28 Day Gavage Study with a Technical Mixture of Lower Poly Brominated Diphenyl Ethers in Sprague-Dawley Rats

Paul Rowsell[1], Al Yagminas[2], Ih Chu[2], Douglas L. Arnold[1]

[1]Health Canada, Health Products and Food Branch, Ottawa, Ontario, Canada K1A 0L2
[2]Health Canada, Healthy Environments and Consumer Safety Branch, Ottawa, Ontario, Canada K1A 0L2

Introduction

Poly Brominated Diphenyl Ethers have recently become a matter of concern due to their low but increasing occurrence and persistence in the environment[1] and in human tissues[2,3]. The major congeners found during residue analysis of human and environmental samples are BDE-47 and BDE-99. One source of these two congeners is the commercial mixture DE-71, used to flame retard polyurethane foam. This mixture contains approximately 0.21% tribromodiphenyl ether, 46 % tetrabromodiphenyl ether, 49% pentabromodiphenyl ether, and 4% hexabromodiphenyl ether.

Materials and Methods

Male and female Sprague Dawley rats (CD IGS, Charles River) ranging from 26 to 28 days in age were randomly assigned to 4 dose groups and control, consisting of 10 males and 10 females each. They were individually housed in polycarbonate cages containing hardwood wood chip bedding located in a specific pathogen free colony with HEPA filtered air. Males and females were housed in separate rooms. Their arrival weights were recorded and statistically analyzed to ensure a similar distribution of body weights among test groups. Animals were acclimated to their new environment for 10 days which included acclimation to gavage dosing with tap water. Each rat was identified by a unique number linked to a transponder implanted subdermally at the nape of the neck. A commercial rat chow and reverse osmosis bottled water were provided ad libitum throughout the study. The environmental conditions were: Temp. 21 ± 2EC; Hum. 55 ± 15% and light/dark 12/12 Hrs.

DE-71 was dissolved overnight with agitation in corn oil. Dose solutions were prepared to deliver 0.05 mg/kg/day, 0.5 mg/kg/day, 5 mg/kg/day or 25 mg/kg/day at a rate of 0.5ml per 100g body weight. Control animals were dosed with corn oil at the same rate. Animals were dosed daily for 28 days. Animals were weighed, the dose calculated and gavaged. The condition of the animals was checked twice daily; at dosing and at the end of the day. A thorough clinical examination was performed weekly. The animals were necropsied on the 29th day. Blood was withdrawn for hematological analysis on an ADVIA 120 hematology analyzer (Bayer, Toronto, Canada) and included a complete blood count with 5 part differential. Serum chemistry analysis was performed on an

EXPRESS PLUS analyzer (Bayer, Toronto, Canada) and included alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase (LDH), total protein, albumin, calcium, inorganic phosphate, urea nitrogen, creatinine, glucose and cholesterol. Heart, brain, kidneys (weighed together), spleen, and liver were weighed. A sample of liver was taken, homogenized in Tris/HCl buffer, centrifuged at 10,000Xg and the supernatant was analyzed for Ethoxyresorufin-*O*-deethylase (EROD), Pentoxyresorufin-*O*-deethylase (PROD) and Benzyloxyresorufin-*O*-deethylase (BROD). Thyroid hormone levels (Total T3, total T4, free T3, and free T4) were analyzed in serum with an ACS180 Plus Immunology Analyzer (Bayer).

Results

There were no clinical signs of toxicity. Growth rates were not significantly different between control and dose groups for either sex (ANOVA on orthogonal polynomial fitted data). Relative and mean absolute liver weights were only significantly different for both sexes in high dose group (Table 1). Microsomal enzymes were significantly induced in both male and female animals by the 5 and 25 mg/kg/d doses (Figure 1). No effect upon serum enzymes was found. Total T4 and free T4 levels were significantly lower in the 25mg/kg/d

Table 1 Liver Weight

	Male		Female	
	Liver weight	% Body Weight	Liver weight	% Body Weight
Control	17.69 ± 0.61	4.30 ± 0.08	8.79 ± 0.34	3.86 ± 0.07
0.05 mg/kg/d	16.07 ± 0.74	4.11 ± 0.10	7.97 ± 0.28	3.70 ± 0.10
0.5 mg/kg/d	17.00 ± 0.74	4.22 ± 0.09	8.77 ± 0.24	3.90 ± 0.08
5 mg/kg/d	17.86 ± 0.74	4.56 ± 0.08	9.27 ± 0.33	4.21 ± 0.07‡
25 mg/kg/d	21.35 ± 0.84*	5.35 ± 0.14†	10.64± 0.50†	4.93 ± 0.11 †

‡ significantly different from control, $p<0.05$ (Tukey test)
* significantly different from control, $p<0.01$ (Tukey test)
† significantly different from control, $p<0.001$ (Tukey test)

male and female groups (Figure 2). Total T3 but not free T3 was significantly reduced in the 25mg/kg/d males only ($p<0.001$, data not shown). There was no difference in the hematological measures in any of the treated groups. The 25 mg/kg/d female animals had significantly higher levels of serum cholesterol and increased levels of glucose. Both males and females in the high dose group had decreased LDH levels ($p<0.05$, data not shown).

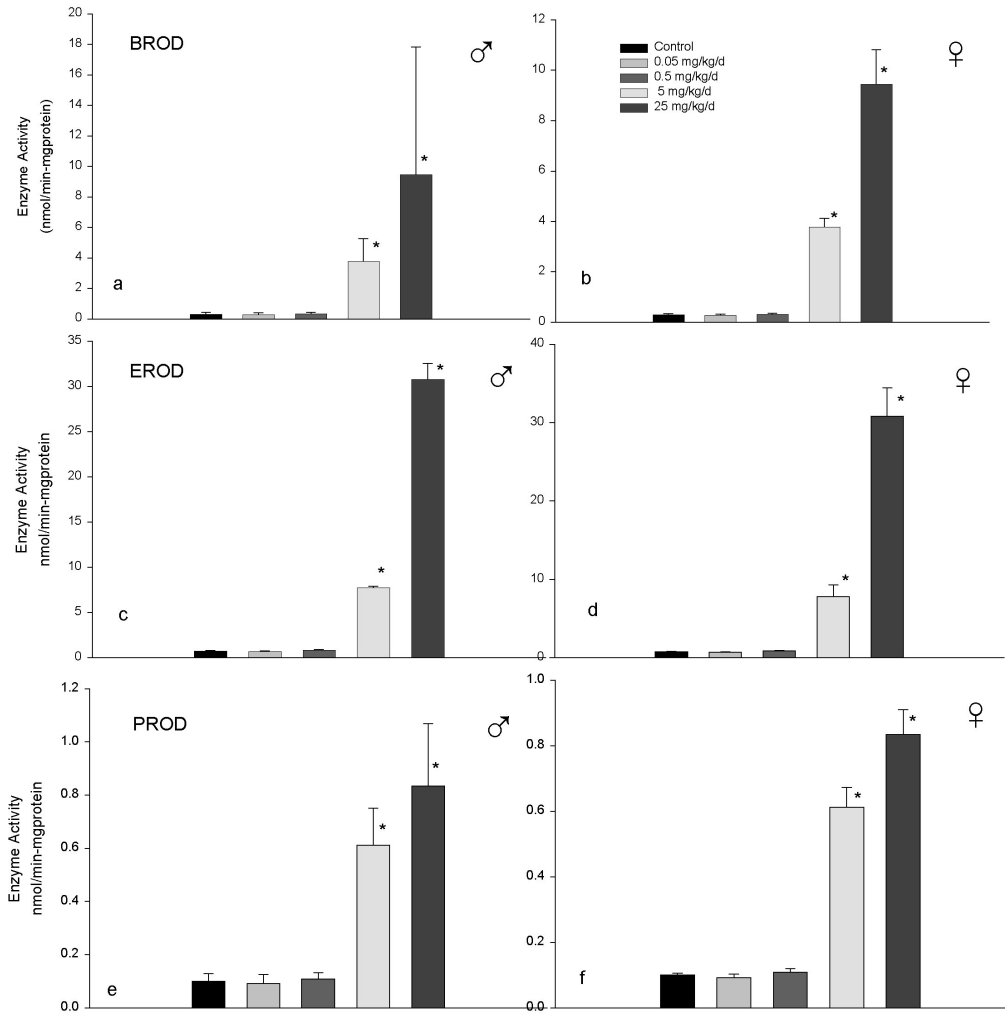

Figure 1 Liver microsomal enzyme induction. Data are presented as group means ±SE. * Significantly different from the respective control (Student's t-test, $p<0.001$).

Discussion

DE-71 ingestion led to significant increases in the relative liver weights in the high dose groups and significant relative liver weight increases in the 5 mg/kg/d female group. Increased cholesterol levels in the high dose females may result from reduced T4 levels[4]. Similar cholesterol effects were seen in both sexes previously at 250 mg/kg/d[5]. PROD, BROD and EROD, indicating cytochrome P450 2B1, 2B2 and 1A1, respectively, activities were induced. The induction of 1A1 may indicate an Ah receptor mediated action, although the literature suggests that the predominant congeners in DE-71 do not activate the Ah receptor[6].

Our study confirms the results of others[7,8] that PBDEs increase relative liver size, induces the ER and reduces the serum levels of T4. Hepatic enzyme induction is believed

to increase the metabolism of T4 and so reduce serum levels of T4 through induction of uridinediphosphate glucuronosyltransferase (UDPGT) and subsequent glucuronidation of T4. Although we did not determine UDPGT induction, this may account for reduced T4 levels. It is unclear what role UPDGT has in T4 clearance as one study[8] explains T4 reduction by UPDGT induction while another study[7] found the level of induction insufficient to explain T4 clearance.

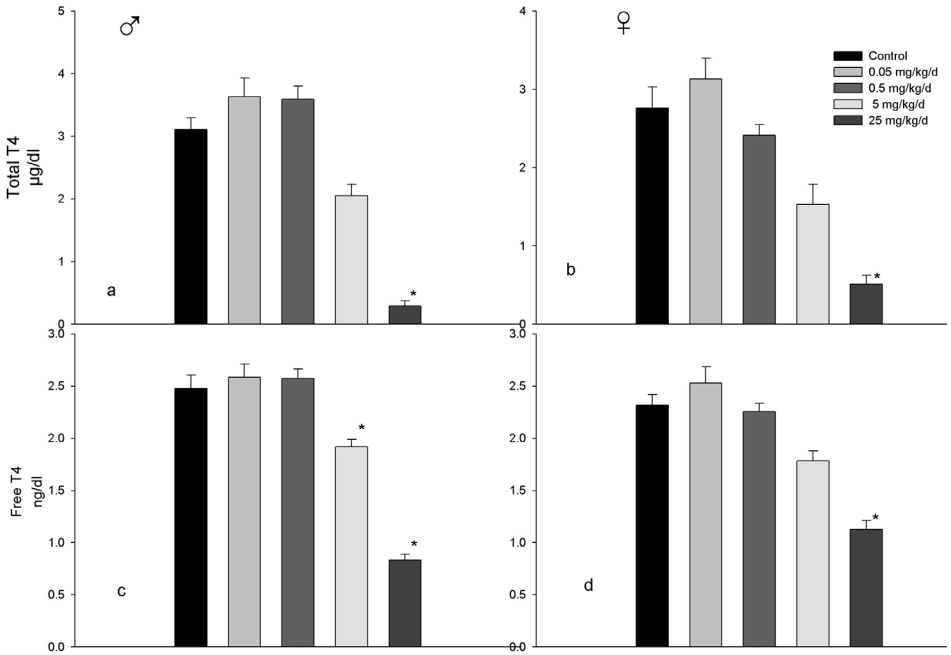

Figure 2. Serum Total T4 and Free T4 levels. Data are presented as group means ±SE.* Significantly different from the respective control (Student's t-test, $p<0.001$).

In conclusion, we have found that 28 day dosing with DE-71 induces EROD, PROD and BROD activity, causes liver hypertrophy and reduces serum T4 levels. Further studies will look at PBDPE's potential effects upon reproduction.

1. Boon JP, Lewis WE, Tjoen-A-Choy MR, Allchin CR, Law RJ, De Boer J, Extra . Ten Hallers-Tjabbes CC, Zegers BN. 2002. Environ Sci Technol 36:4025-32.
2. Ryan JJ, Patry B, Mills P, Beaudoin NG. 2002. Organohalogen Compounds 58:173-176.
3. Petreas M, She J, Brown FR, Winkler J, Windham G, Rogers E, Zhao G, Bhatia R, Charles MJ. 2003.Environ Health Perspect 111:1175-9.
4. Loeb WF.1997, in Clinical biochemistry of domestic animals ed. Kaneko JJ, Harvey JW, Bruss ML. Academic Press p319.
5. Fattore E, Falk-Filipsson A, Hanberg A, Bergendorff A, Håkansson H. 2001. BFR2001 pp 247-248.

6. Chen G, Bunce NJ. 2003. Toxicol Sci 76: 310-20.
7. Hallgren S, Sinjari T, Håkansson, Darnerud PO. 2001. Arch Toxicol 75:200-8.
8. Zhou T, Ross DG, DeVito MJ, Crofton KM. 2001. Toxicol Sci 61:76-82.