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Polychlorinated biphenyls and organochlorine pesticides in human milk samples from two regions in Croatia

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ABSTRACT

We analyzed 20 polychlorinated biphenyls (PCBs) and seven organochlorine pesticides (OCPs) in milk samples collected during 2009–2011 from primiparae living in two different regions in Croatia. *p,p′*-DDE is the dominant organochlorine pesticide. α-HCH/γ-HCH and *p,p′*-DDE/*p,p′*-DDT ratios indicate that there is fresh input of γ-HCH in investigated population on both locations, while this is not applicable to *p,p′*-DDT. The PCB profile was dominated by higher chlorinated congeners. Non-*ortho* PCB congeners which have the highest TEF values were not detected in any of individual samples. Toxic equivalents for mono-*ortho* substituted PCB congeners indicated higher exposure to toxic PCBs in Zadar, but estimated daily intakes for both locations indicate that infants consuming mother's milk are not at risk of adverse effects caused by PCBs and OCPs. Our study builds on the previous research of human milk samples collected in Zagreb and reveals that over 10-year period, levels of investigated organochlorine compounds decreased significantly.

1. Introduction

Polychlorinated biphenyls and organochlorine pesticides are synthetic organic compounds that belong to a group of *Persistent Organic Pollutants* (POPs). POPs are a group of chemicals which have been intentionally or inadvertently produced and introduced into the environment. Due to their stability and transport properties, they are now widely distributed around the world, and are even found in places where they had never been used, such as the arctic regions. Given their long half-lives and fat solubility, POPs tend to bioaccumulate in animals, particularly in long-lived species at the top of the food-chain. POPs appear at higher concentrations in fat-containing foods, including fish, meat, eggs and milk. POPs are also present in the human body and traces can be found in human milk (Lerche et al., 2002).

As a group, POPs are of concern for both environment and human health, most notably, because of their potential effects on the endocrine system (Fisher and Schmidt, 1999). These are the reasons why the production and usage of these compounds have been banned or strictly limited in most countries since the 1970s and 1980s. With the ratification of the Stockholm Convention on POPs in early 2004, the international

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community signaled its commitment to reduce or eliminate production and emission of POPs into the environment and ultimately, the human body.

Due to these reasons and their presence in food, water, air, vegetation, soil/sediment, animals and humans, evaluation of their environmental distribution and human exposure is required.

As mentioned above, POPs accumulate in human tissues which contain fat and elimination is very slow. Because of relatively high fat content, these compounds are excreted in breast milk. Levels in human milk are indicator of human exposure and reflect their levels in adipose tissue as well (Abballe et al., 2008; Bencko et al., 2004; Čajka and Hajšlova, 2003). Organochlorines are transferred from the mother to the child via the placenta and primarily via breast milk. Breastfeeding bears well-known benefits for developing infants and WHO recommended that breastfeeding should be promoted and encouraged despite to content of unwanted POPs considering short breastfeeding period in comparison to whole life (EHE 3, 1996).

In Croatia, PCBs and organochlorine pesticides have been analyzed in human milk for over 40 years (Krauthacker et al., 2009). The early investigations consider analyses of total PCB while recent work include total PCB and results of PCB congener specific analysis.

In the present study, along with organochlorine pesticides and indicator PCB congeners, we also analyzed three non-*ortho* and eight mono-*ortho* substituted PCB congeners that are important from the toxicological point of view. Analyses were performed on two sets of samples: milk from donors from the city of Zagreb, and from Zadar, a coastal city that was exposed to destruction during the war in the former Yugoslavia. In these events, electrical transformer station was destroyed which caused higher PCB levels in air and fish from that area (Ružičkova et al., 2008; Picer, 2000).

2. Materials and methods

2.1. Samples and sample locations

Human milk samples were collected from healthy primiparae that had no history of accidental or occupational exposure to analyzed compounds. Milk was manually expressed 2–31 weeks after the delivery into pre-cleaned glass bottles and stored at −20 °C until analysis. In 2009/2010, milk was collected from 20 mothers living in Zagreb and in 2011 from 18 mothers living in Zadar. Mothers from Zagreb were between 22 and 37, and mothers from Zadar between 20 and 33 years old.

Zagreb is the northwestern capital city of Croatia, with population of about 800 000. Zadar is located in the middle part of Croatian coast and counts about 70 000 inhabitants. According to research conducted in the western Balkan region, the population in this area is exposed to higher levels of PCBs in air due to the warfare in the 1990s. In Croatia, significantly higher levels have been measured in Zadar on the location where electrical transformer station was destroyed, despite the fact that the capacitor was removed and soil remediated (Klanova et al., 2007).

2.2. Analyzed compounds

In this study we analyzed 20 PCB congeners: PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180 (six indicator congeners), PCB-77, PCB-126, PCB-169 (three non-*ortho* congeners), PCB-105, PCB-114, PCB-118, PCB-123, PCB-156, PCB-157, PCB-167, PCB-189 (eight mono-*ortho* congeners), PCB-60, PCB-74 and PCB-170, and the following OCPs: hexachlorobenzene (HCB), α-HCH, β-HCH, γ-HCH (α-, β-, γ-hexachlorocyclohexanes), 1,1-dichloro-2,2-di(4-chlorophenyl)ethylene (*p*,*p*′-DDE), 1,1-dichloro-2,2-di(4-chlorophenyl)ethane (*p*,*p*′-DDD), and 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (*p*,*p*′-DDT).

2.3. Chemical analysis

About 5 g of unfrozen homogenized milk was extracted twice with a mixture of chloroform and methanol (1:1). Chloroform extracts were separated and dried under nitrogen flow. Milk fat was weighed and dissolved in *n*-hexane. It was then cleaned up with sulfuric acid and by adsorption chromatography on a multilayer silica column with 4% diethyl-ether in *n*-hexane as a solvent (described in Herceg Romanić and Krauthacker, 2004). Cleaned extracts were dried under nitrogen flow, dissolved in *n*-hexane and applied on commercial tubes pre-packed with carbon (ENVI-Carb SPE tubes, 3 mL, 0.25 g, Supelco. USA) which were washed with toluene and *n*-hexane prior to use. The first fraction which contained organochlorine pesticides and 17 PCB congeners was eluted with *n*-hexane/toluene (99:1). The second fraction which contained three non-*ortho* PCB congeners was eluted with *n*-hexane/toluene (75:25). After fractionation, the eluates were reduced to dryness and dissolved in *n*-hexane for gas chromatographic analysis.

High-resolution gas chromatography with electron capture detector(s) was performed on *CLARUS 500* chromatograph using two capillary columns (Restek, Bellefonte, PA, USA) simultaneously: (1) 60 m × 0.25 mm, Rtx-5 film thickness of 0.25 μm, and (2) 30 m × 0.25 mm, Rtx-1701 film thickness of 0.25 μm. Column temperature was programmed from 100 °C to 110 °C at 4 °C min^{-1} (isothermally 5 min at 110 °C) and then to 240 °C at 15 °C min^{-1} (50 min isothermally at 240 °C). The carrier gas was nitrogen. The injector and detector temperatures were 250 and 270 °C, respectively.

Qualitative and quantitative analyses were done by comparison with the external standard, and each sample was analyzed on both columns. Only compounds identified on both columns were evaluated. The method determination limits were calculated as the average of all determinations based on signal-to-noise ratio and recovery of compounds. The determination limits for the analyzed compounds were 0.5 ng g^{-1} milk fat for PCB congeners, 0.1 ng g^{-1} milk fat for α-HCH and HCB, 0.2 ng g^{-1} milk fat for *p*,*p*′-DDE, 0.3 ng g^{-1} milk fat for β-HCH, γ-HCH and *p*,*p*′-DDD, and 0.6 ng g^{-1} milk fat for *p*,*p*′-DDT.

The method recovery and reproducibility were determined by adding a known amount of all analyzed compounds to the aliquots of homogenized samples before extraction (method of addition, $n = 5$). Results are presented in Table 1. Average recoveries for PCBs were in the range between 56% and 93% and for organochlorine pesticides in the range between 55%