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Photolysis of highly brominated flame retardants leads to time-dependent dioxin-responsive mRNA expression in chicken embryonic hepatocytes

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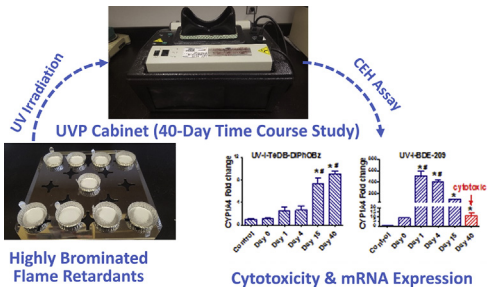
HIGHLIGHTS

- Product fractions from the time-course photolysis of TeDB-DiPhOBz and BDE-209 were exposed to chicken embryonic hepatocytes (CEH).
- Significant cytotoxicity was observed in CEH exposed to the UV-I-BDE-209 fraction collected at 40 days.
- Over the time-course, CYP1A4/5 mRNA induction occurred more rapidly for UV-I-BDE-209 as compared to UV-I-TeDB-DiPhOBz.
- Over the time-course, the fold changes in CYP1A4/5 mRNA induction was greater for UV-I-BDE-209 as compared to UV-I-TeDB-DiPhOBz.
- Environmental concerns are raised on the potential formation of dioxin-like compounds via photolysis in flame retardant-containing products.

GRAPHICAL ABSTRACT



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ABSTRACT

Tetradecabromo-1,4-diphenoxybenzene (TeDB-DiPhOBz) and 2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether (BDE-209) are flame retardant chemicals that can undergo photolytic degradation. The present study compared the time-dependent photolyic degradation of TeDB-DiPhOBz and BDE-209, and dioxin-like product formation as a result of (UV) irradiation (I; irradiation time periods of 0, 1, 4, 15 and 40 days). Photo-degraded product fractions of UV-I-TeDB-DiPhOBz (nominal concentration: 1.9 μM) were administered to chicken embryonic hepatocytes (CEH), and significant induction of *CYP1A4/5* mRNA expression was observed for fractions collected at the day 15 and 40 time points (fold change of 7.3/3.6 and 9.1/4.7, respectively). For the UV-I-BDE-209 fractions (nominal concentration: 10 μM), significant *CYP1A4/5* up-regulation occurred at all time points, and the fraction collected on day 1 induced the

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Time course
Chicken embryonic hepatocytes
Cytotoxicity
mRNA expression

greatest fold change of 510/86, followed by 410/68 (day 4) and 110/26 (day 15), respectively. For the UV-I-BDE-209 fraction collected at day 40, significant CEH cytotoxicity was observed. As a result, *CYP1A4/5* expression was determined at a nominal concentration of 1 μM instead of 10 μM and *CYP1A4/5* fold changes of 11/8.2 (day 40) were observed. Fractions eliciting the greatest *CYP1A4/5* mRNA upregulation were further screened for transcriptomic effects using a PCR array comprising 27 dioxin-responsive genes. A total of 6 and 16 of the 27 target genes were up or down-regulated following UV-I-TeDB-DiPhOBz and UV-I-BDE-209 exposure, respectively. Overall, and regardless of the formation rate, these results raise concerns regarding the potential formation of dioxin-like compounds from flame retardants in products and materials such as plastics, and in natural sunlight irradiation situations in the environment (e.g. in landfill sites or electronic waste facilities).

1. Introduction

Brominated flame retardants (BFRs) are chemicals that have been widely used in various commercial products, i.e. furniture, textiles, plastics, paints and electronic appliances, to reduce flammability (Betts, 2002). Previous studies suggest that several BFRs can bioaccumulate, undergo long-range transport and elicit adverse biological effects, and thus BFRs continue to be a growing environmental concern (Jansson and Asplund, 1987; Liu et al., 2013; Arkoosh et al., 2015; Su et al., 2015). Research on the biological effects of BFRs is generally limited to polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCDD) and tetrabromobisphenol A (TBBPA) (Yu et al., 2010, 2016; Covaci et al., 2011).

Highly brominated BFRs include 2,2′,3,3′,4,4′,5,5′,6,6′-decaBDE (BDE-209) and tetradecabromo-1,4-diphenoxybenzene (TeDB-DiPhOBz). TeDB-DiPhOBz was marketed under the commercial name SAYTEX-120. SAYTEX-120 was generally used in solid plastic and wire/cable products, and as an alternative to BDE-209, which was also used in a variety of polymeric materials (Lassen et al., 2006). BDE-209 is the major congener of deca-BDE products (generally >90%) (La et al., 2006). BDE-209 has been produced and used in large volumes (Zhou et al., 2014), and has been reported in various environmental matrices (Boor et al., 2015; Su et al., 2015).

We previously showed that both SAYTEX-120 and BDE-209 are photolytically unstable and rapidly degrade via stepwise, reductive debromination when dissolved in suitable organic solvents (i.e. tetrahydrofuran (THF), hexane, methanol) (Chen et al., 2013; Su et al., 2014a). Degradation of TeDB-DiPhOBz and BDE-209 in solution occurs when irradiated with natural sunlight, forming a complex mixture of debrominated products and polybrominated polybenzofurans and dibenzofurans. Upon administration of these complex mixtures (formed via photolysis) to chicken embryonic hepatocytes (CEH), alterations in the mRNA expression of various genes occurs; especially aryl hydrocarbon receptor (AhR)-mediated *CYP1A4* gene expression (fold change increase of >1000) (Su et al., 2014a). Photolytic degradation of TeDB-DiPhOBz and BDE-209 and similar gene expression changes were also demonstrated when these compounds were irradiated in their pure solid form (Su et al., 2016). What is not presently known is the time dependency of the photolysis degradation of TeDB-DiPhOBz and BDE-209, and the corresponding changes in mRNA expression by degradation fractions from progressive time periods of the photolysis process. Since TeDB-DiPhOBz and BDE-209 are additive BFRs, we first developed a novel lab-based approach that better simulates the way in which these chemicals would be exposed to sunlight irradiation in the environment (e.g. in plastics at landfills and in biosolids from wastewater treatment facilities). Prior to irradiation, TeDB-DiPhOBz and BDE-209 were put into solution and coated on inert silica gel beads (and allowed to evaporate to dryness) to maximize the BFR solid surface area. The time-dependent degradation samples were subsequently administered to CEH to examine the mRNA expression of 27 dioxin-responsive genes, including *CYP1A4/5*, using a PCR array.

2. Materials and methods

2.1. Chemicals and reagents

The pure standards (in solid powder form) of TeDB-DiPhOBz and BDE-209 were kindly supplied by Wellington Laboratories (Guelph, ON, Canada) (Su et al., 2014a, 2016). The purity of BDE-209 was >98% and the TeDB-DiPhOBz was technical SAYTEX-120 (Lot# 0GN01-$I0). The organic solvents, methanol, hexane and dichloromethane, were provided by Caledon Laboratories Ltd. (Georgetown, ON, Canada) with the exception of dimethyl sulfoxide (DMSO), which was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Time-course study of UV irradiation of BFRs

Our previous studies showed that dioxin-like compounds are formed from photolysis of TeDB-DiPhOBz and BDE-209 (Su et al., 2014a, 2016). The present study aimed to investigate the time dependency of the photolysis degradation process using a more manageable 40 day period, in contrast to our earlier 3 month studies of solid TeDB-DiPhOBz and BDE-209 exposure to natural sunlight (Su et al., 2016). In this earlier study, the *CYP1A* upregulation was attenuated for these BFRs in solid form compared to being in organic solution. For example, for TeDB-DiPhOBz at 5 μM, *CYP1A4* was induced 6-fold for the solid and approximately 2500-fold in organic solution. In the present study, we accelerated the BFR degradation with UVB and UVC to ensure sufficient degradation of the solid to be able to examine, with optimal resolution, a greater fold increase for the time increments over the 40 day irradiation period.

Before the UV irradiation experiment, TeDB-DiPhOBz and BDE-209 were coated on silica gel beads to maximize their exposure surface area for maximum irradiation effect. Firstly, the TeDB-DiPhOBz and BDE-209 solids were dissolved in DMSO with the maximum possible concentrations of 380 μM and 1800 μM, respectively (see details in our previous study (Su et al., 2014a)). Then, an aliquot of 150 μL of the TeDB-DiPhOBz or BDE-209 solution was spiked into 0.5 g of pre-cleaned silica gel (n = 5 replicates for each BFR) in pre-cleaned aluminum foil boats. Similarly, an aliquot of 150 μL of DMSO was spiked into 0.5 g of pre-cleaned silica gel as a blank control for further assessment of the *in vitro* gene expression effects. All aluminum foil boats (n = 11 total; n = 5 for TeDB-DiPhOBz, n = 5 for BDE-209, n = 1 for the DMSO solvent blank) with coated silica gel samples were covered with