PENTABROMINATED DIPHENYLETHER INDUCES CYTOCHROME P450 ENZYMES IN MALE AND FEMALE RATS.

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Introduction

Polybrominated flame retardants (BFRs) represent a broad spectrum of organic compounds used in a variety of commercial products. Their widespread production and use has led to increasing contamination in the environment and in humans, which has raised concern about possible adverse health effects.[1] The commercial pentabrominated diphenylether(Pentamix) is a mixture of several tetra-, penta and hexabrominated diphenyl ethers. The Pentamix was used as an additive flame in polyurethane-based materials including mattresses and furniture until abandoned in Europe in 2004.[2] The polybrominated diphenylethers (PBDEs) are highly lipophilic compounds which are found in indoor air, environmental and human samples. The reported rising levels in human samples over the last decades may be caused by their proposed persistency.

Some BFRs have been suspected to act as endocrine disruptors and/or to affect the development of the unborn. Induction of drug metabolism may play a role in such effects by changing the body's homeostasis of certain hormones such as steroid and thyroid hormones. In particular, induction of drug-metabolising enzymes via the aryl hydrocarbon receptor (AhR), the pregnane X receptor (PXR) or the constitutive androstane receptor (CAR) can interfere with the homeostasis of thyroid hormones and steroids.[3,4,5] In this study we treated male and female rats with a cleaned-up Pentamix, and analysed the effects on the hepatic levels of a number of CYP enzymes.

Materials and Methods

Chemicals. A commercial mixture of Pentamix was provided by Great Lakes Chemical Coorperation (Dr. D. Sanders) and purified from dioxins, dibenzofurans, and any other coplanar molecules (Dr. Åke Bergman). It mainly consists of the congeners tetrabrominated biphenylether 47 (BDE-47), pentabrominated BDE-99 and BDE-100. Minor constituents are the hexabrominated BDE-153 and -154.

Animal treatment. Male and female rats of the WU (CPB) strain were administered Pentamix daily by gavage, seven days a week, over 28 days. The doses were 0, 0.27, 0.82, 2.47, 7.4, 22.2, 66.7 or 200 mg/kg body weight per day. Each dose group consisted of five animals per sex. After the end of exposure, animals were anesthetized and sacrificed, livers were removed, immediately frozen in liquid nitrogen, and stored at -80°C until further processing. The study was approved by the RIVM Committee on Animal Experimentation according to Dutch legislation.

Catalytic activities. Microsomes were isolated according to the protocol of Hoffman et al.[7], and dissolved in 50 mM sodium phosphate buffer, pH 7.4. 7-Ethoxyresorufin O-deethylase (EROD) and 7-pentoxyresorufin O-dealkylase (PROD) activities were measured in triplicates in multiwells using a Fluoroskan Ascent FL microplate reader (Labsystems, Frankfurt, Germany). Total protein was determined according to Kennedy et al.[8] using the fluorescamine reaction. Activity of luciferin benzylether debenzylase (LBD) was carried out with 13 µg microsomal protein using the P450-Glo™ CYP3A4 Assay (Promega, Heidelberg, Germany) according to the manufacturer's protocol.

Results and Discussion

The CYP1A protein level in male and female rat liver increased remarkably with higher doses, the induction being significant from 22.2 mg/kg body weight per day (Figure 1). This finding was consistent with the increased EROD activity in female and male rat liver (Figure 2). For both assays the effects were higher in female than in male animals.

The protein amount of CYP2B1/2 increased remarkably with rising dose in female and male rats, significantly at 7.4 mg/kg body weight per day. This finding may indicate a CAR dependent induction of CYP2B enzymes. The CYP2B mRNA was induced similarly to the enzyme protein level both in males and females. The female rat liver seems to be more responsive to Pentamix treatment at higher doses, i.e., 7.4 – 200 mg/kg body weight per day, than male rat liver (Figure 3). The corresponding hepatic PROD activity was shown to be increased for both female and male rats (Figure 4).

CYP3A is known to be induced via a PXR dependent mechanism. However, CAR may competitively bind to the 5'-untranslated region of the CYP3A gene and thus, also lead to an increase in CYP3A mRNA. Here, Pentamix might act through such a mechanism via CAR or PXR since a significant trend for increased CYP3A1 protein over dose was found in male and female rats (Figure 5). The LBD activity did not give any hint on changes due to Pentamix dose groups which may be caused be the low sensitivity of the assay (Figure 6). Alternatively, Pentamix constituents may inhibit catalytic CYP3A activity.

CYP1A, CYP2B and CYP3A enzymes were induced in female and male rat liver due to oral treatment of Pentamix. If the induction indeed is caused by the proposed pathways via AhR, CAR or PXR remains to be shown. Indications for a CAR-dependent CYP2B induction of the 'phenobarbital-type' suggest that Pentamix may act as a liver tumor promoter in rodents. Since certain CYPs under investigation are involved in the metabolism of steroid hormones the hormonal balance might be influenced through the revealed over-expression. Moreover thyroid hormone levels might be affected by CAR- or PXR-regulated induction of phase II conjugating enzymes such as UDP-glucuronosyl-transferases. It remains to be shown if Pentamix lead to similar induction in human-derived experimental models.

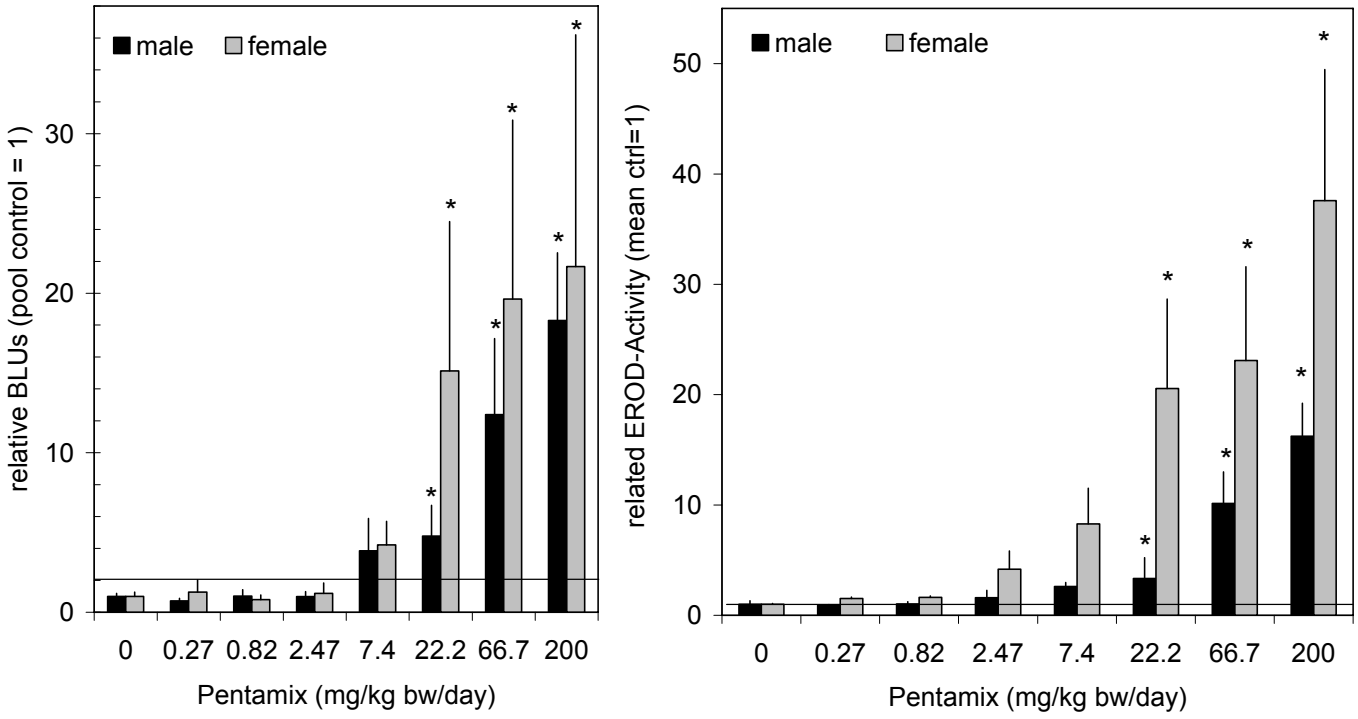

Figure 1: Hepatic microsomal protein level of CYP1A1 in rats orally administered Pentamix.
Figure 2: Hepatic microsomal EROD activity of female and male rats orally administered Pentamix.
Bars represent means ± S.D. from 5 animals.*p<0.1

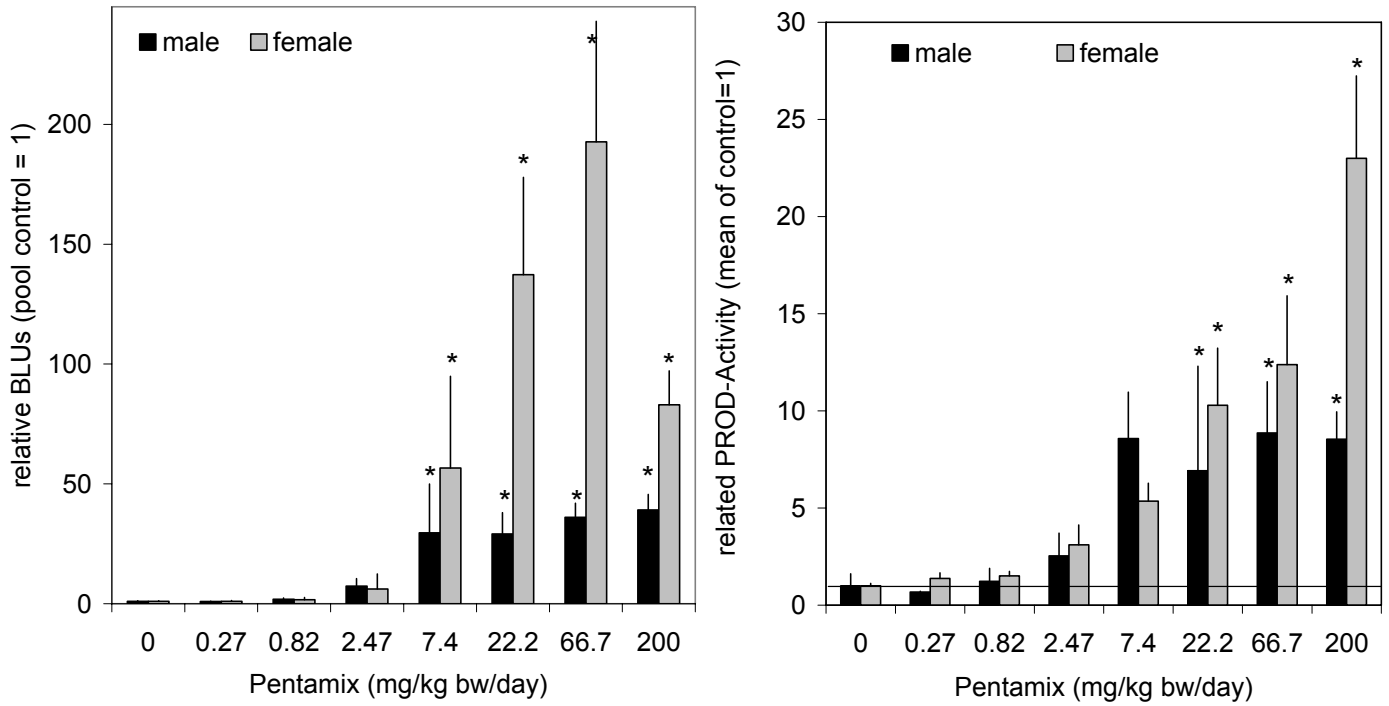

Figure 3: Hepatic microsomal protein level of CYP2B1/2 in rats orally administered Pentamix.
Figure 4: Hepatic microsomal PROD activity in rats orally administered Pentamix.
Bars represent means ± S.D. from 5 animals. *p<0.1

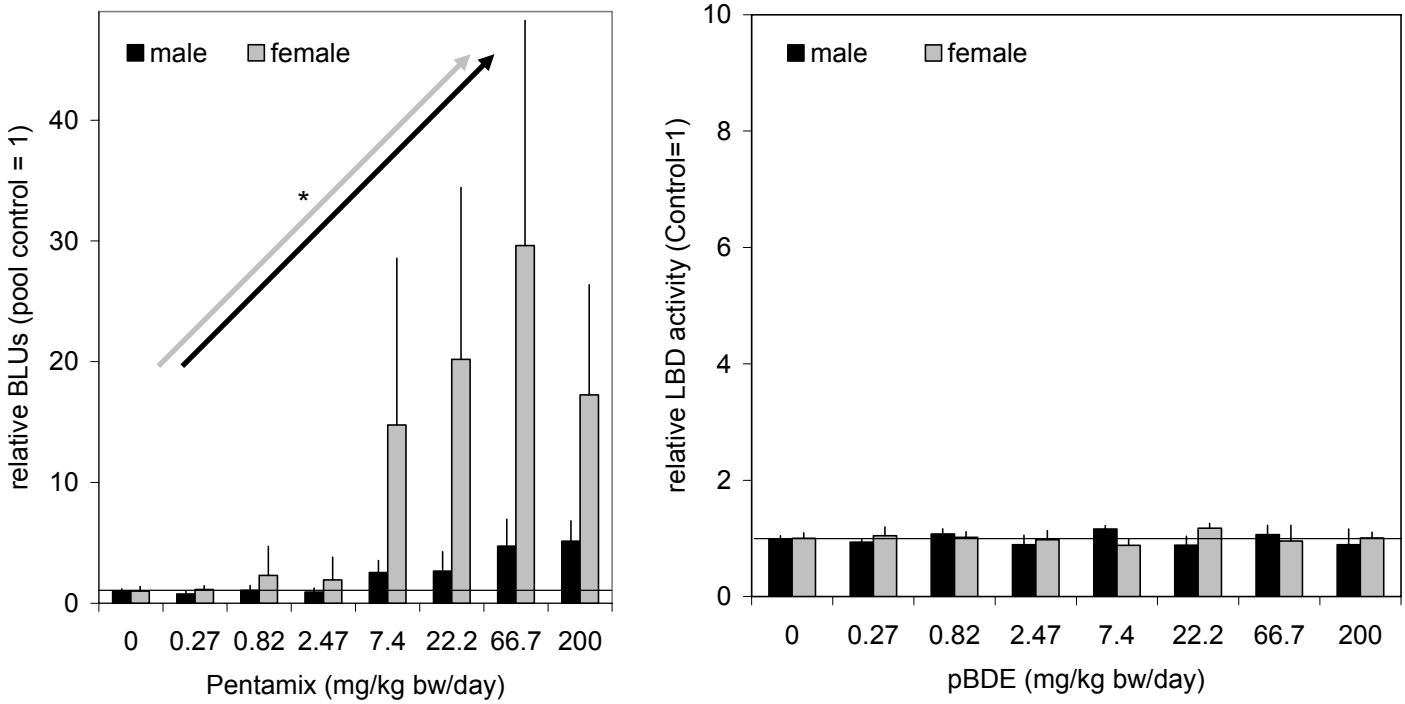

Figure 5: Hepatic microsomal protein level of CYP3A1 in rats orally administered Pentamix.
Figure 6: Hepatic microsomal LBD activity in rats orally administered Pentamix.
Bars represent means ± S.D. from 5 animals. Trend analysis *p<0.1

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References

1. Birnbaum L.S., Staskal D.F. (2004). Brominated flame retardants: cause for concern? *Environ. Health Perspect.* **112**, 9-17.
2. Alaee M., Arias P., Sjödin A., Bergman A. (2003). An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environ. Int.* **29**, 683-689.
3. Mikamo E., Harada S., Nishikawa J., Nishihara T. (2003) Endocrine disruptors induce cytochrome P450 by affecting transcriptional regulation via pregnane X receptor. *Toxicol. Appl. Pharmacol.* **193**, 66-72.
4. Xie W., Yeuh M.F., Radomionska-Pandya A., Saini S.P., Negishi Y., Bottroff B.S., Cabrera G.Y., Tukey R.H., Evans R.M. (2003) Proc. Natl. Acad. Sci. U.S.A. (2003) Control of steroid, heme, and carcinogen metabolism by nuclear pregnane X receptor and constitutive androstane receptor. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 4150-4155.
5. Maglich J.M., Watson J., McMillen P.J., Goodwin B., Willson T.M., Moore J.T. (2004) The nuclear receptor CAR is a regulator of thyroid hormone metabolism during caloric restriction. *J. Biol. Chem.* **279**, 19832-19838.
6. Pfaffl M.W., (2001) A new mathematical model for relative quantification in real-time RT PCR. *Nucleic Acid Research* **29 (9)**, e45
7. Hoffman D.J., Melancon M.J., Klein P.N., Rice C.P., Eisemann J.D., Hines R.K., Spann J.W., Pendleton G.W. (1996) Developmental toxicity of PCB 126 (3,3',4,4',5-pentachlorobiphenyl) in nestling American Kestrels (*Falco sparverius*). *Fund. Appl. Toxicol.* **34**, 188-200.
8. Kennedy S.W., Jones S.P., Bastien L.J. (1995) Efficient analysis of cytochrome P4501A catalytic activity, porphyrins, and total proteins in chicken embryo hepatocyte cultures with a fluorescent plate reader. *Anal. Biochem.* **226**, 362-370.