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Polychlorinated biphenyls in umbilical cord serum of newborns from Rio Grande do Sul state, Brazil

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ABSTRACT

Background: Polychlorinated biphenyls (PCBs) are food-chain contaminants that have been shown to contaminate foods worldwide. The newborn are exposed to these organochlorine compounds across the placenta and through breastfeeding. They are proven to be carcinogenic and may contribute to congenital malformation etiology.
Methods: This study examined levels of five PCB congeners (28, 52, 138, 153 and 180) in umbilical cord serum samples from 148 newborns from Rio Grande do Sul state, Brazil. Serum concentrations of PCBs were analyzed by gas chromatography with electron capture detection and mass spectrometry.
Results: Levels of ∑ PCBs ranged from 0.35 to 55.17 ng/ml in umbilical cord serum positive samples, and PCB 138 was the most prevalent congener. Only 7.4% of samples presented no PCB congener.
Conclusions: Some PCB congener cord serum levels were related to the locale of the mothers' residence, smoking and drinking habits, fruit consumption, and congenital malformation.

1. Introduction

Polychlorinated biphenyls (PCBs) were discovered before the turn of the 20th century and their usefulness for industry, because of their physical properties, was recognized early. They have become widely distributed in the environment throughout the world, and are persistent and accumulate in the food chain. PCBs bioaccumulate in the fatty tissues of humans and other animals and have caused toxic effects in both, particularly if repeated exposure occurs. The results of the studies on rodents suggest that some PCB congeners may be carcinogenic and that they can promote the carcinogenicity of other chemicals [1]. Previous studies suggested that human exposure to PCBs is associated with the occurrence of various types of cancer, such as breast cancer [2–4], colon cancer [5], liver, stomach, intestinal and prostate cancers[6,7] and a recent study showed that poisoning due to PCBs and dioxins can affect quality of life 30 y after the exposure [8]. PCBs were also associated with the occurrence of miscarriages and premature births [9], with damage to the peripheral nervous system [10], with respiratory infections in children from birth to 5 y [11], with reduced immunity in children [12], with diabetes [13] and with asymmetric hearing loss [14].

PCB concentration in umbilical cord plasma is a good indicator of prenatal exposure to PCBs [15]. Their levels detected in maternal and umbilical cord serum showed significant relationships, confirming their efficient transplacental transfer [16–18]. Entering the fetal blood system in this way, PCBs are a health risk to fetuses and newborns, which are much more vulnerable to environmental pollutants. The exposure to persistent organic pollutants in utero can have adverse effects on fetal growth (i.e. birth weight, length, head circumference) and health [19].

The largest number of studies on this subject is found in countries that produced PCBs, such as Italy, Germany, Japan, and the United States. Generally speaking, congeners 138, 153 and 180 are those that process the greatest concentrations of detectable residues [20–23]. These three congeners, along with congeners 28, 52, 101 and 118 are denominated “the seven indicators” and are responsible for the greatest part of environmental contamination.

This paper reports the residue concentrations of PCBs (28, 52, 138, 153, and 180) in umbilical cord serum of newborns from Rio Grande do Sul State, Brazil. The comparison among the PCB concentrations, sociodemographic characteristics and food habits of the mothers and the characteristics from the newborns, were also reported.

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2. Material and methods

2.1. Population and samples

Arterial umbilical cord blood samples were collected from the babies of 148 mothers admitted to the Obstetrics Center of the University Hospital of Santa Maria, Rio Grande do Sul, Brazil, during 2006. Umbilical cord blood samples were taken immediately after the delivery, collected from the section of the umbilical cord next to the placenta, after clamping and cutting. To determine the PCB congeners, serum obtained from blood centrifugation was used. After centrifugation, the samples were stored at −20 °C prior to analysis. The approximate volume of serum collected was 0.5 ml. Due to relatively low sample volumes, lipid analysis was not performed for this set of samples. All participants were asked to complete a questionnaire which detailed their weight, age, height, sociodemographic information as well as food habits. Details of the characteristics of the newborn infants were also documented. Not all participants responded to the questionnaires. The applied protocol was previously approved by the Research Ethical Committee of Federal University of Santa Maria through their Letter of Approval.

2.2. Standards, reagents and materials

A stock solution of PCBs containing 10 μg/ml of congeners 28 (2,4,4′-trichlorobiphenyl), 52 (2,2′,5,5′-tetrachlorobiphenyl), 138 (2,2′,3,4,4′,5′-hexachlorobiphenyl), 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) and 180 (2,2′,3,4,4′,5,5′-heptachlorobiphenyl) was used for the identification method and the validation process. The standard solutions were obtained from SUPELCO Inc. *n*-Hexane for pesticide residue analysis was obtained from Mallinckrodt Baker, analytical grade sulfuric acid from Vetec Química Fina Ltda, and nitrogen 5.0 analytical grade from White Martins. Glassware used in the analyses and to store samples was previously washed following the method by Angulo et al. [24], with distilled water, rinsed with hexane and acetone alternately and dried at 150 °C, to assure chemical cleanliness.

2.3. Serum extraction and clean-up

Details of the procedure have been described previously [25]. Briefly, arterial umbilical cord serum samples were placed in centrifuge tubes. Then, *n*-hexane and sulfuric acid were added and the mixture was stirred in a vortex mixer. The supernatant *n*-hexane layer was separated by centrifugation and transferred to a second centrifuge tube using a Pasteur pipette. This *n*-hexane extraction step of sulfuric acid digested sample was repeated twice. The combination of all *n*-hexane extracts provided a total volume of 7 ml. Additional sulfuric acid was added to this volume and the mixture was vortex stirred and centrifuged as before. The supernatant was transferred to a conical bottomed, graduated tube and the volume was reduced to near dryness under a gentle stream of nitrogen. After this stage, the sample was reconstituted in 0.5 ml *n*-hexane.

2.4. Instrumental analysis

Identification and quantification analyses were performed in an Agilent 6890 Plus gas chromatograph (Agilent Technologiesprovided with a μECD and an Agilent HP-5 (crosslinked 5% phenyl methyl siloxane) capillary column (30 m long × 0.32 mm i.d. × 0.25 μm film thickness). Nitrogen was the carrier gas (1.5 ml/min). The injector and detector temperatures were 280 and 320 °C, respectively. The temperature program was 60 °C (2 min), 30 °C/min to 190 °C (5 min), 5 °C/min to 220 °C (5 min), and 20 °C/min to 300 °C (1 min). Selected samples were analyzed using a Hewlett-Packard GC–MS HP 6890-5973 to confirm the qualitative results with the same column used in the GC–μECD analysis. The carrier gas was helium (1.5 ml/min). The MS detector was used in selected ion monitoring mode (SIM), with electron energy of 70 eV. The temperature program was 60 °C (2 min), 5 °C/min to 220 °C (5 min), and 20 °C/min to 300 °C (2 min). The ions monitored were *m/z* 186, 256 and 258 (PCB 28); *m/z* 220, 222, 290 and 292 (PCB 52); *m/z* 290, 358, 360 and 362 (PCB 153); *m/z* 290, 235, 360 and 362 (PCB 138); *m/z* 324, 394, 396 and 398 (PCB 180). GC–MS analysis confirmed the identity of PCBs (Supplementary Material).

2.5. Quality control

To evaluate the accuracy and reliability of the PCB analysis, the validation method procedure was previously performed [25]. Trueness was evaluated by spiked umbilical cord serum samples, with levels ranging from 5.0 to 20 ng/ml (n = 3) and related to recovery. Mean recoveries for the 5 PCBs ranged from 73 to 119% and the coefficient of variation was below 12.5%, indicating an adequate repeatability for the method. The limit of detection (LOD) was 0.1 ng/ml for all PCBs. The limit of quantification (LOQ) was 0.25 ng/ml for PCB 28 and 0.5 ng/ml for PCBs 52, 138, 153 and 180. The calibration curve (0.25 to 12 ng/ml of each analyte) was prepared in umbilical cord serum samples, linear and characterized by good correlation coefficients (>0.99) for all compounds studied. Intra- and inter-assay variations were calculated and the values were <18%. Blanks were made for each sample lot extracted, with the objective of eliminating possible interference.

2.6. Statistical analysis

Statistical analyses were performed using the Statistica® 7.0 software package. PCBs measured in the umbilical cord serum samples that did not fit normal distribution, were analyzed using non-parametric tests. The Mann–Whitney test was used to compare the means of PCBs between groups of variables. A value equal to half of the LOD was given to each PCB not detectable in a sample. Positive samples were calculated for samples with levels above the LOD. ∑ PCB was calculated as the sum of PCB congeners. All statistical significance was set at $p < 0.05$ and $p < 0.10$.

3. Results and discussion

A description of the main characteristics of mothers and newborns is shown in Table 1. The mean age of pregnant women was 25.5 y, with mean weight gain during the pregnancy of 14.1 kg. The mean weight of the newborns was 3.2 kg, and half of them were boys. Among these pregnant women, an estimated 74% were multiparas.

Although PCBs were banned more than three decades ago, in the present study, we found PCB congeners in umbilical cord serum samples from newborns. Recent studies also have noted the presence of these substances in biological fluids and human tissues worldwide [16,26–28].

Levels of ∑ PCBs ranged from 0.35 to 55.17 ng/ml in umbilical cord serum positive samples, with a mean of 7.04 ng/ml in positive samples and of 6.65 ng/ml in all samples (Table 2). PCB 138 was the most prevalent congener (63.5% of positive samples), while PCB 28 was the least prevalent (18.9%). This pattern was observed due to the biodegradability of PCBs, that is diminished considerably when halogenation degree increases [29]. PCB 138 is hexachlorinated, while PCB 28 is three chlorinated. PCB 138 was also the main contributor to ∑ PCB (36.8%), followed by PCB 52 (25.7%) and PCB 180 (22.4%). A different result was observed by Lopes et al. [18], where the major congener was PCB 153. Umbilical cord samples presented at least one PCB congener in a frequency of 92.6%. The majority of samples had two (29.1%) or three (25.7%) PCB congeners. One PCB congener was detected in 16.2% samples, four in 16.9% and five in 4.7% of the samples. The PCB congeners found were ranked as follows: 138 > 52 > 180 > 153 > 28 (Table 2).

The levels of PCBs found in umbilical cord serum samples for this study were greater than those reported by studies in other countries [18,22,30–32] (Table 3). In the present work, levels of PCBs 52 and 28