Chlorinated and Brominated Organic Contaminants in Human Breast Milk from Different Regions in India

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Introduction

Polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) are industrial chemicals with vast and global use and some of them are still produced in large volumes. Due to their high persistent properties, these chemicals have been detected in all kinds of environmental and biotic matrices (de Wit 2002). These compounds can accumulate in fat-rich tissues of biota and biomagnify through food web. Particularly, higher trophic organisms including human tend to accumulate high levels of these lipophilic chemicals. The presence of toxic chemicals in human milk is of public concern because of their potential health effects to the breast-feed infants. Breast milk has several advantages in human biomonitoring studies: it allows simple, non-invasive collection, monitoring of body burdens in reproductive-age women and estimations of exposure through breast feeding. Although well documented data sets on organohalogen compounds in human breast milk have been reported in North America and Europe, the number of studies in developing countries is limited. India is of particular interest because of its increasing economic development in recent years which may lead to series of environmental problems. During the process of industrial development, the urban population expanded rapidly and environmental pollution became apparent. The present study was therefore carried out to determine the current contamination status and geographical variation of organohalogen compounds such as PCBs, PBDEs and HBCDs in human breast milk collected from several parts of India.

Materials and Methods

Sample Collection

Human breast milk samples *(n=130)* were collected during 2009 from different locations in India such as Chennai, Madurai and Kolkata (urban), Goripalyam in Bangalore (urban slum), Chidambaram (suburban agriculture) and Parangipettai, Thoothukudi and Marakanam (fishing village). Out of these, *(n=55)* samples (Fig 1) from Chennai *(n=10)*, Bangalore *(n=14)*, Kolkata *(n=7)*, Chidambaram *(n=10)*, Parangipettai *(n=7)* and Kolkata (municipal dumping site) *(n=7)* were used in analyzing for PCBs, PBDEs and HBCDs. All the samples were airlifted to Ehime University, Japan, on dry ice and stored in the environmental specimen bank (*es*-BANK) for global monitoring (Tanabe, 2006) at −20 °C until chemical analysis.

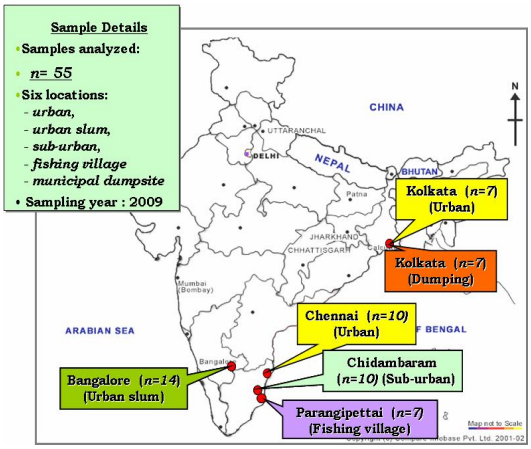

Figure 1. Map showing the sampling locations in India

Chemical Analysis

Briefly, approximately 40 g of the sample was freeze-dried and extracted with a high speed solvent extractor using 50% acetone in hexane. Fat content was determined gravimetrically from an aliquot of the extract. The remaining extract was spiked with $^{13}C_{12}$-labeled PBDEs, PCBs and HBCDs as surrogates and then subjected to gel permeation chromatography (GPC) for fat removal and eluted with a mixture of hexane and dichloromethane (1:1). The GPC fraction containing organohalogen compounds was concentrated and passed through 4 g of activated silica gel packed in a glass column (1cm i.d. x 10cm length). The first fraction was eluted with 5% dichloromethane in hexane and second fraction with 25% dichloromethane in hexane. $^{13}C_{12}$-labeled BDE-139 was added as an internal standard to the first fraction containing PBDEs prior to quantification using a gas chromatograph equipped with a mass selective detector (GC-MSD). Second fraction containing HBCDs was spiked with deuterated HBCDs and analysed using liquid chromatograph coupled with a tandem mass spectrometer (LC-MS/MS), based on the method published elsewhere (Malarvannan et al. 2009).

Quantification of PBDEs (mono- to hepta-BDEs) and PCBs was performed using a GC (Agilent 6890 N) with MSD (Agilent 5973 N), and GC (Agilent 6980 N) coupled with MSD (Agilent 5975) for octa- to deca-BDEs, with electron impact ionization in selective ion monitoring mode (EI-SIM). Fourteen congeners of PBDEs from mono to deca (BDE-3, BDE-15, BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, BDE-196, BDE-197, BDE-206, BDE-207, and BDE-209) were quantified in this study. All the congeners were quantified using the isotope dilution method to the corresponding $^{13}C_{12}$-labeled congener. HBCD isomers (α-, β-, γ-HBCD) were quantified using a LC-MS/MS, based on the method published elsewhere (Malarvannan et al. 2009).

Results and Discussion

Contamination Status of Polychlorinated Biphenyls

Organohalogen compounds were detected in all the samples (data not shown), indicating ubiquitous contamination in people living in India. The residue pattern in human breast milk was dominated by PCBs (mean: 34 ng/g lipid wt.) followed by PBDEs (mean: 1.30 ng/g lipid wt.) and HBCDs (mean: 0.42 ng/g lipid wt.). Among the locations, the municipal dumping site in Kolkata had significantly *($p<0.05$)* high concentration of PCBs (mean: 1700 ng/g lipid wt.) than the general population in other locations in India (range: 3.2 - 160 ng/g lipid wt.) which clearly indicates the presence of prominent sources of PCBs in municipal dumping site. The levels of PCBs from municipal dumping site in the present study are at the highest end in the recent literature reported (Kunisue & Tanabe, 2007). Elevated levels of PCBs and DRCs in human milk, crow and fish samples collected near the dumping site in India have also been reported (Someya et al. 2009, Watanabe et al. 2005). Furthermore, significantly high levels of PCBs were also found in urban (mean: 46 ng/g lipid wt.) areas compared to urban slum (mean: 42 ng/g lipid wt.), sub urban (mean: 14 ng/g lipid wt.) and rural fishing village (mean: 32 ng/g lipid wt.) indicating the need for environmental concern in India, particularly in urban areas. However, in global comparison (data not shown) the levels of PCBs in India were still low when compare to developed countries and slightly higher than some developing countries such as China, Cambodia and Philippines but lower than Vietnam (Malarvannan et al. 2009). In temporal variation, when compared with our previous reports (Tanabe et al. 1990 and Devanathan et al. 2009), the present data showed a significant decreasing trend like other organochlorine compounds including DDTs and HCHs, indicating the implementation of bans/restriction over these chemicals in India. Congener pattern of PCBs was dominated by CB-153 followed by CB-138

and CB-118 from general population in India, whereas the dumping site showed different congener pattern with penta-CB (CB-118) being the dominant followed by CB-138 and CB-153.

Contamination Status of Brominated Flame Retardants

Total PBDE concentrations in the present study varied from 0.10 to 15 ng/g lipid wt. with a mean concentration of 1.3 ng/g lipid wt. in the general population of India. These levels were in the same order with many Asian and European countries but one or two orders of magnitude lower than Canada and USA (Sudaryanto et al. 2008). Similar to PCBs, the concentrations of PBDEs (mean: 5.7 ng/g lipid wt.) in municipal dumping site was significantly ($p<0.05$) higher than the other locations: urban (mean: 1.7 ng/g lipid wt.), urban slum (mean: 0.40 ng/g lipid wt.), suburban (mean: 0.40 ng/g lipid wt.) and fishing village (mean: 3.7 ng/g lipid wt.) indicating presence of prominent sources in dumping site as explained for PCBs. Long term health impacts of POPs and new POPs on human living in and around these dumping sites are probably a major concern for future research in India. The levels of PBDEs were also significantly higher ($p<0.05$) in fishing and urban locations than the slum and suburban. It is apparent that urban populations may more disclosure to PBDEs containing products than the people in rural locations. High levels of other contaminants such as DDTs and HCHs were also previously reported in human milk collected from urban locations in India (Devanathan et al. 2009).

Among the PBDE congeners analysed, except in Bangalore and Kolkata which BDE-47 accounted for larger proportion, higher BDEs such as BDE-197, -207 and -209 were the predominant (Fig 2); the profile of which was similar to the PBDEs pattern reported recently in human milk samples from general population in China (Sudaryanto et al. 2008). However, the profile in this study was different from the general pattern in human milk from various studies worldwide (Sudaryanto et al. 2008), in which lower brominated congeners were generally predominant. High proportion of BDE-209 in this study suggests a possible wide industrial use of commercial deca-BDE in India.

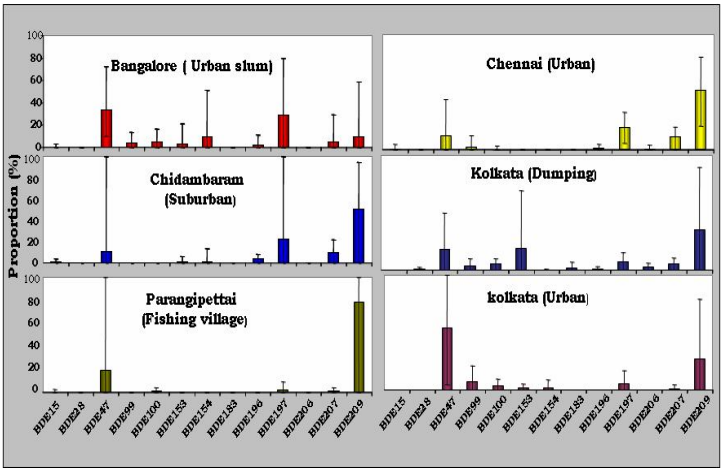

Figure 2. Congener profiles of PBDEs in human milk from India

HBCDs were detected only in municipal dumping site and urban locations, which concentration ranged from 0.50 to 3.5 ng/g lipid wt. with a mean 0.42 ng/g lipid wt. The levels of HBCDs in India were comparable to Asian countries but lower than European countries (Malarvannan et al. 2009). Like PCBs and PBDEs, the levels of HBCDs were also significantly higher ($p<0.05$) in municipal dumping site (mean: 4.2 ng/g lipid wt.) than urban locations (mean: 0.42 ng/g lipid wt.). In this study, the stable isomer, α-HBCD was the predominant in all the samples. Further measurements of organohalogen compounds in food items and environmental samples including water, air and dust in India are necessary to assess their pathways of human exposure.

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