Environment International 88 (2016) 23–29

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Concentrations of polybrominated diphenyl ethers (PBDEs) and 2,4,6-tribromophenol in human placental tissues

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ARTICLE INFO

Article history:
Received 30 June 2015
Received in revised form 30 November 2015
Accepted 2 December 2015
Available online xxxx

Keywords:
Brominated flame retardants
Polybrominated diphenyl ethers
Thyroid hormone
Placenta

ABSTRACT

Legacy environmental contaminants such as polybrominated diphenyl ethers (PBDEs) are widely detected in human tissues. However, few studies have measured PBDEs in placental tissues, and there are no reported measurements of 2,4,6-tribromophenol (2,4,6-TBP) in placental tissues. Measurements of these contaminants are important for understanding potential fetal exposures, as these compounds have been shown to alter thyroid hormone regulation in vitro and in vivo. In this study, we measured a suite of PBDEs and 2,4,6-TBP in 102 human placental tissues collected between 2010 and 2011 in Durham County, North Carolina, USA. The most abundant PBDE congener detected was BDE-47, with a mean concentration of 5.09 ng/g lipid (range: 0.12–141 ng/g lipid; detection frequency 91%); however, 2,4,6-TBP was ubiquitously detected and present at higher concentrations with a mean concentration of 15.4 ng/g lipid (range:1.31–316 ng/g lipid; detection frequency 100%). BDE-209 was also detected in more than 50% of the samples, and was significantly associated with 2,4,6-TBP in placental tissues, suggesting they may have a similar source, or that 2,4,6-TBP may be a degradation product of BDE-209. Interestingly, BDE-209 and 2,4,6-TBP were negatively associated with age ($r_s = -0.16$; $p = 0.10$ and $r_s = -0.17$; $p = 0.08$, respectively). The results of this work indicate that PBDEs and 2,4,6-TBP bioaccumulate in human placenta tissue and likely contribute to prenatal exposures to these environmental contaminants. Future studies are needed to determine if these joint exposures are associated with any adverse health measures in infants and children.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been used as additive flame retardants for decades in a variety of applications from polyurethane foams to high-impact polystyrene (HIPS). The presence of PBDEs in consumer products has led to their accumulation in indoor environments, and subsequent human exposure via inadvertent ingestion and/or inhalation of dust particles (Johnson et al., 2010; Stapleton et al., 2012a). Particular attention has been given to a PBDE commercial mixture known as pentaBDE, which had a primary application in polyurethane foam used in residential furniture (Stapleton et al., 2012b; Zhang et al., 2011). Studies have documented higher serum concentrations of PBDEcongeners associated with pentaBDE in the US population relative to other regions of the world, likely due to the higher use of this mixture in residential furniture to meet a regional (i.e. State of California) flammability standard (Hites, 2004). While the use of pentaBDE has now been banned or phased-out throughout the world, many older products in the home still contain these flame retardants, which will continue to leach into the indoor environment during the product lifetime. As a result, human exposure to PBDEs will continue for years to come, especially with the use of recycled foams and plastics in consumer products that may contain these phased-out chemicals. As such, PBDEs continue to be measured in human tissues such as serum, breast milk, umbilical cord blood, and placental tissues, suggesting that prenatal exposures to PBDEs occurs during pregnancy, and continues during infancy via breast feeding (Zota et al., 2013; Adgent et al., 2014; Abdelouahab et al., 2013; Nanes et al., 2014).

In contrast, 2,4,6-tribromophenol (2,4,6-TBP) is widely used as an industrial chemical with an estimated US production volume of 4500 to 23,000 tonnes in 2006 (Covaci et al., 2011). 2,4,6-TBP has multiple applications, including use as an antifungal agent (e.g. as a replacement for pentachlorophenol) in wood applications, as a reactive brominated flame retardant (BFR), and as an intermediate in the production of other BFRs. 2,4,6-TBP can also be formed as a result of the photolytic degradation of tetrabromobisphenol-A (TBBPA), a widely used reactive BFR, and during the synthesis of 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE) (Suzuki et al., 2008). In addition to the anthropogenic sources

Abbreviations: 2,4,6-TBP, 2,4,6-tribromophenol; BFR, brominated flame retardant; DI, deiodinase; GC/ECNI-MS, electron capture negative ion mass spectrometry; HIPS, high-impact polystyrene; LC–MS/MS, liquid chromatography tandem mass spectrometry; PBDE, polybrominated diphenyl ether; SPE, solid phase extraction; SULT, sulfotransferase; THs, thyroid hormones; TPT, transplacental transfer; TTR, transthyretin.

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http://dx.doi.org/10.1016/j.envint.2015.12.002
0160-4120/

of 2,4,6-TBP, there are natural sources of 2,4,6-TBP and other bromophenols from marine organisms and algae (Gribble, 2000). Few toxicity studies have examined the effects of 2,4,6-TBP in animal models. One study examined oral exposure to 2,4,6-TBP in adult zebrafish and observed reproductive toxicity in addition to perturbed gonadal morphology when exposed to spiked food at concentrations of 3300 μg/g dw (Haldén et al., 2010). Only a few studies have examined environmental levels and human exposure to 2,4,6-TBP. It has been measured in marine sediments at an average concentration of 3.02 ng/g dry weight and in riverine systems at 0.66 ng/g dry weight (Sim et al., 2009). 2,4,6-TBP has also been measured in the indoor environment of Japanese homes, with indoor house dust concentrations ranging from 15 to 30 ng/g and indoor air concentrations between 220 and 690 pg/m^3 (Takigami et al., 2009). Very few biomonitoring studies have included 2,4,6-TBP in the analyses of human tissues such as serum, cord blood, and/or breast milk. One Japanese study collected maternal serum and umbilical cord blood from a cohort of 16 mothers in 2006 for analysis of BFRs and PCBs. This study measured 2,4,6-TBP in maternal blood at a concentration of 22 pg/g wet weight and in cord blood at a concentration of 37 pg/g wet weight (Kawashiro et al., 2008). BFRs were also evaluated in Norwegian individuals working in electronics dismantling facilities, where 2,4,6-TBP was measured in plasma ranging from 0.17 to 81 ng/g lipid (Thomsen et al., 2001). In a study measuring BFRs in a Canadian Inuit population from Nunavik, Quebec, plasma samples contained a geometric mean 2,4,6-TBP concentration of 9.4 μg/kg lipid, however, these concentrations were not correlated with PBDE concentrations (Dallaire et al., 2009). Finally, Qiu et al. measured mean 2,4,6-TBP concentrations of 5.6 ng/g lipid in fetal plasma and 0.8 ng/g lipid in maternal plasma (Qiu et al., 2009).

PBDEs and 2,4,6-TBP share a chemical structure that is similar to endogenous thyroid hormones (THs), and have been demonstrated to disrupt TH homeostasis either in vitro or in animal exposure studies (Noyes et al., 2013; Szabo et al., 2009). Concentrations of PBDEs in human serum have also been found to be significantly correlated with circulating levels of THs in adults, and are associated with adverse neurodevelopmental outcomes in children (Herbstman et al., 2010; Eskenazi et al., 2013). Early childhood represents a developmental period that is vulnerable to endocrine disruption. Development is a hormonally-regulated growth process that is sensitive to perturbations by environmental contaminants, like PBDEs and 2,4,6-TBP. The in utero stage of development also represents a highly vulnerable period of growth that may be even more sensitive to endocrine disruption due to the underdeveloped nature of the fetus' detoxification pathways, in addition to the myriad different growth and developmental processes that are occurring throughout gestation.

The placenta acts to facilitate the materno-fetal transfer of nutrients, gas, waste, and hormones throughout gestation and can act as a protective barrier against toxins and environmental contaminants (James et al., 2007). In the case of PBDEs, passive diffusion and/or active uptake of these chemicals into the placenta occurs, and the placenta can act as a repository for these lipophilic chemicals. For example, one study looked at mother–child pairs in China and compared the placental transfer characteristics of various environmental endocrine disruptors, including PBDEs. Their results indicated that PBDEs can be transferred across the placenta from maternal circulation, and eventually reach the fetus (Li et al., 2013). Additionally, Frederiksen et al. used an experimental ex vivo human placenta perfusion system to show the differences in transplacental transfer of PBDEs based on degree of bromination (Frederiksen et al., 2010a). Thus there is a need to better understand the accumulation of these contaminants in placental tissues, in order to understand fetal exposures. In this study, we present our findings from the analysis of 102 human placental tissues that were collected in North Carolina, USA. Tissue samples were analyzed for a suite of PBDEs and 2,4,6-tribromophenol in order to increase our understanding of exposures during pregnancy and their accumulation within the placenta.

2. Materials and methods

2.1. Participant recruitment

Participants were recruited from within an observational prospective cohort study assessing the joint effect of social, environmental, and host factors on pregnancy outcomes (i.e. the Healthy Pregnancy, Healthy Baby (HPHB) Study conducted by the Children's Environmental Health Initiative) (Miranda et al., 2010; Swamy et al., 2011). The HPHB study enrolled pregnant women from the Duke Obstetrics Clinic and the Durham County Health Department Prenatal Clinic at the Lincoln Community Health Center in Durham, NC. Our analyses included a subset of women from the HPHB study that delivered at the Duke University Medical Center between March 2010 and December 2011. The intentional study design was to oversample women attending the Lincoln Community Health Clinic, in order to explore disparities in pregnancy outcomes by comparing African-American women with good outcomes to those with poor outcomes. As a result, the study population is predominantly African-American women with a lower socioeconomic standing and low educational attainment relative to the general US population. All aspects of this study were carried out in accordance with a human subject research protocol approved by the Duke University Institutional Review Board.

2.2. Sample collection

Consenting women had placenta tissue subsamples taken at the time of delivery at the Duke University Medical Center. Tissues (approximately 5–20 g) were stored in screwtop cryovials at −80 °C until analysis.

2.3. Chemicals

All solvents used for the analysis were HPLC-grade or better. A fluorinated BDE standard, 2,3′,4,4′,6-tetrabromodiphenyl ether (FBDE-69) (Chiron Inc., Trondheim, Norway), ^{13}C labeled 2,2′,3,4,5,5′-hexachlorinated diphenyl ether (CDE-141) (Cambridge Isotope Laboratories, Andover, MA), and labeled ^{13}C-2,2′,3,3′,4,4′,5,5′,6,6′-decabromodophenyl ether (BDE-209) were used as internal and recovery standards for the BFR extractions. PBDE calibration standards were purchased from AccuStandard and 2,4,6-tribromophenol was purchased from Cambridge Isotope Laboratories, Andover, MA.

2.4. BFR analysis and lipid determination

Extractions were performed using between 2 and 17 g of placenta tissue, depending on the sample and the amount collected during delivery. Tissues underwent 24 h of lyophilization in order to completely dry the samples. The freeze-dried tissue samples were then homogenized into a fine powder with a pre-cleaned mortar and pestle before adding 15 mL of 1:1 hexane/dichloromethane (DCM) and letting the samples sit overnight, in order to allow for full solvent penetration. Samples were spiked with 1 ng of FBDE-69 and ^{13}C-BDE-209 as internal standards. All glassware used for BFR analysis were cleaned by muffle furnace, in addition to triple-rinsing with hexane, DCM, and methanol solvents in order to minimize background contamination. Samples then underwent 20 min of water bath sonication followed by centrifugation, after which the solvent was decanted to a separate tube. The extraction step was then repeated twice (three times total), and the solvent extracts were combined in a clean 50 mL glass centrifuge tube. Following extraction, the samples were blown down under a gentle stream of N_2 to a volume of 1 mL. A small aliquot of the extract was used for gravimetric lipid analysis and the remaining extract was passed through acidified silica columns for sample clean-up. Deactivated silica (4.0 g) was acidified using 40% by mass H_2SO_4, shaken, and loaded into a glass chromatography column. The columns were pre-cleaned