Toxicological mechanisms of current flame retardants

Boris V. Krivoshiev[1], Freddy Dardenne[1], Adrian Covaci[2], Ronny Blust[1], Steven J. Husson[1]

[1]Systemic Physiological & Ecotoxicological Research, University of Antwerp, Antwerp, Belgium
[2]Toxicological Centre, University of Antwerp, Antwerp, Belgium
E-mail contact: boris.krivochiev@uantwerpen.be

1. Introduction

There is an ever-increasing exposure to complex mixtures of chemicals in our daily lives. One such family of compounds that are produced at high volume are flame retardants (FRs). FRs are introduced into materials with the purpose of preventing the initiation and spread of fire. They are present in nearly all manufactured items and are able to diffuse out of materials and contaminate surrounding environments [1]. Accordingly, many FRs have been detected in household dust [2], and have been detected in human breast milk and serum as a consequence of daily exposure [3]. More worryingly, FRs are able to be transferred from mother to infant during pregnancy and breast-feeding stages [4]. In addition, previously-used FRs have been shown to elicit a wide range of toxicological effects and have thus been banned from use. Considering the structural similarity to their toxic predecessors, their persistence, bioaccumulation, and lack of insight of toxicological and molecular mechanisms, currently-used FRs pose a significant risk.

We employed a bacterial gene profiling assay to investigate *in vitro* effects of 12 currently-used FRs on a selection of general bacterial stress responses. Such responses included responses to oxidative stress, DNA damage, membrane damage, and general cell lesions such as protein degradation and growth arrest [5]. Bacterial biosensors are frequently used to assess ecotoxicological impacts of compounds since they are particularly useful in compound screening and classification according to mode of action [6].

2. Results and discussion

2.1. Gene inductions

A majority of FRs significantly affect multiple stress genes (Table 2). However, only a few genes were induced at any significant level (>2-fold) (data not shown). These genes included *ClpB*, *RecA*, and *MicF*, indicating possible effects on protein, DNA, and membrane integrity associated with these compounds. Additionally, these effects could be a result of reactive oxygen species (ROS) production attributed to these compounds, as evidenced by the significant induction of *KatG*, *Zwf*, *Soi28*, and *Nfo*.

	Oxidative Stress				General Cell Lesions		DNA Damage					Membrane Damage	
Flame Retardant	***KatG***	***Zwf***	***Soi28***	***Nfo***	***ClpB***	***UspA***	***RecA***	***UmuDC***	***Ada***	***SfiA***	***Nfo***	***MicF***	***OsmY***
TCPP	**	**		***		**	*	**	**	*	***	**	*
TPP	**				**	*	****					**	**
TBEP	***	*	*		*	***			**			**	****
TDCPP	**	*			**	**			**			**	*
TCEP	**	**		**	**		**	**	****		**	***	**
TnBP	*	*	*		*	*						**	****
TEP			**	**	*			**	**	**	**		
DOPO		****	**	****		**	**		***	**	****		***
HBCD		**	*		**	*	*		*	***			
TBBPA	**	**			**		****	*				**	
TBPH	*		*		**	**	*						**
TBC	***	***	**	**		****	***		**		**	**	***

Table 1: Significance of inductions of stress genes by FRs at highest concentration. *p<0.05 **p<0.01 ***p<0.001 ****p<0.00001.

2.2. Clustering

Clustering analysis identified two additional clusters involving *OsmY*, and *UspA* stress genes, responsive to membrane damage and growth arrest respectively (Figure 1). These FRs therefore affect multiple toxicological modes of action which include protein, membrane, and DNA damage, along with growth arrest. Additionally, ROS production could be the underlying mechanism resulting in these effects, in agreement with previous studies showing ROS induction in response to FRs [7].

3. Conclusions

FRs effect several toxicological modes of action on prokaryotic cells. Given that many of these bacterial genes have eukaryotic homologues, such results are relevant to higher biological systems [8]. Finally, the lack of any notable gene induction following DOPO treatment along with its excellent fire-retardation supports its increasing interest as an alternative to halogenated FRs [9]. Full details of this work have recently been published [10].

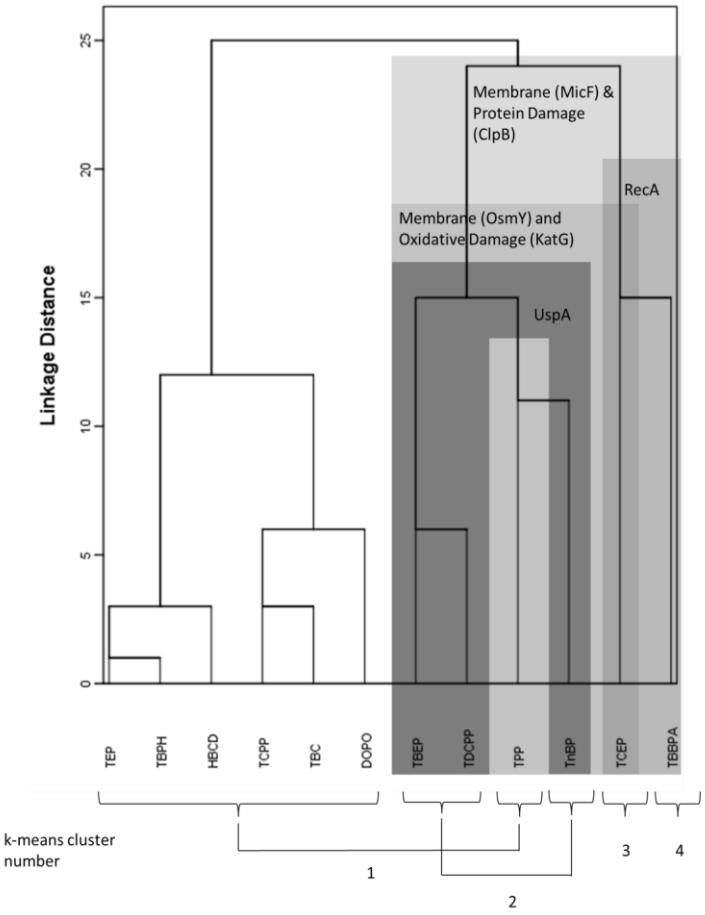

Figure 1: Clustering using hierarchical and k-means algorithms

4. References

1. Covaci A, Gerecke AC, Law RJ, Voorspoels S, Kohler M, Heeb N V, et al. Critical Review Hexabromocyclododecanes (HBCDs) in the Environment and Humans : A Review. Environ Sci Technol. 2006;40: 3679–3688.
2. Dodson RE, Perovich LJ, Covaci A, Van den Eede N, Ionas AC, Dirtu AC, et al. After the PBDE phase-out: a broad suite of flame retardants in repeat house dust samples from California. Environ Sci Technol. 2012;46: 13056–66.
3. Fujii Y, Nishimura E, Kato Y, Harada KH, Koizumi A, Haraguchi K. Dietary exposure to phenolic and methoxylated organohalogen contaminants in relation to their concentrations in breast milk and serum in Japan. Environ Int. Elsevier Ltd; 2014;63: 19–25.
4. Kim U-J, Oh J-E. Tetrabromobisphenol A and hexabromocyclododecane flame retardants in infant-mother paired serum samples, and their relationships with thyroid hormones and environmental factors. Environ Pollut. Elsevier Ltd; 2014;184: 193–200.
5. Orser C, Foong FC., Capaldi S., Nalezny J, Mackay W, Benjamin M, et al. Use of prokaryotic stress promoters as indicators of the mechanisms of chemical toxicity. In Vitro Toxicol. 1995;8: 71–85.
6. Nobels I, Spanoghe P, Haesaert G, Robbens J, Blust R. Toxicity ranking and toxic mode of action evaluation of commonly used agricultural adjuvants on the basis of bacterial gene expression profiles. PLoS One. 2011;6: e24139.
7. Su G, Crump D, Letcher RJ, Kennedy SW. Rapid in Vitro Metabolism of the Flame Retardant Triphenyl Phosphate and Effects on Cytotoxicity and mRNA Expression in Chicken Embryonic Hepatocytes. Environ Sci Technol. 2014;48: 13511–13519.
8. Dardenne F, Smolders R, Coen WDE, Blust R. Prokaryotic Gene Profiling Assays to Detect Sediment Toxicity : Evaluating the Ecotoxicological Relevance of a Cell-Based Assay. Environ Sci Technol. 2007;41: 1790–1796.
9. Salmeia K, Gaan S. An overview of some recent advances in DOPO-derivatives: Chemistry and flame retardant applications. Polym Degrad Stab. 2015;113: 119–134.
10. Krivoshiev BV, Dardenne F, Blust R, Covaci A, Husson SJ. Elucidating toxicological mechanisms of current flame retardants using a bacterial gene profiling assay. Toxicol Vitr. 2015;29: 2124-32.