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Detection of polybrominated diphenyl ethers in culture media and protein sources used for human in vitro fertilization

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HIGHLIGHTS

- PBDE levels were measured in human IVF medium and protein source samples.
- PBDEs were found in 23 of the 30 samples analyzed.
- Dominant PBDE congeners were BDE-47, -100, -99, and -153.
- PBDE levels were relatively high in the protein source samples (7.5–385 pg g^{-1}).

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ABSTRACT

The concentrations of 10 polybrominated diphenyl ethers (PBDEs) in commercial culture media and protein sources (PSs) for in vitro fertilization (IVF) of human ova were investigated. Samples of 15 IVF media (IVFM), nine sperm preparation media (SPM), and six PSs were analyzed. PBDEs were detected in 10 IVFM, seven SPM, and all PS samples in ranges of 0.6–35, 0.9–31, and 7.5–385 pg g^{-1}, respectively. A dominant PBDE congener BDE-47 was detected in the PS and PS-supplemented samples. Our findings suggested that PS supplementation was the potential cause of PBDE-contamination of IVFM and SPM.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a group of flame retardants used in the production of common consumer products such as electronics, furniture, and textiles. Since PBDEs are not chemically bound to the materials, they can leak into the environment during the production, use, and disposal of the product (Rahman et al., 2001). Because of the marked increase in the levels of PBDEs in the environment and in humans over the past several decades, they are currently recognized as environmental pollutants of global concern (Hites, 2004). Because of their persistence, bioaccumulation, potential for long-range environmental distribution, and toxicity, PBDEs with 4–7 bromine atoms are currently banned and restricted internationally as the "new" Persistent Organic Pollutants (POPs) under the Stockholm Convention on Persistent Organic Pollutants (UNEP, 2009). Animal studies suggested that PBDEs might disrupt thyroid homeostasis (Zhou et al., 2002) and neonatal brain development (Viberg et al., 2004). Schreiber et al. (2010) reported that PBDE congeners BDE-47 and BDE-99 adversely affect the development of primary fetal human neural progenitor cells in vitro via endocrine disruption of cellular thyroid hormone signaling. In addition, several studies suggested that PBDEs may adversely affect human fertility. Harley et al. (2010) reported that increase in the concentration of 4 PBDE congeners in women's blood [BDE-47, -99, -100, and -153], was associated with decrease in the odds of becoming pregnant each month. Johnson et al. (2012) recently reported increased odds (10-fold) of failed embryo implantation associated with elevated levels of BDE-153 in the follicular fluid of women undergoing in vitro fertilization (IVF).

IVF is one of the most widely used treatments for infertility. In recent years, the birth rate using IVF has increased remarkably because of the progress of reproductive medicine. In Japan, IVF was performed in 548 registered institutions, and approximately 26 000 newborn babies, accounting for 2.5% of all Japanese births,

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were born by IVF in 2009 alone (Saito et al., 2011). In IVF treatment, the ova and the embryo are incubated in appropriate IVF media (IVFM), and the sperm are incubated in the sperm preparation media (SPM); these media are routinely supplemented with a protein source (PS). In the field of IVF, there is a growing concern about the potential adverse effects of exposure to the different chemical substances that form a part of the artificial cultivation environment on the fertilized ovum (Market-Velker et al., 2010). Recent studies by our group suggest that certain PS products and PS-supplemented IVFM and SPM were inadvertently contaminated with ubiquitous pollutants such as phthalates (Takatori et al., 2012) and perfluorinated compounds (Iwasaki et al., 2012). However, to the best of our knowledge, there has been no report on the analysis of PBDEs in IVF culture media and related products. The aim of this study was to provide a preliminary assessment of the concentration of PBDEs in the commercial culture media and PS products for IVF.

2. Materials and methods

2.1. Chemicals

Standard solutions containing a mixture of native PBDEs (BDE-MXE) and $^{13}C_{12}$-labeled PBDEs (MBDE-MXE and MBDE-139) were purchased from Wellington Laboratories (Ontario, Canada). In this study, 10 PBDE congeners with 3–10 bromine atoms (BDE-28, -47, -99, -100, -153, -154, -183, -197, -207, and -209) were monitored. Ethanol and *n*-hexane of pesticide analysis grade, ammonium sulfate of biochemistry grade, 44% sulfuric acid-impregnated silica gel, and *n*-nonane of dioxin analysis grade were purchased from Wako Pure Chemicals (Osaka, Japan). Water was deionized and purified using a Milli-Q cartridge system (Millipore, Bedford, MA).

2.2. Samples

A total of 30 samples—15 IVFM, 9 SPM, and six PSs—were commercially obtained from 5 global manufacturers. The five manufacturer names are randomly and anonymously labeled as A, B, C, D, and E in Table 1. The IVFM and SPM samples were available as two different types of products: one was a ready-to-use medium that already contained sufficient amounts of PS and the other was a PS-free medium that generally requires protein supplementation manually prior to clinical use. These two variations of the media are shown as PS "+" or "−", respectively in Table 2.

2.3. Analytical procedure

The accepted method for analyzing PBDEs in human serum (Akutsu et al., 2008) was modified and used in this study. The sample (2.5 g) was extracted using ethanol/*n*-hexane (1:3 v/v, 6 mL) in a 20-mL test tube after adding $^{13}C_{12}$-labeled surrogate standards (MBDE-MXE) and 1.5 mL of saturated ammonium sulfate solution. The test tube was shaken for 30 min and then centrifuged for 10 min at 1500 rpm at room temperature. The *n*-hexane phase was collected, and the aqueous phase was re-extracted twice with 5 mL of *n*-hexane, as mentioned earlier. The three *n*-hexane phases were combined and evaporated to obtain a final volume of 1 mL. The solution was transferred to a column packed with 44% sulfuric acid-impregnated silica gel (2 g). The column was eluted with 30 mL of *n*-hexane, and the eluate was evaporated to 1 mL under reduced pressure. The solution was transferred to a concentration tube with the injection standard (MBDE-139) and keeper solvent (20 μL of *n*-nonane). The extract was finally evaporated to approximately 20 μL under a gentle stream of nitrogen.

The sample extract was assayed using a gas chromatography/mass spectrometry (GC/MS) system (Agilent 6890A GC coupled with JEOL JMS-700D, Tokyo, Japan) with a fused silica capillary column (Rtx-1MS, 15 m, 0.25 mm i.d., 0.1-μm film thickness; Restek, Bellefonte, PA). For each compound, two ions of the molecular ion or fragment ion cluster were monitored. Quantitation was based on the isotope dilution method using $^{13}C_{12}$-labeled internal standards. The PBDE concentrations were expressed in picogram per gram (pg g^{-1}).

The limits of detection (LODs) were defined as the mean plus 3 times the SD values obtained from the analysis of six blank samples. However, for congeners that could not be detected in the blank samples, 3 times the SD values obtained from the analysis of five replicates of the lowest calibration standard were used as LOD. The LOD values for the target PBDE congeners ranged from 0.5 pg g^{-1} to 10 pg g^{-1}. Determination of recovery was made by quantification of the target PBDE congeners added to the IVFM samples at low (10–50 pg g^{-1}, $n = 3$), middle (50–250 pg g^{-1}, $n = 3$), and high (100–500 pg g^{-1}, $n = 3$) concentrations. The recoveries of $^{13}C_{12}$-labeled internal standards were within the range of

Table 1
Samples of media and protein sources used in the study.

Sample code	Sample type	Description	Manufacturer	Type and conc. of protein content[a]
A011110	IVFM	Fertilization medium	A	HSA 5 mg mL^{-1}
A021110	IVFM	Fertilization medium	B	HSA 3 mg mL^{-1}
A021210	IVFM	Fertilization medium	B	–
A031110	IVFM	Fertilization medium	C	SSS 6 mg mL^{-1}
A031210	IVFM	Fertilization medium	C	–
A012110	IVFM	Cleavage medium	A	HSA 5 mg mL^{-1}
A022110	IVFM	Cleavage medium	B	SSS 5 mg mL^{-1}
A022210	IVFM	Cleavage medium	B	–
A032110	IVFM	Cleavage medium	C	SSS 6 mg mL^{-1}
A032210	IVFM	Cleavage medium	C	–
A013110	IVFM	Blastocyst medium	A	HSA 5 mg mL^{-1}
A023110	IVFM	Blastocyst medium	B	SSS 5 mg mL^{-1}
A023210	IVFM	Blastocyst medium	B	–
A033110	IVFM	Blastocyst medium	C	SSS 6 mg mL^{-1}
A033210	IVFM	Blastocyst medium	C	–
A017110	SPM	Sperm dilution medium	A	HSA 10 mg mL^{-1}
A018110	SPM	Sperm separation medium	A	HSA 10 mg mL^{-1}
A019110	SPM	Sperm separation medium	A	HSA 10 mg mL^{-1}
A068110	SPM	Sperm separation medium	B	HSA 5 mg mL^{-1}
A038210	SPM	Sperm separation medium	C	–
A039210	SPM	Sperm separation medium	C	–
A027110	SPM	Sperm washing medium	B	HSA 5 mg mL^{-1}
A067110	SPM	Sperm washing medium	B	HSA 5 mg mL^{-1}
A037110	SPM	Sperm washing medium	C	HSA 5 mg mL^{-1}
A024110	PS	HSA solution	B	HSA 100 mg mL^{-1}
A034110	PS	HSA solution	C	HSA 100 mg mL^{-1}
A044110	PS	HSA solution	D	HSA 100 mg mL^{-1}
A054110	PS	HSA solution	E	HSA 100 mg mL^{-1}
A025110	PS	SSS solution	B	SSS 50 mg mL^{-1}
A035110	PS	SSS solution	C	SSS 60 mg mL^{-1}

Abbreviations: IVFM, in vitro fertilization medium; SPM, sperm preparation medium; PS, protein source; HSA, human serum albumin; SSS, serum substitute supplement.
Each SSS was composed of 84–88% HSA and 12–16% human immunoglobulin.
The five manufacturer names are randomly and anonymously shown as A, B, C, D, and E.
[a] –, PS-free product.