PERSISTENT ORGANOHALOGEN COMPOUNDS IN HUMAN BREAST MILK COLLECTED FROM DIFFERENT REGIONS IN INDIA

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1. Abstract:
The present study reports the concentrations of organohalogen compounds such as DDTs, HCHs, PCBs, PBDEs and HBCDs in human breast milk samples collected from different locations in India, including urban, suburban, urban slum, rural and municipal dumping site. The levels of organohalogen compounds were significantly higher in urban and municipal dumping sites than urban slum, sub urban and rural locations. DDTs and HCHs were the predominant contaminants followed by PCBs, PBDEs and HBCDs. In worldwide comparison, DDTs and HCHs were relatively high in human milk from India. However, the levels of PBDEs and HBCDs were comparable to many Asian and European countries but far lower than in America and Canada. Contamination pattern in human milk was different between southern and northern India, indicating regional specific exposure routes and variable sources. PBDE congener profiles in this study were different from the general pattern found in worldwide human milk, indicating specific exposure to PBDEs in Indian population.

2. Introduction:
Organohalogen compounds such as dichlorodiphenyl trichloroethane (DDT), hexachlorocyclohexanes (HCHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) have been extensively used and determined in all environmental and biological matrices. Exposure to organohalogen compounds has also been associated with an increased health risk. Particularly, infants are extremely vulnerable to pre- and post-natal exposure, resulting in a wide range of adverse health effects including possible long-term impacts on intellectual function[1] and delayed effects on central nervous system functioning[2], neuro-developmental effects, effects on learning behavior, etc. 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 Asia/Asian developing countries is limited.

Environmentalists, scientists and public health advisors have become interested on developing countries in tropical and subtropical regions with respect to finding the potential sources of the organohalogen compounds originating from these regions and contaminating the global environment and biota. India is of particular interest because of its widespread, past and ongoing use of organochlorine pesticides such as DDT and HCHs and the introduction of bans rather later than in many other countries. In addition, its strong agriculture based economy and rapid industrial development may also lead to series of environmental problems. During the process of industrial development, the urban population expanded rapidly and environmental pollution became apparent. Indeed, organohalogen compounds may become the biggest environmental concern in India, especially in major cities and their suburbs. The present study was therefore carried out to determine the current contamination status and geographical variation of organohalogen compounds in human breast milk collected from several parts of India.

3. Materials and Methods:
3.1 Sample collection
Human breast milk samples *(n=140)* were collected during 2009 from different locations in India such as Chennai, Madurai and Mumbai (urban), Goripalyam in Bangalore (urban slum), Chidambaram (suburban agriculture) and Parangipettai, Thoothukudi and Marakanam (fishing village) in India. Informed consent was obtained from all donors. Samples were collected manually in chemically cleaned containers and stored in dry ice.

All the samples were shipped to Ehime University, Japan, in dry ice and stored in the environmental specimen bank (*es*-BANK) for global monitoring[3] at −20 °C until chemical analysis. Out of these, *(n=26)* samples from Chennai (*n=7*), Bangalore *(n=7)*, Chidambaram *(n=7)* and Kolkata (municipal dumping site) *(n=5)* were used for analyzing the PBDEs and HBCDs. Data for organochlorine compounds (OCs) such as DDTs, HCHs and PCBs in human breast milk collected during 2000-2006 from different locations in India were cited from previous studies reported by our group[4, 5, 6].

3.2 Chemical analysis

Briefly, approximately 40g of the sample was freeze-dried and extracted with a 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 BDEs and $^{13}C_{12}$–labeled HBCDs ($^{13}C_{12}$-labeled BDE-3, BDE-15, BDE-28, BDE-47, BDE-99, BDE-153, BDE-154, BDE-183, BDE-209 and α-, β-, γ- isomers) and as surrogates and then subjected to gel permeation chromatography (GPC) for fat removal and eluted with a mixture of 50% hexane/dichloromethane (1:1). The lipid removed 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 for clean-up. $^{13}C_{12}$-labeled BDE-139 was added as an internal standard to the final solution prior to quantification using a gas chromatograph equipped with a mass selective detector (GC-MSD).

Quantification of PBDEs was performed using a GC (Agilent 6980 N) with MSD (Agilent 5973 N) for mono- to hepta-BDEs and GC (Agilent 6980 N) coupled with MSD for octa- to deca-BDEs, having an electron impact with selective ion monitoring mode (EI-SIM). Fourteen congeners of PBDE 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 and isomers were quantified using the isotope dilution method to the corresponding $^{13}C_{12}$-labeled congener or isomers. Whereas for HBCDs, isomers (α-, β-, γ-HBCD) were quantified using liquid chromatograph with tandem mass spectrometry detector (LC-MS - MS), based on the method published elsewhere[7]. OCs such as DDTs (*p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD), HCHs (*α-, β-* and *γ-* HCH isomers) and PCBs were analyzed and quantified by gas chromatograph equipped with ECD (electron capture detector), based on the method published elsewhere[4].

3.3 Statistical analysis

Statistical analysis of the results was performed with the SPSS software (SPSS for Windows: SPSS Inc., 2001). The Mann–Whitney U-test was used to compare each group, and Spearman rank correlation was used to examine the strength of associations between parameters. A probability value of <0.05 was considered statistically significant.

4. Results and discussion:

4.1 Contamination status & composition of organochlorine compounds

Among the OCs, HCHs & DDTs (880 & 740 ng/g lipid wt.) were the predominant contaminants followed by PCBs (62 ng/g lipid wt.), in human breast milk in India. Contamination patterns of OCs varied among different regions of India. DDTs were the predominant contaminants in New Delhi, Mumbai, Kolkata and Kolkata dumping site located in the northern, western and eastern parts of India, whereas HCHs were the dominant contaminants in Chennai, Chidambaram, Parangipettai and Perungudi located in the southern part in India. This result indicates location specific exposures to OCs, due to variations in their usage patterns in different regions of India, particularly for DDTs and HCHs.

Among the locations, concentrations of OCs were significantly higher in urban locations (New Delhi, Kolkata and Chennai) than the other locations such as semi-urban (Chidambaram) and rural fisheries (Parangipettai) **(Fig 1).** This may probably be due to ongoing usage of organochlorine pesticide such as DDT for the eradication of vector borne diseases, applied largely in urbanized areas. In India, the use of DDT in agriculture was banned in 1989, but has been permitted for public health purposes (restricted use up to 10,000 tons/year) due to its cost effectiveness, multi-spectrum applicability and persistence. In addition, differences in dietary habits between urban and rural people may also be responsible for the observed higher concentrations of OCs in urban milk

samples, because the urban residents are relatively richer, and consume more animal products and lipid rich food that are having more of these lipophilic contaminants.

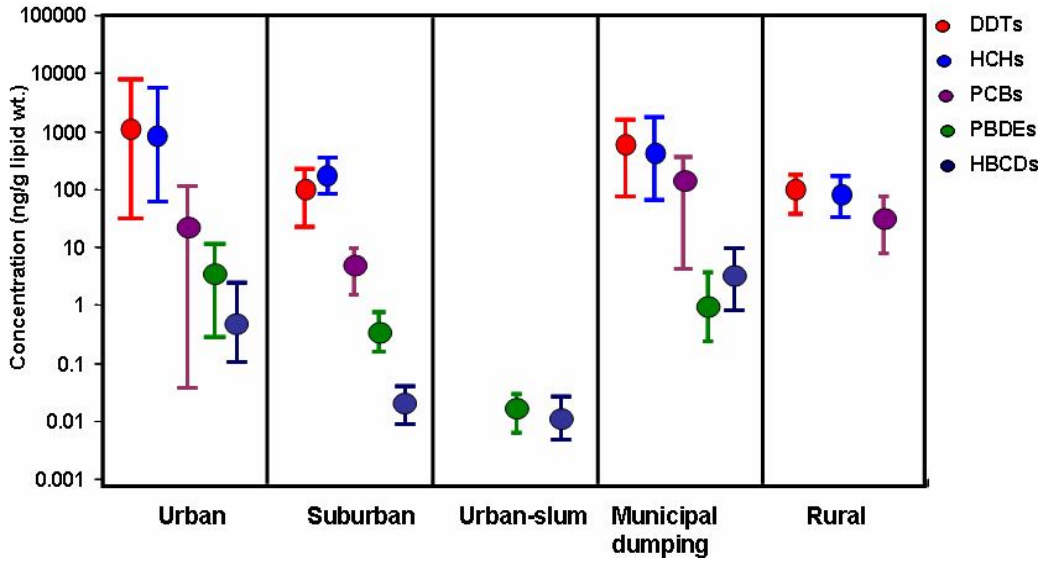

Figure 1. Concentrations of organohalogen compounds in human breast milk from different regions in India

Generally, levels of OCs in the present study were lower than those in the same locations observed previously, indicating that the concentrations of OCs have been declining in the Indian environment. For example, DDTs and HCHs in human breast milk from different locations in India are lower when compared with the values in previous studies [8,9,10]. However, there is an exception, an increase in DDTs and HCHs concentrations in Chennai between 1988 and 2003. Past and on-going usage of these two pesticides for controlling vector borne diseases and continuous intake of contaminated foods may be the plausible reasons for this. The general declining trends in other areas found in the present study confirms the positive effects of governmental and voluntary restrictions and prohibitions on the usage of DDT and HCH and other measures to minimize their pollution.

Worldwide comparison indicates that India is still at the top of DDT and HCH contamination levels. The elevated levels of DDTs and HCHs in developing countries in the tropical region, including India are due to local usage as considerable quantities of these compounds are still being applied for disease control and sanitary purposes, while developed nations stopped using DDTs several years ago. At the same time, global contamination patterns imply that developing countries in the tropical region play a major role as a source of OCs.

Among the DDTs, *p,p'*- DDE was the main compound found in the present study, accounting for 74-82% of the total DDTs concentrations, suggesting wide usage in the past and long–term accumulation of DDTs in humans. However, *p,p'*- DDT contributed 13-20% of the total DDT concentrations in human milk which could also be due to fresh exposure to DDT. Among HCH isomers, the most persistent and bioaccumulative isomer, β-HCH was predominant contributing 85-96% of the total HCHs. However, in some individuals of the present study, α-HCH exceeded over 50% of the total HCHs, indicating that specific donor(s) are still exposed to recent application of technical HCH.

4.2 Contamination status & composition of brominated flame retardants

The total PBDE concentrations varied widely from 0.090 to15 ng/g lipid wt with a mean concentration of 2.2 ng/g lipid wt. Levels of HBCDs also varied widely, from 0.014 to 15 ng/g lipid wt., with an overall mean of 1.8 ng/g lipid wt. Concentrations of PBDEs in the urban location (Chennai) (6.1 ng/g lipid wt) was significantly (*$p<0.05$*) higher than urban slum (Bangalore) and semi urban (Chidambaram) locations (0.020 and 0.70 ng/g lipid wt., respectively) **(Fig 1)** indicating the presence of prominent sources in urban locations. It is apparent that urban populations may more exposure to PBDEs containing products than the slum population. Relatively high levels of PBDEs and HBCDs present in municipal dumping site (Kolkata) compared with urban slum and semi urban areas indicate that the municipal open dumping sites in developing countries act as potential sources for many chemical contaminants. In global comparison the PBDE levels in India were comparable to many European and Asian countries but far lower than North America and Canada. The HBCDs levels were comparable with Philippines[10] and Russia but lower than in Japan[11]. The levels of PBDEs and HBCDs in India are low. But the biological effects and potential risks of PBDEs in human even in low levels are yet to be determined.

Among PBDEs, higher brominated congeners such as BDE-197, -207 and -209 were the predominant congeners found in this study (data not shown), the profile of which is more or less similar to the PBDEs profile reported recently in human milk samples from general population in china[7]. The profile in this study was different from the general pattern in human milk from various studies worldwide, in which lower brominated congeners were generally predominant. High proportion of BDE-209 in this study suggests the possible wide industrial use of commercial deca-BDE in India. In the present study, the PBDEs profile between the donors of different professions (teacher, pharmasist, nurse, typist, sweeper, etc) and hosue wives was similar (data not shown), indicating occupational exposure may not be a significant determinant of the profile of PBDEs in human. In this study, the stable isomer, α-HBCD was the predominant in all the samples. Further measurements of organohalogen compounds in food items such as dairy products, meat, seafood, fruits, vegetables and environmental samples including water, air and dust in India are necessary to assess the pathways of human exposure to organohalogen compounds.

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