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Impact of non-occupational exposure to polybrominated diphenyl ethers on menstruation characteristics of reproductive-age females

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs) have documented effects on thyroid functions and rodent behavior *in vivo*. Epidemiological studies, however, have revealed only limited information about associations between PBDE exposure and menstruation characteristics. Our goal was to examine whether high breast milk PBDE levels in reproductive-age females lead to interference with menstruation characteristics. We analyzed 15 PBDE congeners in 46 breast milk samples. Fifteen PBDE congeners (BDE-15, 28, 47, 49, 99, 100, 153, 154, 183, 196, 197, 203, 207, 208, and 209) were analyzed using a gas chromatograph equipped with a high resolution mass spectrometer. The mean sum of PBDEs (ΣPBDEs) in breast milk was 3.42 ng/g lipid. Women's age at menarche was not correlated with breast milk PBDE levels. Increased BDE-208 and 209 levels were significantly associated with the prolonged length of average and the longest menstrual cycle independent of age, pre-pregnant BMI, and parity. Higher concentrations of ΣPBDEs and the higher brominated PBDEs from BDE-183 to 209, except 197, were significantly linked to women whose menstruation periods were still coming irregularly at the sampling time. Age-adjusted odds ratios (ORs) of BDE-153, 183, 207, 208, and ΣPBDEs were significantly higher in women with length of average menstrual cycle >32 days, compared to the control. Women whose menstruation periods still came irregularly when they were 18 years old had higher age-adjusted ORs of BDE-207, 208, 209, and ΣPBDEs than those whose periods came regularly at the same age. Although ΣPBDEs and certain higher brominated PBDEs appear to have potential to prolong length of average menstrual cycle and delay the age when menstruation periods begin coming regularly, these findings are not conclusive because our sample size is small and more scientific evidence is needed.

1. Introduction

Polybrominated diphenyl ethers (PBDEs)—including pentaBDE, octaBDE, and decaBDE—are the brominated flame retardants (BFRs) used in electronic circuit boards and cases, cables, textiles, vehicles, synthetic building materials, and carpet lines. PBDEs, mixed into polymers as flame-retardant additives, are easily leached from BFR products and then released into the environment. PBDEs, structurally similar to polychlorinated biphenyls (PCBs), exhibit characteristics similar to those of PCBs, such as persistence and bioaccumulation (Darnerud et al., 2001). PBDEs are widespread in the environment and easily accumulated in biota (Darnerud et al., 2001); and they have been recognized as both lipophilic endocrine disruptors (Darnerud, 2008; Mercado-Feliciano and Bigsby, 2008) and as persistent organic pollutants (de Wit, 2002). PBDEs have been found in household dust (Schecter et al., 2005a; Wu et al., 2007; Stapleton et al., 2009), meat (Huwe and Larsen, 2005), foodstuffs in the supermarkets (Ohta et al., 2002; Bocio et al., 2003; Schecter et al., 2006; Martí-Cid et al., 2008; Schecter et al., 2010), fish (Zhu and Hites, 2004; Hites et al., 2004), aquatic animals (Hermanussen et al., 2008), and human specimens,

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including breast milk, cord blood, and serum (Norén and Meironyté, 2000; Schecter et al., 2003; Mazdai et al., 2003; Sjodin et al., 2004; Schecter et al., 2005b; Wang et al., 2008). Although the manufacture and use of PBDEs have been banned in the European Union since 2003, PBDEs are still imported into Taiwan.

The endocrine-disrupting potential of several PBDE congeners has been demonstrated mostly in their adverse health effects on the functions of thyroid hormones (Julander et al., 2005). PBDEs may be able to interfere with thyroid homeostasis (Darnerud et al., 2007). Some predominant BDE congeners, i.e., BDE-99 and BDE-153, have been demonstrated to induce developmental neurotoxicity (Eriksson et al., 2001) and neurobehavioral alterations (Viberg et al., 2007). Occupational exposure to PBDEs has resulted in an increased level of thyroid hormone in workers (Julander et al., 2005; Darnerud, 2008). Recent studies have also linked PBDE exposure to induction of estrogenic activity *in vitro* (Mercado-Feliciano and Bigsby, 2008) and reproductive effects *in vivo*, including sexual development and behavior (Stoker et al., 2004; Lilienthal et al., 2006; Talsness et al., 2008). A weak estrogenic activity of the PBDE mixture DE-71 has been demonstrated by using MCF-7 cells (*in vitro*), as well as BALB/c and C57BL/6 female mice (*in vivo*) (Mercado-Feliciano and Bigsby, 2008). High levels of BDE-209 (500 or 1500 mg/kg/day) exposure to adult mice had potential for reductions of sperm velocity of motion, decreases in sperm count with mitochondrial membrane potential, and increases in generation of hydrogen peroxide in sperm (Tseng et al., 2006). By administration of ^{14}C-BDE-99 to C57BL mice, the whole-body autoradiography showed that radioactivity of ^{14}C-BDE-99 was accumulated in ovary (Darnerud and Risberg, 2006). In addition, higher BDE-47 exposures have been significantly correlated with elevated levels of testosterone in adult male sport fish consumers (Turyk et al., 2008). However, epidemiological studies on the association between PBDE exposure and female reproductive functions remain limited.

Although several PBDE congeners have been demonstrated to have the potential to cause adverse effects on female reproductive functions in rodents, our recent epidemiological study showed borderline significances between predominant PBDEs (i.e., BDE-153 and BDE-209) exposure and menstruation characteristics (i.e., menstrual cycle length and duration of menstrual bleeding per cycle) (Chao et al., 2007a). In the present study, by using a new cohort of women with non-occupational PBDEs exposure, we examined the associations between PBDE levels and menstruation characteristics.

2. Materials and methods

2.1. Recruitment of study participants and method of sample collection

This report is part of ongoing research on mothers and their offspring with non-occupational PBDE exposure to examine whether PBDEs exposure affects hormone secretion and child development. The participants in the present study were healthy pregnant women recruited from four hospitals from southern Taiwan between April 2007 and April 2008 (Chao et al., 2010). The study protocol was reviewed and approved by the institutional review boards of the Human Ethical Committee of Pingtung Christian Hospital, Taiwan, in 2007. Ethical standards formulated from the Helsinki Declaration of 1964 and revised in 2000 were followed. We also obtained agreement from the four hospitals' departments of gynecology and obstetrics. Prior to enrollment all participants gave informed consent after receiving detailed explanations of the study and potential consequences. Our subjects were interviewed by well-trained researchers at obstetric clinics during routine health check-ups.

We invited more than 220 pregnant women to join this program; of these, 125 agreed to answer the detailed questionnaire. Questions included women's age, pre-pregnant BMI, parity, reproductive and pregnancy histories, occupational and non-occupational exposure, socioeconomic status (i.e., annual household income), smoking and dietary habits (including the frequency and amount of consumption of fish), alcohol consumption, medical history, and possible exposure to electronics. The study participants were selected mainly based on compliance with the following criteria: minimum residency of 3 years in the sampling area, a supply of sufficient breast milk samples (>120 ml), and eagerness to strictly obey our protocol to prevent contamination prior to chemical analyses. A total of 98 participants were enrolled based on healthy outcome and voluntary donation of two specimens (cord blood and breast milk). Of the 98 participants, 35 women who did not offer breast milk were excluded. Until April 2008, 63 milk samples were obtained. Finally, 46 breast milk samples were randomly selected from the 63 milk samples for further chemical analysis.

Breast milk samples (120–360 ml) were collected in 3 n-hexane-washed glass-bottles and frozen at home (−4 °C) within one month after delivery. Participants telephoned us after they finished breast milk collection. Our researchers went to the women's homes and immediately delivered milk samples to our laboratory in the Department of Environmental Science and Engineering, National Pingtung University of Science and Technology, where the samples were stored at −20 °C. Milk samples of 25 ml each were packaged in chemically clean containers and transported to the Supermicro Mass Research and Technology Center at Cheng Shiu University in southern Taiwan for chemical analysis.

2.2. Chemical analysis

Chemical analysis for PBDEs in breast milk has been described in our previous reports (Koh et al., 2010; Chao et al., 2010). Fifteen PBDE standards (BDE-15, 28, 47, 49, 99, 100, 153, 154, 183, 196, 197, 203, 207, 208, and 209) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). ^{13}C-labeled standard of 15 PBDEs was sourced from Wellington Laboratories (Guelph, Canada). The highest-quality sodium sulfate, alumina oxide, potassium oxalate, and silica gel were obtained from Merck (Darmstadt, Germany).

Fifteen ml milk samples were extracted by sonication for 20 min with 15 ml of hexane after the addition of 45 ml of acetone; then the mixture was centrifuged. These steps were repeated three times. The extract was pooled and evaporated to dryness with anhydrous sodium sulfate. The milk lipid content was determined using a gravimetric method. ^{13}C-labeled internal standards were spiked in 15 ml of breast milk before the extraction and the cleanup procedure for the following PBDEs analyses. The extract was cleaned up using a multi-layered silica column with a plug of glass wool, an activated silica gel, a silver nitrate silica gel (10%, w/w), and anhydrous granular sodium sulfate; the extract was then passed through an alumina oxide column. 50 µl of ^{13}C-labeled BDE-139 was added as a recovery standard after the cleanup and prior to injection. The final extract was concentrated in volume under a stream of nitrogen. Measurements of 15 PBDEs were performed using a high resolution gas chromatography (Hewlett-Packard 6970) and a high resolution mass spectrometer (Micromass Autospec Ultima). Separation was performed by splitless injection onto a DB-5HT column (Koh et al., 2010; Chao et al., 2010). The two most abundant isotope masses were measured for each component.

Solvents and reagents were tested before each procedure. All glassware was washed with HPLC ultra-grade hexane or acetone (Merck, Darmstadt, Germany) before use. The blank tests of solvents and glassware were regularly checked. Recovery measured for 10 $^{13}C_{12}$-labeled PBDE internal standards was added in breast milk before the extraction to ensure recovery in the whole process of chemical analysis to be calculated the recovery rates between 70 and 130%. Limits of detection (LODs) were predetermined so that the signal to noise ratio for both ions of a specific congener should be above 3. For