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Comparative oxygen radical formation and toxicity of BDE 47 in rainbow trout cell lines

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Abstract

The polybrominated diphenyl ethers (PBDEs) constitute a class of flame retardants whose residues have markedly increased in fish and human tissues during the last decade. In particular, the levels of certain PBDE congeners in salmon have raised concern regarding potential risks associated with dietary PBDE exposures. However, little is known regarding PBDE-mediated cell injury in relevant *in vitro* cell models. We conducted a comparative study of oxyradical production and cell injury in rainbow trout gill (RTgill-W1) and trout liver cells (RTL-W1) exposed to 2,2',4,4'-tetrabromodiphenyl ether (BDE 47), a predominant BDE residue found in fish tissues such as salmonids. Exposure to low micromolar concentrations of BDE 47 elicited a significant loss in RTgill-W1 and RTL-W1 cell viability as measured by alamarBlue assay. The dose-response of BDE toxicity differed among the two cell lines, with the RTL-W1 liver cells showing greater resistance to toxicity at lower BDE 47 doses, but a more dramatic loss of viability relative to gill cells when challenged with higher (50 μ M) doses. The sensitivity of the trout liver cells at higher BDE 47 exposures was reflected by a higher basal production of oxygen radical production by 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescence that was markedly enhanced in the presence of BDE 47, suggesting an overwhelming of trout liver cell antioxidant defense pathways. Collectively, our data indicate that RTgill-W1 and RTL-W1 liver cells are sensitive to BDE 47-mediated cell injury through a mechanism that may involve oxidative stress. Our data also provide an *in vitro* basis for potential tissue differences in BDE 47-mediated cell injury.

Keywords

2,2',4,4'-Tetrabromodiphenyl ether; Rainbow trout; Cell lines; Reactive oxygen species

The polybrominated diphenyl ethers (PBDEs) represent an important group of flame retardants that are extensively used in plastics, textiles, furniture and electronic devices (McDonald, 2002). Due to their high lipid solubility, low vapor pressure and resistance to degradation, PBDE congeners can be transported through environmental media and bioaccumulate in aquatic and wildlife species (Darnerud, 2003). In addition, there is cause for concern regarding the presence of PBDE residues that have been shown to bioaccumulate in human tissues, including serum and breast milk (McDonald, 2002). Of the various congeners, 2,2',4,4'-tetrabromodiphenyl ether (BDE 47) is a lower molecular weight PBDE congener that is often the major congener detected in fish tissues (Darnerud,

2003). BDE 47 has been shown to be a rodent developmental neurotoxicant that may also elicit oxidative stress as a potential mechanism of toxicity (Fernie et al., 2005). In the current study, we examined the comparative toxicity and oxygen radical production by BDE 47 in gill and liver-derived cells from a model salmonid, the rainbow trout (*Oncorhynchus mykiss*).

RTgill-W1 cells were purchased from American Type Culture Collection (Manassas, VA) and were maintained without CO₂ incubation in free gas exchange with air at 19 °C in Leibovitz's L-15 media supplemented with 10% FBS (v/v), 2 mM L-glutamine, 100 IU/ml penicillin, 100ug/ml streptomycin. RTL-W1 cells were maintained under similar conditions as RTgill-W1 but in L-15 containing 5% FBS (v/v). The RTgill-W1 cell line was originally prepared from a histologically normal gill lamella (Bols et al., 1994), whereas the RTL-W1 cells were derived from normal adult trout liver (Lee et al., 1993). The cells were allowed to attach to culture flasks and acclimate for 48 hours prior to chemical exposures. BDE 47 was dissolved in DMSO and prepared in L-15/ex solution for the final concentrations of 0–50 μ M (or vehicle control) as described in the figure legends. The effect of BDE 47 on cell viability was determined by alamarBlue assay (Ahmed et al., 1994) using a 96-well SpectraMax fluorometric plate reader, and the assay was optimized with respect to cell number and incubation time. The level of oxygen radical stimulation was measured fluorometrically using 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) (Grzelak et al., 2001). Treatment-related effects were evaluated statistically using ANOVA with significance assessed at $p < 0.05$.

RTgill-W1 cells exposed to BDE 47 showed signs of toxicity when exposed to high nanomolar concentrations of the compound (27% loss of viability observed at 0.8 μ M BDE 47, Fig. 1), and toxicity became more dramatic at 12.5 μ M and 50 μ M BDE 47 concentrations (34% and 35% decreases in cell viability, respectively, Fig. 1). However, a statistically significant increase in oxygen radical production was observed only at the highest concentration tested (50 μ M BDE 47, Fig. 2), and there was not a clear concentration- dependent increase in oxygen radical formation in the gill cells. In contrast, the RTL-W1 liver cells were resistant to BDE 47 toxicity at lower concentrations, but were more dramatically injured than the gill cells at higher BDE 47 concentrations (58% and 88% losses of viability at 12.5 μ M and 50 μ M, respectively, Fig. 1). The loss of cell viability in the liver cell line was better reflected by the ability of the cell lines to stimulate oxygen radical production in the presence of BDE 47. Interestingly, higher basal oxygen radical production was observed in the non-exposed trout liver cells (3.5-fold higher than the gill cell lines, Fig. 2), which was consistent with the comparative sensitivity of the liver cells to BDE 47 toxicity.

The results of our study are generally consistent with oxidative stress as a potential mechanism of BDE 47-mediated cell injury in wildlife species. Although there is not an extensive body of data regarding mechanisms of PBDE toxicity in fish, the structurally-related polychlorinated biphenyls induce oxidative stress as a mechanism of toxicity (Twaroski et al., 2001). Others have shown that hepatocytes isolated from rainbow trout are relatively sensitive to BDE 47 toxicity, and it has been hypothesized that such sensitivity may be partially dependent upon the poor detoxification capabilities of the cells (Nakari and Pessala, 2005). Although we did not investigate the effects of BDE 47 on expression of biotransformation or antioxidant genes in the trout liver or gill cells, others have demonstrated that a number of genes critical to cellular protection are down-regulated in the liver of trout exposed to BDE 47 *in vivo* (Hook et al., 2006).

Of note is the fact that the alamarBlue assay is based upon alamarBlue being a redox sensitive dye that reflects metabolic activity associated with the mitochondria (Ahmed et al.,

1994). In addition to reflecting viability status, the alamarBlue reduction assay reflects the integrity of oxidation-reduction activity of respiratory chain components in mitochondria, a major intracellular source of reactive oxygen species (ROS). Accordingly, our observations of increased ROS generation at lower micromolar concentrations of BDE 47 preceding cell death in the liver cells is consistent with the pathogenesis of oxidative stress. Furthermore, our observations of higher basal level of ROS production in the liver cells that was markedly enhanced in the presence of high levels of BDE 47 and that coincided with cell toxicity, is consistent with saturation of RTL-W1 cell antioxidant defenses. Ongoing studies in our laboratory are directed towards characterizing the role of oxidative stress and mitochondrial injury in BDE 47 toxicity in cells derived from salmonid and human tissues that are targets of PBDEs.

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References

- Ahmed SA, Gogal RM Jr, Walsh JE. *Journal of Immunological Methods*. 1994; 170:211–224. [PubMed: 8157999]
- Bols NC, Barlian A, Chirino-Trejo M, Caldwell SJ, Goegan P, Lee LEJ. *Journal of Fish Diseases*. 1994; 17:601–611.
- Darnerud PO. *Environmental International*. 2003; 29:841–853.
- Fernie KJ, Shutt JL, Mayne G, Hoffman D, Letcher RJ, Drouillard KG, Ritchie IJ. *Toxicological Sciences*. 2005; 88:375–383. [PubMed: 16120752]
- Grzelak A, Rychlik B, Bartosz G. *Free Radicals in Biology and Medicine*. 2001; 30:1418–1425.
- Hook SE, Skillman AD, Small JA, Schultz IR. *Aquatic Toxicology*. 2006; 77:372–385. [PubMed: 16488489]
- Lee LEJ, Clemons JH, Bechtel DG, Caldwell SJ, Han KB, Pasitschniak-Arts M, et al. *Cell Biology and Toxicology*. 1993; 9:279–294. [PubMed: 8299006]
- McDonald TA. *Chemosphere*. 2002; 46:745–755. [PubMed: 11999798]
- Nakari T, Pessala P. *Aquatic Toxicology*. 2005; 74:272–279. [PubMed: 16024102]
- Twaroski TP, O'Brien ML, Larmonier N, Glauert HP, Robertson LW. *Toxicology and Applied Pharmacology*. 2001; 171:85–93. [PubMed: 11222084]

Fig. 1. BDE 47 mediated cellular toxicity as indicated by alamarBlue assay. Top panel: effect of BDE 47 on RTgill-W1 cell viability. Bottom panel: RTL-W1 liver cells. Data represent mean $\pm$ S.E.M. of three experiments, with asterisks indicating significant differences at $p < 0.05$ by ANOVA.

Fig. 2. Comparative stimulation of reactive oxygen species by BDE 47 in rainbow trout RTgill-W1 and RTL-W1 liver cells. Cell numbers were determined prior to a 30 min incubation with DCFH-DA in the presence of the various concentrations of BDE 47. Data represent mean $\pm$ S.E.M. of three experiments, with asterisks indicating significant treatment related effects at $p < 0.05$ by ANOVA.