis sample of deca-BDE under the existence of 0.1% HCl or $CuCl_2$

Figure 6 showed EI-SIM chromatogram of unknown Co-PXBs peaks observed in pyrolysis sample of deca-BDE under the existence of 0.1%HCl or $CuCl_2$. It could find and identified many peaks of Co-PXBs as expected on SIM-chromatogram by the pyrolysis of deca-BDEs. Especially, by the pyrolysis experiment in the existence of $CuCl_2$, we certainly could recognize Co-PXBs #77 and #126B using their standard.

Further study is needed to search other Co-PXBs family congeners in the pyrolysis or photolysis samples of deca-BDE or flue gas from incinerator, showing high concentration, by using HRGC/HRMS scanning method. Then, it needs to evaluate their toxicity of Co-PXBs congeners as newly contaminants

Acknowledgements

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