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Levels and profiles of PCDD/Fs, PCBs in mothers' milk in Shenzhen of China: Estimation of breast-fed infants' intakes

Bo Deng [a,b], JianQing Zhang [a,*], Lishi Zhang [c], YouSheng Jiang [a], Jian Zhou [a], Daokui Fang [a], Huiming Zhang [a], HaiYan Huang [a]

[a] Department of POPs Lab, Shenzhen Center for Disease Control and Prevention, No. 21, 1st Road Tianbei, Luohu District, Shenzhen, Guangdong 518020, PR China
[b] North Sichuan Medical College, Nanchong, Sichuan, 637000, PR China
[c] West China University of Medical Sciences, Sichuan University, Chengdu, Sichuan, 610041, PR China

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ABSTRACT

Sixty breast milk samples were collected in Shenzhen, China from July to November in 2007. The samples were analyzed of the concentrations of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs). The range of upper-bound for ∑TEQ-(PCDD/Fs + PCBs) in the samples was 4.10–35.3 pg TEQ g^{-1} lipid (median: 10.6 pg TEQ g^{-1} lipid; mean: 11.9 pg TEQ g^{-1} lipid). The levels of the measured contaminants in the breast milk had significant correlations with the length of inhabitation period in Shenzhen ($r = 0.487$, $p < 0.05$ for PCDD/Fs, $r = 0.431$, $p < 0.05$ for PCBs and $r = 0.478$, $p < 0.05$ for ∑TEQ-(PCDD/Fs + PCBs)), and the consumption rate of fish ($r = 0.366$, $p < 0.05$ for PCDD/Fs, $r = 0.486$, $p < 0.05$ for PCBs and $r = 0.416$, $p < 0.05$ for ∑TEQ-(PCDD/Fs + PCBs)), respectively. Moreover, significant positive correlations were also detected between the participant's age ($r = 0.305$, $p < 0.05$ for ∑TEQ-PCBs and $r = 0.275$, $p < 0.05$ for ∑TEQ-(PCDD/Fs + PCBs)) and the body burdens of these contaminants respectively. It is estimated that the daily intake (EDI) of the sum of PCDD/Fs and DL-PCBs by the breast-fed infants was 5.60–161 pg TEQ kg^{-1} bw per day (mean: 48.2 pg TEQ kg^{-1} bw per day; median: 42.2 pg TEQ kg^{-1} bw per day). The result showed that both the body burdens of PCDD/Fs and PCBs of the recruit population and the calculated EDI of the breast-fed infants were higher than those in the non-exposed areas in mainland China. This suggests that continuous surveillance on PCDD/Fs and PCBs levels in human milk is critical to more precisely evaluate the human health risk posed by the negative environmental impact in Shenzhen in the future.

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1. Introduction

Persistent organic pollutants (POPs), such as polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs), are a family of lipophilic stable toxic compounds that occur widely in the environment. Humans, due to their unique position on the food chains, may be exposed to higher levels of environmental pollutants through intake of contaminated food. Because of the ubiquitous occurrence, long-range transportation, bioaccumulation and metabolic persistence, and potent toxic effects of some of the congeners (including immunological, neurological, reproductive, carcinogenic effects, endocrine-disruption effects (Bláha et al., 2006), and thyroid disorders (Wang et al., 2005; Turyk et al., 2007)), they are classified into controlled and eliminated POPs. Since 1980s, many countries have banned the usage of some types of POPs such as PCBs, and have been monitoring levels and changing trend of PCDD/Fs and PCBs in organisms and environment regularly in order to control and eliminate their pollution (Hannu et al., 1999; Fürst, 2006).

Human milk reflects the body burden of dioxin and it is the main way of transporting dioxin from mothers to infants (Malisch and van Leeuwen, 2003). Moreover, collection of human milk is convenient and noninvasive and is easily replicated. Since 1987, the World Health Organization (WHO) has coordinated four rounds of exposure studies on dioxins using human milk (Malisch and Moy, 2006). China participated in the fourth WHO coordinated global survey of POPs pollution levels in breast milk in 2007. The survey included 12 representative areas in the mainland China, but unfortunately the city of Shenzhen was not included (Li et al., 2009). Shenzhen is a special economic zone in the Pearl River Delta Area of Guangdong, China, possessing around 2000 km^2 of area in the vicinity of Hong Kong. The population is about 12 million, and the residents are mostly emigrants from the other areas of mainland China and obviously have diversified diet habits.

Although Shenzhen is near to Hong Kong SAR which participated in the 3rd round of the WHO study initiated in 2000 (Hedley et al., 2006), no data have been reported on the levels of PCDD/Fs and PCBs in human breast milk in this city. In the present study, 60 milk

* Corresponding author.
E-mail address: zhjianqing95@gmail.com (J. Zhang).

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samples from the mothers living in Shenzhen were collected, and the levels of PCDD/Fs and PCBs in these samples were firstly examined by the isotopic dilution HRGC/HRMS method. Furthermore, the exposure risk and intake for breast-fed infants were also estimated. Thus, the major objective of this study is to investigate the body burdens of dioxins of Shenzhen maternal and carry out a health risk assessment for breast-fed infants.

2. Materials and methods

2.1. Chemicals and reagents

US EPA Method 1613 and 1668 standard solutions for determining PCDD/F and PCB congeners (CS1 to CS5, window defining and isomer specificity, labeled compound Stock solution (IS), clean up standard, internal standard spiking solution (ISS)) were purchased from Cambridge Isotope Laboratories Co. (Andover, MA, USA). Organic solvents for trace residual analysis (acetone, n-hexane, dichloromethane, ethyl acetate, benzene, methanol, and toluene) were purchased from Merck (Darmstadt, Germany).

2.2. Donor selection and sample collection

The approach for participator selection and sample collection was based on the 'Guidelines for Developing a National Protocol' of the Fourth WHO-Coordinated Survey of Human milk for Persistent Organic Pollutants in Cooperation with UNEP (WHO, 2007). Some modifications were made for the special situation of Shenzhen. The participators (n = 60) were vaginal delivery primiparas aging from 20 to 34 years old (average: 28 years) and have lived in Shenzhen non-directly POPs polluted areas for 5–28 years until the sampling (average: 10 years). Personal questionnaire data were collected, including information on birth weight and length of the infants, age of participators, length of inhabitation period in Shenzhen, residence environment record, dietary habits, and consumption of animal origin food before pregnancy, including aquatic food, meat, egg and milk. Information on smoking habit and indoor usage of DDT were also obtained for all the participators. The sampling was conducted during July–November, 2007. About 50–100 ml breast milk sample was self-collected by each participator after delivery 3 weeks to 2 months. Each sample (from one participator) was stored in one pre-washed collecting jar at −20 °C right after the samplings until chemical analyses.

The Human Ethical Committee of Shenzhen Center for Disease Control & Prevention inspected, reviewed and approved the study protocol. Each of the participants was provided informed consent form after receiving a detailed explanation of the study and its potential consequences.

2.3. Sample preparation

The samples were freeze-dried and mixed well individually. After spiking with ^{13}C-labeled internal standards, about 10 g for each sample were extracted individually by ASE system (Accelerate solvent extractor equipment, ASE300, USA) with a mixture reagent of n-hexane and dichloromethane (1:1) for 10 min × 2 times under pressure (2000 psi) and temperature (150 °C). Gravimetric lipid determination was performed after solvent evaporation. The concentrated sample extract was cleaned by a acid-modified silica column before subjecting it to carbon column fractionation by an automated system (Fluid Management Systems, Waltham, MA, USA). The fraction containing PCDD/Fs, PCBs congeners was then concentrated by vacuum evaporation and further cleaned by an alumina column. The eluate was dried by nitrogen evaporation to nearly dryness, and the residue was further reconstituted in 20μL of ^{13}C-labeled injection standard in nonane waiting for HRGC/HRMS analysis.

2.4. Sample analysis

Concentrations of seven 2,3,7,8-substituted PCDDs, ten 2,3,7,8-substituted PCDFs, and the six indicator PCB (non dioxin-like PCBs) congeners (No. 28, 52, 101, 138, 153 and 180), the coplanar PCB congeners (No. 77, 81, 126 and 169), as well as the mono-ortho PCB congeners (IUPAC No. 105, 114, 118, 123, 156, 157, 167, 189) were analyzed by a high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) (MAT95XL Thermo Finnigan, Germany) with a DB-5MS capillary column (60 m × 0.25mmi. d. × 0.25 μm). Further details of the experimental procedure were presented elsewhere (Zhang et al., 2008).

The minimum detection limit (DL) and the minimum quantification limit (QL) were determined. And each compound in the standard solution for calibration curve of the minimum concentration was quantified 5 times, and then the standard deviation (SD) was calculated. Threefold of the SD was taken as DL for the instrument (IDL) and tenfold of the SD was taken as QL for the instrument (IQL). Then, blank test carried out 5 times, each compound was quantified and then the SD was calculated. Threefold of the SD was taken as DL for the method (MDL) and tenfold of the SD was taken as QL for the method (MQL). For some congeners, system blank level was subtracted in order to get MDL, then the sample detect limit and quantification limit were calculated from MDL and MQL, respectively.

2.5. Data report and estimation PCDD/Fs and PCBs intake for breast-fed infants

Toxic equivalents (TEQ) of PCDD/F, PCBs were calculated on the basis of the Toxic Equivalency Factor (TEF) published by the World Health Organization (WHO) in 2005 (Van den Berg et al., 2006). In case the concentrations of some congeners in the samples were not detectable, the MDL (Method Detect Limit) values were used as the values of the concentrations of the congeners respectively.

Estimation of the intake of PCDD/Fs, PCBs for breast-fed infants employed the upper–bound of TEQ concentration. The calculation was based on the assumption published previously (Chan et al., 2007), that infant's daily milk consumption was 700 ml and infant weight was 5 kg. Therefore, the calculation formula was:

$$\text{PCDD / Fs and PCBs intake of infants}\left(\text{pg kg}^{-1}\cdot\text{bw}\cdot\text{day}\right) = \frac{700}{5} * F * C$$

whereas F: concentration of fat in milk, %; C: concentrate of PCDD/Fs and PCBs in milk, pg/g fat.

2.6. QA/QC

Method blank and quality control samples were included with each batch of 12 samples. Certified Reference Material was served as the quality control sample to validate the long determination process. Chicken samples, purchased from the Norwegian Institute of Public Health, were measured to confirm the laboratory performance and the method validation. The laboratory is accredited to ISO/IEC 17025 by CNACL of China (No. L2154). And our laboratory regularly and successfully participated in international interlaboratory comparison study on PCDD/Fs and PCBs in human milk, beef, butter, egg yolk and herring, organized by the Norwegian Institute of Public Health since 2005.

2.7. Statistical analysis

All data analyses were performed using SAS9.1 (SAS Institute Inc., Cary, NC). Normality test (Shapiro–Wilks test) was executed for all the continuous variables. The concentration of $\sum$TEQ-(PCDD/Fs + PCBs) was beyond normal distribution, thus, Spearman correlation analysis

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