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HIGHER BROMINATED CONGENERS OF COPLANAR POLYBROMINATED AND/OR CHLORINATED BIPHENYLS EXHIBIT HIGH CYTOCHROME P450 INDUCTION

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Introduction

Polybrominated chlorinated dioxins were generated from BFRs during production, using, and recycling of plastics. As a result, these dioxins have become ubiquitous environmental pollutants. So, it is necessary to investigate the pollution of these dioxins on environment and human being and to assess the toxicity. Polybrominated chlorinated biphenyls (PXBs) are recently reported new environmental pollutant 1), and belong to a class of structurally similar chemicals known as polyhalogenated aromatic hydrocarbons, which includes human homeostasis disruptors such as polychlorinated biphenyls (PCBs). PCBs were historically used in a large number of industrial applications, including coolants, plasticizers, lubricants, and insulators. Their high chemical stability have resulted in environmental persistence, and coupled with their highly lipophilic nature raises the potential for bioaccumulation in higher mammals, including humans. Consequently, it is suggested that PXBs derived from PCBs are widespread. In fact, we firstly reported the occurrence of PXBs in market fish sample collected from various global regions 1). However, there are only a few reports with PXBs contamination until present 2). One of the reasons is that there are a limited number of PXBs commercially available and those are all coplanar. The introduction of a second halogen into the polychlorinated biphenyl moiety increases the number of possible congeners from 209 to 9180. Therefore, little is currently known about PXBs contamination sources, and contamination levels of PXBs in environment, food, and human being, and there is no report with their toxicities.
In present study, we investigated whether coplanar PXBs-induced CYP P450 expression and activity in HepG2 cells and C57BL/6 mouse is altered by the number of introduction of bromine into PCBs.

Material and methods

1) Cell culture
HepG2 cells, a human hepatocarcinoma cell line, and Caco-2 cells, a human intestinal cell line, were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) at 37 ˚C.
2) Chemicals
2,3,7,8-TCDD, 2,3,7,8-TBDD, 3,3',4,4',5-PeCB (#126), 4'-Br-3,3',4,5-PCB (Co-PXB-1Br), 3,4-Br-3',4',5'-Cl-PXB (Co-PXB-2Br), 3'4'5'-Br-3,4- PCB (Co-PXB-3Br) and 3,3',4,4',5'-PBB purchased from Cambridge Isotope Laboratories (MA, USA).
3) In vitro EROD activity
Cell-mediated cytocrome P450-1A activities were determined by ethoxyresorufin-O-deethylase (EROD) activity. Confluent HepG2 cells and Caco-2 cells, in 96-well microplate, were exposed to various organic environmental pollutants for 24 h at the indicated concentration. The cells were washed with PBS and incubated for 1 h at 37 ˚C with 5 mM 7-ethoxyresorufin in DMEM supplemented with 10% FBS. Resorufin-associated fluorescence was measured at 550-nm excitation and 595-nm emission using a SPECTRA FLUOR (TECAN).The EROD activity was normalized to the cell protein content, determined with the Bradford regent.
4) In vivo EROD activity
Female C57BL/6 mouse (6-8 weeks old) were administered a single intraperitoneal injection of dioxins and their related compounds in the saline containing 1% ethanol and 10% Tween20. Day 2 after the administration, mouse was sacrificed under the anesthesia. Liver were homogenized in 0.1 M Tris-acetate buffer (pH 7.4). Homogenates were centrifuged at 9,000 ×g for 15 min at 4 ˚C, and the supernatant was centrifuged at 105,000 ×g for 45 min at 4 ˚C. The resulting pellet was homogenized in 0.1 M potassium pyrophosphate buffer (pH7.4). The homogenate was centrifuged at 105,000 ×g for 45 min at 4 ˚C. The microsomal pellet was homogenized in 10 mM Tris-acetate buffer (pH 7.4) and stored at -80 ˚C until use. Protein concentrations in microsomes were determined according to the Lowly method with bovine

serum albumin as the standard. Diluted liver microsomes and the reaction mixture (50 mM KPO4, 5 mM MgCl2, 0.5 mM NADP+, 1 IU/ml G-6-P dehydrogenase and 2.4 #M 7- ethoxyresorufin) were preincubated for 5 min at 37 ˚C. The reaction was initiated by adding 5 mM glucose-6-phosphate and carried out at 37 ˚C for 10 min. Resorufin production was measured a SPECTRA FLUOR.

Result and discussion

To determine whether Co-PXBs induced the activity of CYP P450, we performed EROD, PROD and CYP2C9 activity assays. As shown in Fig. 1, a dose-dependent increase in EROD activity was observed. When the dose-response curves were compared among the Co-PXB congeners, the patterns of #126-3Br and PBB were clearly different from those of PCB, #126-Br and #126-2Br. The EC50 of EROD activity with TCDD, PCB, #126-Br, #126-2Br, #126-3Br and PBB were 0.21, 223.4, 192.8, 182.0, 12.52 and 9.72 nM, respectively. On the other hand, Co-PXBs had no effect on PROD and CYP2C9 activities (data not shown). These results indicated that the introduction of bromine into the chemical frame of a polychlorinated biphenyl tends to result in the expression of higher CYP1A activity.
To confirm the difference in cell-based CYP activities by Co-PXBs, we assayed in vivo EROD activity using liver microsomes collected from C57BL/6 mice. As shown in Fig. 2, EROD activity due to Co-PXBs increased in a dose-dependent manner. No significant difference in activity was observed between the congeners at 50 nmol/kg, whereas at 5 and 10 nmol/kg, #126-3Br and PBB more significantly increased EROD activity than the other Co-PXB congeners, providing a 5.2- and 4.9-fold relative increase in activity of PCB-treated mouse microsomes at 10 nmol/kg, respectively. Similar to the results of the in vitro assay, more highly brominated congeners provided higher CYP1A activity.
In conclusion, we showed that Co-PXBs, comprised of only five congeners, contributed highly to the level of toxins in breast milk collected from donors in Japan. We also showed that the toxic potency as reflected in CYP1A activity induced by Co-PXBs was higher than that by Co-PCB. If the toxicity of Co-PXBs is higher than that of Co-PCBs, our investigation strongly suggests that the TEQ level of Co-PXBs is not a negligible human health risk, especially in infants whose host defense system is poor. Infants exposed to high levels of these contaminants via breast-feeding may suffer adverse effects, such as delayed development, neural function impairment and the development of allergies. Further studies are required to clarify the details of the molecular mechanisms by which human homeostasis is disrupted by Co-PXBs.

Acknowledgements

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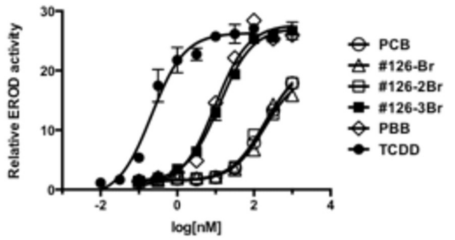

Fig. 1 Induction of cytochrome P450 activity in Co-PXB-treated HepG2 cells.
HepG2 cells were incubated with Co-PXBs at various concentrations for 24 h. After incubation, ERODactivities were measured. The relative activity is expressed as the fold induction as compared to the vehicle. The data are presented as means ± SD (n=5).

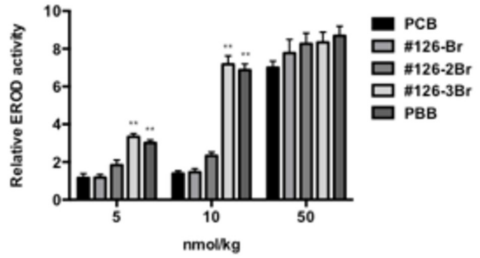

Fig. 2 Enhancement of CYP1A activity in Co-PXB-treated mouse liver microsomes.
Mice were orally administered Co-PXBs at 5, 10 and 50 nmol/kg. After 2 days, EROD activity in the liver microsomes was measured. The relative activity is expressed as the fold induction as compared to the vehicle. The data are presented as means ± SE (n=10). $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the same concentration of PCB.