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Toxicity assessment of air-delivered particle-bound polybrominated diphenyl ethers

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ABSTRACT

Human exposure to polybrominated diphenyl ethers (PBDEs) can occur *via* ingestion of indoor dust, inhalation of PBDE-contaminated air and dust-bound PBDEs. However, few studies have examined the pulmonary toxicity of particle-bound PBDEs, mainly due to the lack of an appropriate particle-cell exposure system. In this study we developed an *in vitro* exposure system capable of generating particle-bound PBDEs mimicking dusts containing PBDE congeners (BDEs 35, 47 and 99) and delivering them directly onto lung cells grown at an air–liquid interface (ALI). The silica particles and particles-coated with PBDEs ranged in diameter from 4.3 to 4.5 μm and were delivered to cells with no apparent aggregation. This experimental set up demonstrated high reproducibility and sensitivity for dosing control and distribution of particles. ALI exposure of cells to PBDE-bound particles significantly decreased cell viability and induced reactive oxygen species generation in A549 and NCI-H358 cells. In male Sprague-Dawley rats exposed *via* intratracheal insufflation (0.6 mg/rat), particle-bound PBDE exposures induced inflammatory responses with increased recruitment of neutrophils to the lungs compared to sham-exposed rats. The present study clearly indicates the potential of our exposure system for studying the toxicity of particle-bound compounds.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are commonly used as flame retardants that are added to a wide variety of consumer products, such as upholstered furniture, carpet, building materials, toys and electronic goods (Allen et al., 2008; Birnbaum and Staskal, 2004; Harrad et al., 2006). Since 2004, the penta- and octa-brominated diphenyl ether mixtures, two of the three main commercial formulations, have been banned in the European Union (EU) and voluntarily phased out of the USA market because of concerns over the persistence and toxicity of PBDEs. Deca-BDE, the third main technical formulation, is currently being phased out in the EU and its production, importation and use in the USA will cease by the end of 2013 (EPA, 2010). Despite efforts to ban commercial PBDE mixtures, PBDEs will remain in the environment and in biological matrices because of their persistence and ability to bioaccumulate. Thus, human exposure to PBDEs will likely continue for decades similar to polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) even if their production and use are discontinued (Watkins et al., 2011).

PBDEs are persistent, bio-accumulative and have some structural similarities to PCBs and PBBs that can disrupt the immune, reproductive, nervous and endocrine systems in animals (EPA, 2010; Gao et al., 2009; He et al., 2009). PBDEs interfere with the endocrine system (thyroid hormone) (Ren et al., 2013), impair neurobehavioral development (Dingemans et al., 2011; He et al., 2009) and induce DNA damage (Gao et al., 2009; He et al., 2008; Lai et al., 2011) in animals and human cells *in vitro*. Data show that BDE47 and BDE99 disturb the development of primary fetal human neural progenitor cells *in vitro via* disruption of cellular thyroid hormone signaling (Schreiber et al., 2010). Co-exposure to BDE47 (1–2.5 μM) and BDE99 (5–30 μM), in particular at low

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doses, induced synergistic oxidative stress-mediated neurotoxicity in human neuroblastoma cells (SK-N-MC cell lines) (Tagliaferri et al., 2010). An *in vitro* study showed that BDE47 (4 μg/mL) inhibited cell viability, increased lactate dehydrogenase (LDH) leakage, induced reactive oxygen species (ROS), DNA damage and cell apoptosis in human neuroblastoma (SH-SY5Y) cells (He et al., 2008).

PBDEs are not permanently bound to the products and can be released from the products into the environment as dust (particle-bound) or as vapor (de Wit, 2002). Therefore, PBDEs have been commonly detected in indoor air, house dust and human tissues such as serum and breast milk (Allen et al., 2006; Batterman et al., 2009; Schecter et al., 2003; Vorkamp et al., 2011). Human exposure pathways to PBDEs remain unclear, even though the indoor environment is an important source of exposure to PBDEs used in household products (Allen et al., 2008; Harrad et al., 2006; Vorkamp et al., 2011). The main routes of human exposure to PBDEs appear to occur *via* food consumption, ingestion of dust and inhalation of PBDE-contaminated air and particle-bound PBDEs, principally in indoor exposure scenarios (Harrad et al., 2006; Huwe et al., 2008; Vorkamp et al., 2011; Wilford et al., 2008). PBDEs were found at high concentrations in house dust (BDE47 and BDE99 were 16.9 and 13.6 ng/g, respectively) and residential indoor air (BDE47 and BDE99 were 134 and 63.7 pg/m^3, respectively) (Vorkamp et al., 2011). It has been widely accepted that indoor air and dust concentrations were higher in North America than in continental Europe (Frederiksen et al., 2009). BDE47 and BDE99 were the dominant congeners in indoor air and dust collected from USA urban residences as well as in human tissues (Allen et al., 2006; Batterman et al., 2009; EPA, 2010). Interestingly, strongly elevated blood levels of PDBEs among aircraft crew and passengers were associated with inhalation exposures (Christiansson et al., 2008). Inhaled PBDEs in dust and corn oil were readily bioavailable and are biologically active in male rats, as indicated by increased transcription of hepatic enzymes. PBDEs and structurally similar semi volatile organic contaminants, such as PCBs and PAHs, are more enriched in the fine indoor particles than coarse particles. Chemicals bound to smaller particles are more bioavailable and have a longer pulmonary residence time (Hwang et al., 2008; Meeker et al., 2009; Paustenbach et al., 1997; Shoeib et al., 2012). These observations support the significance of dust in exposure to particle-bound contaminants.

Few studies have examined pulmonary toxicity of particle-bound PBDEs using *in vitro* models mainly due to the lack of an appropriate particle-cell exposure system. In some experimental designs, particles are directly added to the cell culture medium for the assessment of particle toxicity. However, these approaches have limitations, including poor reproducibility, changes of particle size due to the aggregation, interactions of particles with components of the medium (*e.g.*, albumin), and dissolution of particles by the medium (Fatisson et al., 2012; Savi et al., 2008). These limitations may account for poor correlation between toxicity of particle-types tested by *in vivo* insufflation *versus in vitro* cell culture exposures (Sayes et al., 2007). Differences in cell types, media compositions, exposure concentrations, and particle delivery techniques make comparisons between *in vitro* toxicity studies difficult.

Inhaled particles first interact with pulmonary surfactants, which are produced by epithelial type II cells and cover the alveolar region serving to prevent alveolar collapse among other functions. Coating with surfactant may alter surface characteristics and subsequent toxicity of the inhaled particle. The most commonly used *in vitro* model for assessing pulmonary toxicity of inhaled particles, the liquid cell culture method does not replicate the *in vivo* conditions of lung cells. In contrast, air–liquid interface (ALI) models more closely reflect *in vivo* conditions by providing an air-facing surface of epithelial cells with a thin layer of airway surface liquid at the air interface (Jayaraman et al., 2001). Moreover, epithelial cells grown at the ALI have well-differentiated structures and functions compared to cells grown immersed in the medium (Kameyama et al., 2003). These features account for the increase in the use of ALI models for the *in vitro* toxicity study of particles (Bitterle et al., 2006; Kim et al., 2013; Lenz et al., 2009; Savi et al., 2008; Stringer et al., 1996; Tippe et al., 2002).

The goal of this research was to develop a realistic inhalation exposure model for airborne particle-bound PBDEs. We first coupled an ALI cell exposure system with a particle aerosolizer and exposure chambers allowing the airborne particles to directly interact with the lung cells cultured on commercially available semipermeable membranes. A preliminary study was carried out to validate the reproducibility of the distribution and dose of particles and the impact on biological endpoints, cytotoxicity and ROS using two types of human lung cell lines (A549 and NCI-H358) as a precursor to studies of particle-bound PBDE toxicity. We also evaluated pulmonary responses of the same PBDE-bound particles delivered to rats *via* intratracheal insufflation to compare the results from *in vitro* studies to *in vivo* toxicity.

2. Materials and methods

2.1. Materials

Three PBDE congeners – 3,3′,4-tribromodiphenyl ether (BDE35), 2,2′,4,4′-tetrabromodiphenyl ether (BDE47), and 2,2′,4,4′,5-pentabromodiphenyl ether (BDE99) – were chosen as study compounds on the basis of their prevalence, origin and toxicity (Klösener et al., 2008). BDEs 35, 47 and 99 were synthesized by nucleophilic aromatic substitution of the diphenyliodonium salts with bromophenols as described previously (Klösener et al., 2008). A spherical silica gel particle (Nucleosil, pore size 100 Å, Macherey-Nagel, Inc., Bethlehem, PA, USA) was used as the carrier material.

2.2. Cell culture

Two types of human lung cells (A549 and NCI-H358, American Type Culture Collection, Manassas, VA, USA) were used to evaluate cellular responses of particle-bound PBDEs. A549 cells are transformed human type II alveolar epithelial cells with endocytic properties. Type II cells continuously regenerate the airways epithelium (Stringer et al., 1996). NCI-H358 cells are Clara (club)-type cells that detoxify harmful substances inhaled into the lungs, being one of the few lung cell types in which cytochrome P-450 enzymes are expressed. Cells were cultivated in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Atlanta Biologicals, Lawrenceville, GA) and 100 mg/mL penicillin and streptomycin (Invitrogen). The cells were grown in T-cell culture flasks (75 cm^2 of growth area) and incubated at 37 °C in a humidified atmosphere containing 5% CO_2. The culture medium was changed 24 h after subculturing and every 2 days thereafter (up to passage 30). The cells were split 1:10 during each passage.

2.3. Preparation of particle-bound PBDEs

PBDEs are often applied as flame-retarding coatings to a parent backbone material that may range from an inorganic material to an organic polymer (de Wit, 2002). PBDEs from these products are then released into the air by volatilization. We therefore generated and characterized coatings of our PBDE congeners on silica particles (Nucleosil) as a model of airborne particle-bound PBDEs to mimic inhalation exposure of mixtures of dust and PBDEs. Spherical silica particles coated with PBDEs were generated as described previously (Klösener et al., 2008). Briefly, for the *in vitro* study, a stock solution of individual PBDE congeners (BDEs 35, 47 or 99) was prepared for each PBDE congener to a concentration of 2 mg/mL diethyl ether. Spherical silica particles (as support materials, 40 mg), stock solution (5 mL) and diethyl ether (5 mL) as a solvent were mixed in a round bottom flask (each PBDE congener represented 20% of the weight of the silica particles). The solvent was evaporated under reduced pressure in a rotary evaporator with tumbling at 30 rpm and outside warming to 36 °C.

For the *in vivo* study a stock solution was prepared with a total PBDE concentration of 2 mg/mL diethyl ether, containing equal molar amounts of each congener, BDEs 35, 47 and 99. The PBDEs represented 20% of the weight of the silica particles. The silica particles (40 mg), stock solution of PBDE mixture (5 mL) and diethyl ether (5 mL) were mixed together as described above.

2.4. In vitro exposure system for air-delivery of particle-bound PBDEs

An *in vitro* exposure system was designed for assessing the toxicity of air-delivered particle-bound PBDEs to human lung cells. Fig. 1 shows a cross-section representation of our *in vitro* exposure system. The exposure model consists of two major chambers: (1) a delay chamber to mix particles with air, reduce particle