



Health risks posed to infants in rural China by exposure to short- and medium-chain chlorinated paraffins in breast milk



Dan Xia^{a,b}, Li-Rong Gao^{a,*}, Ming-Hui Zheng^a, Jing-Guang Li^c, Lei Zhang^c, Yong-Ning Wu^{c,*}, Lin Qiao^{a,b}, Qi-Chang Tian^a, Hui-Ting Huang^{a,b}, Wen-Bin Liu^a, Gui-Jin Su^a, Guo-Rui Liu^a

^a State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

^b University of Chinese Academy of Sciences, Beijing 100085, China

^c National Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, 29 Nanwei Road, Beijing 100050, China

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ABSTRACT

Chlorinated paraffins (CPs) are complex mixtures of synthetic chemicals found widely in environmental matrices. Short-chain CPs (SCCPs) are candidate persistent organic pollutants under the Stockholm Convention. There should be great concern about human exposure to SCCPs. Data on CP concentrations in human breast milk is scarce. This is the first study in which background SCCP and medium-chain CP (MCCP) body burdens in the general rural population of China have been estimated and health risks posed to nursing infants by CPs in breast milk assessed. The concentrations of 48 SCCP and MCCP formula congeners were determined in 24 pooled human milk samples produced from 1412 individual samples from eight provinces in 2007 and 16 provinces in 2011. The samples were analyzed by comprehensive two-dimensional gas chromatography electron capture negative ionization high-resolution time-of-flight mass spectrometry. The median SCCP and MCCP concentrations were 303 and 35.7 ng g⁻¹ lipid weight, respectively, for the 2007 samples and 360 and 45.4 ng g⁻¹ lipid weight, respectively, for the 2011 samples. The C₁₀ and C₁₄ homologs were the dominant CP carbon-chain-length groups, contributing 51% and 82% of the total SCCP and MCCP concentrations, respectively. There are probably multiple CP sources to the general Chinese population and numerous exposure pathways. The median estimated daily SCCP and MCCP intakes for nursing infants were 1310 and 152 ng kg⁻¹ d⁻¹, respectively, in 2007 and 1520 and 212 ng kg⁻¹ d⁻¹, respectively, in 2011. SCCPs do not currently pose significant risks to infants in China. However, it is necessary to continuously monitor CP concentrations and health risks because CP concentrations in Chinese human breast milk are increasing.

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1. Introduction

Chlorinated paraffins (CPs) are high-production-volume synthetic chemicals that have been widely used as metal-working fluids and flame retardants for plastics (Sverko et al., 2012). Commercial CPs are classified, according to their carbon chain lengths, as short chain CPs (SCCPs; with carbon chain lengths of C_{10–13}), medium chain CPs (MCCPs; with carbon chain lengths of C_{14–17}), and long chain CPs (with carbon chain lengths of C_{18–30}), and the degree of chlorination of each class of CP ranges from 30% to 70% (Tomy et al., 1998). SCCPs and MCCPs are persistent and can bioaccumulate, so there is concern about the potential adverse effects of SCCPs and MCCPs on human health (Bayen et al., 2006; Houde et al., 2008; Li et al., 2016). SCCPs at high concentrations have been found to be hepatotoxic in trout and

rats (Cooley et al., 2001). SCCPs have also been found to be carcinogenic in rats and mice (ECB, 2000). SCCPs have therefore been classed as candidate persistent organic pollutants under the Stockholm Convention (Persistent Organic Pollutants Review Committee, 2009).

Production of CPs started in the 1930s, and it has been estimated that 7.5–11.3 kt of CPs were produced in Canada, Europe, and the USA in 2007 and that about 600 kt of CPs were produced in China in 2007 (van Mourik et al., 2015; Wei et al., 2016). The production and use of CPs is currently not restricted in China, and annual CP production is continually growing. The rapid increase in the amounts of CPs produced and the wide use of CPs in China have led to CPs being ubiquitous in the Chinese environment. Relatively high SCCP concentrations (1000–3500 ng g⁻¹ dry weight (dw)) have been found in fish samples from a lake that receives sewage treatment plant effluent in Beijing, China, and such concentrations could pose risks to humans and other organisms (Zeng et al., 2011). Extremely high CP concentrations have been found in wild animal tissues from the Yangtze River Delta (8000–

* Corresponding authors.

E-mail addresses: gaolr@cees.ac.cn (L.-R. Gao), wuyongning@cfsa.net.cn (Y.-N. Wu).

340,000 ng g⁻¹ lipid weight (lw)) (Zhou et al., 2016) and in terrestrial bird tissues from the Pearl River Delta (620–17,000 ng g⁻¹ lw) (Luo et al., 2015). There should be great concern about the exposure of Chinese people to CPs because of the potential for CPs to bioaccumulate and biomagnify.

There is concern about the presence of environmental pollutants in human breast milk because the pollutants could negatively affect the health of breastfeeding infants. A fetus will be exposed to potentially harmful chemicals transferred through the placenta, but breast milk is the main route through which infants are exposed to potentially harmful chemicals (Lyche et al., 2015). Infants are more vulnerable than older humans to potentially harmful chemicals because they have immature metabolism systems and because some development processes are sensitive to the presence of potentially harmful chemicals (Damerud et al., 2001). Children appear to be exposed to larger amounts of CPs than are adults via the diet (Iino et al., 2005). In biomonitoring studies, breast milk offers several advantages over other tissues. Breast milk is simple to collect and collection is non-invasive, and it allows the body burdens of reproductive age women, in utero exposure of fetuses, and exposure of infants through breast feeding to be estimated. High SCCP and MCCP concentrations have been found in breast milk collected in urban areas in China, indicating that the production and use of CPs may be important sources of CP exposure in urban areas (Xia et al., 2017). China is one of the largest and most populous countries. The rural population was 48% of the total population of China in 2011, but the incomes of people living in rural areas accounted for only 25% of the total incomes of people in China. This means there are significant differences in the lifestyles and dietary habits of people living in urban and rural areas in China. Breast milk produced by women living in urban and rural areas may contain different CP concentrations, and the sources of CPs found in breast milk produced by women living in urban and rural areas may be different. To understand the occurrence, congener profile, and sources of CPs in breast milk produced by women living in rural areas in China, the study described here was focused on SCCP and MCCP concentrations in breast milk in Chinese rural population.

A total of 1412 individual human breast milk samples were collected from rural areas in eight provinces across China in 2007 and from 16 provinces in 2011. The samples were pooled to give 24 samples, and 48 SCCP and MCCP formula congeners were determined in the pooled samples by comprehensive two-dimensional gas chromatography coupled to electron capture negative ionization high-resolution time-of-flight mass spectrometry (GC × GC-ECNI-HRTOFMS).

The aim of the study was to estimate background CP body burdens in the general population of rural areas in China. Daily CP intakes by infants were estimated, and the potential health risks posed to nursing infants by CPs in breast milk were assessed. Possible sources and pathways of CPs and associations between CP exposure and maternal characteristics were also evaluated.

2. Materials and methods

2.1. Donor selection and sample collection

Breast milk samples were collected from rural areas in eight Chinese provinces in 2007 and 16 Chinese provinces in 2011. The donor selection, sampling, and pooling protocols were based on the 'Guidelines for Developing a National Protocol' developed as part of the 'Fourth WHO-Coordinated Survey of Breast milk for Persistent Organic Pollutants in Cooperation with UNEP' (WHO, 2007). The breast milk samples were collected using a multistage random cluster sampling method. First, the provinces were divided into "northern" and "southern" groups depending on both their geographical locations and the dietary patterns of the residents of the provinces. Samples were collected from eight provinces in 2007 and 16 provinces in 2011, and the women samples were selected at random. The 16 provinces samples were collected from were Fujian, Guangdong, Guangxi, Hebei, Heilongjiang, Henan,

Hubei, Jiangxi, Jilin, Liaoning, Neimenggu, Ningxia, Qinghai, Shanghai, Shanxi, and Sichuan (see Fig. S1 in Supplementary material). >50% of the total population of China live in these provinces. Two rural sites in each province were randomly selected in each province, then 30 breast milk donors were randomly selected at each rural site. Each donor was primiparous, and the donors were 17–39 y old (mean 26 y) and all lived in areas in which occupational exposure did not occur. Each donor supplied her age, weight, residence record, and dietary habits before pregnancy. The donor's ages ranges and sample collection numbers in each province are detailed in Table S1. The characteristics of the donors in 2007 were described in previous publications in which studies of dioxin-like compounds and polybrominated diphenyl ethers in breast milk were described (Li et al., 2009; Zhang et al., 2011). At least 50 mL of milk was collected from each of 60 donors in two rural areas in each selected province. Each sample was collected directly into a pre-washed jar. A total of 452 samples were collected from eight provinces in 2007 and 960 samples were collected from 16 provinces in 2011. Each sample was stored at -20 °C until it was pooled and analyzed. The 60 individual samples from each province were pooled to give one sample, so there were eight pooled samples in 2007 and 16 pooled samples in 2011.

2.2. Sample preparation

Approximately 30 mL of a pooled sample was freeze-dried and then spiked with 2.5 ng of ¹³C₁₀-labeled *trans*-chlordane. The sample was then extracted with a 1:1 mixture of dichloromethane and *n*-hexane using an ASE 350 extraction unit (Dionex, Sunnyvale, CA, USA). The extract was evaporated to dryness to allow the lipid content to be determined gravimetrically. The residue was then redissolved and the lipids removed by gel permeation chromatography, then the extract was cleaned by passing it through a multi-layered column. The cleaned extract was concentrated, solvent exchanged into cyclohexane, evaporated to 50 µL in a vial containing 2.5 ng of ϵ -hexachlorocyclohexane, and analyzed by GC × GC-ECNI-HRTOFMS. The sample extraction and clean-up procedures are described in detail in the Supplementary material.

2.3. Instrumental analysis and quantification

The sample extracts were analyzed by GC × GC-ECNI-HRTOFMS. The gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) was fitted with a ZX2004 loop cryogenic modulator (Zoex Corporation, Houston, TX, USA) and was connected to a HRTOFMS instrument (Tofwerk, Thun, Switzerland). The first column was a DB-5MS column (30 m long, 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies) and the second column was a BPX-50 column (1 m long, 0.1 mm i.d., 0.1 µm film thickness; SGE Analytical Science, Ringwood, Australia). A single aliquot of a sample was injected into the gas chromatograph to analyze 48 SCCP (C_{10–13}Cl_{5–10}) and MCCP (C_{14–17}Cl_{5–10}) formula congeners. The procedures used to identify and quantify the CPs are described in a previous publication about the development of the GC × GC-ECNI-HRTOFMS method (Xia et al., 2016).

2.4. Quality assurance/quality control

To ensure the validity of quantifications method, all glassware was heated to 450 °C before use to minimize the risk of contamination. Three replicate CP-free cow milk samples spiked with a SCCP standard (55.5% CI, 1 µg) and a MCCP standard (57% CI, 1 µg), were cleaned up and analyzed using the same method as was used for the breast milk samples. The mean SCCP and MCCP recoveries were 89% and 85%, respectively. The samples were analyzed in batches of seven, and one procedural blank was included with each batch. The estimated method detection limits were 5.6 ng g⁻¹ lw for the SCCPs and 2.0 ng g⁻¹ lw for the MCCPs. The procedural blanks were extracted and analyzed in the same way as the samples. The SCCP concentrations in the blanks

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