Chemosphere 75 (2009) 634–639

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Levels of persistent organic pollutants in human milk in two Chinese coastal cities, Tianjin and Yantai: Influence of fish consumption

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ARTICLE INFO

Article history:
Received 4 November 2008
Received in revised form 1 January 2009
Accepted 5 January 2009
Available online 10 February 2009

Keywords:
POPs
Pesticide
Dioxins
Human milk
Fish
China

ABSTRACT

In 2006–2007, we collected human milk from 60 and 48 donors in the Chinese coastal cities of Tianjin and Yantai, respectively, in accordance with the WHO/UNEP global milk survey. We determined the concentrations of organochlorine pesticides by GC/MS/MS and dioxins by XDS-CALUX bioassay in the individual milk specimens. The geometric mean concentrations (GMs) of β-HCH (586.7 ng g^{-1} fat), total DDTs (855.9 ng g^{-1} fat), and dl-PCBs (4.4 pg CALUX-TEQ g^{-1} fat) in the milk from Yantai were higher than those from Tianjin (254.4 ng g^{-1} fat, 654.7 ng g^{-1} fat, 1.9 pg CALUX-TEQ g^{-1} fat, respectively). However, the GMs of HCB (41.1 ng g^{-1} fat) and PCDD/Fs (13.1 pg CALUX-TEQ g^{-1} fat) from Tianjin were higher than those from Yantai (15.7 ng g^{-1} fat, 9.9 pg CALUX-TEQ g^{-1} fat). The low ratios of DDT/(DDE+DDD) in milk from both areas suggested that past exposure contributed to the total DDTs body burden. The dl-PCBs body burden in the high sea fish intake group was higher than that in the low intake group, both with and without adjustments for potential influencing factors. For β-HCH, a marginal *P* value ($P = 0.063$) was observed between high and low sea fish consumption groups after adjusting for potential influencing factors. Donors in the high freshwater fish group showed higher PCDD/Fs and HCB levels than those in the low intake group, both with and without adjustments. Further monitoring studies of POPs contamination in human milk and foods are needed in China.

1. Introduction

Persistent organic pollutants (POPs) have been found in human tissues because of their long-term persistence and bioaccumulation through the food chain. Being a large agricultural country, a substantial amount of organochlorine pesticides (OCPs) has been produced and applied in China during the past several decades. Although both production and use of most OCPs were banned in China in 1983, dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene (HCB), chlordane and mirex have still been produced and used in a limited fashion due to specific exemption from the Stockholm Convention (Wei et al., 2007). In addition, illegal small-scale production and usage still exist in China. Past excessive production and use and current illegal use have given rise to high environmental background levels of OCPs in China. In addition, with rapid economic development and urbanization, the issue of dioxins has attracted more public concern in China in recent years. Due to similarities in structure and biological properties to dioxins, dioxin-like polychlorinated biphenyls (dl-PCBs) are recognized as dioxin-like compounds. PCB contamination in the environment is becoming a great concern, as are issues surrounding disposal of PCB-containing devices, such as obsolete transformers, in China.

Human milk has been used worldwide as a matrix for monitoring POPs levels. The contaminant status of OCPs and dioxin-like compounds in milk from some regions in mainland China has been reported (Wong et al., 2002; Yu et al., 2003; Kunisue et al., 2004; Sun et al., 2006), but the donor selection criteria did not completely match those of the World Health Organization (WHO) human milk survey. These earlier studies had limited value for making international comparisons, since the studies used different enrollment criteria.

Fish consumption is considered a major source of dietary POPs exposure. POPs residues such as DDT, HCB, hexachlorocyclohexane (HCH), chlordane, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs), and PCBs have been found in marine fish, mollusks, crabs and shrimp in the coastal areas of China (Yang et al., 2006; Jiang et al., 2007; Liu et al., 2007b). Residents of coastal areas who have a habit of consuming sea fish are vulnerable to POPs exposure. Therefore, the present study measured concentrations of OCPs and dioxin-like compounds in human milk from two typical coastal cities along the eastern coast of China, Tianjin and Yantai.

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doi:10.1016/j.chemosphere.2009.01.008

Tianjin is the largest open port city in northern China, with an area of about 12000 km^2 and a population of approximately 9.5 million. It is close to the west of Bohai Bay and the lower reaches of the Hai River, where OCPs and dioxins were detected in river sediments (Ding et al., 2005; Liu et al., 2007a). More importantly, DDT, HCH and HCB were historically produced in large quantities at two large chemical companies, the Tianjin Chemical Plant and the Dagu Chemical Plant, located in the city. In addition, large amounts of DDT and HCH were used agriculturally in Tianjin from 1952 to 1983 and from 1952 to 1992, respectively (Li et al., 2006a,b). The Tianjin Chemical Plant has been annually producing a quantity of 4–6 kt (Wei et al., 2007) and is the only manufacturer in China approved by the government to produce, sell and export DDT. The Dagu Chemical Plant produced 3522 tons per year of HCB, an intermediate of pentachlorophenol (PCP), under exemption, and terminated the operation in 2003. Agricultural application and wastewater discharged from chemical plants contributed to the high OCPs levels in the environment and biota of Tianjin, e.g., soil, fallout, water, sediment, seafood, and vegetables (Gong et al., 2004; Tao et al., 2005; Wu et al., 2005; Yang et al., 2006; Tao et al., 2007). Yantai, with a well-developed fishing industry, is a coastal city with a population of approximately 6.5 million people in northern China, and covers an area of about 13000 km^2. It is situated on the eastern tip of the Shandong Peninsula and borders the Bohai Bay and the Yellow Sea, which were reported to be polluted by OCPs and dioxins (Wan et al., 2005; Liu et al., 2008; Pan et al., 2008). Human milk from these two areas would be expected to have high OCPs and dioxin-like compounds levels.

The present study aimed at assessing the extent of contamination with OCPs and dioxin-like compounds in human milk of donors selected by the criteria of the WHO human milk study in the two regions and at examining the association between levels of POPs in milk and dietary habits, specifically fish consumption.

2. Materials and methods

2.1. Selection of donors and milk sampling

From November 2006 to April 2007, 60 donors from Tianjin and 48 donors from Yantai participated in this study. In accordance with the entrance criteria recommended by the fourth round of the WHO/UNEP-Coordinated Global Survey of Human Milk for Persistent Organic Pollutants and in view of the multi-nationality of China, eligible donors had to (a) be primipara, (b) be of Han ethnicity, (c) be younger than 30 yrs old (y.o.), (d) have given birth to only one child and primarily breastfed the child, (e) be healthy and have a healthy child, (f) born in the sampling area (either urban or rural district), and (g) have resided in the sampling area for at least the previous 5 yrs. Informed consent was obtained from all of the donors. Every donor provided 60 mL of breast milk 2–8 weeks after childbirth. The milk was stored at −20 °C and placed in a box with colloid ice bags when transported.

2.2. Data collection

All donors were interviewed by a single interviewer using a translated questionnaire of the fourth round WHO/UNEP global human milk survey, including an additional questionnaire that was used by our team in a previous POPs study in China (Sun et al., 2006). The additional questions included breastfeeding period of the donor, socioeconomic status (education and monthly household income), obstetric information (gestational age, baby gender and birth weight), residence history, occupation, past disease history, physiological information (including age at menarche and number of spontaneous abortions), past occupational exposure to POPs, drinking alcohol, tea, and coffee, smoking (active and passive), and dietary habits before pregnancy. Semi-quantitative dietary history questions were designed to ask the frequencies and portion sizes of food items before pregnancy, including eight species of sea fish, five species of freshwater fish, other seafood, other freshwater food, meat (pork, beef, mutton, and chicken), egg, offal, and milk (including yogurt), which were potential contributors to dietary exposure to POPs. Fish consumption was the focus of the study due to reports of pollution of aquatic environments in China by POPs (Liu et al., 2008; Zhou et al., 2008), and residents in both areas consumed more fish than the average Chinese population.

2.3. Chemical measurements of POPs

We measured the concentrations of OCPs by gas chromatography/mass spectrometry/mass spectrometry (GC/MS/MS) and dioxins by xenobiotic detection systems – chemically activated luciferase expression (XDS-CALUX) bioassay at Hiyoshi Corporation (Omiyahachiman, Shiga, Japan). Twenty grams of each individual milk sample were treated and cleaned up using the previously described method (Denison et al., 1996). The extracts were evaporated to concentrate under nitrogen, and the dried residues were weighed to determine percent organic extractables (milk lipid). An aliquot (75%) of the sample was used for dioxins/dl-PCBs measurement by XDS-CALUX bioassay. The remainder (25%) of the sample was used for measurement of OCPs.

The CALUX bioassay uses a recombinant cell line (H1L6.1c2) which is generated by transfecting the plasmid pGudLuc6.1 into mouse hepatoma (Hepa1c1c7) cells. The pGudLuc6.1 plasmid contains the firefly luciferase gene under aryl hydrocarbon receptor (AhR)-dependent control of dioxin-responsive elements (DREs) and responds to dioxin-like compounds with the induction of luciferase gene expression in a time-, dose-, and chemical specific manner. When the cells are exposed to dioxin-like compounds, the luciferase reporter protein is produced and quantified by measuring luminescence to compare to the 2,3,7,8-TCDD standard curve. As described previously (Garrison et al., 1996), the extract was passed through an acid–silica gel column and an XCARB carbon (Xenobiotic Detection Systems: XDSI Inc., USA) column. The dl-PCBs fraction was eluted with 15 mL of hexane/toluene/ethyl acetate (8:1:1), and the PCDD/Fs fraction was eluted with 20 mL of toluene.The purified sample extracts and 2,3,7,8-TCDD standard solutions in dimethyl sulfoxide (DMSO) were diluted in 400 μL of modified RPMI1640 culture medium prior to dosing monolayers cells. The cells were grown on 96-well microplates and cultured in a humidified 5% CO_2 incubator at 37 °C. After incubation, the medium was removed and the cells were lysed. The induced luciferase activity was quantified using a Lucy I microplate luminometer (Antos, Salzburg, Austria). The concentrations of PCDD/Fs and dl-PCBs were reported as pg CALUX-TEQ g^{-1} fat.

Five ng of surrogate internal standard (Persistent Organic Pollutants Clean-up Spike ($^{13}C_1$, 99%): ES-5261-1.2, Cambridge Isotope Laboratories, Inc.) was added to each extract sample to monitor both clean-up and GC/MS/MS analysis processes and to calculate recovery rate in clean-up process. Then, the extract was replaced with acetonitrile and cleaned up by ODS mini-column: Bond Elut C18 (1 g) (Varian, Inc., Palo Alto, CA, USA) and by PSA mini-column: PSA (500 mg; Sigma–Aldrich Corp., St. Louis, MO, USA) and concentrated up to 500 μL. Two microliters of the concentrated sample were injected into GC/MS/MS (TSQ Quantum GC; Thermo Fisher Scientific with Column HT8 30 m, 0.25 mmID, and Detector: SRM method, Waltham, MA, USA), for analysis of 19 OCPs, including DDT and its metabolites (o,p′-DDT, p,p′-DDT, o,p′-DDE, p,p′-DDE, o,p′-DDD, and p,p′-DDD), HCH isomers (α-HCH, β-HCH, γ-HCH, and δ-HCH), HCB, heptachlor and its epoxides (trans-heptachlor-epoxide and cis-heptachlor-epoxide), and chlordane compounds