28-DAYS ORAL DOSE TOXICITY STUDY OF A PURIFIED PENTABDE MIXTURE DERIVED FROM DE-71 IN WISTAR RATS: EVALUATION OF FEMUR LENGTH AND DENSITY AND APOLAR LIVER RETINOID LEVELS

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Introduction

There are more than 175 chemicals classified as flameretardants. Brominated diphenyl ethers, BDEs, are frequently used in combustible materials to increase fire resistance. Several BDEs are found in quantifiable levels in wildlife, as well as in humans. The focus of this study is on pentaBDE, which is an additive flame retardant. PentaBDE is bioaccumulating and has the potential of being transported far by air[1]. It shows structural similarities to dioxins and PCBs. Food, is possibly the largest source of human pentaBDE exposure. Inhalation[2] and dermal[3] routes may also contribute significantly, notably in occupational exposure. Sufficient evidence demonstrates that pentaBDE is toxic to the developing brain and interferes with hormonal pathways[4-6]. PentaBDE as well as octaBDE are, since August 2004, banned in the EU[7]. In this study we investigated the effects of a purified pentaBDE mixture, derived from DE-71, on femur length and density, as well as apolar liver retinoid levels in female and male rats.

Materials and Methods

Study design: The study was a 28 day repeat dose toxicity study. The institutional Committee on Animal Experimentation approved experiments, according to Dutch legislation. Female and male 8-week old Wistar rats (5/group) received either vehicle or dosing solutions daily. The pentaBDE mixture, provided by Great Lakes Chemical Cooperation, was freed of brominated dibenzodioxins, dibenzofurans and other coplanar molecules by purification prior to use. The compound was given by gavage, dissolved in corn oil in doses of 0.27, 0.82, 2.47, 7.4, 22.2, 66.7 and 200 mg pentaBDE/kg bw with a vehicle control group. Femur and livers were collected and stored at -20 and -70°C, respectively until analysis.

Bone measurements: Left femur was dissected and stored in Ringer solution, (1 l contains 0.3 g Tris, 0.24 g $CaCl_2$ $(H_20)_2$, 0.4 g KCl; 2.05 ml 1M HCl, pH 7.4) to prevent the bone tissue from drying. On the day of analysis the bones were thawed at room temperature and stored moistened in closed plastic tubes until examination. The bone length was measured using an electronic sliding caliper to the nearest 0.01 mm (IP65, Sylvac SA, Crissier, Switzerland). The femoral bone length was measured from the top of the caput femoris to the distal point of the condylus medialis. The excised femur was scanned with a pQCT system (Stratec XCT Research SA+).

Retinoid analyses: Analyses of apolar liver retinoid levels, including retinol and retinylesters, were carried out in duplicate as described previously [8].

Statistics: Statistical analyses were conducted by one-way analysis of variance (ANOVA) and differences between treatment groups and controls were established with the Kruskal-Wallis test, Mann-Whitney U test and Dunnett 2-sided t-test, as appropriate, using the SPSS statistical software. A significance level of $*p<0.05$ was used.

Results

Body and organ weights: No significant differences were seen between female groups regarding body weight gain, while, compared to control animals a significant decrease in body weight gain was observed among male rats exposed to 7.4 and 200 mg pentaBDE/kg (data not shown). Increased liver-to-body weight ratios were observed in both female and male rats (Figure 1).

Retinoid analyses: A dose-dependent decrease in the liver content of apolar retinoids (µg) was observed for female and male rats (Figure 2). The decreases in the high dose groups were 29% and 33%, respectively, when comparing with the corresponding controls.

Bone measurements: No pentaBDE related effects were seen on femur length in either sex (data not shown). A treatment-related decrease in total bone mineral density at diaphysis was seen among male but not among female rats. However, there were no significant changes when comparing individual male groups with the corresponding control animals (Figure 3). There were no pentaBDE-related effects at femur metaphysis in either sex (data not shown).

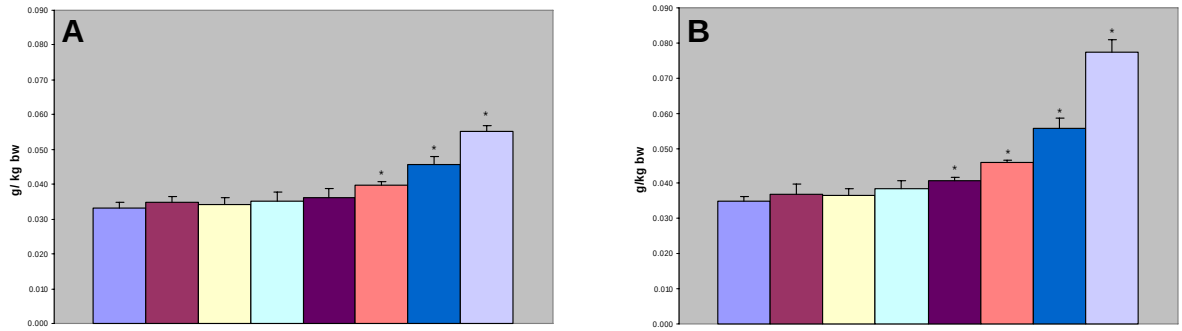

Figure 1 Liver-to-body weight (g/kg bw) ratios in female (A) and male (B) Wistar rats treated with daily doses of 0, 0.27, 0.82, 2.47, 7.4, 22.2, 66.7 and 200 mg pentaBDE/kg bw for 28 days. Values represent the mean ± SD of 4-5 animals. * Indicates significant differences between treatment groups and the corresponding controls.

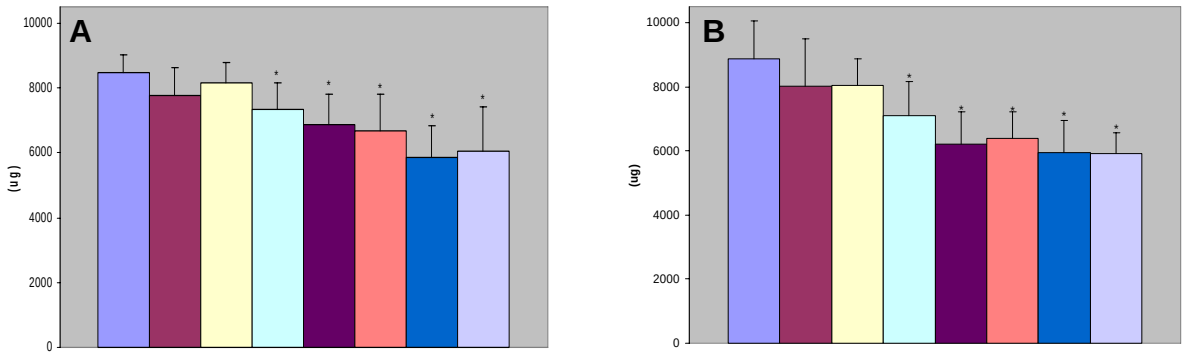

Figure 2 Apolar liver retinoids (µg) in female (A) and male (B) Wistar rats treated with daily doses of 0, 0.27, 0.82, 2.47, 7.4, 22.2, 66.7 and 200 mg pentaBDE/kg bw for 28 days. Values represent the mean ± SD of 4-5 animals. * Indicates significant differences between treatment groups and the corresponding control animals.

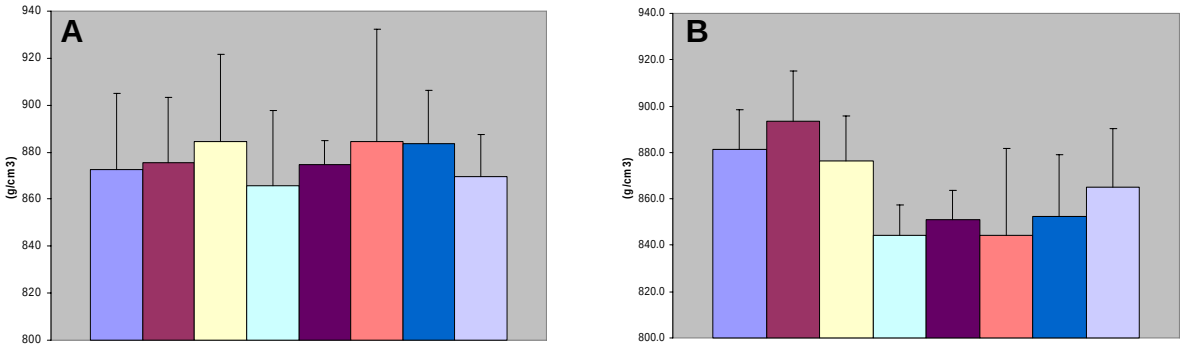

Figure 3 Total bone mineral density (g/cm^3) at femur diaphysis in female (A) and male (B) Wistar rats treated with daily doses of 0, 0.27, 0.82, 2.47, 7.4, 22.2, 66.7 and 200 mg pentaBDE/kg bw for 28 days. Values represent the mean ± SD of 4-5 animals.

Conclusions

In the present study we observed that pentaBDE exposure of female and male Wistar rats caused markedly increased liver weights and decreased hepatic vitamin A content. NOAELs for increased liver-to-body weight ratios of 7.4 and 22.2 mg pentaBDE/kg bw were established for female and male rats, respectively. These data are consistent with previous observations in rats[4,9-11] and mice[4,11]. NOAELs for liver retinoid reductions were established at 0.82 mg pentaBDE/kg bw in both female and male rats, i.e. at dose levels well below the dose-level where liver weight changes were observed. Decreases of liver retinoid levels have also been reported in rodent studies by Hallgren and coworkers, when using the pentaBDE mixture, Bromkal 70-5 DE[11]. Further evaluation of bone data using benchmark dose modelling are planned and it seems likely that studies of longer duration are needed to clarify the biological significance of the bone observations in the present study.

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