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Organohalogen and Metabolite Contaminants in Human Serum Samples from Indian E-Waste Recycling Workers

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Abstract—Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) share many physicochemical properties and toxic effects, including immunological, reproductive and endocrine/cytotoxic effects in various species. Despite their ban in many countries (PCBs in the 1970s, most PBDE technical mixtures in 2004), both types of contaminants are still found in the global environment and biota. PCBs and PBDEs may undergo metabolic transformation resulting in hydroxylated PCBs (OH-PCBs) and hydroxylated PBDEs (OH-PBDEs). The structural similarity to thyroid hormone of OH-PCBs and OH-PBDEs allows strong affinity to transthyretin (TTR) and disrupts thyroid hormone homeostasis. In the present study, PCBs, PBDEs and their hydoroxylated metabolites were determined in the serum of workers in an Indian e-waste recycling factory and compared with residents from a rural area (control group). PCBs and OH-PCBs concentrations were not significantly different between the e-waste recycling workers and the control group. PBDE concentrations were also not significantly different between the e-waste recycling workers and the control group, but OH-PBDEs in the control group were significantly higher than in e-waste recycling workers. This result suggests that exposure source of PBDEs and OH-PBDEs may be different and also the e-waste workers in the present study area are not highly exposed to these chemicals than the control group. Concentrations of PCBs, PBDEs, OH-PCBs, and OH-PBDEs were lower than those reported in the United States, Nicaragua, Canada, the Netherlands and Slovakia.

Keywords: polychlorinated biphenyls, polybrominated diphenyl ethers, metabolites, e-waste

INTRODUCTION

Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) share several physicochemical properties and toxic characteristics, including immunological, reproductive and endocrine/cytotoxic effects in various species

(Safe, 1994; de Wit, 2002). PCBs and PBDEs may undergo metabolic transformation resulting in hydroxylated PCBs (OH-PCBs) and hydroxylated PBDEs (OH-PBDEs) (Letcher *et al.*, 2000; Stapleton *et al.*, 2009). The structural similarity to thyroid hormone of OH-PCBs and OH-PBDEs allows strong affinity to transthyretin (TTR) and potential to disrupt thyroid hormone homeostasis. In recent years, about 50 metric tons of electronic-waste (e-waste) are produced annually in the world and transported in massive quantities to developing countries. During the crude recycling of e-waste, PCBs and PBDEs may be released into the environment. Because of such observations, investigations on residue levels of OH-PCBs and OH-PBDEs in human blood are increasing. Moreover, research to date suggests that OH-PBDEs are formed by hydroxylation of PBDEs and by demethylation of the MeO-PBDEs (Wan *et al.*, 2009). In the present study, PCBs and PBDEs and their hydroxylated metabolites were determined in the serum of workers in an Indian e-waste recycling factory and compared with residents from a rural area (control group).

MATERIALS AND METHODS

Human serum samples were collected from Bangalore (E-waste recycling worker group) and Chidambaram (Rural area: control group) in India during the year 2007. The serum samples of 10 males (Control: $n = 5$, Age, 25–35; E-waste recycling workers: $n = 5$ Age, 26–33) were stored in the Environmental Specimen Bank for Global Monitoring (*es*-BANK) of Ehime University at –25°C until analysis (Tanabe, 2006).

PCBs, PBDEs, MeO-PBDEs, OH-PCBs and OH-PBDEs were analyzed as follows. Briefly, the serum sample (3 g) was denatured with hydrochloric acid (HCl). 2-propanol was added, and then OH-PCBs and OH-PBDEs were extracted thrice with 50% methyl *t*-butyl ether (MTBE)/hexane. Nine $^{13}C_{12}$-labeled OH-PCBs, three $^{13}C_{12}$-labeled OH-PBDEs, twenty $^{13}C_{12}$-labeled PCBs and ten $^{13}C_{12}$-labeled PBDEs were spiked as internal standards. The organic phases were combined, evaporated and exchanged to hexane. 1 M potassium hydroxide (KOH) in 50% ethanol/water was added and shaken. The partition process was repeated two times and the alkaline phases were combined. The remaining organic phase was concentrated and lipid was removed by gel permeation chromatography (GPC), and then passed through activated silica-gel packed in a glass column (Wako DX, Wako Pure Chemical Ind. Japan). PBDEs were eluted with 5% DCM/hexane and concentrated for gas chromatograph (GC; Agilent 7890A)-mass spectrometry (MS; Agilent 5975C) analysis. PCBs and MeO-PBDEs were eluted with 5% DCM/hexane and concentrated for high resolution gas chromatograph (GC; Agilent 6890N)-mass spectrometry (MS; JEOL JMS-800D) analysis (HRGC/HRMS). The combined alkaline phase was acidified with sulfuric acid, and then OH-PCBs and OH-PBDEs were extracted twice with 50% MTBE/hexane. The organic phases were combined, evaporated and exchanged to hexane, and then passed through deactivated silica-gel (Wako S-1, 5% water, w/w; Wako Pure Chemical Ind. Japan) packed in a glass column. OH-PCBs and OH-PBDEs were eluted with 50% dichloromethane (DCM)/hexane, concentrated and

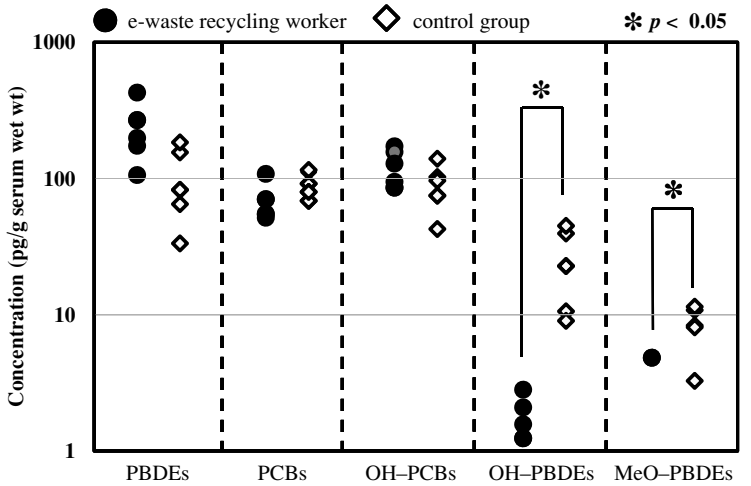

Fig. 1. Concentrations of ∑PBDEs, ∑PCBs, ∑OH-PCBs, ∑OH-PBDEs, and ∑MeO-PBDEs in the serum of workers in an Indian e-waste recycling factory and residents from a rural area (control group).

exchanged to hexane. OH-PCBs and OH-PBDEs in hexane were methylated by reaction with trimethylsilyldiazomethane. The derivatized solution was lipid was removed by GPC and then passed through activated silica-gel packed in a glass column (Wako S-1, Wako Pure Chemical Ind. Japan). MeO-PCBs and MeO-PBDEs were eluted with 10% DCM/hexane and concentrated. Identification and quantification of OH-PBDEs were performed using HRGC/HRMS.

RESULTS AND DISCUSSION

Residue levels of PCBs and OH-PCBs

The median concentrations of total PCBs in control and e-waste recycling group were 75 pg/g wet wt, and 86 pg/g wet wt and total OH-PCBs were 80 pg/g wet wt. and 55 pg/g wet wt. respectively (Fig. 1). Concentrations of total PCBs and OH-PCBs were not significantly different between the e-waste recycling workers and control group. Moreover, concentrations of total PCBs and OH-PCBs were lower than those reported in Japan (Mean of PCBs; 3200 pg/g wet wt, Mean of OH-PCBs 690 pg/g wet wt), Sweden (Mean of PCBs; 450 ng/g lipid wt, Mean of OH-PCBs 340 ng/g lipid wt), Canada (Mean of PCBs; 130 ng/g lipid wt, Mean of OH-PCBs 60 ng/g lipid wt) and Slovakia (Mean of PCBs; 570 ng/g lipid wt, Mean of OH-PCBs 270 ng/g lipid wt) (Sjodin *et al*., 2000; Hovander *et al*., 2002; Sandau *et al*., 2002; Nomiyama *et al*., 2010). The similarity in PCB levels in e-waste recycling sites as also seen in the reference site indicates that the former were not major sources of PCBs.

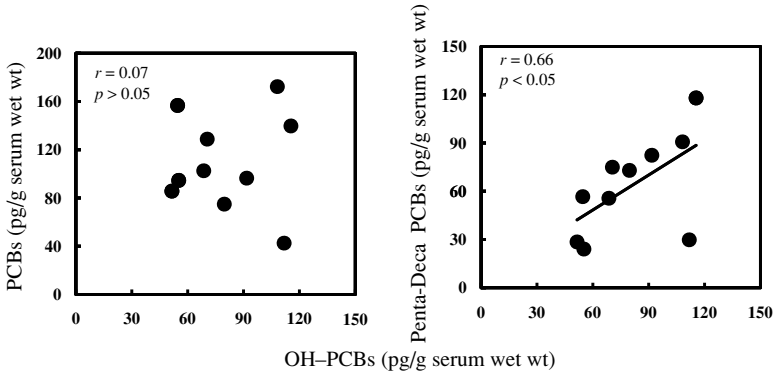

Fig. 2. Relationships between concentrations of ∑PCBs, $\sum_{5-10}$PCBs and ∑OH-PCBs.

Accumulation patterns of PCBs and OH-PCBs

Among PCB isomers, CB28 was dominant, followed by CB74 and CB70 in e-waste recycling workers; however in the control groups, CB138, CB153, CB118 and CB180 were the dominant congeners. Isomer profiles of control groups were similar to general profiles in human serum, but E-waste recycling workers had relativity higher concentrations of lower chlorinated isomers. In a previous study, CB-28 was detected at significantly higher levels in the human milk samples of females living in and around the e-waste recycling site than the reference site in Vietnam (Tue *et al.*, 2010). The source of these congeners may be old electric devices such as capacitors and small transformers which contain PCBs as heat transfer and dielectric fluids. In this study, the OH-PCBs congeners, 4OH-CB107/4′OH-CB108, 4OH-CB146, 4OH-CB138, 4OH-CB187 and 4OH-CB172 were as predominant congeners in the serum of human. The structure activity relationship data on binding affinity to TTR with OH-PCBs, shows that chlorine-substitution on the phenolic ring adjacent to the *meta*- and *para*-OH group enhances the binding of OH-PCBs to TTR (Rickenbacher *et al.*, 1986). The dominant OH-PCB isomers found in the serum of the present study had these specific chemical structures. In this study, 4OH-CB107/4′OH-CB108 was relativity highly accumulated (40–50%) than these found in some other previous reports (10–30%) (Sandau *et al.*, 2000; Sjodin *et al.*, 2000; Fangstrom *et al.*, 2002; Hovander *et al.*, 2002; Sandau *et al.*, 2002; Nomiyama *et al.*, 2010). Same type of results was reported in the plasma from males in Sweden and Latvia (Sjodin *et al.*, 2000). 4OH-CB107/4′OH-CB108 was detected in cord plasma and children. It is suggested that 4OH-CB107/4′OH-CB108 can be transferred from maternal to fetal blood. From these results, it can be suggested that the specific isomer pattern observed in this study reflect the sexual difference in OH-PCBs accumulation.

No significant correlation was observed between the concentrations of PCBs

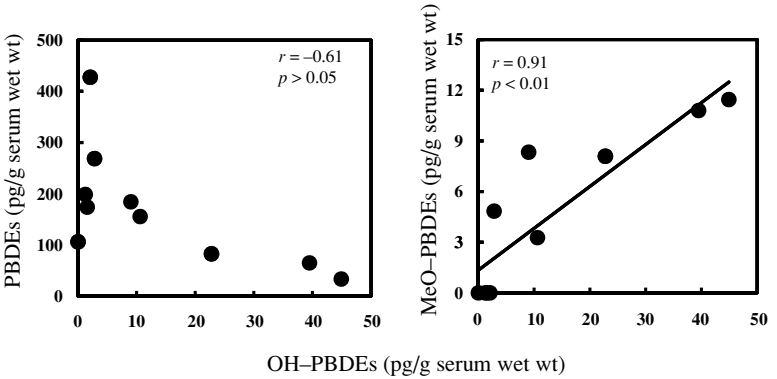

Fig. 3. Relationships between concentrations of ∑PBDEs, ∑MeO-PBDEs and ∑OH-PBDEs.

and OH-PCBs in serum. However, significant correlation was observed between the concentrations of $\sum_{5-10}$PCBs (Sum of Penta to Deca PCBs) and OH-PCBs in serum ($p < 0.05$) (Fig. 2). In a human study it was found that the concentrations of lower chlorinated OH-PCBs were relatively lower than higher chlorinated OH-PCBs (Nomiyama *et al*., 2010), which may be due the easy excretion of lower chlorinated.

Residue levels of PBDEs, MeO-PBDEs and OH-PBDEs

The median concentrations of total PBDEs were 100 pg/g wet wt, and 240 pg/g wet wt in the control and e-waste recycling groups, respectively (Fig. 1). The highest concentration of PBDEs was 430 pg/g wet wt., found from an e-waste recycling worker. PBDEs concentrations of e-waste recycling workers were slightly higher than the control group, but not significant.

The median concentrations of total OH-PBDEs were 25 pg/g wet wt and 1.5 pg/g wet wt in control and e-waste recycling groups, respectively (Fig. 1). The highest concentration of OH-PBDEs was 45 pg/g wet wt, in a sample from control group. These PBDEs and OH-PBDEs levels were lower than those reported from United States (Mean of PBDEs; 100 ng/g lipid wt, Mean of OH-PBDEs 52 ng/g lipid wt) (Qiu *et al*., 2009) and Nicaragua (Mean of PBDEs; 840 pmol/g lipid wt, Mean of OH-PBDEs 42 pmol/g lipid wt) (Athanasiadou *et al*., 2008). Interestingly, OH-PBDEs in the control group were significantly higher than in e-waste recycling workers. In an *in vitro* or *in vivo* exposure study, concentrations of PBDEs were generally great (μg/g level), and the resultant products of PBDEs, OH-PBDEs, occurred at trace concentrations (Stapleton *et al*., 2009). The levels of PBDEs are lower in the present study and hence there might not have been much metabolism resulting in less than detectable levels of metabolites.

MeO-PBDEs were detected in all samples from control group, but only in one sample from e-waste recycling group. The median concentration of total

MeO-PBDEs was 8.4 pg/g wet wt in the control group and one of the samples of e-waste recycling group contained 0.97 pg/g (Fig. 1). The highest concentration of total MeO-PBDEs was 12 pg/g wet wt, in a sample from the control group.

In average, OH-PBDEs were three times higher than MeO-PBDEs. The present result is different from a previous report on human breast milk from Spain (Lacorte and Ikonomou, 2009). This result suggested that OH-PBDEs, due to their polarity, tend to partition more favorably than MeO-PBDEs in serum.

Accumulation patterns of PBDEs, MeO-PBDEs and OH-PBDEs

Among PBDE isomers, BDE209 was dominant in all samples. The reasons for this pattern may be the fact that Asian developing countries are predominantly using deca-BDE commercial mixture containing mostly BDE-209. In fact, previous studies on PBDEs profile in the e-waste recycling workers in China showed similar results (Bi *et al.*, 2007). The results confirmed a significantly enhanced exposure to BDE-209 in the e-waste recycling workers. In the above study, lower brominated compounds were not detected.

6OH-BDE47 was the dominant OH-PBDEs isomer in e-waste and control group. In another report, 6OH-BDE47 was detected from marine animals like salmon, mussel, the red algae (Marsh *et al.*, 2004; Malmvarn *et al.*, 2005). Because of control site is near the coast, there might be a lot of fish consumption. It is reasonable to believe that dietary intake plays an important role in the exposure to 6OH-BDE47 in human, especially in the control group.

No significant correlation was observed between concentrations of PBDEs and OH-PBDEs indicating that the sources of PBDEs and OH-PBDEs were different. Biotransformation ratio of PBDE to OH-PBDEs was found to be very low (<0.01–1% of PBDEs) (Qiu *et al.*, 2007; Stapleton *et al.*, 2009). Thus, it can be suggested that OH-PBDEs have other human exposure routes.

In this study, among the MeO-PBDEs isomers, only 6MeO-BDE47 was detected in human serum samples. Based on radiocarbon measurements, 6MeO-BDE47 has been reported as a natural product (Teuten *et al.*, 2005).

Significant positive relationships were found between concentrations of MeO-PBDEs and OH-PBDEs ($p < 0.01$) (Fig. 3). Similar relationship was found in human breast milk collected from Catalonia, Spain (Lacorte and Ikonomou, 2009). No significant relationship was found between concentrations of PBDEs and OH-PBDEs, but positive relation between MeO-PBDEs and OH-PBDEs was seen (Fig. 3). In a previous report, demethoxylation of MeO-PBDEs to OH-PBDEs at environmentally relevant concentrations was suggested (Wan *et al.*, 2009). Thus, our results suggest that OH-PBDEs were the metabolites of MeO-PBDEs.

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