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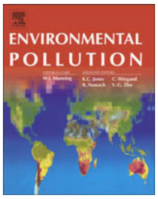

Organohalogen compounds in human breast milk from mothers living in Payatas and Malate, the Philippines: Levels, accumulation kinetics and infant health risk

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First comprehensive study on organohalogen contaminants in human breast milk from the Philippines.

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ABSTRACT

Human breast milk samples ($n = 33$) from primipara and multipara mothers from Payatas a waste dump site, and Malate a reference site in the Phillipines were collected in 2004 and analyzed for eight organohalogen compounds, viz., PCBs, DDTs, CHLs, HCHs, HCB, TCPMe, PBDEs and HBCDs. DDTs and PCBs were predominant in all the samples. Overall mean concentrations of PBDEs found in our study were higher (7.5 ng/g lipid wt.) than those reported for Japan and many other Asian countries. Primipara mothers had significantly higher levels of DDTs, CHLs and HCHs than multipara mothers, but not PBDEs and HBCDs. A few individuals accumulated CHLs close to or even higher than the tolerable daily intake guidelines proposed by Health Canada.

1. Introduction

During the last few decades, numerous studies have been conducted on global contamination and toxic effects of persistent organochlorines (OCs) such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane and its metabolites (DDTs), chlordane compounds (CHLs), hexachlorocyclohexane isomers (HCHs) and hexachlorobenzene (HCB) (Tanabe and Kunisue, 2007). These are highly bioaccumulative and have serious effects on environmental quality as well as wildlife and human health (Tanabe and Kunisue, 2007). Due to their historically high rate of use, with only a recent ban on their production or restrictions on use, tropical regions in developing countries are considered to be major sources of these pesticides (Jaward et al., 2005).

Because of their environmental stability, persistence and high production volume, polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) are among the most abundant brominated flame retardants (BFRs) detected in the environment and in wildlife and humans (Alaee et al., 2003). Concentrations and distribution of BFRs in environmental and human samples have been well documented in North America and Europe (de Wit, 2002; Sjödin et al., 2003). However, the number of studies in Asia/Asian developing countries are limited (Tanabe et al., 2008). By 2001 Asia consumed a large quantity of the total commercial PBDEs (24,650 tons, 37%) ranking second after America (33,100 tons, 49%), whereas consumption in Europe was the lowest (8360 tons, 12%) (BSEF, 2007). Thus, there is an urgent need to identify sources of exposure to PBDEs in Asian countries, quantify emission and document their potential environmental fate in the region. In fact, there is an important difference in exposure routes of PBDEs from chlorinated persistent organic pollutants (POPs). Diet was regarded as a major pathway of exposure to PBDEs like chlorinated POPs, because they also bioaccumulate in food chains (Bocio et al., 2003), but recent studies discovered that house dust, not diet, was the main exposure route of PBDEs (Wu et al., 2007) and that humans are exposed to them through direct inhalation, ingestion and dermal exposure (Lorber, 2008).

HBCDs are used as an alternative for PBDEs in some applications (Sellström et al., 1998) and consist mainly of three diastereomeric

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pairs of enantiomers; α-, β-, and γ-HBCD, with the γ-isomer being the major constituent (>70%). However, biological samples such as marine organisms and human serum contain mainly the α-isomer (Janak et al., 2005). By 2001 world market demand for HBCDs was 16,700 metric tons, of which 23% was sold in Asia (BSEF, 2007). Information on the concentration and distribution of the different diastereomers in human milk seems to be crucial for understanding the fate and behavior of these compounds as well as for assessing the health risk associated with exposure to HBCDs.

The Philippines is one of the archipelagic countries in the Southeast Asian region, with a population of 85 million. Because of the lack of advanced facilities, large amount of municipal wastes are directly dumped at Payatas without proper management, causing probably several adverse environmental consequences and increased human health risk to local communities. The data on human exposure to organohalogen compounds in the Philippines is rather scarce and comprehensive studies have not been made. The present study was therefore carried out to determine concentrations of persistent organohalogens such as PCBs, OC pesticides, PBDEs and HBCDs in human breast milk samples from primipara and multipara mothers living in two locations in the Philippines, viz., one near a waste dumpsite, Payatas and a reference site Malate. In addition, the present study examined relationships between contaminant levels and parity/age of the mothers and assessed intake of contaminants by infants through breast milk consumption.

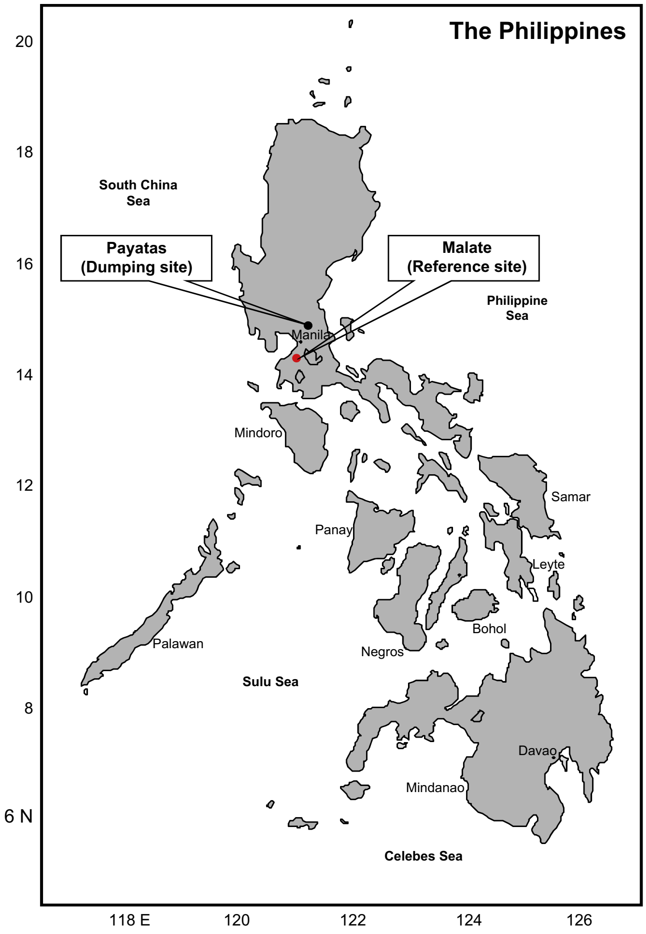

Fig. 1. Map showing the sampling locations.

2. Materials and methods

2.1. Sample collection

Human breast milk samples ($n = 33$) from primipara and multipara mothers living in two locations in the Phillipines (Fig. 1) were obtained in 2004 and analyzed for eight organohalogen (PCBs, DDTs, CHLs, HCHs, HCB, TCPMe, PBDEs and HBCDs) compounds. Of these, twenty-two samples were from the mothers living in the periphery of a dumping site at Payatas [primipara ($n = 4$) and multipara ($n = 18$)] and eleven samples were from the reference site at Malate [primipara ($n = 2$) and multipara ($n = 9$)]. About 100 ml of breast milk was collected using a breast pump expressing the milk into pre-washed glass containers prepared for every individual. These samples were shipped frozen to the laboratory in Japan and stored at −20 °C in the Environmental Specimen Bank (*es*-Bank) of Ehime University (Tanabe, 2006) until analysis. Almost all the randomly selected breast milk donors were housewives in the age range of 17–41, their demographic characteristics and relevant information of sampling sites are given in Table 1. Informed consents were obtained from all the donors and the answers for the questionnaire on dietary aspects were recorded.

2.2. Chemical analysis

Analysis of PBDEs was performed following Ueno et al. (2004), with slight modification, and that of HBCDs following Tomy et al. (2004). Briefly, a 40 g sample of human breast milk was spiked with 2 ng of internal standards of PBDEs ($^{13}C_{12}$-labeled BDE-3, -15, -28, -47, -99, -153, -154, -183, -197, -207 and -209), and 10 ng of internal standards of HBCDs (α-, β- and γ-$^{13}C_{12}$-HBCD) as surrogates and extracted using column extraction on the absorber, 40 g pre-cleaned diatomite earth (Merck, Damstadt, Germany) and eluted with 250 ml of diethyl ether. The extract was then subjected to gel permeation chromatography (GPC; Bio-Beads S-X3, Bio-Rad Laboratories, CA, 2 cm i.d. and 50 cm length) for lipid removal and eluted with mixture of 50% hexane/dichloromethane (1:1). The GPC fraction containing organohalogens was concentrated and passed through 4 g of activated silica gel (Wakogel DX, Wako Pure Chemical Industries Ltd., Japan). The first fraction of 5% dichloromethane in hexane contained PBDEs, while the second fraction of 25% dichloromethane in hexane contained HBCDs.

To the fraction of PBDEs, $^{13}C_{12}$-BDE-139 was added as an internal standard and concentrated prior to GC–MS analysis. Quantification was done using a GC (Agilent 6890N) equipped with MSD (Agilent 5973N) for mono- to hepta-BDEs and GC (Agilent 6890N) coupled with MSD (JEOL GC mate II) having an electron ionization with selective ion monitoring (EI-SIM) mode for octa- to deca-BDEs. Fourteen congeners of PBDE from mono to deca (BDE-3, -15, -28, -47, -99, -100, -153, -154, -183, -196, -197, -206, -207, and -209) were quantified in this study. All the congeners were quantified using the isotope dilution method to the corresponding $^{13}C_{12}$-labeled congener. The fraction of HBCDs was transferred, evaporated, and spiked with 10 ng of HBCD-d_{18} (α-, β- and γ-HBCD-d_{18}) as an internal standard prior to LC–MS–MS analysis. Sample extract was quantified with Quattro Micro API triple-quadrupole mass spectrometer (Waters/Micromass, Tokyo, Japan) equipped with Alliance 2795 LC separation module (Waters, Tokyo, Japan). LC separation of three isomers (α-, β- and γ-) of HBCDs was achieved with an Extend-C18 column (2.1 mm i.d. × 150 mm, 5 μm). An MS–MS analysis, which was operated in negative mode of electro spray ionization (ESI), was performed in multiple reactions monitoring mode (MRM). Quantification of native HBCDs was achieved from mean value of the response of two MRM transitions (i.e., m/z 640 → 81, m/z 642 → 81) corrected with response of $^{13}C_{12}$-HBCDs (i.e., m/z 652 → 81 MRM transition).

Organochlorine compounds were analyzed following Minh et al. (2004). Briefly, a 10 g sample of human breast milk was extracted in an absorber, 10 g pre-cleaned diatomite earth packed in a glass column and extracted with 200 ml of diethyl ether. The extract was subjected to GPC for lipid removal. The fraction containing OCs was concentrated and passed through 12 g of activated Florisil for separation. The first fraction eluted with hexane contained PCBs, hexachlorobenzene (HCB), dichlorodiphenyldichloroethylene (p,p'-DDE) and *trans*-nonachlor, and the second fraction eluted with 20% dichloromethane in hexane contained dichlorodiphenyltrichloroethane (p,p'-DDT), dichlorodiphenyldichloroethane (p,p'-DDD), hexachlorocyclohexane (HCH) isomers (α-, β- and γ-), *cis*-nonachlor, *trans*-nonachlor, *cis*-chlordane, *trans*-chlordane, oxychlordane and *tris*(4-chlorophenyl) methane (TCPMe). OCs except TCPMe were quantified by a GC–ECD (Agilent 6890 series) equipped with an auto-injection system. The GC column employed was DB-1 (J&W Scientific, Folsom, CA, USA) fused silica capillary (0.25 mm i.d. and 30 m length) coated with 100% dimethylpolysiloxane at 0.25 μm film thickness. OC concentrations were calculated from the peak area of the sample to the corresponding external standard. The PCB standard used for quantification was an equivalent mixture of 62 PCB isomers and congeners (BP-MS) obtained from Wellington Laboratories Inc. (Ontario, Canada) with known PCB composition and content. Concentrations of individually resolved peaks of PCB isomers and congeners were summed to obtain total PCB concentrations. For the quantification of TCPMe, a GC–MSD (Agilent 6890 series GC coupled with 5973 mass selective detector) was employed. Lipid contents were determined gravimetrically by measuring the total nonvolatile solvent extractable material on sub samples taken