

Endocrine Modulating Activity of Some Brominated Flame Retardants in Rats

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Introduction

Brominated flame retardants (BFRs) are substances which are used in a wide variety of consumer products to prevent unintended inflammation. These compounds enter the environment at various stages of production and application in consumer products. Humans are exposed either directly, through emission from these products, or through food, particularly fish, meat, and dairy products. Structural analogy with natural hormones, particularly thyroid hormones, suggests that at least some BFRs may interfere with endocrine systems in humans. To expand the toxicology database on this particular issue, we conducted 28d repeated oral dose studies in Wistar rats with hexabromocyclododecane (HBCD), tetrabromobisphenol-A (TBBPA), and two commercial mixtures of polybrominated diphenyl ethers (PBDEs) indicated as pentaBDE (pBDE) and decaBDE (dBDE). TBBPA was also studied in a one generation reproduction study in rats.

Materials and Methods

Wistar rats were purchased from Harlan (Horst, NL), or bred at the RIVM facilities. TBBPA, HBCD and dBDE were industrial mixtures obtained through BSEF (dr. Klaus Rothenbacher), the industrial pBDE mixture (DE-71) was provided by Great Lakes Chemical Corporation (Dr. D. Sanders) and purified to remove dioxins, dibenzofurans, and any other coplanar molecules (dr. Åke Bergman). TBBPA was mixed in the diet, HBCD and pBDE were dissolved in corn oil, then given by daily gavage, dBDE was administered by gavage in an emulsion. The compounds were tested in 28d repeated oral dose toxicity studies (OECD407 protocol), TBBPA also in a one-generation reproduction study (OECD415). The protocols were enhanced for endocrine and immunological endpoints (Andrews et al. 2001). For precise assessment of dose-response relationships, the animals were distributed among eight dose groups (including control). This setup enables benchmark dose (BMDL) calculations (Slob 2002), i.e. the 5% lower confidence bound of the critical effect dose at a critical effect size, which was defined at a 10 % change for most parameters. Exposure started after at least one week of acclimatization in animals 8-12 w of age. Dose ranges were 3-3000 mg per kg body weight for TBBPA, 0.3-200 mg/kg for HBCD, 0.27-200 mg/kg for pBDE, and 1.87-60 mg/kg for dBDE.

Materials were collected during necropsy of all animals in the 28d studies and the same number of F1 animals in the reproduction studies, that is, five animals per sex per dose group for assessments of cauda epididymis sperm, of (immune) cell subpopulations and/or NK activity in whole blood, bone marrow, and/or part of the spleen (de Jong et al. 1980), and of weight and histopathology of a conventional set of organs. TSH, FSH, LH and prolactin in the adenohypophysis were analyzed by immunohistochemistry when indicated by other effects. (Olausson et al. 2006). Various organ and tissue samples were stored under appropriate conditions for further analysis, including routine plasma clinical chemistry and hormone analysis (mainly thyroid hormones; Friedrichsen et al. 2003). Effects on drug metabolism

were assessed by analysing hepatic P450 at the level of mRNA, protein, and enzyme activity (Germer et al. 2006a, 2006b), and in the HBCD study only by analysis of hepatic uridine diphosphate glucuronyl transferase (UDPGT) activity (Schoor et al. 1997). Effects on the production of sex steroid hormones were assessed by analysing the activity of CYP19 (aromatase; key enzyme for estrogen synthesis) by a radioactive assay (Lephart & Simpson 1991), in ovaries, and by measuring DHEA, which is the product of CYP17 (17-hydroxylase/17,20lyase; key enzyme of androgen synthesis) with a RIA (Immunotech, Bechman Coulter, Mijdrecht NL) in adrenals. Control positive and negatives were included in both assays. Additional parameters in the reproduction study included neonatal status and viability, and developmental endpoints in pups. Young adult F1 animals were also used for immunocompetence (Van Loveren et al. 1991) and neurobehavioural (reported separately (Lilienthal et al. 2006) assessments. Internal dosing was verified by compound analysis in liver samples by LC-MS/MS after gradient separation in a HPLC column. Experiments were approved by the institutional Committee on Animal Experimentation, according to Dutch legislation.

Results and Discussion

Some of the BFRs induced effects of general toxicity (e.g. reduced food intake, reduced growth, kidney pathology; see Table 1), at high doses. Effects were also observed in immune parameters. Regarding endocrine system, there appeared to be two clusters of modulating effects, one related to the thyroid hormone system, the other related to the sex steroid hormone system.

The thyroid hormone system appeared to be affected by several of these compounds. Reduction of circulating thyroxine (TT4) was observed with HBCD (28d study only), TBBPA, and pentaBDE, while increased levels of circulating triiodothyronine (TT3) was observed with TBBPA and with decaBDE. Decreases of TT4 are probably secondary to induction of hepatic metabolic activity, which some of these BFRs share with other persistent organic pollutants. Increased TT3 is possibly due to competitive binding of the concerned BFRs with T4 carrier proteins (Hamers et al. 2006), thus increasing availability of T4 for deiodination. Effects associated with T4 hypothyroidy were in lipid metabolism (increased cholesterol; HBCD and pentaBDE 28d studies), bone physiology (in the 28 days HBCD-study; Olausson et al. 2006, Van der Ven et al. 2006), and developmental effects (in the TBBPA reproduction study; Lilienthal et al. 2006), including neurobehavioural effects at young adult age. Such effects may be modulated by direct interaction of BFRs with TH receptors.

A second set of effects appeared to be related to the sex steroid domain. With TBBPA there was an increase of gonad weights in F1 males, which in turn was statistically correlated with female sex organ weights, circulating testosterone in males, aromatase activity in the ovary, and pituitary weights in males. With pentaBDE, there was a decrease of male accessory reproductive organs and induction of androgen synthesis in female adrenals, and similar effects were observed with decaBDE. Particularly in the case of pentaBDE, such anti-androgenic effects may be explained by direct interaction with steroid receptors, as observed in bioassays.

These results can be used to refine risk assessment. Effects of HBCD and TBBPA appear to be induced at least 3-4 orders of magnitude above human exposure levels, based on comparison of internal concentrations in the rat livers of these studies and human plasma levels. The BMDL levels for sensitive effects of penta- and decaBDE are at or within a factor 100 above human exposure levels.

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Table 1 – Effects of four brominated flame retardants in Wistar rats

		<i>TH related</i>		<i>steroid hormone related</i>		<i>immune, general</i>		
		<i>BMDL</i>		<i>BMDL</i>		<i>BMDL</i>		
		<i>m</i>	<i>f</i>	<i>m</i>	<i>f</i>	<i>m</i>	<i>f</i>	
<i>TBBPA</i>	pituitary weight	0.6		d21 weight testis	468			
	plasma total T3	(124) *	2	testis weight L+R	0.5			
	plasma total T4	31 (48)	16	pnd21 female gonad weight		c		
	pituitary gland weight	0.6		uterus weight		c		
	plasma albumin	3752		endometrium thickness		c		
	litters with mortality		33	relative saccharine intake f		c		
	threshold 0.5 kHz		42	testosterone m	c			
	threshold 2 kHz f		0.9	plasma total T3	(124)	2		
	latency peak 2, 0.5 kHz		33					
	latency peak 4, 0.5 kHz		8					
	latency peak 4, 0.5 kHz		7					
	latency peak 4, 2 kHz		56					
	saccharin intake	c						
	anogenital distance d7 f		2736					
	day vagina open		2745					
<i>HBCD</i>	liver weight		23			thymus	104	
	T4-UGT		4.1			total cells per spleen	1.7	
	TT4		56			CD4 (Th)	0.3	
	pituitary weight		30			CD161a (NK)	6.3	
	thyroid weight		1.6					
	cholesterol	66	7					
	alkaline phosphatase		19					
	glucose	57	71					
<i>pBDE</i>	TT4	1.1	1.8	epididymis weight	28	body weight gain week 1-4	9.7	
	body growth w 1-4	3.8		seminal ves. / coag. gland	51	% body weight gain w 1-4	6.5	
	liver weight	18	17	prostate weight	43	kidney weight	109 85	
	cholesterol	8.5	12	% deformed sperm heads	9.6	urea	30 22	
	total protein		13	adrenal CYP17 f		<0.22		
	alanin aminotransf.	16		large unstained cells (LUC)	42.8	thymus weight	110	
	glucose	67		% large unstained cells (LUC)	9.8	monocytes	0.7	
						% monocytes	3	
					% eosinophilic granulocytes	NA 22		
					erythrocyte volume	467		
					erythrocyte hgb (HDW)	294 85		
<i>dBDE</i>	TT3		33	CYP17 adrenal f		0.18	brain weight	69
				epididymis weight ^a	NA		thymus weight	43
	alkaline phosph.		0.6	seminal ves. / coag. gland	0.2		erythrocyte volume (RDW)	254
							erythrocyte hgb (HDW)	111

BMDLs are in mg/kg bw; c, statistically correlated variables (in the TBBPA reproduction assay), these showed no significant dose-responses. Effects were measured in 28-d repeated oral dose studies (HBCD, pentaBDE, decaBDE) or a reproduction study (TBBPA); * BMDLs in brackets for TBBPA were measured in a 28-d repeated oral dose study. NA, no BMDL calculated in a significant dose-response.