DETERMINATION OF PCDD/Fs AND DL-PCBs IN HUMAN BREAST MILK FROM CHINA IN 2011

Zhang Lei[1], Yin Shuang-xing[1,2], Li Jing-guang[1]*, Zhao Yun-feng[1], Wu Yong-ning[1]

[1] The Key Laboratory of Food Safety Risk Assessment, Ministry of Health (CFSA) and China National Center for Food Safety Risk Assessment, 7 Panjiayuannanli, Beijing, 100021, China; [2]Wuhan Polytechnic University, Wuhan, 430023, China

Introduction

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) are ubiquitous and persistent environmental pollutants. Due to their stability and lipophilic properties, PCDD/Fs and dl-PCBs can be stored in fat tissues and bioaccumulate through food chain. Some experimental studies and human epidemiology studies have suggested neurodevelopmental toxicity, immunotoxicity, reproductive effects and endocrine disturbing effects of PCDD/Fs and dl-PCBs indicating that they could have strictly adverse effect on human health[1]. Bio-monitoring of breast milk recommended by WHO/UNEP is an important and convenient non-invasive method for assessing the body burden on the mother and the exposure of the infant[2]. Background body burdens of PCDD/Fs and dl-PCBs have been estimated for the Chinese population in 2007[3]. Here we present the results of the second national survey of breast milk in China conducted in 2011. In the present study, levels of PCDD/Fs and dl-PCBs in human milk from 16 provinces of China were determined to evaluate the body burden and analyze the temporal trend since 2007.

Materials and methods

The design of the survey was followed the fourth WHO-coordinated survey of human milk for persistent organic pollutants, with slight modification for the special condition in China. The selection criteria for participating mothers included: (1) primiparous, breastfeeding one child only; (2) age <35 years; (3) apparently healthy mother and child, and normal pregnancy; (4) resident within the study area for the last 10 years; (5) not reside in local areas where emissions of POPs are known or suspected to result in elevated levels of POPs in the local population. Milk samples were collected within 3 to 8 weeks of delivery. Mothers donating milk were informed the nature and purpose of the study and signed the informed consent forms. The milk samples were collected from 16 province including Heilongjiang, Liaoning, Hebei, Henan, Shanxi, Ningxia, Jiangxi, Fujian, Shanghai, Hubei, Sichuan, Guangxi, Jilin, Neimenggu, Qinghai, and Guangdong in 2011. In each province, one urban site and two rural sites were selected for sampling. In each urban area, 50 donors were selected. And in each rural area, 30 donors were selected. For each province the urban individual human milk samples were pooled into one composite sample and rural samples were pooled into another composite sample. Finally, these samples were divided into 32 pools based on mother's residence.

Analysis of PCDD/Fs dl-PCBs were described elsewhere[3], with slightly modification. Briefly, about 50 mL milk sample was freeze-dried. Then, after spiking $^{13}C_{12}$-labeled internal standards, samples were extracted by an ASE 350 (Thermo Scientific) using n-hexane / dichloromethane mixture (1:1, v/v) as solvent. Gravimetric lipid determination was performed after solvent evaporation. The

bulk lipid was removed by shaking with acid-modified silica-gel and further cleanup was achieved using a Power Prep instrument (Fluid Management Systems, Waltham, MA, USA) with multiple commercial silica-gel columns, alumina columns and carbon columns. Two fractions containing PCDD/Fs and dl-PCBs were collected and concentrated to approximately 20 μL, respectively. After adding $^{13}C_{12}$-labeled injection standard, the final extract was analyzed by a high-resolution gas chromatograph – high resolution mass spectrometer (HRGC-HRMS) on a Trace 1300 Gas Chromatography (Thermo Scientific, Italy) equipped with a DB-5MS capillary column (60 m × 0.25 mm i.d. × 0.25 μm) and coupled to a DFS High Resolution Mass Spectrometer (Thermo Scientific, Germany).

In the present study, TEFs scheme recommended by WHO in 1998 was applied to calculate the TEQ concentration of PCDD/Fs and dl-PCBs in milk samples[4]. The concentration for non-detectable congeners was set as 'zero', i.e. ND=0. Procedural blank sample was performed every eight samples. The laboratory performance was validated by successfully participating in participating in Bi-ennial Global Interlaboratory Assessment on Persistent Organic Pollutants in human breast milk in 2012/2013 organized by UNEP.

Results and discussion:

14 of 17 congeners of PCDD/Fs were detected in almost all pooled samples (≥31/32), including the most toxic congener, 2,3,7,8-TCDD, within the range of 0.19 – 1.25 pg g^{-1} lipid weight (lw). The other three congers, 1,2,3,7,8,9-HxCDF, 1,2,3,4,7,8,9-HpCDF and OCDF, were detected in 10, 17 and 18 samples, respectively. Fig.1 illustrated the pattern of average concentrations of PCDD/F congeners and PCB congeners in Chinese human milk. By mass concentration, OCDD was the predominant congener in all samples with the mean percentage contribution of 71.3% with a range of 47.4 – 95.8% of total PCDD/Fs followed by 1,2,3,4,6,7,8-HpCDD (6.2%), 2,3,4,7,8-PeCDF (5.5%), and 1,2,3,6,7,8-HxCDD (2.9%). For PCB congeners, with the exception of PCB-169, the other congeners were found in all samples. PCB-118, PCB-156 and PCB-105 were the most predominant congeners, accounting for 52.5%, 16.8%, and 15.5%, respectively. However, by TEQ concentration, 2,3,4,7,8-PeCDF made the highest contribution to the total TEQ of PCDD/Fs and dl-PCBs, accounting for 32.1%, followed by PCB-126 (17.0%), 1,2,3,7,8-PeCDD (16.8%), and 2,3,7,8-TCDD (8.1%). In all samples, the contribution of PCDD/Fs was about two times more than that of PCBs. A significant correlation between PCDD/Fs-TEQ and PCBs-TEQ was found (r=0.767, p<0.01), suggesting simultaneous exposure as well as similar emission of these contaminants in China.

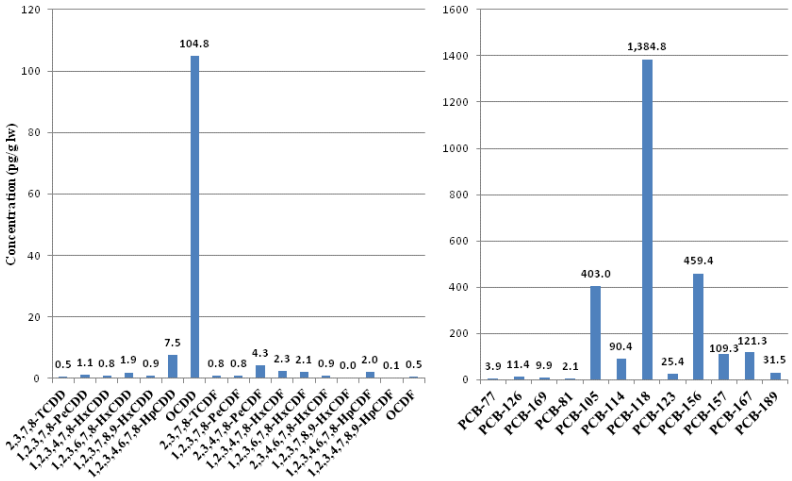

Fig.1 Pattern of average concentration of PCDD/Fs and dl-PCBs in Chinese human milk.

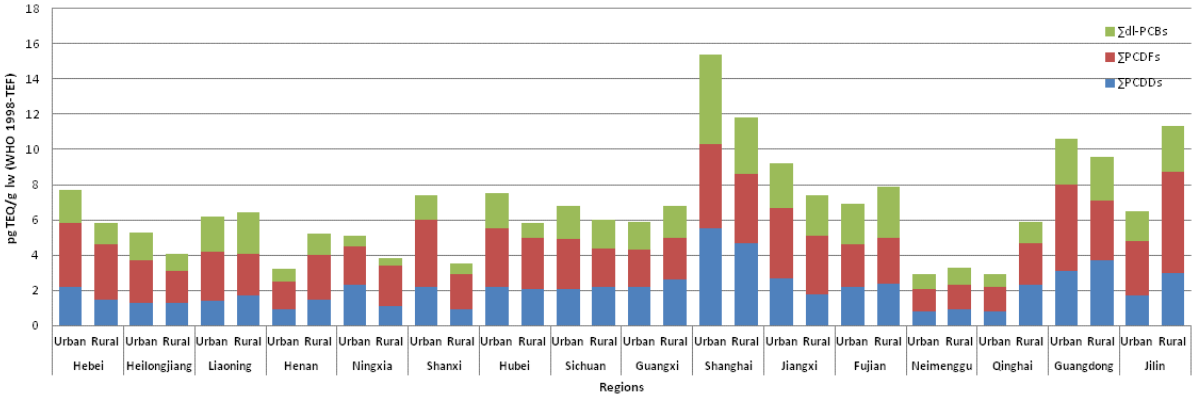

Fig.2. The TEQ concentrations of PCDD/Fs and dl-PCBs in pooled milk samples from 32 regions of China.

The TEQ concentration of PCDD/Fs and dl-PCBs in pooled samples from 32 regions of China was shown in Fig.2. The range of total TEQ was from 2.9 pg TEQ g^{-1} lw (urban area of Neimenggu, urban area of Qinghai) to 15.4 pg TEQ g^{-1} lw (urban area of Shanghai) with a mean of 6.7 pg TEQ g^{-1} lw, indicating large variation of human body burden of PCDD/Fs and dl-PCBs as well as environmental pollution among regions in China. The geographical distribution of human body burden in current study was consistent with that of the emission inventory in China[5]. For example, the maximum and minimum of TEQ in human milk was from Shanghai, and Neimenggu and Qinghai, respectively, which have the highest and lowest emission of these contaminants, respectively. Some previous studies have indicated that some geographical factors, like urban/rural, could affect the human body level[6]. In the present study, there was no significantly statistical difference between urban and rural samples (*paired t-test*, $p>0.05$). The average total TEQ concentration in breast milk from urban area and rural area was 6.8 pg TEQ g^{-1} lw and 6.5 pg TEQ g^{-1} lw, respectively. However, some geographical difference was also observed, concentrations of PCDD/Fs and dl-PCBs determined in human milk samples from relative less developed northwest inland area, like Ningxia, Neimenggu, Qinghai, were notably lower than that from more developed east area of China, like Shanghai and Guangdong, suggesting high industrialization might result in high exposure to PCDD/Fs and dl-PCBs.

It has been indicated that over 90% of exposure to PCDD/Fs and dl-PCBs of general population, non-occupational, results from dietary intake[7]. Thus, the level of dietary intake of these pollutants can strongly influence the body burden. In our previous study, the dietary intake of PCDD/Fs and dl-PCBs has been evaluated from the 5th round Chinese Total Diet Study (TDS) conducted in 2011. There are significantly statistical correlation between levels of PCDD/Fs and dl-PCBs in breast milk samples, i.e. human body burden, and the dietary intake, and the statistical relation was independent of the lipid adjustment. But, lipid adjustment could lead to more significant correlation. PCDD/Fs-TEQ, dl-PCBs-TEQ, and total TEQ in human milk, expressed as pg TEQ g^{-1} lw, significantly correlated with dietary intake of PCDD/Fs ($r=0.76$, $p<0.01$), dl-PCBs ($r=0.70$, $p<0.01$), and total TEQ ($r=0.78$, $p<0.01$), respectively. Interestingly, there were also significantly correlation between human body burden of PCDD/Fs, dl-PCBs, and PCDD/Fs plus dl-PCBs and dietary intake from the earlier Chinese TDS conducted in 2007 ($r=0.78$, $p<0.01$; $r=0.64$, $p<0.01$; $r=0.75$, $p<0.01$, respectively), approving that levels of PCDD/Fs and dl-PCBs in human milk originate from the chronic bio-accumulation from dietary intake.

Some national survey and global survey of POPs including PCDD/Fs and dl-PCBs in human milk have been conducted since 1980s, and the decreasing trend of PCDD/Fs and dl-PCBs in human milk has been found in last decades in some countries probably due to the effort and strict regulation on emission[8-10]. However, there was limited information on PCDD/Fs and dl-PCBs in human breast milk from general population in China. The 1st national survey of human milk in China was carried out in 2007 to assess the background of body burden of various POPs. In our previous study, PCDD/Fs and dl-PCBs were determined in these milk samples[3]. Based on this study, it is possible to calculate and present temporal trends of the determinants in Chinese human breast milk from 2007 to 2011. There were significant difference of total TEQ, ∑PCDDs-TEQ, ∑PCDFs-TEQ in human milk between 2007 and 2011 (paired t-test, $p<0.01$), and the increase for average total TEQ, ∑PCDDs-TEQ, and ∑PCDFs-TEQ are 27.1%, 29.8%, and 42.2%, respectively. Although the ∑dl-PCBs-TEQ slightly increased about 7.5% from 2007 to 2011, no statistical difference was found. For PCDD/Fs, with the exception of OCDF and 1,2,3,4,7,8-HxCDF ,the concentrations of the other 15 congeners increased, and there was significant difference of three PCDD congeners, including 1,2,3,7,8,9-HxCDD ($p<0.05$), 1,2,3,4,6,7,8-HpCDD ($p<0.01$), and, OCDD ($p<0.05$), and seven PCDF congeners, including 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, and 1,2,3,4,7,8,9-HxCDF($p<0.01$). Moreover, the sum of PCDDs and PCDFs significantly increased about 111.2% from 56.6 pg g^{-1} lw to 119.5 pg g^{-1} lw, and 36.6% from 10.1 pg g^{-1} lw to 13.8 pg g^{-1} lw. For dl-PCBs, there was no significant difference of the sum of dl-PCBs found, and the average concentrations were comparable. However, the average concentrations of the non-ortho PCB congener, including PCB-77, PCB-81, PCB-126, and PCB-169, decreased from 2007 to 2011, and statistical difference was only found for PCB-81 ($p<0.01$). For mono-ortho PCB congeners, there was significant elevation of the concentration of PCB-114, PCB-156, PCB-157, PCB-167, and PCB-189 in Chinese human milk from 2007 to 2011. These results might suggest increasing trends of emission of PCDD/Fs and dl-PCBs probably due to rapid industrialization and urbanization in China. Therefore, more efforts should be done to reduce the dioxin emission to protect the environment and human health.

Acknowledgements:
We would like to express our gratitude to all participant mothers in this study. This study was support by the National Science Foundation of China (Grant No. 81472986)

References:

1. De Coster, S.; van Larebeke, N., (2012). Journal of environmental and public health. <http://dx.doi.org/10.1155/2012/713696>
2. WHO, Fourth WHO-coordinated Survey of Human Milk for Persistent Organicpollutants in Cooperation with UNEP. Guidelines for Developing a National Protocol, World Health Organization. 2007.
3. Li, J.; Zhang, L.; Wu, Y.; Liu, Y.; Zhou, P.; Wen, S.; Liu, J.; Zhao, Y.; Li, X., (2009). Chemosphere, 75, (9), 1236-1242.
4. Van den Berg, M.; Birnbaum, L.; Bosveld, A. T.; Brunström, B.; Cook, P.; Feeley, M.; Giesy, J. P.; Hanberg, A.; Hasegawa, R.; Kennedy, S. W., (1998), Environment Health Perspectives, 106, (12), 775-792.
5. Zheng, M., Sun, Z., Liu, w., (2008). China Environmental Science Press, Beijing

6. Mannetje, A. t.; Coakley, J.; Bridgen, P.; Brooks, C.; Harrad, S.; Smith, A. H.; Pearce, N.; Douwes, J., (2013). Science of The Total Environment, 458–460, 399-407.
7. Liem, A. K. D.; Furst, P.; Rappe, C., (2000). Food Additives and Contaminants, 17, (4), 241-259.
8. Ryan, J. J.; Rawn, D. F. K., (2014). Chemosphere, 102, , 7-86.
9. UNEP, Results of the global survey on concentrations in human milk of persistent organic pollutants by the United Nations Environment Programme and the World Health Organization. Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants Sixth meeting. Geneva, 28 April–10 May 2013. http://www.who.int/foodsafety/chem/POPprotocol.pdf. 2013.
10. Fång, J.; Nyberg, E.; Bignert, A.; Bergman, Å., (2013). Environment International, 60, ,224-231.