

# SERUM CONCENTRATIONS OF DIOXINS AND DIOXIN-LIKE COMPOUNDS AMONG RUSSIAN BOYS AND THEIR MOTHERS: THE ROLE OF BREASTFEEDING

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## Introduction

The current analysis utilizes data collected in our ongoing longitudinal cohort study on male pubertal development and exposure to dioxins, furans and polychlorinated biphenyls (PCBs). The study is being conducted in Chapaevsk, a city of 72,000 people, located approximately 950 kilometers southeast of Moscow in the Samara region of Russia. Several chemical industries, of which Khimprom Chemical Plant was the largest, in the past produced chemical warfare agents, and more recently agricultural and industrial chemicals<sup>1</sup>, including organochlorines. Release of byproducts from manufacturing and incineration from these industries has contributed to substantial environmental contamination of Chapaevsk with dioxins, furans and PCBs. The high levels of dioxins and PCBs were reported not only in environmental samples and local food products in Chapaevsk<sup>2,3</sup> but in human breast milk<sup>2</sup> and blood serum<sup>4</sup> of Chapaevsk residents. In the current analysis we assessed the serum concentrations of PCDDs/PCDFs, coplanar PCBs (C-PCBs) and mono-ortho PCBs (M-PCBs) in 427 pairs of 8- to 9-year-old boys and their mothers, and evaluated the impact of breastfeeding on their serum concentrations. To the best of our knowledge, this is the largest study describing the levels of multiple congeners of dioxins, furans and PCBs in a cohort of peri-pubertal boys and their mothers.

## Materials and Methods

The study participants are 499 peri-pubertal boys in Chapaevsk, Russia, and their mothers, who took part in the ongoing longitudinal Russian Children's Study of environmental contaminants and male growth and sexual development. The present analysis includes complete data on serum concentrations of dioxins, furans, and PCBs in 427 boys, ages 8 and 9 years, and their biological mothers. 37 mothers had missing concentration of one PCB, PCB-77, therefore we restricted our analysis of dioxin like compound concentrations to 390 mother/son pairs. For the analysis of toxic equivalencies (TEQs) we used 427 mother son pairs because missing PCB-77 did not impact Total TEQs (it contributes < 0.1% to Total TEQs). At study entry, the boys had a physical examination and provided fasting blood samples for the analyses of dioxins and PCBs. Along with their mothers they completed nurse-administered health, lifestyle and dietary questionnaires. Mothers' fasting blood was also collected at baseline on the same day as their sons. Blood samples were centrifuged and the serum was stored at -20° C until shipment on dry ice to the Centers for Disease Control and Prevention (CDC) for analysis. The chemical analyses were performed by the National Center for Environmental Health (NCEH), CDC, Atlanta, GA. The list of dioxin-like congeners (referred to as dioxins in this manuscript) included 7 PCDDs, 10 PCDFs, 4 C-PCBs, and 6 M-PCBs. The samples were spiked with a mixture of <sup>13</sup>C<sub>12</sub>-labeled dioxins and PCBs and extracted by a C<sub>18</sub> solid phase extraction (SPE) followed by a multi-column automated cleanup and enrichment procedure<sup>5</sup>, and analyzed using high resolution mass spectrometry in selective ion monitoring<sup>6</sup>. M-PCBs were extracted by SPE method<sup>7</sup> using Waters

Oasis HLB cartridge, followed by automated acid and neutral silica gel SPE. Serum total cholesterol and triglycerides were determined using enzymatic methods and the total lipid content was then calculated using the Phillips equation.<sup>8</sup> We calculated basic descriptive statistics (mean, standard deviation (SD), and percentiles) for the boys' and mothers' serum concentrations, 2005 Toxic Equivalencies (TEQs) and Spearman correlations between the boys' and mothers' levels. There were 7 pairs of twins and siblings in this cohort, but for each sibling pair only the older sibling was included in the correlation analysis.

## **Results and Discussion**

### ***Study Population***

The boys were 8 (64%) and 9 (36%) years old at study entry, and 66% were the first born child. 70% of mothers were < 25 years old at their son's birth, and 88% of families resided in Chapaevsk for 6 years or more. Most of the mothers (58%) breastfed their sons for 1–6 months, 30% for more than 7 months, and 12% of mothers did not breastfeed their sons. The duration of breastfeeding was highly skewed, with a median of 4 months (25<sup>th</sup>, 75<sup>th</sup> percentiles: 2, 10). Mothers who did not breastfeed were slightly older on average (median=25 versus 24 years old, respectively). There were no significant differences in breastfeeding history by maternal parity, parental education, or household income. However, among the women who lived in Chapaevsk for 6 or more years, 13% did not breastfeed their son, while among women who lived in Chapaevsk for less than six years 4% did not breastfeed (p-value=0.07).

### ***Serum dioxins and PCBs among the boys and their mothers***

The ranges of total serum PCDD/PCDF/C-PCB concentrations were wide and higher than those reported in the U.S.<sup>9</sup> The median serum total 2005 TEQs of the 8- to 9-year-old Chapaevsk boys was triple the geometric mean from the U.S. National Health and Nutrition Examination Survey for males 12–19 years of age (there were no data on children < 12 years of age)<sup>9</sup>. As expected, total serum PCDDs/PCDFs/C-PCBs concentrations and 2005 TEQs were higher in mothers than in boys (Table 1). Median concentrations of PCDD/PCDF/C-PCBs in mothers and in boys were 383 and 362 pg/g lipids, respectively. Median 2005 TEQs for mothers and boys were 24.5 and 20.9 pg TEQ/g lipid, respectively. This finding was expected because serum dioxins and PCBs accumulate with increased age.

### ***Breast feeding and the levels of serum dioxins and PCBs in boys and their mothers***

Figures 1a and 1b show median serum 2005 TEQ levels (using summary measures for dioxins, furans, C-PCBs and M-PCBs) for mothers and for sons by breastfeeding duration category. The median levels of 2005 TEQs for the mothers decreased with the length of breastfeeding, especially for the sum of PCDDs. Total median PCDD/PCDF/C-PCB concentrations for mothers decreased slightly with the duration of breastfeeding, from 371 pg/g lipids for non-breastfeeding mothers to 362 pg/g lipids for mothers who breastfed for 7 or more months. In sons, the median total 2005 TEQs levels increased with breastfeeding duration for all summary measures from no breastfeeding up through  $\geq 7$  month breastfeeding. Similarly, the median total PCDD/PCDF/C-PCB concentrations for sons also increased with the duration of breastfeeding, from 307 pg/g lipids for non-breastfeeding to 352 pg/g lipids for 1-6 months breastfeeding to 415 pg/g lipids for  $\geq 7$  month breastfeeding. Although mothers who never breastfed were slightly older, the decrease in the levels of serum dioxins is likely attributable to the transfer of these compounds through breastfeeding<sup>10</sup>. Likewise, the increase in levels of dioxins and PCBs among breastfed boys was likely due to transfer of dioxins from breast milk to sons as appears to be the case in other breastfed populations<sup>11,12</sup>.

### ***Correlations between concentrations of dioxins and PCBs in mothers and sons by breastfeeding category.***

The correlations for total PCDD/PCDF/C-PCB serum concentrations between mother and son pairs were stronger among the mothers who breastfed their sons as compared to those who did not, 0.37 and 0.19, respectively. Similar results were observed for total 2005 TEQ, 0.32 among breastfed pairs and 0.18 among those who did not. Figure 2 shows the correlations for total TEQs for mother son pairs stratified into 5 categories based on the length of

breastfeeding. The correlation increased only modestly after the first few months of breastfeeding. The results were unchanged by the exclusion or inclusion of all seven sibling-pairs.

### Conclusions

Serum total PCDDs/PCDFs/C-PCBs concentrations and 2005 TEQs were higher among mothers than among boys. Mothers' levels of total PCDD/PCDF/C-PCB and 2005 TEQs decreased with the length of breastfeeding. Both sons' levels of total PCDD/PCDF/C-PCB and 2005 TEQs increased with breastfeeding duration. Correlations between both total PCDD/PCDF/C-PCB concentrations and 2005 TEQs of mother son pairs was higher among the mothers and sons who breastfed than among those who did not. Despite evidence of likely transfer of dioxins from mother to son via breast milk, among the general population the beneficial effects of breastfeeding are believed to substantially outweigh the risk of possible breast milk contaminant exposure<sup>13</sup>.

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Table 1. Distribution of serum dioxin-like (PCDD/PCDF/C-PCB) concentrations and 2005 TEQs (pg/g lipid) among 8-9 year old boys and their mothers in the Russian Children's Study							
Boys				Mothers			
PCDD/PCDF/C-PCB concentrations (N=390)		2005 TEQs (N=427)		PCDD/PCDF/C-PCB concentrations (N=390)		2005 TEQs (N=427)	
Mean (SD)	Median (25 <sup>th</sup> , 75 <sup>th</sup> %)	Mean (SD)	Median (25 <sup>th</sup> , 75 <sup>th</sup> %)	Mean (SD)	Median (25 <sup>th</sup> , 75 <sup>th</sup> %)	Mean (SD)	Median (25 <sup>th</sup> , 75 <sup>th</sup> %)
419 (246)	362 (278, 493)	27.6 (21.8)	20.9 (14.2, 33.5)	438 (232)	383 (297, 499)	29.8 (20.9)	24.5 (17.2, 36.0)

<sup>1</sup>N=390 due to missing values for C-PCB #77

Figure 1a. Mothers' median serum 2005 TEQs (pg/g lipid) by breastfeeding category

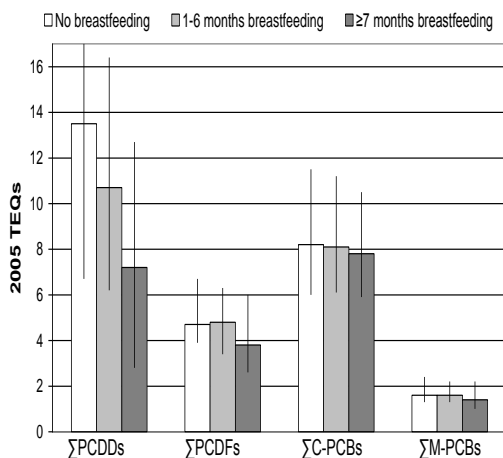


Figure 1b. Sons' median serum 2005 TEQs (pg/g lipid) by breastfeeding category

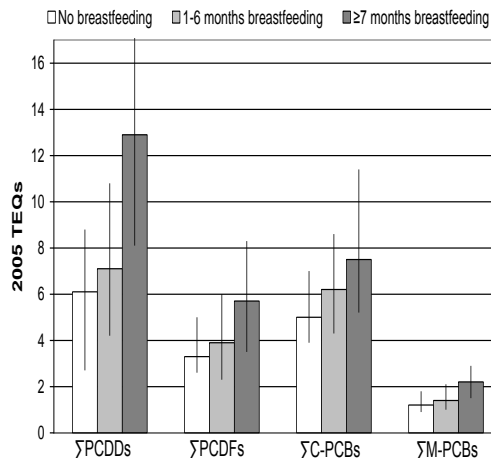


Figure 2: Spearman correlation between the mother and son's serum 2005 TEQ, by duration of breastfeeding

