Effects of polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) on thyroid hormone levels and enzyme activities in rats

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Introduction

Several persistent organohalogen compounds, among these PBDEs, PCBs and CPs, have been shown to affect the thyroid hormonal system (e.g. 1-3) and to cause altered thyroxin (T4) levels, although the mechanism behind this effect is not elucidated. Among the proposed mechanisms are induction of UDP-glucuronosyltransferas (UDPGT) and the following increase in T4 deactivation by conjugation, the interference with T4 transport proteins and increased T4 excretion, and direct effects on the thyroid gland leading to decreased T4 synthesis (4-6). The possibility of several, simultaneous mechanisms cannot be disregarded.

Effects on thyroid hormonal systems are important to follow, as these hormones play a crucial role in the development of many organs, e.g. the brain. Thus, alterations in CNS development might be an effects of exposure to T4-modulating chemicals. Indeed, those effects have been observed in animals experiments, and indicated in humans exposed to PCB and dioxins (7-9) and they form part of the basis for the Swedish dietary advice on consumption of fatty Baltic fish (10).

In the environment, animals and man are exposed to a mixture of persistent organic chemicals. However, almost all data, e.g. on toxicity, are produced from studies on single compounds. Therefore, in the present study, several environmental chemicals with known or suggested thyrotoxic effects were studied, as well as the interaction between them.

Materals and Methods

2,2',4,4'-Tetrabromodiphenyl ether (TBDE; >98% pure) was obtained from Eva Jakobsson and coworkers, Environmental Chemistry, Stockholm University. Aroclor 1254 and Witaclor 171P (technical grade PCB and CP, respectively, with chlorine degree of 54 and 71 %; the CP preparation has a 10-13 carbon chain length range) were obtained from commercial sources (purity unknown). The substances were dissolved in corn oil.
Female Sprague-Dawley rats (7 weeks old) were used. The animals were given the substances by daily gastric intubation, accordning to the scheme presented in Table 1. In groups exposed to mixtures of compounds, the TBDE concentration of 6 mg/kg /day was

chosen. After a 14 days administration period, the rats were killed 24 hr after the last gavage, the bodies were dissected and blood and tissue samples were taken for subsequent analyses. Thyroid hormone analysis on free and total T4 (FT4, TT4), and of TSH, were performed by standard RIA techniques (Amersham, USA). Ethoxy-, methoxy- and pentoxy-resorufin-O-deetylase (EROD, MROD, and PROD) activities were measured by analysing the resorufin product, formed in vitro during incubation with rat liver microsomes, by spectrofluorometer. UDPGT activities were determined by microsomal incubations with radioactive T4 as substrate and UDPGA as cofactor, counting the formed glucuronyl product after chromatographic separation.

Table 1. *Dosing scheme for the substances of the present study. Substances were administered orally to female rats once a day for two weeks*

Substance	n	Dose (mg/kg body wt./day)
Control (corn oil)	6	-
TBDE	6	1
TBDE	6	6
TBDE	6	18
PCB (Aroclor 1254)	6	4
CP (Witaclor 171P)	6	6,8
TBDE + PCB	6	6 + 4
TBDE + CP	6	6 + 6,8
PCB + CP	6	4 + 6,8
TBDE + PCB + CP	6	6 + 4 + 6,8

Results and Discussion

The effect of treatment on free T4 levels in rats are shown in Figure 1. Significantly decreased levels were found after exposure to TBDE (18 mg/kg /day), PCB, TBDE + PCB, PCB + CP, and TBDE + PCB + CP. The total T4 gave a similar, although less distinct, answer. The treatments did not significantly change the TSH levels.

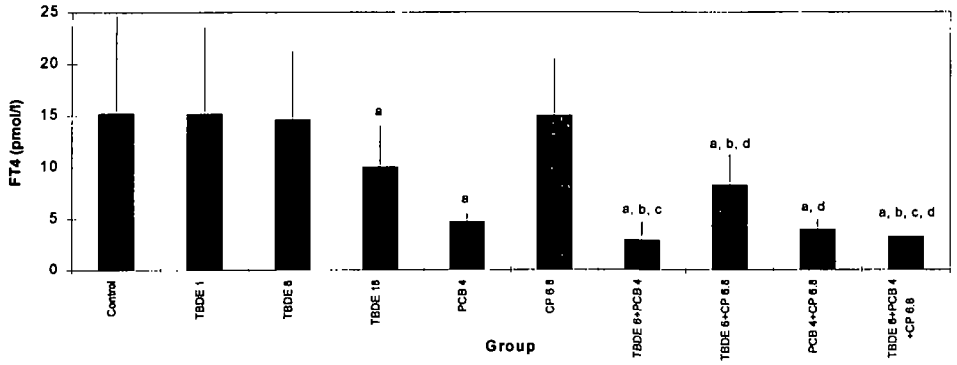

Figure 1. *Plasma levels of free thyroxine (T4) expressed in nmol/l in female rats exposed to TBDE, PCB and/or CP. Median values; vertical lines represent min-max values. Letters a, b, c and d in figure denote a significant difference from control, TBDE (6 mg/kg), PCB, and CP, respectively ($p<0.05$)*

The effects on microsomal enzymes were the following: EROD activities (Figure 2 a,b) were highly induced (more than 100 times) after treatment with all oil solutions containing PCB (alone or as mixtures with TBDE, CB, or TBDE and CP). Significant, although much smaller, induction levels were seen after exposure to TBDE (6 or 18 mg/kg /day) or to TBDE + CP. The result from MROD induction studies were similar to those of EROD, but the levels of induction were smaller. The PROD induction pattern (Figure 3) was quite different from EROD and MROD, and the dose-related effects of TBDE were more prominent: the TBDE 18 mg/kg/day-exposure resulted in enzyme activities as high as those found in the PCB groupsAs regards UDPGT, the induction of glucuronide conjugates is low and the largest increase in activities (TBDE + PCB + CP) was 140% of control (data not shown). Notably, TBDE (18 mg/kg /day) and all mixed groups containing TBDE gave significantly induced levels. In our model, CP alone did neither induce microsomal enzymes nor alter T4 levels to any significant extent.

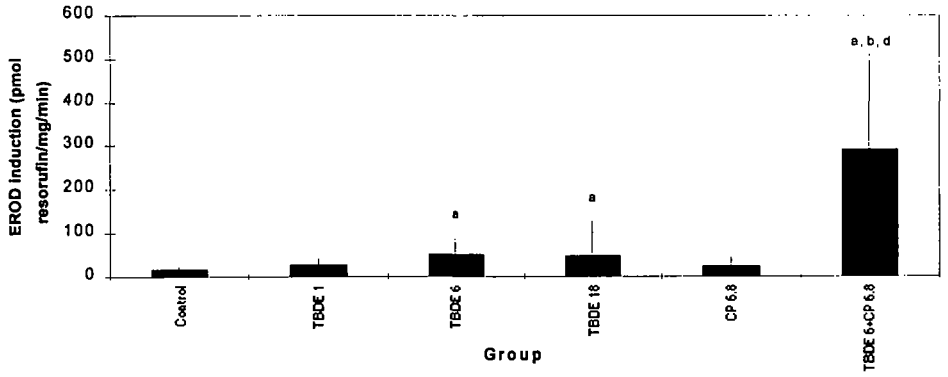

Figure 2a. EROD induction expressed as pmol resorufin/mg/min in female rats exposed to TBDE and/or CP (for letter symbols in figure, see text to Figure 1)

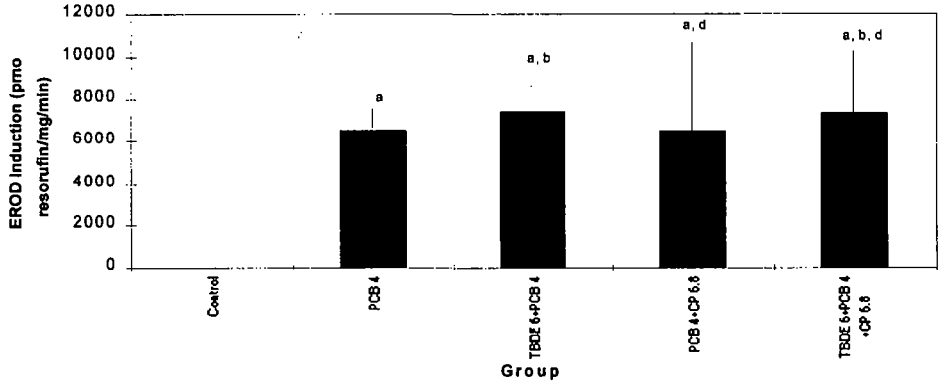

Figure 2b. EROD induction expressed as pmol resorufin/mg/min in female rats exposed to TBDE, PCB and/or CP. Note the difference between the scales in diagram a and b.

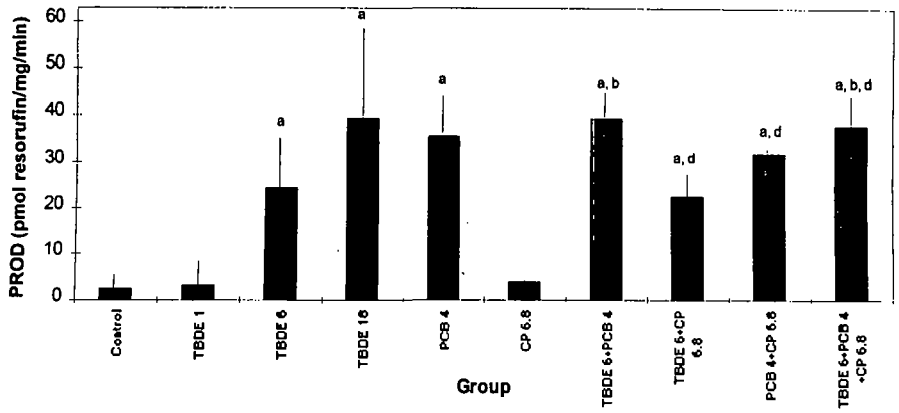

Figure 3. *PROD induction expressed as pmol resorufin/mg/min in female rats exposed to TBDE, PCB and/ or CP (for letter symbols in figure, see text to Figure 1)*

The results show that PCBs (Arochlor 1254) and PBDEs (BDE-47) significantly reduce the T4 levels in rats, in the actual exposure interval, and that Aroclor 1254 results in the strongest effect, when administrating the substances orally in isomolar concentrations. EROD and MROD, but to a lesser extent PROD and UDPGT, activites correlated to T4 effects, which could indicate that glucuronidation of T4 is not a major factor to explain the observed decrease in T4 plasma levels. Regarding the mixed TBDE + CP group, a synergistic decrease in free T4 levels is observed; this may be a result of several mechanisms working simultaneously.

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