

Some recent developments in BFR toxicology

- Evidence, arguments and implications for risk assessment –

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During the last decades scientific and public interest in brominated flame retardants (BFRs), especially polybrominated diphenylethers (PBDEs), has strongly increased. Clearly this was caused due to increasing levels of these compounds in both humans and wildlife. Although many of the presently used BFRs have already been produced as early as the 60's, it was especially in the last decade that scientist, regulators and the public became more interested in these groups of compounds.

Originally, the approach in toxicology for e.g. PBDEs was to consider these as analogues of PCBs, or even dioxin like compounds. However, conflicting information became available with respect to the mechanism of action for which at first a dioxin like mechanism of action was not excluded (Chen and Bunce 2003). Though, when using more mechanistic specific assays and highly purified PBDE congeners it became clear that these compounds were not analogues of halogenated dioxins (PCDDs), dibenzofurans (PCDFs) or non ortho substituted PCBs (NO-PCBs) (Peters *et al.* 2006; Peters *et al.* 2004). Based on the non planar configuration of PBDEs and increasingly environmental common hexabromocyclododecane (HBCD) it is now accepted, that PBDEs might at best have toxicological similarity with non dioxin, ortho substituted PCBs like PCB 153.

Consequently, PBDEs are no longer considered to be dioxin like compounds and are not included in its toxic equivalency concept (Van den Berg *et al.* 2006). Nevertheless, there are clearly a number of non Ah receptor mediated endpoints that require the attention of the toxicologist and risk assessor. During the last five years it has become clear that the more sensitive endpoints of PBDEs are of endocrine, neurotoxic and neurobehavioral nature. *In vivo* postnatal experiments with mice from the groups of Viberg *cs.* and Lilienthal clearly indicate that during this life stage is rather sensitive with respect to neurobehavioral effects (Dingemans *et al.* 2007; Lilienthal *et al.* 2006; Viberg *et al.* 2002, 2003a, 2006; Viberg *et al.* 2003b). Several PBDE congeners were found to induce neurobehavioral and learning deficits in mice and rat, including the presently commonly used DecaBDE. Although the results of these neurobehavioral studies are by itself conclusive and all point in the same direction a distinct structure activity relationship is not directly obvious. In contrast with PCDDs, PCDFs and NO-PCBs for which with larger molecular size the lowest effective dose increases, this phenomenon can not be clearly observed for PBDEs (Viberg *et al.* 2002, 2003a; Viberg *et al.* 2003b). Thus, it can be questioned if the observed effect is a specific mechanism of action for PBDEs and/or ortho substituted PCBs. The fact that similar neurobehavioral effects have also been reported for the structural completely different HBCD seems to contradict this (Mariussen and Fonnum 2003). In the future *in vitro* neurotoxicological studies can possibly clarify the existence of a possible structure-activity relationship (SAR). *In vitro* effects e.g. with the dopamine receptor and calcium uptake in neuronal cells can give additional mechanistic support for *in vivo* neurobehavioral effects (Kodavanti and Derr-Yellin 2002; Kodavanti and Ward 2005; Mariussen and Fonnum 2003, 2006) Nevertheless, a mechanistic similarity between non dioxin and *ortho* substituted PCBs and PBDEs appears evident from a number of recent studies.

In addition, endocrine related effects have been reported for PBDEs and again more specifically their hydroxylated metabolites during the last decade. *In vitro* effects of OH-PBDEs have been observed with the estrogen receptor (ER), thyroid hormone transporting protein (transthyretin; TTR) and with the steroidogenic enzymes CYP17 and 19 (aromatase) (Canton *et al.* 2005; Canton *et al.* 2006; Ceccatelli *et al.* 2006; Hamers *et al.* 2006; Meerts *et al.* 2000; Schuur *et al.* 1998). Initially, experiments were done with *in vitro* models, recent semi-chronic studies (e.g. EU-FIRE project) with several BFRs, including PeBDE and DecaBDE show that similar effects can also be found *in vivo*. These endocrine effects, especially on thyroid hormone levels and steroidogenic enzymes (CYP17), were found to be the more sensitive endpoints (van der Ven *et al.* 2006).

Based on the above results it is obvious, that several PBDEs and/or their metabolites are *in vivo* endocrine disruptors and neurotoxic agents. From a toxicological and mechanistic point of view it is interesting and intriguing to which extent the observed effects are actually caused by their parent compounds or their metabolites. Obviously, the hydroxylated metabolites of PBDEs are good candidates for many of the observed *in vivo* endocrine effects. This has been shown by *in vitro* experiments that prove a significant role of the OH group in thyroid, estrogen and steroidogenic related effects [ref]. This role is further supported by the fact that when the position of the OH was changed or replaced with a -OCH₃ group endocrine activities could change significantly (Canton *et al.* 2005; Canton *et al.* 2006).

Although the significant role of metabolites of PBDEs has been known for years, it is remarkable how little attention this has been given here to support a more adequate risk assessment. Human data on OH-PDBEs are relatively scarce and clearly inadequate to establish e.g. the internal variation of these metabolites in humans at background and occupational levels. This argument is probably most true for decaBDE, which is at present commonly used as a flame retardant all over the world. DecaBDE is a BFR with a remarkable short half live in experimental animals and humans in view of its high hydrophobicity. Rat experiments indicated that major breakdown products of decaBDE are of polar nature, most likely lower hydroxylated PBDEs with still unknown structure (Morck *et al.* 2003). Consequently, some of the (higher dose) effects of decaBDE have been ascribed to possible biological active metabolites. DecaBDE is clearly not a BFR that bioaccumulates through the food chain and direct exposure appears a much more likely exposure pathway for humans. From a scientific and risk assessment point of view it is a gross negligence, that after all these years not more thorough and detailed experiments have been funded and performed to establish their possible relevance for decaBDE exposure. Clearly, identification and quantization of the human levels of decaBDE metabolites should have first priority to finally determine if this BFR is safe for use in the human environment. If decaBDE metabolite structures a properly identified in human blood and tested in a number of relevant *in vitro* experiments, a comparison of (internal) blood and *in vitro* medium concentration could give sufficient information to indicate a possible margin of safety.

In relation to the common use of decaBDE as a BFR it has also been suggested, that this compound might be responsible for the formation lower PBDEs that accumulate more in the food chain. Although, rodent experiments showed that lower brominated PBDEs can be formed from DecaBDE, such a biotransformation process appears to be a minor pathway in experimental mammals compared to the formation of more polar metabolites (Morck *et al.* 2003). This possible lack of *in vivo* formation in humans is indirectly supported by the very limited presence of nonaBDEs, that might originate from decaBDE. In fact it is still

debatable, if the observed presence of nonaBDEs originates from decaBDE metabolism, or is a consequence of being present in the commercial decaBDE or octaBDE mixtures.

In addition, it has been brought forward that decaBDE can be responsible for the formation of brominated dibenzofurans (PBDFs) and dioxins (PBDDs) during ignition and burning processes. Such a risk comparison of decaBDE with these PBDD/Fs seems unjustified, since this compound is actually meant to be an inhibitor of ignition processes and in almost every combustion process with relatively low temperatures dioxin-like compounds are easily formed in significant quantities.

Finally, it can be discussed if regulatory authorities are using the recent *in vivo* and *in vitro* toxicological and biochemical data adequately. When looking at the EU risk assessments for PBDEs including decaBDE, the focus seems to be on classical long-term rodent studies. Without any doubt these standard studies provide an adequate method to do a risk assessment for long-term background exposure if adequate safety factors are applied. However, regulatory authorities might certainly be a bit more progressive when using various more modern *in vivo* studies that focus on sensitive life stages. In addition, results of a wide array of mechanistic *in vitro* studies with human cells could also be better used for risk assessment, when concentration – effect relationships would be linked to actual human blood or tissue concentrations. By doing this risk assessors would on the one hand use the wealth of recent scientific information for these compounds in a better way. Furthermore, when linking human *in vitro* results with blood or tissue concentrations the need for large safety factors would be less, because a difference in safety factor between species (rodents to humans) could be avoided or at least smaller. In addition, such alternative risk assessment approaches would allow a better discrimination between different life stages and exposure situations and provide more certainty.

In this presentation the above considerations will be further elaborated and reviewed from a retrospective as well as prospective point of view.

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