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Crystallographic Analysis and Mimicking of Estradiol Binding: Interpretation and Speculation

<http://dx.doi.org/10.1289/ehp.1307987>

In their recent article, Gosavi et al. (2013) presented the results of a crystallographic analysis of the binding of tetrabromobisphenol A (TBBPA) and 3-hydroxy-2,2',4,4'-tetrabromodiphenyl ether (3-OH-BDE-47) to estrogen sulfotransferase (SULT1E1). The authors demonstrated that the tested molecules fit into the same binding pocket as estradiol. However, although the study's methodology and interpretation of the crystallographic analysis provide insight into how binding might occur in isolated and *in vitro* systems, they did not provide evidence that the tested molecules would initiate any biological activity with the relevant estrogen receptors (ERs) or proteins in a human body. For example, the ability of TBBPA to interact with the ER and estrogen-related receptors has been evaluated in recombinant yeast strains, mammalian cell-based assays, and tests developed by the Organisation for Economic Co-operation and Development (Lee et al. 2012; Nakagawa et al. 2007; Ogunbayo et al. 2007, 2008; Reistad et al. 2005, 2007; Strack et al. 2007). Those studies found that TBBPA either did not interact with ERs or that it acted as a weak ER agonist/antagonist with a potency orders of magnitude below that of natural ER ligands. In addition, the data presented by Gosavi et al. (2013) did not include the use of controls to validate the methods. The use of both positive controls (such as diethylstilbestrol and ethinyl estradiol) and a negative control (such as testosterone) would provide validation of the analysis and allow for the quantification and comparison of the two test substances in relation to the binding potentials of the controls.

The authors also speculated about the possible additivity of the various brominated flame retardants and their metabolites and suggested that low-dose exposure to multiple low-affinity binding compounds may result in endocrine disruption. However, none of the data presented directly addressed this point.

It is highly complex, not well understood, and speculative to extrapolate data on inhibition of enzymes such as SULT1E1 in *in vitro* assay systems to endocrine-system modulation of selective gene expression, receptor binding, and activation and the

production of adverse effects that would characterize endocrine disruption *in vivo* by additivity of different chemicals competing on the same receptors. Only through a more complete understanding of target tissue dosimetry, potency of interaction of the chemical of interest with the macromolecule of interest (e.g., SULT1E1), and subsequent events can one address the likelihood of *in vivo* additivity.

This work was supported by the North American Flame Retardant Alliance (NAFRA) Panel of the American Chemistry Council, which previously provided funding for travel expenses and honoraria to the authors as members of NAFRA's Science Advisory Council. The researchers' scientific conclusions and professional judgments were not subject to the funder's control.

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REFERENCES

- Gosavi RA, Knudsen GA, Birnbaum LS, Pedersen LC. 2013. Mimicking of estradiol binding by flame retardants and their metabolites: a crystallographic analysis. *Environ Health Perspect* 121:1194–1199; doi:10.1289/ehp.1306902.
- Lee HK, Kim TS, Kim CY, Kang IH, Kim MG, Jung KK, et al. 2012. Evaluation of *in vitro* screening system for estrogenicity: comparison of stably transfected human estrogen receptor- α transcriptional activation (OECD TG455) assay and estrogen receptor (ER) binding assay. *J Toxicol Sci* 37(2):431–437.
- Nakagawa Y, Suzuki T, Ishii H, Ogata A. 2007. Biotransformation and cytotoxicity of a brominated flame retardant, tetrabromobisphenol A, and its analogues in rat hepatocytes. *Xenobiotica* 37:693–708.
- Ogunbayo OA, Jensen KT, Michelangeli F. 2007. The interaction of the brominated flame retardant: tetrabromobisphenol A with phospholipid membranes. *Biochim Biophys Acta* 1768:1559–1566.
- Ogunbayo OA, Lai PF, Connolly TJ, Michelangeli F. 2008. Tetrabromobisphenol A (TBBPA), induces cell death in TM4 Sertoli cells by modulating Ca²⁺ transport proteins and causing dysregulation of Ca²⁺ homeostasis. *Toxicol In Vitro* 22:943–952.
- Reistad T, Mariussen E, Fonnum F. 2005. The effect of a brominated flame retardant, tetrabromobisphenol-A, on free radical formation in human neutrophil granulocytes: the involvement of the MAP kinase pathway and protein kinase C. *Toxicol Sci* 83:89–100.
- Reistad T, Mariussen E, Ring A, Fonnum F. 2007. In vitro toxicity of tetrabromobisphenol-A on cerebellar granule cells: cell death, free radical formation, calcium influx and extracellular glutamate. *Toxicol Sci* 96:268–278.
- Strack S, Detzel T, Wahl M, Kuch B, Krug HF. 2007. Cytotoxicity of TBBPA and effects on proliferation, cell cycle and MAPK pathways in mammalian cells. *Chemosphere* 67:S405–S411.

Crystallographic Analysis and Mimicking of Estradiol Binding: Pedersen et al. Respond

<http://dx.doi.org/10.1289/ehp.1307987R>

Previous studies have addressed the biological effects of brominated flame retardants (Birnbaum and Staskal 2004; Koike et al. 2013; Mariussen and Fonnum 2003; Ogunbayo et al. 2008), including a 2-year bioassay study performed by the National Toxicology Program (NTP), which demonstrated that tetrabromobisphenol A (TBBPA) can induce aggressive uterine tumors in rats (NTP 2013). As pointed out by Osimitz et al., TBBPA has been shown to bind poorly to the estrogen receptor, providing the impetus to study other pathways such as disruption of steroid transport and metabolism. Other groups have demonstrated the ability of TBBPA and flame retardant metabolites to inhibit estrogen sulfotransferase (SULT1E1), with IC₅₀ (median inhibitory concentration) values near the K_m for estradiol (Hamers et al. 2008; Kester et al. 2002; Zhang et al. 1998). Our work (Gosavi et al. 2013) was focused solely on understanding the structural mechanism by which these compounds bind to and inhibit SULT1E1's ability to metabolize estradiol. The results of our work demonstrate that TBBPA and the 3-OH metabolite of BDE-47, although structurally different, bind in a similar manner at the estradiol binding site. This work suggests that these compounds could have an additive effect on the inhibition of this enzyme. We wholeheartedly agree with Osimitz et al. that the results of our work warrant future studies addressing the potential additive effect of these compounds on steroid metabolism in target tissues.

The authors declare that they have no actual or potential competing financial interests.

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REFERENCES

- Birnbaum LS, Staskal DF. 2004. Brominated flame retardants: cause for concern? *Environ Health Perspect* 112:9–17; doi:10.1289/ehp.6559.
- Gosavi RA, Knudsen GA, Birnbaum LS, Pedersen LC. 2013. Mimicking of estradiol binding by flame retardants and their metabolites: a crystallographic analysis. *Environ Health Perspect* 121:1194–1199; doi:10.1289/ehp.1306902.
- Hamers T, Kamstra JH, Sonneveld E, Murk AJ, Visser TJ, Van Velzen MJM, et al. 2008. Biotransformation of

- brominated flame retardants into potentially endocrine-disrupting metabolites, with special attention to 2,2',4,4'-tetrabromodiphenyl ether (BDE-47). *Mol Nutr Food Res* 52(2): 284–298.
- Kester MHA, Bulduk S, van Toor H, Tibboel D, Meil W, Glatt H, et al. 2002. Potent inhibition of estrogen sulfotransferase by hydroxylated metabolites of polyhalogenated aromatic hydrocarbons reveals alternative mechanism for estrogenic activity of endocrine disrupters. *J Clin Endocr Metab* 87(3):1142–1150.
- Koike E, Yanagisawa R, Takigami H, Takano H. 2013. Brominated flame retardants stimulate mouse immune cells *in vitro*. *J Appl Toxicol* 33(12):1451–1459.
- Mariusssen E, Fonnum F. 2003. The effect of brominated flame retardants on neurotransmitter uptake into rat brain synaptosomes and vesicles. *Neurochem Int* 43(4–5):533–542.
- NTP (National Toxicology Program). 2013. Toxicology Studies of Tetrabromobisphenol A in F344/NTac Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of Tetrabromobisphenol A in Wistar Han [CrI:WI(Han)] Rats and B6Cf1/N Mice. TR-587. http://ntp.niehs.nih.gov/NTP/About_NTP/TRPanel/2013/October/DRAFT_TR-587.pdf [accessed 12 March 2014].
- Ogunbayo OA, Lai PF, Connolly TJ, Michelangeli F. 2008. Tetrabromobisphenol A (TBBPA), induces cell death in TM4 Sertoli cells by modulating Ca²⁺ transport proteins and causing dysregulation of Ca²⁺ homeostasis. *Toxicol in Vitro* 22:943–952.
- Zhang HP, Varmalova O, Vargas FM, Falany CN, Leyh TS. 1998. Sulfuryl transfer: the catalytic mechanism of human estrogen sulfotransferase. *J Biol Chem* 273(18):10888–10892.
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