# Polybrominated diphenyl ethers and organochlorine pesticides in human breast milk from Massachusetts, USA†

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*Received 19th March 2007, Accepted 26th July 2007*
*First published as an Advance Article on the web 10th August 2007*
**DOI: 10.1039/b711409p**

Concentrations of polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs; DDTs, HCHs, CHLs, and HCB) were measured in human breast milk samples collected across Massachusetts, USA, in 2004. Seventeen PBDE congeners were found in the samples, ranging in concentration from 0.06 to 1910 ng $g^{-1}$ lipid wt. BDE-47 (2,2′,4,4′-tetraBDE), BDE-99 (2,2′,4,4′,5-pentaBDE), and BDE-100 (2,2′,4,4′,6-pentaBDE) were the major congeners detected in breast milk samples. Overall mean (±SD) concentrations of DDTs, HCHs, CHLs, and HCB were 64.5 ± 75, 18.9 ± 19, 32.4 ± 36, and 2.3 ± 2.2 ng $g^{-1}$ lipid wt, respectively. Concentrations of PBDEs were strongly correlated with concentrations of OCPs in the samples. Based on the concentrations of organohalogens and the intake rates of breast milk by infants in the United States, daily ingestion rates of contaminants were calculated. The median ingestion rates for PBDEs, HCHs, DDTs, CHLs, and HCB were 4.0, 212, 141, 44, and 5.79 ng $kg^{-1}$ body wt $day^{-1}$, respectively. The estimated daily intake of organohalogens by infants was compared with threshold reference values suggested by the United States Environmental Protection Agency (USEPA) and the Agency for Toxic Substances and Disease Registry (ATSDR), for calculation of hazard quotients (HQs). HQs for individual organohalogens and the sum of HQ for all organohalogens were calculated as HQ indices (HQI). The results suggest that one or more of the contaminants analyzed in this study exceeded the threshold reference values in at least 26% of the breast milk samples.

## Introduction

Polybrominated diphenyl ethers (PBDEs) are persistent, bioaccumulative and toxic organic contaminants found in humans and wildlife. PBDEs are additive flame retardants used to meet requirements of the manufactured products to prevent combustion;[1] these compounds are incorporated into electronics, furniture, building materials, textiles, and polyurethane foams.[2] PBDEs have been commercially produced as pentaBDE mixture (tri- to hexa-BDE congeners), octaBDE mixture (hexa- to nona-BDE congeners) and decaBDE mixture (nona- and deca-BDE congeners). Manufacture of the pentaBDE and octaBDE mixtures ceased in 2001 in European Union countries, and in 2004 in the United States. During the last decade, a number of studies have reported the occurrence of PBDEs in human tissues. Concentrations of PBDEs have been increasing exponentially, with a doubling time of ~5 years.[3] Furthermore, tissue concentrations of PBDEs in the United States population are much higher than those reported for European and Asian populations.[4] Such elevated concentrations, coupled with an exponential increase of PBDEs in human tissues in the United States, underscores the need to identify the sources of exposures. In this context, exposure of newborn babies to PBDEs and organochlorine pesticides (OCP) through the ingestion of breast milk is a concern. Analysis of PBDEs in breast milk provides a means not only to assess the contaminant burden in mothers but also to assess potential exposure of neonates feeding on breast milk. In an attempt to characterize exposure pathways of PBDEs, we have analyzed human breast milk samples collected in 2004 from Massachusetts, USA. In addition to PBDEs, OCPs, including chlordane and its derivates (CHLs), dichlorodiphenyl trichloroethane and its derivates (DDTs), hexachlorocyclohexane isomers (HCHs), and hexachlorobenzene (HCB), were determined. Demographic and biological factors that influence the concentrations were examined, and daily intake rates of organohalogen contaminants by infants through the ingestion of milk were calculated. While earlier studies have reported PBDE concentrations separately from other contaminants, this study reports concurrent analyses of PBDEs and OCPs, enabling (a) comparisons among these organohalogens, and (b) estimates of relative risks to infants, from exposure to organohalogens in breast milk.

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## Material and methods

### Samples

Human milk samples were obtained from volunteer donors ($n$ = 38) from 23 towns situated across the state of Massachusetts, USA, between June and November, 2004 (Fig. 1). Demographic information, including age, and number of infants previously breast fed, was obtained from all participants. Institutional Review Board (IRB) approval was obtained for collection and analysis of breast milk samples. Samples were stored in pre-cleaned and sterile glass bottles, and then transported to the laboratory where they were diluted 1 : 1 with phosphate buffered saline. Samples were centrifuged at 100 $g$ for 15 min to pelletize cells in the milk. Samples were held at −20 °C; then after 1 week, the frozen milk samples were stored at −80 °C until analysis.

### Chemical analysis

PBDEs and OCPs were analyzed following the method described elsewhere.[4,5] A detailed description of the analytical methods used is given in the ESI†. Briefly, the measurement of tri- to hexa-BDE congeners was accomplished by use of a Thermo Finnigan Trace GC Ultra gas chromatograph-MAT95XP high resolution mass spectrometer (HRGC-HRMS). Measurements were carried out at a resolution of >9000–10 000. PBDE congeners were monitored using the two most abundant masses of the ion clusters $[M]^+$ ($m/z$ 405.8026 and 407.8006) for tri-BDE, $[M]^+$, ($m/z$ 283.6955 and 285.6935) for tetra-BDE, $[M\text{-}Br_2]^+$ ($m/z$ 403.7800 and 405.7800) for penta-BDE, and $[M\text{-}Br_2]^+$ ($m/z$ 481.6975 and 483.6955) for hexa-BDE. A six-point calibration curve (0.2–100 ng $mL^{-1}$) was prepared every time when samples were measured.

The analysis of decabromodiphenyl ether (BDE-209), 2,2′,3,4,4′,5,6-heptabromodiphenyl ether (BDE-183), and 2,2′,3,4,4′,5,5′,6-octabromodiphenyl ether (BDE-203) was performed using an Agilent Technologies 6890N gas chromatograph-electron capture detector (GC-ECD). The commercial octaBDE (Great Lakes DE79) and decaBDE (Great Lakes DE83) products (Great Lakes Chemical Corporation, West Lafayette, IL, USA) were used as qualitative standards for the identification of octa- and deca-BDE congeners, respectively. Composition and retention times of commercial octa- and deca-BDE specific congeners were confirmed by GC-MS. A calibration curve was prepared for each congener, to quantify concentrations in samples. The term total PBDE (∑PBDEs) denotes the sum of all of the identified tri- to deca-BDE congeners.

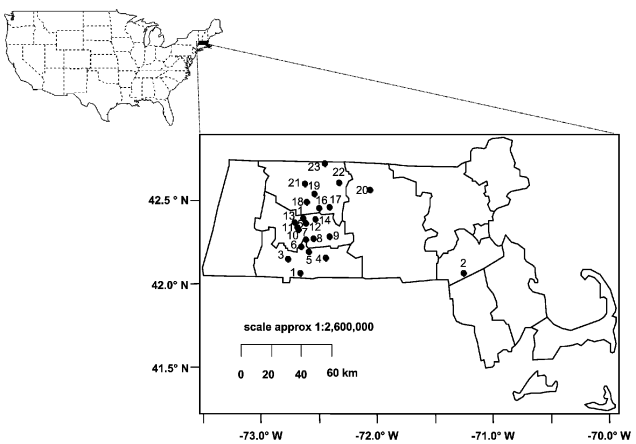


**Fig. 1** Map of Massachusetts, USA showing human breast milk sampling locations. Town locations on the map are: Agawam (1), Mansfield (2), Westfield (3), Wilbraham (4), Chicopee (5), Holyoke (6), South Hadley (7), Granby (8), Belchertown (9), Florence (10), Northampton (11), Hadley (12), Leeds (13), Amherst (14), West Hatfield (15), Leverett (16), Shutesbury (17), South Deerfield (18), Montague (19), Templeton (20), Greenfield (21), Orange (22), Northfield (23).

Organochlorine pesticides were analyzed on an Agilent Technologies 6890N gas chromatograph with electron capture detector. An external six-point standard calibration curve was used to quantify the sample concentrations from the peak areas for each OCP. The term DDTs refers to the sum of $o,p'$-DDE, $p,p'$-DDE, $p,p'$-DDT, and $p,p'$-DDD; The term CHLs refers to the sum of *trans*-chlordane, *cis*-chlordane, *cis*-nonachlor, *trans*-nonachlor, and oxychlordane; The term HCHs refers to the sum of α-, β-, γ-, and δ-HCH isomers. The identities of the detected OCPs were confirmed by GC-MS. Concentrations of PBDEs and OCPs are reported on a lipid weight basis. PBDEs are identified by the IUPAC numbering system.

### Quality assurance and quality control

Details of quality assurance and quality control are given in the ESI†. Briefly, the entire analytical procedure, including extraction, cleanup, and fractionation steps, was evaluated by the spiking of $^{13}C$-labelled internal and native surrogate standards. Procedural blanks were analyzed for every set of 10 samples, to check for interferences and contamination. The concentrations of signals lower than the limit of detection (calculated as 3 times the standard deviation of blank measurements) were reported as below the limit of detection. Retention time, molecular ion ratios, and the abundance of the monitored ions were used for the identification. The reported concentrations were not corrected for recoveries of internal standards.

### Statistical analysis

Data are presented as mean ± standard deviation. The non-parametric Mann–Whitney or Wilcoxon rank test was used to compare organohalogen concentrations between mothers who have nursed one or more infants previously and mothers who have not nursed before. Linear regression was performed to obtain relationships between PBDE and OCP concentrations and age of nursing mothers. For all comparisons, a value of $P < 0.05$ was considered significant. The distribution of all contaminant concentrations was examined using the Shapiro–Wilk goodness-of-fit test.[6] Skewed data were log transformed for further analysis. Values that fell at 1.5 to 3 times the distance from the upper or lower boundaries of the interquartile range of all values were considered outliers, and values that fell at 3 times the distance from the interquartile range were considered as extreme values. Upper and lower

 

boundaries represent the highest and lowest values.[7] The statistical software package R (release 2.4.1) was used for all calculations.[8]

## Results and discussion

Mean age of the volunteer nursing mothers was 35 ± 5 years (mean ± SD), and ranged from 24 to 45 years. Most mothers (79%; $n = 30$) enrolled in this study were nursing their first child, and only 21% ($n = 8$) were those who had nursed one or more previous children. While the concentrations of organohalogen compounds detected in breast milk samples varied from sample to sample, some general trends were observed in the data. PBDEs were detected in all samples. PBDEs were the most prevalent contaminants in human milk samples followed by DDTs (Table 1). Overall, the distribution pattern of contaminant concentrations analyzed in the breast milk samples from Massachusetts was PBDEs > DDTs > CHLs > HCHs > HCB.

### PBDE concentrations and profiles

Seventeen PBDE congeners were measured in the breast milk samples; included were BDE-28, BDE-47, BDE-66, BDE-77, BDE-85, BDE-99, BDE-100, BDE-118, BDE-138, BDE-153, BDE-154, BDE-183, BDE-203, BDE-209, and three unidentified congeners. Two of the tri-BDE congeners and one tetra-BDE congener were unidentified due to the lack of pure reference standards; however, the fragmentation pattern and ratios of molecular ion masses were characteristic of the corresponding BDE homologues. The concentrations of the sum of the 17 congeners ($\sum$PBDEs) found in breast milk samples varied widely, from 0.06 to 1910 ng $g^{-1}$ lipid wt, with an overall mean of 75 ng $g^{-1}$, lipid wt (median: 19.8 ng $g^{-1}$; Table 1). The mean concentration of PBDEs found in our study was of the same order of magnitude as concentrations reported in other studies for breast milk samples collected from Texas (mean: 73.9 ng $g^{-1}$),[9] the Pacific Northwest of the United States and Canada (mean: 95.6 ng $g^{-1}$),[10] and Massachusetts (mean: 42.7 ng $g^{-1}$).[11] Our concentrations were twice as high as the concentrations reported for breast milk collected in 2001–2002 in Canada (mean: 42.8 ng $g^{-1}$).[12] Studies from Sweden reported a mean PBDE concentration of 4.01 ng $g^{-1}$ for the breast milk collected in 1996–1999;[13] that value was 19-fold lower than concentrations determined in our study. Similarly, mean concentrations of PBDEs found in our study were greater than values reported for samples collected in 2004 in Japan (mean: 2.5 ng $g^{-1}$),[14] in 2004 in Poland (mean: 2.5 ng $g^{-1}$),[15] and in 1999 in the Faroe Islands, North Atlantic (mean: 7.2 ng $g^{-1}$).[16] We reported in our earlier study that the concentrations of PBDEs in human adipose tissues

**Table 1** Concentrations of PBDEs and OCPs (ng $g^{-1}$, lipid wt) in human breast milk samples collected in 2004 from Massachusetts, USA

| | Mean ± SD | Median | Range |
|---|---|---|---|
| Age of mothers | 35.3 ± 5 | 35 | 24–45 |
| Lipid content (%) | 2.2 ± 2.1 | 2.1 | 0.4–5.9 |
| Compounds | | | |
| PBDEs | | | |
| Tri-BDE | 1.97 ± 7.1 | 0.6 | <0.02–44.2 |
| Tetra-BDE | 42.1 ± 185 | 8.1 | <0.84–1140 |
| Penta-BDE | 19.9 ± 98 | 1.7 | <0.46–611 |
| Hexa-BDE | 6.11 ± 18 | 1.5 | <0.03–108 |
| Hepta-BDE | 6.22 ± 7.8 | 4.1 | <6–33.2 |
| Octa-BDE | <7 | <7 | <7 |
| Deca-BDE | <204 | <204 | <204 |
| Sum PBDEs | 76.3 ± 308 | 19.6 | 0.06–1910 |
| DDTs | | | |
| *o*,*p*′-DDE | 1.3 ± 4 | <0.6 | <0.6–8.9 |
| *p*,*p*′-DDE | 53.3 ± 64 | 35.3 | <0.6–177 |
| *p*,*p*′-DDT | 6.7 ± 13 | <0.6 | <0.6–29.1 |
| *p*,*p*′-DDD | 3.1 ± 3 | 2.7 | <0.6–6.3 |
| Sum DDTs | 64.5 ± 75 | 41.0 | <0.6–211 |
| HCHs | | | |
| α-HCH | 1.7 ± 1.9 | 1.4 | <1.6–9.1 |
| β-HCH | 7.7 ± 10 | 4.4 | <1.6–54.0 |
| γ-HCH | 7.6 ± 5.1 | 5.1 | <1.6–35.1 |
| δ-HCH | 1.9 ± 3.8 | <1.6 | <1.6–15.1 |
| Sum HCHs | 18.9 ± 19 | 13.4 | <1.6–74 |
| CHLs | | | |
| Oxy-CHL | 17.4 ± 23 | 3.8 | <1–80.4 |
| *Trans*-CHL | 1.1 ± 1.9 | <1 | <1–6.3 |
| *Cis*-CHL | 2.7 ± 4.4 | 1.2 | <1–20.5 |
| *Trans*-nona | 7.4 ± 8.8 | 4.2 | <1–31.3 |
| *Cis*-nona | 3.8 ± 7.5 | 2.6 | <1–44.7 |
| Sum CHLs | 32.4 ± 36 | 14.0 | <1–126 |
| HCB | 2.3 ± 2.2 | 1.6 | <0.8–10.6 |

**Table 2** PBDE congener concentrations (ng $g^{-1}$, lipid wt) in human breast milk samples collected in 2004 from Massachusetts, USA

| PBDE congeners | IUPAC number | Mean | SD | Median | Range |
|---|---|---|---|---|---|
| U3BDE1[a] | | 0.08 | 0.19 | <0.02 | <0.02–0.9 |
| U3BDE2[a] | | 0.02 | 0.09 | <0.02 | <0.02–0.55 |
| 2,4,4-tri-BDE | 28 | 1.80 | 7.05 | 0.47 | <0.02–43.6 |
| U4BDE[b] | | 0.43 | 1.58 | 0.84 | <0.84–9.64 |
| 2,2′,4,4′-tetra-BDE | 47 | 40.7 | 178 | 7.69 | <0.84–1100 |
| 2,3′,4,4′-tetra-BDE | 66 | 1.07 | 4.73 | <0.84 | <0.84–29.3 |
| 3,3′,4,4′-tetra-BDE | 77 | 0.02 | 0.11 | <0.84 | <0.84–0.66 |
| 2,2′,3,4,4′-penta-BDE | 85 | 1.02 | 5.50 | <0.46 | <0.46–34.0 |
| 2,2′,4,4′,5-penta-BDE | 99 | 11.8 | 57.7 | 1.46 | <0.46–357 |
| 2,2′,4,4′,6-penta-BDE | 100 | 6.91 | 34.6 | <0.46 | <0.46–611 |
| 2,3′,4,4′,5-penta-BDE | 118 | 0.20 | 1.06 | <0.46 | <0.46–6.54 |
| 2,2′,3, 4,4′,5′-hexa-BDE | 138 | 0.16 | 0.92 | <0.03 | <0.03–5.68 |
| 2,2′,4,4′,5,5′-hexa-BDE | 153 | 5.15 | 13.7 | 1.06 | 0.04–82.3 |
| 2,2′,4,4′,5,6-hexa-BDE | 154 | 0.63 | 3.18 | 0.05 | <0.03–19.7 |
| 2,2′,3, 4,4′,5′,6-hepta-BDE | 183 | 6.22 | 7.78 | 4.13 | <6–33.2 |
| 2,2′,3,4,4′,5,5′,6-octa-BDE | 203 | <7 | | <7 | <7 |
| 2,2′,3,3′,4,4′,5,5′,6,6′-decaBDE | 209 | <204 | | <204 | <204 |
| ∑PBDEs (sum of 17 PBDE congeners) | | 75.0 | 304 | 19.8 | 0.06–1914 |

[a] U3BDE1 and U3BDE2 = unidentified tribromodiphenyl ether. [b] U4BDE1 = unidentified tetrabromodiphenyl ether.

from the United States[4] were 10- to 100-fold greater than concentrations reported from European countries. Similarly, the concentrations that we have determined here in breast milk are 10- to 100-fold greater than those reported for Asian and European countries. Elevated concentrations of PBDEs in the United States' samples can be explained by the country's great market demand for PBDEs[1]. Flame retardants have become important additives in a number of products used in industrial and household products in the United States, due to the regulations that require flame retardants to be incorporated in many products in order to make them less combustible.

The highest concentration of ∑PBDEs in breast milk (1910 ng $g^{-1}$, lipid wt) was found in a sample from a 32-year old woman who was nursing for the first time. BDE-47 was the most abundant congener, accounting for 53.4% of the total PBDE concentration, followed in decreasing order by the congeners BDE-99, BDE-100, BDE-183, and BDE-153, accounting for (on average) 16%, 9.1%, 8.2%, and 6.8%, respectively, of the total PBDE concentration (Table 2). Together, these five congeners accounted for 93% of the total PBDE concentrations (Fig. 2). In addition to the above-mentioned congeners, the occurrence of PBDE-209 was examined in the breast milk samples, but it was not detected in any samples at concentrations above the detection limit of 204 ng $g^{-1}$.

### OCP concentrations and profiles

DDTs, CHLs, HCHs, and HCB were found in the breast milk samples in our study. DDTs showed the highest concentration among the OCPs with an overall mean concentration of 64.5 ng $g^{-1}$, lipid wt (Table 1), followed by CHLs (mean: 32.4 ng $g^{-1}$ lipid wt). The use of DDT was prohibited in the United States in 1972; at that time, the concentrations of DDT in breast milk ranged from 4000 to 13 040 ng $g^{-1}$ lipid wt.[17] In 1987, the mean concentration of DDTs in breast milk samples collected from New York was 563 ng $g^{-1}$, lipid wt.[18] Reported concentrations of DDTs in breast milk samples analyzed across the United States during the last three decades have been compiled to permit the temporal relationships in concentrations to be assessed. A plot of the year of collection *versus* the concentrations of DDTs showed that these concentrations are decreasing with one half-life of ~2 years (Fig. 3). The DDE : DDT ratio in our study was 8, which is an indicative that women have not recently been exposed to technical DDT product, but rather that DDT is from aged sources.

Among CHLs, oxychlordane (oxy-CHL), which is the most stable metabolite arising from the biotransformation of technical chlordane, was the most abundant compound in breast milk samples, accounting for 54% of total CHL concentration (Table 1). *Cis*- and *trans*-nonachlor isomers accounted for 12% and 23% of total CHL concentration, respectively (Fig. 2). CHL concentrations found in this study were lower than those reported for breast milk samples collected in Arkansas in 1986, (mean: 62 ng $g^{-1}$ lipid wt) for *trans*-nonachlor and (51 ng $g^{-1}$ lipid wt) for oxychlordane.[19] The average ratio of oxy-CHL : *trans*-nonachlor determined for breast milk samples from the Arkansas study (0.8) was lower

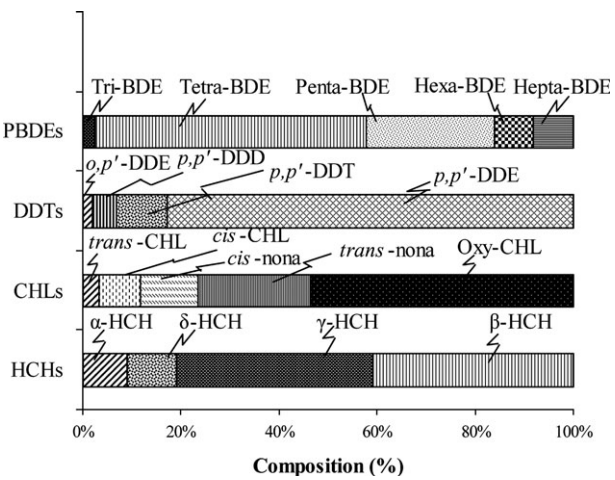


**Fig. 2** Profiles of homologue/congener/isomer patterns of PBDEs, and OCPs (DDTs, HCHs, HCB, and CHLs) in human breast milk samples collected in 2004 from Massachusetts, USA.

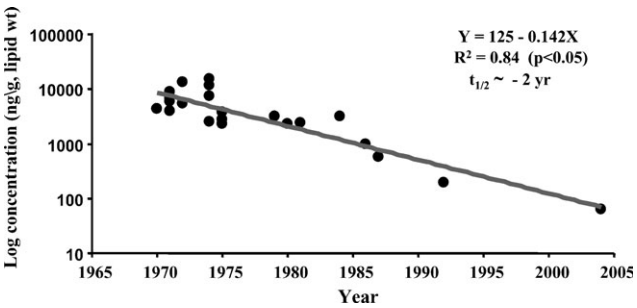


**Fig. 3** Log concentrations of DDTs (DDT + DDE) and PCBs in human breast milk samples collected across the United States (ng $g^{-1}$, lipid wt) shown as a function of time (year of sample collection). The overall regression is shown as log concentrations on the *Y* axis. DDT data collected from 1970 to 1980 were from Jensen and Slorach.[17] Other DDT data were included from additional references (ref. 18, 19, 35–37).

than ratio determined in the present study (2.4), implying that recent exposures to technical chlordane mixtures are at lower levels than in the study from two decade ago.

Among HCH isomers, β-HCH and γ-HCH (lindane) were the most abundant isomers in our study, collectively accounting for 81% of total HCH concentration (Fig. 2). The ratio of α-HCH to β-HCH in breast milk samples was 0.22 (Table 1); this low value implied a lack of recent exposure to technical HCH. However, the proportion of γ-HCH to β-HCH was 1 : 1, implying ongoing exposure to γ-HCH. γ-HCH is still being used in the United States as an insecticide. In addition, γ-HCH is available as a prescription medicine, in topical lotion, cream, and shampoo forms to treat pediculosis (*i.e.*, louse infestation) and scabies (mites) in humans.[20]

HCB was used as a fungicide but was banned in the mid-1970s by most countries, including the United States. However, HCB occurs as an impurity or is formed as a by-product in many chlorination processes, including wastewater treatment and production of chlorinated solvents and pesticides.[5] The mean HCB concentration in breast milk samples in the United States during 1979–1980 was 30 ng $g^{-1}$, lipid wt.[17] Breast milk samples collected from New York State during 1986–1992 contained HCB concentrations ranging from 1.7 to 14.4 ng $g^{-1}$, lipid wt.[21] HCB concentrations have decreased by 10-fold over the last two decades in the United States (2.3 ng $g^{-1}$, lipid wt).

In general, mean concentrations of OCPs in breast milk from the United States are lower than those reported for Japan and European countries (Table 3). However, mean concentrations of OCPs in breast milk samples from less

**Table 3** Mean concentration of organohalogens (ng $g^{-1}$, lipid wt) in breast milk samples collected from various countries

| Country (regions) | | *n* | Collection/yr | PBDEs | DDTs | HCHs | HCB | CHLs | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Asia | China | | | | | | | | |
| | Dalian | 20 | 2002 | | 2100 | 1400 | 81 | 16 | 44 |
| | Guangzhou | 27 | 2005 | 3.5 | | | | | 39 |
| | Shenyang, | 20 | 2002 | | 870 | 550 | 56 | 6.7 | 44 |
| | Japan | | | | | | | | |
| | Fukuoka | 93 | 2001–2004 | | 285 | 90 | 13 | 62 | 45 |
| | Kyoto | 30 | 2004 | 2.4 | | | | | 14 |
| | Shimane | 20 | 2004 | 1.6 | | | | | 14 |
| | Vietnam | | | | | | | | |
| | Hanoi | 42 | 2000 | | 2100 | 58 | 3.9 | 2 | 46 |
| | Hochiminh | 44 | 2001 | | 265 | 13.5 | 7.2 | 6.9 | 46 |
| Europe | Czech Republic | | | | | | | | |
| | Olomouc | 103 | 2003 | 1.93 | | | | | 43 |
| | Faroe Islands | | | | | | | | |
| | Tórshavn | 9 | 1999 | 7.2 | | | | | 16 |
| | Belgium | | | | | | | | |
| | Liege | 14 | 2000–2001 | 2.73 | | | | | 48 |
| | Italy | | | | | | | | |
| | Venice | Pool | 2000–2001 | 2.3 | | | | | 41 |
| | Rome | Pool | 1998–2000 | 4.1 | | | | | 41 |
| | Netherland | 103 | 1998 | 4.01 | | | | | 38 |
| | Poland | | | | | | | | |
| | Wielkopolska | 22 | 2004 | 2.5 | 868 | 14.1 | 32.2 | 3.3 | 15 |
| | Russia | | | | | | | | |
| | Republic of Buryatia | 35 | 2004 | 0.5 | 620 | 905 | 115 | 15 | 27 |
| | Sweden | | | | | | | | |
| | Stockholm | Pool | 1997 | 4.02 | 143 | | 12 | | 47 |
| | Uppsala | 93 | 1996–1999 | 4.01 | | | | | 13 |
| | Turkey | | | | | | | | |
| | Kaharamanmaras | 37 | 2003 | 0.63 | 2571 | 175 | 24 | 31 | 40 |
| | United Kindom | | | | | | | | |
| | Lancaster | 27 | 2001–2003 | 7.29 | 112 | 14.2 | 15 | 0.23 | 42 |
| | London | 27 | 2001–2003 | 11.1 | 330 | 59.6 | 24.6 | 0.46 | 42 |
| Americas | Canada | | | | | | | | |
| | Vancouver | 20 | 2001–2002 | 42.8 | | | | | 12 |
| | United States | | | | | | | | |
| | Massachusetts | **38** | **2004** | **76.3** | **64.5** | **18.9** | **2.3** | **32.2** | **This study** |
| | Texas | 47 | 2002 | 73.9 | | | | | 9 |

highly-industrialized countries were notably higher than those found for the United States, Japan or various European countries (Table 3).

### Correlations among organohalogens

The Shapiro–Wilk goodness-of-fit test for normality suggested that the transformed data are not statistically different from a normal distribution at a 95% confidence interval for PBDEs and DDTs (Fig. 4, histograms placed on right and top). However, data for HCHs, HCB, and CHLs were not normally distributed, despite logarithmic transformation of concentrations (Fig. 4; histograms on the panels above of the plot). Lack of a normal distribution of HCHs, HCB, and CHLs can be explained by low concentrations of these contaminants, and the existence of several non-detectable determinations. The highest concentration of PBDEs, 1910 ng $g^{-1}$ lipid wt, was an outlier and removed from the data set in the statistical analysis. Pairwise correlations among log transformed concentrations of PBDEs, HCHs, HCB, CHLs, and DDTs in breast milk samples were investigated. The coefficient of correlation was strong, statistically significant ($P < 0.05$) and positive, between PBDEs and each of the other type of organohalogens analyzed (Fig. 4 and ESI Fig. 1S†). All breast milk samples (Fig. 4; open circle) from mothers who had previously nursed infants were grouped at or below the regression lines. When these samples are removed from the dataset, stronger correlations were observed among OCP and PBDE concentrations (results not shown). The positive and strong correlations suggest the existence of common sources of human exposure to PBDEs and OCPs. Although a variety of sources of exposures to PBDEs, including indoor air, house dust, computer dust, meat, and fish have been suggested, dust and dietary exposure[22] appear to be an important and common pathway for all of the contaminants analyzed here. Meat, chicken, pork, and fish are important sources of lipophilic contaminants such as PBDEs[23] and OCPs. In addition, women are advised during pregnancy to increase the consumption of protein-rich food. The effect of a change in dietary habits can increase the levels of lipophilic contaminants in pregnant women. Recently, it has been shown that house dust is an important source of PBDEs[11] and OCPs.[24–26] Although concentrations of OCPs in house dust are expected to be lower than PBDE concentrations, a strong correlation among the contaminants analyzed in breast milk suggests some common sources of exposure.

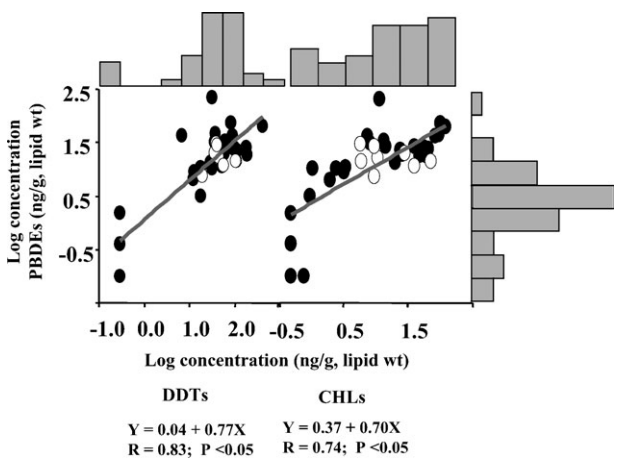


**Fig. 4** Distribution of concentrations (histograms: PBDEs on the right panel; OCPs in the top panel) and pairwise correlations between concentrations of PBDEs with those OCPs (DDTs, and CHLs) in human breast milk samples. Filled circles (●) represent mothers who have not previously nursed infants. Open circles (○) represent mothers who have previously fed infants.

### Organohalogen concentrations and demography

A lack of correlation was found between either original or log-transformed concentration, for organohalogen compounds, and age of the nursing mother (correlation coefficients ranged from −0.002 to 0.28; $P > 0.05$). This is consistent with findings of a previous report from human adipose tissue samples collected from New York City, in which PBDE concentrations were not correlated with age of female donors.[4] Lack of age-dependent increase in concentrations of organohalogens in adult females can be explained by excretion of these contaminants *via* breast milk. We compared the median concentrations of organohalogen compounds in the breast milk samples from women who had previously nursed one or more infants with the women who had not nursed before. No significant difference was found between the two groups of nursing mothers for all organohalogen contaminants analyzed ($P > 0.05$), which is similar to that reported earlier.[27]

### Organohalogen intake by infants and risk assessment

Daily intake of contaminants by infants *via* breast milk can be calculated by the equation $DI_i = C_i FMb$, where $DI_i$ is the daily intake estimated for each sample (μg $kgbw^{-1}$ $day^{-1}$); $C_i$ is the average concentration of organohalogen in each milk sample (μg $g^{-1}$ lipid wt); $F$ is the lipid content in each milk sample (g lipid per 100 g milk); and $Mb$ is the average daily consumption of milk per kg body weight (g $kgbw^{-1}$ $day^{-1}$). For this calculation, guidelines suggested by the USEPA,[28] such as the daily intake rate of breast milk and body weight for a one-month old infant were on average 702 mL of milk $day^{-1}$ (723 g of milk $day^{-1}$) and 4.14 kg, respectively, were used to calculate an infant's average milk consumption; which was ($Mb$) 175 g of milk $kgbw^{-1}$ $day^{-1}$. The infant's daily intake rates estimated for PBDEs, DDTs, HCHs, CHLs, and HCB, based on median concentrations in breast milk were 4.0 ng for selected PBDE congeners (BDE-47 + BDE-99 + BDE-153), 212 ng for HCHs (α-HCH + β-HCH + γ-HCH), 141 ng for *p,p′*-DDTs, 44 ng for CHLs, and 5.8 ng for HCB per kgbw $day^{-1}$. We also calculated the hazard quotient (HQ) for organohalogen contaminants, by dividing the daily intake (DI) by the corresponding threshold reference values (TRV) suggested by the USEPA or the Agency for Toxic Substances and Disease Registry (ATSDR). The TRVs are estimated as the minimal oral daily human exposure to hazardous substances for no-observed-adverse, non-carcinogenic effects. TRVs are reported as reference doses (RfD) by the USEPA and oral minimal risk levels (MRL) by the ATSDR. Particularly, TRVs considered in this study for *p,p′*-DDT, γ-HCH (lindane), HCB, BDE-47 and BDE-99, and CHLs were derived from neuro-developmental toxicological effects, which

can be directly related to the normal growth and development of infants. The TRVs used were: *p,p′*-DDT – 0.5 μg $kg^{-1}$ $day^{-1}$;[29] α-HCH – 8 μg $kg^{-1}$ $day^{-1}$;[30] β-HCH – 50 μg $kg^{-1}$ $day^{-1}$;[30] γ-HCH – 0.3 μg $kg^{-1}$ $day^{-1}$;[29] BDE-47 – 0.2 μg $kg^{-1}$ $day^{-1}$;[31] BDE-99 – 0.1 μg $kg^{-1}$ $day^{-1}$;[32] BDE-153 – 0.2 μg $kg^{-1}$ $day^{-1}$;[33] CHLs – 0.5 μg $kg^{-1}$ $day^{-1}$;[29] and HCB – 0.8 μg $kg^{-1}$ $day^{-1}$.[29] HQs were determined only for the congener/isomer for which RfD or MRLs were available. The median HQ values for all contaminants were lower than one (Fig. 5a; horizontal dashed line for HQ = 1) but the proportion of samples with HQ values above the median was 50% for PBDEs, CHLs, HCHs, and HCB and 42% for *p,p′*-DDT. The median (range) HQ values were 0.24 (0.001–35.6) for PBDEs, 0.64 (0–4.4) for HCHs, 0.01 (0–0.05) for HCB, 0.1 (0–0.93) for CHLs, and 0.0 (0–0.43) for *p,p′*-DDT. However, the median HQ as a central point estimate is prone to a high degree of uncertainty, due to the fact that individual samples can contain elevated concentrations of organohalogens, such that individuals with high concentrations may have HQ values greater than one. For instance, the percent of samples with HQ values >1 for PBDEs, HCHs, CHLs, *p,p′*-DDT, and HCB were 7.9% (3/38), 34.2% (13/38), 0% (0/38), 0% (0/38), and 0% (0/38), respectively (Fig. 5a). In addition, the proportion of samples with HQ > 1 for one or more organohalogens analyzed here is expressed as a cumulative hazard quotient index (HQI), which is calculated as:

$$HQI = HQ_{PBDEs} + HQ_{p,p'\text{-DDT}} + HQ_{CHLs} + HQ_{HCHs} + HQ_{HCB}$$

where $HQ_{PBDEs}$, $HQ_{p,p'\text{-DDT}}$, $HQ_{CHLs}$, $HQ_{HCHs}$, and $HQ_{HCB}$ are HQ for individual contaminants. A value of 1 is assigned when the HQ value exceeded 1 and a value of 0 is assigned for the samples with HQ between 0 and 0.99. The proportion of breast milk samples with HQ >1 for one or more of the organohalogens analyzed is shown in Fig. 5a. Sixty-six percent of the samples recorded HQ values of <1 for all of the organohalogens (*i.e.*, HQI = 0). At least one of the 6 contaminants analyzed showed an HQI value of >1 (HQI = 1) in 26% of the breast milk samples. HQI was 2 in 8% of the samples, and none of the breast milk samples analyzed had an HQI of 3–5. The exposure of an infant to one or more contaminants, simultaneously, at levels above the threshold reference values in the breast milk is an issue of concern (Fig. 5b). Despite the ban on OCPs over three decades ago, concentrations of these contaminants in breast milk have persisted at or above the threshold reference values suggested by the USEPA and the ATSDR. However, breast milk is the unique food that offers optimal and balanced ingredients (nutrients and immune protection factors) to supply the needs of growing infants. Therefore, the World Health Organization strongly recommends full breast feeding, at least during the first six months of an infants' life.[34] In our opinion, minimizing the levels of contaminants in breast milk would further enhance the benefits of breast feeding and the overall well being of children.

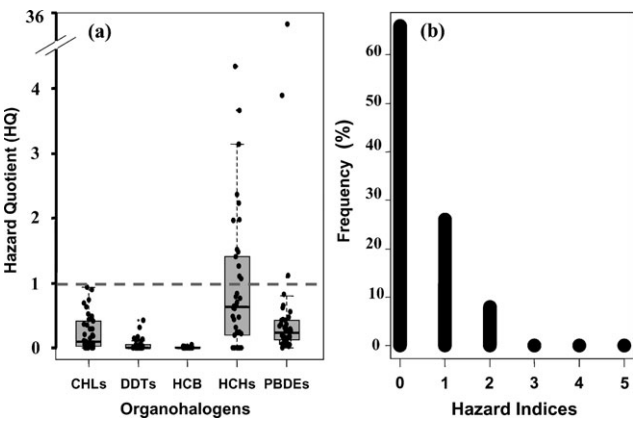


**Fig. 5** (a) Hazard quotients (HQ) for organohalogens estimated as a ratio between concentrations in human breast milk samples from Massachusetts, USA, and the threshold reference values suggested by the ATSDR and USEPA. Values of HQ over 1 exceed the daily intake guideline (μg $kgbw^{-1}$ $day^{-1}$). The box is delimited by the interquartile range (between 25th and 75th percentiles) with a line that crosses the box at the median (50th percentile). All data point determinations are plotted in the box plot as filled circles. (b) Distribution of hazard quotients for organohalogen compounds for the consumption by infants of mother's milk from Massachusetts, USA.

In summary, the results of this study suggest that the concentrations of PBDEs in breast milk of nursing women from Massachusetts are 10- to 100-fold greater than concentrations that have been reported for European and Japanese populations. Concentrations of HCHs, CHLs, HCB, and DDTs have declined in breast milk samples over the past two decades in the United States, although the residue levels of OCPs in certain individuals remained at or above HQ values, based on the reference doses suggested by the USEPA and the ATSDR. In this context, PBDEs, and HCH are critical contaminants in breast milk samples; the percent of the breast milk samples analyzed in this study that exceeded the threshold reference values for one and two contaminants were 26% and 8%, respectively. We have suggested a risk assessment based on a cumulative exposure approach, as indicated by HQ indices. HQ indices are useful to calculate cumulative risk from multiple contaminant exposures. In addition, HQ indices elucidate the proportion of one or more contaminants above the threshold for adverse effects that a population is exposed to. Significant correlation among organohalogens in breast milk suggests the existence of a common source of exposure, which is thought to be through the diet and home dust.

## Acknowledgements

The analytical portion of this study was funded by a grant from the Centers for Disease Control and Prevention U59/CCU223392 Public Health Laboratory Biomonitoring Implementation Program to Wadsworth Center (KK). We also thank to Dr Jan Storm, Mr Henry Spliethoff, Judy Abbott, and Kevin Gleason from Bureau of Toxic Substance Assessment of New York State Department of Health for comments on the earlier draft of the manuscript.

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