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Using placenta to evaluate the polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) exposure of fetus in a region with high prevalence of neural tube defects

Jin Ma [a], Xinghua Qiu [a,*], Aiguo Ren [b,**], Lei Jin [b], Tong Zhu [a]

[a] State Key Joint Laboratory for Environmental Simulation and Pollution Control, College of Environmental Sciences and Engineering and Center for Environment and Health, Peking University, PR China

[b] Institute of Reproductive and Child Health, Peking University Health Science Center and National Reference Laboratory on Reproductive and Child Health, Ministry of Health, PR China

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ABSTRACT

Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are persistent organic pollutants suspected to have various toxic effects, including reproductive toxicity. The aim of this study was to determine the concentrations of PCBs and PBDEs in human placentas and to examine the potential association between *in utero* exposure to these pollutants and the risk of neural tube defects. Subjects were recruited from a birth defects surveillance program in a rural area of Shanxi Province, China, from 2005 to 2007. 80 placental samples from fetuses/neonates with neural tube defects and 50 samples from healthy newborn infants were analyzed for PCBs and PBDEs using electron-capture negative-ionization gas chromatographic mass spectrometry. The median concentrations were 0.89 and 0.54 ng/g lipid for the eight PCB congeners and six PBDE congeners detected, respectively. The median concentration of total PCBs was slightly higher in the case samples than in the controls (0.91 vs. 0.89 ng/g lipid), but the difference was not significant ($P=0.46$), as also found for the median concentration of total PBDEs (0.55 vs. 0.54 ng/g lipid, $P=0.61$). For both PCBs and PBDEs, when their placental concentration was above the median of all samples, it was associated with a non-significantly higher or equal risk of neural tube defects. Low levels of PCBs and PBDEs are not likely risk factors for neural tube defects in this population.

1. Introduction

Polychlorinated biphenyls (PCBs), once widely used as insulators in electrical equipment, are ubiquitous environmental pollutants. PCBs are persistent in the environment, accumulate in biota, and have various health effects in humans (Ross et al., 2000). Animal experiments demonstrated that PCBs have a variety of toxic effects on the nervous and reproductive systems (Grandjean and Landrigan, 2006). Especially, neonatal deficits have been reported in infants exposed prenatally to PCBs (Fein et al., 1984). Similar to PCBs in structure, polybrominated diphenyl ethers (PBDEs) belong to a group of emerging pollutants used worldwide as flame retardants (Hites, 2004). PBDEs have neurotoxicity, thyroid toxicity, carcinogenicity, and reproductive toxicity (McDonald, 2002).

PCBs and PBDEs are lipid soluble and resistant to metabolism (Ross et al., 2000; Hites, 2004). They can cross the placenta, resulting in fetal exposure (Doucet et al., 2009), and can interact with and enhance developmental neurobehavioral defects when the exposure occurs during a critical stage of neonatal development (Eriksson et al., 2006). Effects on cell viability, DNA damage, chromosomal abnormalities, and DNA-protein crosslinks induced by BDE-47 combined with CB-153 have also been observed (He et al., 2010). Hence, we suspected that exposure to PCBs and PBDEs *in utero* could be associated with neural tube defects (NTDs), one of the most common human congenital malformations (Copp et al., 2003).

During mammalian embryo/fetus development, the placenta is responsible for nutrient and oxygen transport, lipophilic pollutants such as PCBs and PBDEs can accumulate in the placenta, and hence the level of pollutants in placenta is a biomarker of prenatal exposure of the embryo/fetus to these chemicals (Myren et al., 2007). Compared with pollutants in blood or urine samples, pollutants in the placenta could reflect the exposure of the fetus in utero throughout the pregnancy (Reichrtova et al., 1998). In addition, pollutants in the placenta are also biomarkers of

* Corresponding author. Fax: +86 10 62760755.
** Corresponding author. Fax: +86 10 8280 1141.
E-mail addresses: xhqiu@pku.edu.cn (X. Qiu), renag@bjmu.edu.cn (A. Ren).

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maternal internal exposure to these chemicals (Myren et al., 2007).

However, there are few data on PCBs and PBDEs in the placenta. Indeed, no study has used placental levels of PCBs and PBDEs as exposure biomarkers to reveal the exposure of a Chinese population to these pollutants, and to study their health effects. In this study, we measured PCBs and PBDEs in 130 placental samples collected from rural areas of Shanxi, China, with a reported NTD prevalence rate as high as 13.9 per 1000 births (Li et al., 2006). 80 placental samples were from fetuses/neonates with NTDs and 50 samples were from healthy newborn infants. The samples were used to examine the potential association between *in utero* exposure to PCBs and PBDEs and the risk of NTDs. To our knowledge, this is the first such study.

2. Materials and methods

2.1. Chemicals

Analytical standard mixtures of PCBs and PBDEs were obtained from Wellington Laboratories (Guelph, ON, Canada). Individual standards for CB-65, CB-155, and BDE-71 were from AccuStandard (New Haven, CT, USA). All solvents used for the extraction and cleanup procedures were residue-analysis grade or its equivalent and were obtained from Fisher Scientific (Fair Lawn, NJ, USA) or from Tedia (Fairfield, OH, USA).

2.2. Sample collection

Sample collection has been introduced by Ren et al. (2011). Briefly, subjects were recruited from rural counties in Shanxi Province, China, from 2005 to 2007. Cases with NTDs were identified through a population-based birth defects surveillance program. Once a fetus or newborn with such defects was identified as a case, a healthy newborn with no congenital malformation in the same hospital was selected as a control. The control was matched for gender and the mother's county of residence, and the date of the mother's last menstrual period being as close as possible to that of the case. Placentas were collected at delivery or the termination of neural tube defect-affected pregnancies, placed in polyethylene bags, and kept at -20 °C. The study protocol was approved by the Ethics Committee of the Centre of Health Sciences, Peking University. Informed consent was obtained from the mothers before the study.

The case–control status of the placental samples was masked from those who performed the subsequent sample preparation and instrumental analysis.

2.3. Sample preparation

In this study, 80 placental samples from fetuses/neonates with NTDs, including 36 anencephaly and 44 spina bifida, and 50 placental samples from healthy newborn infants were analyzed. For each sample, ~10 g of placental tissue were taken from within 2 cm of the point of cord attachment for chemical analysis. The placental tissue was cut into pieces and transferred into a 50-mL round-bottom centrifuge tube, homogenized at 33,000 rpm in 15 mL of *n*-hexane/acetone mixed solvent (1:1 by volume). After spiked CB-65 as recovery surrogate standard, samples were ultrasonicated and vortexed. The organic extract was separated after centrifuging, and the residue was extracted twice more. Approximately 10% of the combined extract was removed for gravimetric determination of lipid mass. The rest was blown down to 2 mL with nitrogen for gel permeation chromatography (GPC) cleanup.

A Bio-beads S-X3 (Bio-Rad Laboratories, Hercules, CA, USA) GPC column (35 g of beads swollen and packed in a 3-cm i.d. glass column) was used to remove most of the lipid with *n*-hexane/dichloromethane (1:1 by volume) as the elution solvent. The 80–210 mL of eluent was collected and concentrated to 1 mL. Further cleanup was performed on a silica gel column (10 cm length, 1 cm i.d., with 1 cm of anhydrous sodium sulfate on the top), using 25 mL *n*-hexane/dichloromethane (1:1 by volume) as the elution solvent. After concentration, CB-155 and BDE-71 were added as internal standards for gas chromatographic mass spectrometry (GC/MS) analysis.

To prevent potential photodegradation, throughout the process the tubes and flasks were wrapped with aluminum foil, or amber vials were used.

2.4. Instrumental analysis

We analyzed all the target compounds by GC/MS (Agilent 7890 A/5975C) with an electron-capture-negative ionization (ECNI) ion source. GC injection (1 μL) was made in splitless mode, with the temperature of the injection port set at 250 and 280 °C for PCBs and PBDEs, respectively. An Rxi-5 ms column (15 m length; 250 μm i.d.; 0.10 μm film thickness; Restek, Bellefonte, PA, USA) was used to separate the analytes. The GC oven temperature programs were as follow: for PCBs, held at 70 °C for 1 min; 6 °C/min to 235 °C; 25 °C/min to 300 °C; held for 2 min; and for PBDEs, held at 110 °C for 1 min; 20 °C/min to 200 °C; 5 °C/min to 300 °C; held for 5 min. The following ions (m/z) were monitored: 324, 326, and 328 for penta-CBs; 358, 360, and 362 for hexa-CBs; 394, 396, and 398 for hepta-CBs; 4428, 430, and 432 for octa-CBs; 462, 464, and 466 for nona-CBs; 496, 498, and 500 for deca-CB; and 79 and 81 for all the congeners of PBDE.

2.5. Quality control

Several quality control criteria were used to ensure the correct identification and quantification of PCBs and PBDEs. First, the GC retention times matched those of the standard compounds within ± 0.1 min. Second, the signal-to-noise ratio was higher than 5:1. Third, the isotopic ratios of all pairs were within $\pm 15\%$ of the theoretical values. The recovery (mean $\pm$ standard deviation) was $95.5 \pm 5.3\%$ for the surrogate standards of CB-65. In addition to the placental tissue samples, one procedural blank sample was prepared with each batch of seven samples. Pollutants were rejected if the concentration in placental samples was not significantly higher than that in the blank. For the remaining target pollutants, the values in the placental samples were all more than four-fold higher than those in the blanks. The data reported here were not blank or recovery corrected, and were expressed on a lipid basis because of their lipid solubility.

2.6. Statistical analysis

Since most of the PCBs and PBDEs were not normally distributed, the median and range were used to describe the skewed distributions of the PCB and PBDE concentrations. Nonparametric analysis was used to compare the medians between the case and control groups. Statistical analyses were conducted using SPSS ver. 13.0 (SPSS, Chicago, IL). A two-sided P-value <0.05 was considered statistically significant.

3. Results

In this study, we assessed 27 PCB and 39 PBDE congeners. However, most of these congeners were not detected because of their low concentrations, and data for some detectable congeners were rejected after the blank test. Table 1 lists the data for PCB congeners -105, -118, -156, -157, -167, -189, -206, and -209, and PBDE congeners -47, -66, -99, -100, -153, and -154 for the NTD group, control group, and the two groups combined.

The eight PCB congeners were detected in 71.3–97.5% of the case placental samples and 66–100% of the control placental samples. The six PBDE congeners were detected in 53.8–97.5% of the case placental samples and 34–100% of the control placental samples. The concentrations of ΣPCBs in placental samples ranged from not detectable to 9.8 ng/g lipid, with a median of 0.89 ng/g lipid. The concentrations of ΣPBDEs in placental samples ranged from not detectable to 11.1 ng/g lipid, with a median of 0.54 ng/g lipid. As shown in Table 1, all individual congeners except for CB-209, and the sum of PCBs and PBDEs were slightly higher in the placental samples for the case group than in those of the control group; however, the differences were not significant.

The risks of NTDs associated with a higher level of a specific pollutant or a group of pollutants were analyzed (Table 2). In general, we found no evidence of a positive association between the risk of NTDs and the placental levels of PCBs and PBDEs. For almost all the PCB and PBDE congeners, the odds ratios (ORs) were slightly higher than 1.0, except for CB-209 (0.81). The ORs of most pollutants were not statistically significant, except for BDE-154 (OR=1.60, P=0.01), although its concentration and detectable ratio were both low.