BDE-209 VERSUS TETRADECABROMODIPHENOXYBENZENE: DIFFERING RATES OF PHOTOLYTIC DEGRADATION TO DIOXIN-LIKE PRODUCTS AND TOXICOGENOMIC EXPRESSION IN CHICKEN EMBRYONIC HEPATOCYTES

Su G[1,2], Letcher RJ[1,2*], Crump D[1], Farmahin R[1,3]

[1] Ecotoxicology and Wildlife Health Division, Environment and Climate Change Canada, National Wildlife Research Centre (NWRC), Carleton University, Ottawa, Ontario, Canada; [2]Department of Chemistry, Carleton University, Ottawa, Ontario, Canada; [3]Department of Biology, University of Ottawa, Ottawa, Ontario, Canada

Introduction:

Brominated flame retardants (BFRs) are man-made chemicals which have been widely used in various commercial products, i.e. furniture, textiles, plastics, paints and electronic appliances, to reduce flammability and hinder fire ignition[1,2]. Given their bioaccumulation, long-range transport, and biological effects, alternative BFRs to e.g. PBDEs continue to be environmental concerns[3-5]. Tetradecabromo-1,4-diphenoxybenzene (TeDB-DiPhOBz, also known as 4'-PeBPO-BDE208 and SAYTEX 120, CAS No: 58965-66-5) and 2,2',3,3',4,4',5,5',6,6'-decaBDE (BDE-209, CAS No: 1163-19-5) are two current-use BFRs. TeDB-DiPhOBz is generally used in solid plastic and wire/cable products, and as an alternative FR to BDE-209, which is used in a variety of polymeric applications[6]. BDE-209 is the major congener of currently produced deca-BDE products (generally >90 %)[7] that are commercially available in some districts around the world,[8,9] and it has been frequently detected in various environmental matrices[4,10].

Our previous studies showed that both TeDB-DiPhOBz and BDE-209 are photolytically unstable and rapidly degraded via stepwise, reductive debromination when dissolved in suitable organic solvents (i.e. tetrahydrofuran (THF), hexane, methanol)[8,11]. Degradation of TeDB-DiPhOBz and BDE-209 in solution occurs when irradiated with natural sunlight to a complex mixture of debrominated products but also to polybrominated polybenzofurans and dibenzofurans. Also, when these complex product mixtures from photolysis were exposed *in vitro* to chicken embryonic hepatocytes (CEH), alterations in the mRNA expression of various genes occurred and especially the aryl hydrocarbon receptor (AhR)-mediated *CYP1A4* gene[11,12]. What is not presently known is the time dependency of dioxin-like degradation product formation from this photolysis process, and the influence of chemical structure of these highly brominated BFRs. The present study addresses this time-dependency knowledge gap. Using a novel analytical approach, BFR-coated beads were UV irradiated for 0, 1, 4, 15 and 40 days and the UV-irradiated(I)-TeDB-DiPhOBz / UV-I-BDE-209 fractions were collected at each time point, and subjected to a CEH assay to examine the comparative alteration of expression of *CYP1A4/5* and 27 other dioxin-responsive genes.

Materials and Methods:

Time-course Study of UV Irradiation of BFRs

Before the UV irradiation experimentation, and unlike our previous studies[11,12], TeDB-DiPhOBz and BDE-209 powder was coated, using a unique approach, on silica gel bulk to maximize the BFR exposure surface area and distribution for maximum analyte irradiation. Firstly, the TeDB-DiPhOBz and BDE-209 solids were dissolved into DMSO with the maximum possible concentrations of 380 µM and 1800 µM, respectively. Then, an aliquot of 150 µL of the TeDB-DiPhOBz or BDE-209 solutions was spiked into 0.5 g of pre-cleaned silica gel (n=5 replicates for each BFR) in pre-clean aluminum foil boats. Similarly, an aliquot of 150 µL of fresh DMSO solvent

was spiked into 0.5 g pre-cleaned silica gel in pre-clean aluminum foil boat, which was the blank control for further assessment of the *in vitro* gene expression effects. All aluminum foil boats with coated silica gel samples were covered with foil paper and placed into a dark hood for 48 hours to let DMSO evaporate. The sample boats were then transferred into a dark UVP cabinet (Upland, CA, USA) equipped with both UV B (wavelength: 302 nm; 8 watts) and C (wavelength: 254 nm; 8 watts) lamps. Two of the boats (one for TeDB-DiPhOBz, one for BDE-209) were taken out of the UVP cabinet at each of the time points of 0, 1, 4 and 15 days, and for the time point at day 40 the final three boats (one for TeDB-DiPhOBz; one for BDE-209; one for solvent blank) were taken out. After the irradiation period, the spiked silica gel sample in the boats was transferred into a borosilicate glass tube (16×125 mm; Fisher Scientific Inc; Waltham, MA, USA), and 2 mL of 1:1 hexane:dichloromethane solvent mixture[13] was added into the tube. The sample was placed into an ultrasonic-cleaner (1.9 L, 35 kHz, 140 W from VWR, Mississauga, Canada) for 10 min at room temperature. Separate chemical analysis experiments were also conducted for the silica gel spiked with TeDB-DiPhOBz and BDE-209 for quality control purposes, and showed > 80 % recovery efficiency for TeDB-DiPhOBz and BDE-209 based on the observed Agilent 6520A Q-TOF-MS responses as described previously[11,12].

CEH Assay & Cell Viability and Real Time PCR & PCR array

Protocols for the chicken embryonic hepatocyte cell culture, cell viability and real-time RT-PCR & PCR arrays have been fully described in detail elsewhere[12,14].

Data Analysis

Analyses of data from real-time RT-PCR and PCR arrays were conducted using MxPro v4.10 software and the cycle threshold (Ct) was set to 0.1. The fold change of target gene mRNA abundance relative to the vehicle control was calculated using the $2^{-\Delta\Delta Ct}$ method and significant differences in fold change of different time points compared to the solvent control were determined using unpaired *t*-test and visualized by use of GraphPad Prism 5. The gene expression visualization of PCR array data was performed on R 3.0.2 version using “gplots” package, and the data points with non-significant fold changes ($p > 0.05$) and those less than 1.5 were set to 0 to minimize noise.

Results and Discussion:

Since TeDB-DiPhOBz and BDE-209 are additive BFRs to e.g. plastics, we developed and report here on a novel approach that better simulates the solid form of the chemicals in relevant environmental compartments e.g. in landfills and in biosolids used for agricultural applications. That is, prior to irradiation TeDB-DiPhOBz and BDE-209 were put into solution and used to coat inert silica gel beads with a very thin layer of the BFRs, and as a result maximized the BFR solid surface area exposed to photolytic irradiation.

Cell viability of CEH exposed to the complex product mixtures resulting from BFR photolysis was quantified by measuring the cytoplasmic adenosine triphosphate levels, which would decrease rapidly following any form of cell injury/death. A significant cytotoxic effect, and a compromise of the cell viability, was only observed in CEH after exposure to the UV-I-BDE-209 fraction (nominal concentration: 10 µM) collected at the 40 day time point. There was no cytotoxic effects to the CEH after exposure to the UV-I-TeDB-DiPhOBz fractions (nominal concentration: 1.9 µM) collected at any time point.

The greatest levels of gene expression of *CYP1A4/5* mRNA from CEH exposure were observed for UV-I-TeDB-DiPhOBz fractions from the 15 and 40 day time points, whereas for UV-I-BDE-209 fractions such expression change was much earlier at the 1 and 4 day time points, although the fold increase of gene expression

was much higher for UV-I-BDE-209 (Figure 1). These fractions with the highest *CYP1A4/5* expression were subsequently assayed by another array of 27 dioxin-responsive genes, and the mRNA expression 6 and 15 genes were altered in the CEH following exposure to UV-I-TeDB-DiPhOBz or UV-I-BDE-209, respectively.

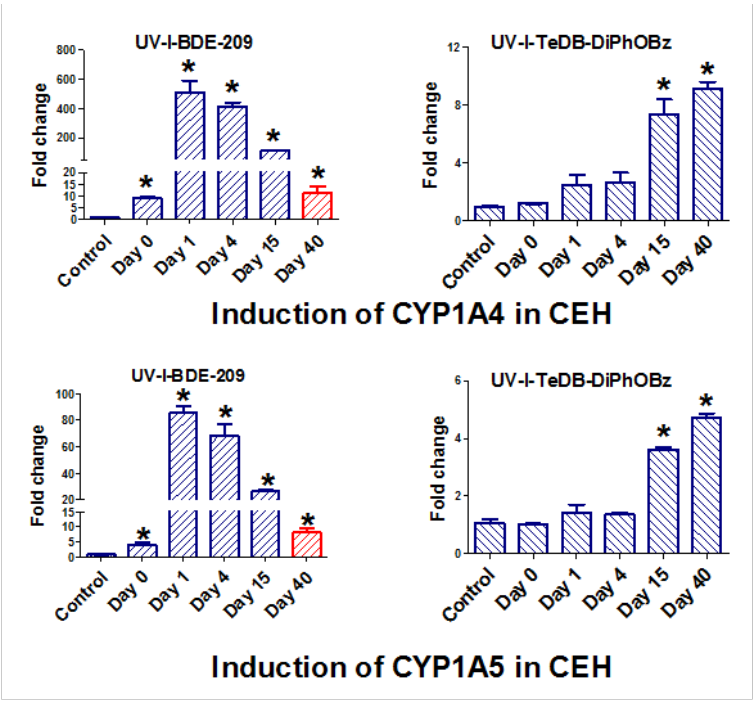

Figure 1. Time-course (time points: 0, 1, 4, 15 and 40 days) investigation of the photolytic degradation of TeDB-DiPhOBz and BDE-209 and for the product fractions formed, and the resulting effects on *CYP1A4* and *CYP1A5* mRNA expression in exposed (24 h) CEH. Error bars for each point represent the standard deviation of three replicates. Treatments that were significantly (unpaired *t*-test, $p < 0.05$) different from solvent controls are identified by *. The blue bars represent chemical fraction mixtures assayed at the original concentrations, for UV-I-BDE-209: 10 µM (nominal); for UV-I-TeDB-DiPhOBz: 1.9 µM (nominal). The red bars represent that the UV-I-BDE-209 fraction collected at the time point of 40 days was assayed at a concentration of 1 µM (nominal) due to its cytotoxic effects.

This time-course study showed that photolytic degradation to dioxin-like products is substantially more rapid for BDE-209 relative to TeDB-DiPhOBz. With respect to environmental implications, the result of this study raises concerns from time-dependent, dioxin-like product formation as a function of natural sunlight photolysis with respect to flame retardant-containing source materials such as plastics in landfills and wastewater treatment plant derived biosolids that are used in field applications for agricultural purposes. Regardless, the photolytic degradation of these BFRs to dioxin-like products in the environment likely represents a major source of exposure to biota and with potential deleterious effects.

Acknowledgements:

Environment and Climate Change Canada's Chemicals Management Plan (CMP) (to R.J. Letcher, D. Crump) provided major funding for this project. Supplemental funding was from the Natural Science and Engineering Research Council (NSERC) of Canada (to R.J.L.).

References:

1. Betts KS. 2002. Environ Sci Technol 36: 50A-52A.
2. U.S. PBDE milestones. 2003. Environ Sci Technol 37: 384A.
3. Arkoosh MR, Van Gaest AL, Strickland SA, Hutchinson GP, Krupkin AB, Dietrich JP. 2015. Environ Sci Technol 49: 6974-6981.
4. Su G, Letcher RJ, Moore JN, Williams LL, Martin PA, de Solla SR, Bowerman WW. 2015. Environ Res 142: 720-730.
5. Jansson B, Asplund L. 1987. Chemosphere 16: 2343–2349.
6. Danish ministry of the environment. 2006. Deca-BDE and alternatives in electrical and electronic equipment.
7. La Guardia MJ, Hale RC, Harvey E. 2006. Environ Sci Technol 40: 6247-6254.
8. Chen D, Letcher RJ, Gauthier LT, Chu S. 2013. Environ Sci Technol 47: 1373-1380.
9. Zhou SN, Buchar A, Siddique S, Takser L, Abdelouahab N, Zhu J. 2014. Environ Sci Technol 48: 8873-8880.
10. Boor BE, Liang Y, Novoselac A, Xu Y. 2015. Environ Sci Technol Lett 2: 89–94.
11. Su G, Letcher RJ, Crump D, Farmahin R, Giesy JP, Kennedy SW. 2014. Environ Sci Technol 48: 12039-12046.
12. Su G, Letcher RJ, Crump D, Farmahin R, Giesy JP, Kennedy SW. 2016. Environ Sci Technol 50: 2318–2327.
13. Trouborst L, Chu S, Chen D, Letcher RJ. 2015. Chemosphere 118: 342-349.
14. Porter E, Crump D, Egloff C, Chiu S, Kennedy SW. 2013. Environ Toxicol Chem 33: 573-582.