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Delineation of 3D dose-time-toxicity in human pulmonary epithelial Beas-2B cells induced by decabromodiphenyl ether (BDE209)☆

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ABSTRACT

Due to frequent detection in environment as well as in the human body, the adverse effects of decabromodiphenyl ether (BDE209) have been extensively studied in the past few years. However, information regarding the inhalation toxicity of BDE209 to humans is currently limited. In this study, the cytotoxicity, cell damage, and inflammation markers including IL-6, IL-8, and TNF-α in the Beas-2B cell line induced by BDE209 were measured using a central composite design. Results showed that as BDE209 concentrations (5–65 μg mL^{-1}) and exposure time (6–30 h) were increased, cell viability sharply decreased from 99.7% to 29.7% and LDH activity increased from 0.1% to 13.1%. Furthermore, expression of IL-6, IL-8 and TNF-α transcripts were enhanced from 4.7 to 29.1 fold, 3.4–68.9 fold, and 2.8–47.0 fold, respectively, and the concentration of IL-6 and IL-8 proteins increased from 5.4 to 16.7 pg mL^{-1} and 71.0 −550.0 pg mL^{-1}, respectively. Results indicate that BDE209 exposure can inhibit cell viability, increase LDH leakage, and upregulate the transcript (mRNA) and protein levels of inflammatory markers of IL-6 and IL-8 in Beas-2B cells. Moreover, these effects were both dose- and time-dependent, and dose and time had a synergistic effect – enhancing toxicity when in combination. Cell density affected both LDH activity and IL-8 release but had little effect on cell activity and IL-6 release in the Beas-2B cells. In contrast, TNF-α protein was not detected but its mRNA expression level was upregulated. This study will provide a reference for human health risk assessment, especially for the toxic damage that BDE209 exposure can elicit in the respiratory tract.

1. Introduction

Since pentabromodiphenyl ether and octabromodiphenyl ether were prohibited, decabromodiphenyl ether (BDE209) had become the most widely used polybrominated diphenyl ether (PBDE) flame retardant around the world (Verslycke et al., 2005; Li et al., 2018; Li et al., 2015). However, due to its persistence, bioaccumulation and toxicity, BDE209 was also banned by Stockholm Convention last year. Indeed, BDE209 currently accounts for over 82% of total PBDE usage across the globe (Sarkar and Singh, 2017; Martellini et al., 2016). As such, BDE209 has been very popular to use as an experimental compound in the study of the environmental behavior and biological effects of PBDEs. For example, BDE209 was previously found to be the only dominant PBDE congener in the particulate matter in an electronic waste (e-waste) dismantling workshop in China (An et al., 2011b). In a survey of the air, dust and window wipes in 63 homes in Canada, also it was found that the highest PBDE concentrations were generally BDE209 (Venier et al., 2016). Due to its long half-life, BDE209 has accumulated in environmental matrices (i.e. water, soil, atmosphere and organisms) (Ma et al., 2017; Chu and Letcher, 2017; Costa et al., 2016) and in human beings (including the serum, placenta, and fetal cord blood) (Tang and Zhai, 2017; Jeong et al., 2018), raising significant concerns about the effects of BDE209 on the environment and human health (Xiong et al., 2015; Wu et al., 2017b).

Previous studies have shown that BDE209 could induce toxicity in both wildlife and humans. For example, exposure to BDE209 can induce mitochondrial dysfunction and cell death in rat liver (Pereira et al., 2017). And the addition of BDE209 to nanoscale zero-

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valent iron exposure significantly affected reproduction in earthworms (*Eisenia fetida*) (Liang et al., 2017). BDE209 exposure has also been shown to induce various toxic effects in human beings including neurotoxicity, immune toxicity, reproductive toxicity, and hepatotoxicity (Martin et al., 2017; Den Hond et al., 2015; Liang et al., 2017; Zhang et al., 2013). BDE209 exposure increased levels of reactive oxygen species (ROS) and induced cell apoptosis in Neuro-2a cells through two pathways: the mitochondrial signaling pathway and the death-receptor signaling pathway (Chen et al., 2016). A study in human men showed that BDE209 could alter hormone levels and increase the risk of subfertility (Den Hond et al., 2015). Typically, the inhalation of air and atmospheric particles is an important exposure pathway for organic pollutants, especially at sites with heavy air pollution, such as chemical manufacturers and e-waste disassembly sites (An et al., 2011b). However, the effects of BDE209 exposure via inhalation, and the associated respiratory problems in humans are rarely investigated. Therefore, it is necessary to explore the toxicity of BDE209 to the human respiratory system.

The human lung epithelial cell (Beas-2B) is a more widely accepted model cell for assessing the effects of external pollutants on the respiratory system. Indeed, previous studies have used Beas-2B cells to better understand the responses of the human respiratory system to atmospheric organic aerosols (i.e. cell viability and inflammatory cytokine IL-8 expression) (Yu et al., 2017), and for air quality risk assessments (for arsenic, nickel, benzene, fine and coarse particulate matters) (Teoldi et al., 2017). The Beas-2B cell line has also been used to study the effects of PM2.5 extracts on cytokine expression. Toxicological assays showed that both IL-8 and MCP-1 (monocyte chemotactic protein-1) were inhibited at the aqueous extracts at 20% and 50% (Gioda et al., 2011). In addition, Koike et al. examined the effects of PBDEs in air and dust on Beas-2B cells and showed that BDE71 (pentabromodiphenyl ether) and BDE79 (octabromodiphenyl ether) but not decabromodiphenyl ether (BDE83R) exposure could damage respiratory cells by inducing an increase in inflammatory cytokines IL-6 and IL-8 (Koike et al., 2014). However, the effects of inhalation of BDE209 (the most used PBDE in China) on lung Beas-2B cells have yet to be elucidated. Other factors, such as exposure time and dose, and any synergistic or antagonistic effects between them, also need to be considered in the toxicological assessment of these contaminants. Therefore, it is necessary to determine the influences of exposure concentration, exposure time and their combinations on cytotoxicity. Often a single-factor-at-a-time optimization method is used, where one factor is changed and all other variables are kept static. However, there are two significant drawbacks to this approach. One is that this approach is time-consuming and laborious, and the other is that the interactions between these various variables are absent. To overcome these shortcomings, the central composite design (CCD) based on response surface methodology (RSM) can be employed. The CCD is a compilation of statistical and mathematical techniques including factorial design and regression analysis, that can be employed to study the influence of various factors for various purposes (Rajendran et al., 2017; An et al., 2011a; Ou et al., 2017; Fakhri et al., 2017). It is carried out through a sequence of designed experiments to predict the response values and perform regression analysis on the results (Chen et al., 2014).

In this study, the cytotoxicity, oxidative stress and inflammatory cytokine responses in Beas-2B cells induced by BDE209 exposure were investigated. CCD was applied to describe the interactions of different variables (e.g. dose and time) on cell activity, lactate dehydrogenase (LDH) activity, and release of IL-6, IL-8 and TNF-α. The results of the study will help characterize the toxicity of BDE209 to Beas-2B cells and will also facilitate human health risk assessment.

2. Materials and methods

2.1. Experimental design

The CCD model, based on RSM, was adopted to study the cytotoxicity of BDE209 (98% purity, Sigma-Aldrich) exposure to Beas-2B cells (from State Key Laboratory of Respiratory Disease, Guangzhou Medical University, China). In the experimental design, three important factors that affect cytotoxicity including BDE209 concentration (A), cell density (B) and exposure time (C) were chosen as the variables of the function at low value (−1), central (0) and high (+1) levels. The variables and levels involved in the design in this study are summarized in Table S1. Accordingly, 20 experiments included 8 (2^n, n represents the factors of CCD experimental design.) full factorial points, 6 axial points and 6 center points designed (Fig. S1), as described in our previous work (Zu et al., 2013). Five responses were selected as cell viability (Y1), LDH activity (Y2), inflammatory cytokine IL-6 (Y3), IL-8 (Y4) and TNF-α (Y5). Three-dimensional response surface plots and contour plots were constructed by drawing the responses to the Z-axis and plotting them against any two independent variables.

2.2. Cell culture

Beas-2B cells were cultured in Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 (DMEM/F-12, Gibco), containing 5% fetal bovine serum (FBS, Gibco) and a 1% double antibody mixture (Pen Strep containing 10000 Units mL^{-1} Penicillin and 10000 μg mL^{-1} Streptomyvin, Gibco), at 37 °C and 5% CO_2 in a humidified incubator. After 2–3 d of incubation, cells covered approximately 80% of the petri dishes (100 mm). The media was then discarded, and the cells were washed twice with sterile phosphate buffered solution (PBS), trypsinized and transferred to sterile centrifuge tubes. Cells were then centrifuged at 1000 g for 5 min. The supernatant was discarded and harvested cells were re-suspended in DMEM/F-12 complete culture medium.

2.3. BDE209 exposure

BDE209 was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich) to prepare stock solutions at concentrations of 1, 4, 7, 10 and 13 mg mL^{-1}. After counting, for cytotoxicity assays, the above exponentially growing cells were seeded into 6-well plates (35 mm) at the CCD experimental design required cell density in a 3 mL complete culture medium per well. After culturing the Beas-2B cells for 24 h, the media was discarded, and the cells were washed twice with sterile PBS. Cells were then cultured with the same volume of DMEM/F-12 medium for 4 h to synchronize the cell cycle and keep the cells growth in the same state. Then, the medium was discarded again and DMEM/F-12 medium containing 0.1% FBS was added. At the same time, 15 μL of each of the BDE209 stock solutions was added into the corresponding well. The final exposure concentrations of BDE209 were 5, 20, 35, 50 and 65 μg mL^{-1}. The final DMSO working concentration was 0.5%. After exposure to BDE209 for 6, 12, 18, 24 and 30 h, supernatants were collected for analysis of cellular response, including cytotoxicity (LDH activity, Y2), inflammatory cytokines IL-6 (Y3), IL-8 (Y4) and TNF-α (Y5). The harvested cells were then re-suspended in complete culture medium to evaluate cell viability (Y1). All experiments were carried out in triplicate, and control wells received only 0.5% DMSO. The experimental design framework for Beas-2B cellular damage induced by BDE209 exposure is shown Fig. S2.

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