EFFECTS OF BROMINATED DIPHENYL ETHERS ON THYROID HORMONES IN A SHORT-TERM SCREEN AND IN DEVELOPMENTAL STUDIES.

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Introduction

Polybrominated diphenyl ethers (PBDEs)are produced commercially as flame-retardants for numerous consumer products, especially electronic equipment. While theoretically, there are 209 possible PBDEs which vary by the number and position of the bromine, the commercial mixtures contain only a limited number of congeners. Evidence has been accumulating over the past several years that PBDEs are accumulating in the environment. In addition, human milk concentrations of PBDEs are also increasing at an alarming rate. The predominate PBDE congeners found in biota and human samples are 2,2',4,4'-tetraBDE (PBDE-47), 2,2',4,4',5-pentaBDE (BDE-99) and 2,2',4,4',6-pentaBDE (BDE-100).

Recent studies from experimental animals indicate that PBDEs can alter serum concentrations of thyroid hormones. PBDE-47 and commercial mixtures, such as DE-71, DE79 and Bromkal 70 have been shown to decrease thyroxin concentrations in rats and mice following short-term exposures[1-4]. In addition, the commercial mixtures and PBDE-47 increase CYP1A1 and CYP2B activities[1,2]. The mechanism by which the PBDEs decrease thyroid hormones has not been worked out. However, several studies have demonstrated increased T4-glucuronidation following exposure to either PBDE-47 or the DE-71 and DE-79 mixtures, suggesting that increased T4 elimination may play a role in the effects of these chemicals on serum TH[1-3]. The present study examines the dose response relationship of DE-71, a commercial mixture of predominately tetra- and pentabrominated PBDE's, DE-71, on serum thyroid hormones and hepatic enzyme induction in fetal, neonatal, weanling and pregnant rats.

Methods

Animals: All animal procedures were approved in advance by our facilities Institutional Animal Care and Use Committee. Weanling female and Time-pregnant Long-Evans female rats were obtained from Charles River Laboratories Inc. on gestation day (GD) 2, and were allowed 4 days acclimation prior to treatment. Dams were housed individually and maintained at 21∀2 ^{O}C with 50∀10% humidity on a 12L:12D (0600-1800 hr) photoperiod, with free access to food (Purina Rodent Chow, Barnes Supply Co., Durham, NC) and tap water *ad libitum*.

On GD21, dams were checked for the number of pups delivered at 0800, 1000, 1200, 1500 1700 hr, and pups were aged postnatal day (PND) 0 on the date of birth. On PND 4, 7, 14 and 21 offspring were counted and sexed, and then group weighed by sex. Average pup weight by

sex was calculated by dividing the group weight by the number of pups. Litters were culled to either 8 or 10 pups per dam. Pups were checked daily for eye opening (pups with at least one eye open) from PND11 through PND18. Pups were weaned on PND21, and genders were separately housed two or three per cage.

Chemicals and Treatment: DE-71 (penta-BDE, lot 7550OK20A) was given generously by the Great Lakes Chemical Corporation (West Lafayette, IN). DE-71 is a mixture that consists primarily of tetra and penta congeners[5]. A stock DE-71 solution (300 mg/ml) was prepared by mixing the compound with corn oil and sonicating for 30 min at 40°C.

Dams were assigned to treatment groups in a semi-random, weight-balanced fashion before being treated. They were orally dosed via gavage with DE-71 (0,1,10,30 mg/kg/day) from GD6 through PND21, except for PND0 (day of birth) when dams were left undisturbed. The dams (GD20 and PND22) and offspring (GD20, PND 4, 14, 36, and 90) were decapitated for collection of trunk blood. Liver samples were removed immediately and frozen in liquid nitrogen. Samples collected for GD20, PND 4 and 14 pups, serum or liver samples within a litter were pooled. For pups at PND36 and PND90, one male and one female pup per litter were randomly sampled for body weight measurement and collection of serum and liver samples. All serum and liver samples for each age point were obtained from a minimum of eight litters, and were stored at -80°C until analysis for thyroid hormone (T4 and T3) concentrations and hepatic enzyme (EROD, PROD and UDPGT) activities. Weanling rats (28 days old) were exposed for four consecutive days with DE-71 (0.3, 1,3,10, 30, 300 mg/kg/d) and killed on day 5.

Thyroid Hormone Assay: Serum total T4 and T3 were measured in duplicate by using standard radioimmunoassay (RIA) kits (Diagnostic Products Corporation, Los Angeles, CA) as described by Goldy et al[6]. The sensitivity for our T4 assay was 2.99 ng/ml any result below this was recorded as 2.99 ng/ml.

Hepatic Enzyme Activities Assay: Liver microsomal fractions were prepared as described previously[7]. Microsomal protein concentrations, EROD activity (a marker for CYP1A1 activity) and PROD activity (a marker of CYP2B activity) were assayed using the method of DeVito et al[7]. Hepatic microsomal T4-UDPGT activity was assayed as described in Zhou et al[8]..

Data Analysis: All statistical analyses were performed on SAS©6.12 (SAS Institute Inc., Cary, NC). The litter was the statistical unit for all analyses of the developmental study. Analysis of variance (ANOVA) was used to analyze for effects of treatment and interactions. For significant effects of treatment, the Duncan's Multiple Range Test was used for mean contrast comparisons with a significance level of 0.05. Benchmark dose (BMD) estimates were determined for alterations in thyroid hormones and hepatic enzyme activities using the U.S. EPA Benchmark Dose Software (BMDS Version 1.3). For each endpoint data were fit to age which demonstrated the greatest potency and efficacy. The EROD and PROD data, as well as the T4 and UDPGT data for neonates data were fit with the Hill model as this function best describes the biological response. The T4 data and UDPGT data were fit using the power model and a second order polynomial, respectively, due to a lack of significant fit for the Hill model. The benchmark effect levels were set at 20% decreases for the thyroid hormone data and 50% increases for the liver enzyme data[8].

Results and Discussion

Reproductive Parameters: There was no evidence of any treatment-related effects of developmental exposure to DE-71 on any reproductive parameter. No treatment-related effect was detected for gestation length, litter size, gender or viability.

Body and Organ Weights: No treatment-related effects were found for maternal offspring or weanling body weights. There were no dose-related effects on eye opening. Exposure to DE-71 caused an increase in liver weight in both pregnant and lactating dams as well as neonatal and weanling rats. The increase in neonatal and weanling rat liver/body weight ratios were as great as 39% above controls while the dams were only 8% above control

Thyroid Hormones: Exposure to DE-71 caused a decrease in serum total T4 in dams, fetuses, offspring and weanlings. The effects of maternal exposure to DE-71 on T4 concentrations in fetuses and offspring were age-dependent. There were small, yet significant decreases in fetal T4 concentrations on GD20 in the 10 and 30 mg/kg/day groups. On PND4 and PND14, significant dose-dependent decreases were observed in the two highest dose groups with maximal decreases. Serum total T4 concentrations returned to control levels by PND36 and remained unaffected on PND90. The lowest dose (1mg/kg/day) did not significantly affect T4 concentrations at any sampled age. Serum T4 concentrations were decreased 30 mg/kg/d and higher by DE-71 in the weanling rats.

There were no treatment-related effects of developmental DE-71 exposure on serum total T3 concentrations in either the dams or the offspring. Serum T3 concentrations were decreased in weanlings exposed to 100 and 300 mg/kg/d. T3 was decreased only 25-30% at these doses.

Hepatic Enzymatic Activities: Maternal exposure to DE-71 resulted in significant increases in hepatic EROD, PROD and UDPGT activities in both dams and offspring. Hepatic EROD activity was slightly increased in dams on GD20 compared to PND22 (Figure 5A). There were increases in EROD activity upto 3.7-fold on GD20, and 2.9-fold on PND22. There was no effect of the 1mg/kg/day dose on EROD activity. Fetal EROD activity was significantly increased 2.5-fold in the high dose group on GD20. Offspring EROD activity was significantly increased 39-fold and 95-fold on PND4 and 20-fold and 57-fold on PND14, in the 10 and 30 mg/kg/day groups, respectively. There was a much smaller, yet significant, increase of 0.5-fold in the high dose group on PND36. There were no treatment-related changes in EROD activity on PND90. Hepatic EROD activity was increased in weanling rats at doses of 10 mg/kg/d or higher and reached a maximum of approximately 20-fold.

Hepatic PROD activity was increased up to 19-fold on GD20 and up to 24-fold on PND22. There was no effect of the 1mg/kg/day dose on PROD activity. Offspring PROD activity was significantly increased up to 26-fold on PND4 and up to 21-fold on PND14. There was a significant increase of 10 fold in the high dose group on PND36. There were no treatment-related changes in PROD activity on PND90.

Exposure to DE-71 caused increases in the rate of glucuronidation of T4 in both dams and offspring. In dams there was a similar increase of about 1.6-fold in UDPGT activity only in the high dose groups on both GD20 and PND22. There were no effects on UDPGT activity detected in the two lower doses. Hepatic UDPGT activity in offspring was increased as a result of maternal exposure to DE-71. Offspring UDPGT activity was significantly increased 1.9-fold and 4.7-fold on PND4 and PND14, respectively, in the 30 mg/kg/day group. There was no significant effect of any lower doses on PND4 or 14. There were no effects of exposure on PND36 or PND90. Hepatic T4-glucuronidation was increased at doses of 30 mg/kg/d or higher in the weanling rats.

NOELs and model estimates for BMDs are shown in Table 1. The relationship for NOELs and BMDs vary by endpoint. These differences are likely due to the effects of variability and dose spacing on the NOEL estimates. Based on visual inspection of the data BMD estimates appeared to be better approximations of potency. BMD estimates for all endpoints were lower for neonates compared to weanlings and dams. BMDs for EROD and PROD were up to an order of

magnitude lower in neonates compared to dams, whereas there was only a 2 - 4 fold difference for T4 and UDPGT (Table 1).

These data indicate that DE-71 alters thyroid hormone homeostasis during development and that these chemicals can be considered endocrine modulators. In addition, the neonates appear more sensitive to the effects of DC-71 compared to the adult animals. The data also demonstrate that the dose response relationship for the effects of DE-71 as a modulator of thryroid hormone homeostasis in weanling rats provide reasonable estimates of the dose response relationships for developmental exposures.

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Table 1. NOEL and BMD estimates for the effects of weanling and developmental exposure to DE-71.[a]

	Neonate[b]		Weanling		Dam[c]	
	NOEL	BMD	NOEL	BMD	NOEL	BMD
Serum T4	1	2.4	10	12.7	10	6.1
EROD	1	0.4	3	2.9	1	4.0
PROD	1	0.5	3	0.8	1	3.8
UDPGT	10	5.5	10	9.5	10	21.1

[a] All values are in mg/kg/day.