BIOCHEMICAL EFFECTS OF HYDROXYLATED PCB METABOLITES

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Introduction

Polychlorinated biphenyls (PCBs) are metabolized by hepatic microsomal mixed function-oxidases (CYP1A1/1A2 and CYP2B1/2B2)[1,2]. The major products are hydroxylated PCB metabolites[3] which are eliminated chiefly *via* the bile as conjugates.
These phenolic metabolites have been detected in plasma from mammals, including man[4]. Earlier studies at our laboratory have shown that administration of 3,4,3′,4′-tetrachlorobiphenyl (CB-77) to pregnant mice results in a high accumulation of hydroxylated metabolite(s) in the fetal compartment[5]. These metabolites are suggested to affect thyroxine levels, probably by transthyretin (TTR) binding and interference with plasma transport of thyroxine (T4)[6,7], and to bind to the estrogen[8] and Ah-receptor[9] and to protein type 1- deiodinase[10]. Result from a study by Klasson-Wehler et al.[9] showed mortality in chick embryos exposed to 4-hydroxylated metabolites of CB-77. There is no previous in vivo data about effects of exposure to pure phenolic PCB metabolites on T4 levels in fetal and pregnant animals.
In this paper we study the endocrine and enzyme kinetic effects of three 4-OH-PCB metabolites (of CB-77 and 3,4,2′,3′,4′-pentachlorobiphenyl - CB-105) that have been found in animal and human plasma.

Materials and methods

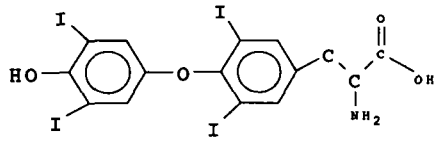
Chemical and animals: ^{14}C-labelled 4-hydroxy-3,5,3′,4′-tetrachlorobiphenyl (4-OH-TCB); spec. act. 26.5 mCi/mmol; diluted with unlabelled 4-OH-TCB to desired molar dose, and unlabelled 4-hydroxy-3,5,2′,3′,4′- and 4-hydroxy-2,3,5,3′,4′-pentachlorobiphenyl (4-OH-$PeCB_A$ respective 4-OH-$PeCB_B$) (purity ≥ 98%) were kindly provided by Dr. Eva Klasson-Wehler and co-workers, Stockholm University[11]. C57BL mice were purchased from Bomholtgård, Denmark. The animals were given free access to commercial pelleted food (R3, EWOS AB, Södertälje) and tap water and were kept at 22 °C under a 12 hr light-dark cycle. The mice were mated over-night and the presence of a vaginal plug on the following day was defined as day 0 of pregnancy.
Dosage. The compounds were dissolved in DMSO and injected i.v. in a tail vein in near equimolar dose of 50 μmol/kg b.wt. in three groups of pregnant C57BL mice at day 16 of gestation. Two other groups at the same stage of pregnancy were treated i.v. with a single dose of 5 or 20 μmol ^{14}C-4-OH-TCB/kg b.wt. Control animals were injected with DMSO. All animals were sacrificed at day 17 of pregnancy. Whole blood (collected by cardiac puncture) and liver were obtained from dams, and whole blood (collected in heparinized

capillary tubes), and liver were collected from fetuses. Blood was centrifuged to separate plasma. About 25-50 µl of the plasma were taken for measurements of levels of the total thyroxine (TT4) and radioactivity (liquid scintillation counting). The maternal liver and pooled foetal livers were placed in ice-cold 0.1 M HCl-Tris buffer (pH 7.8; buffer A).
Analysis of TT4 in plasma: The level of TT4 in plasma of exposed and control animals were measured by Amerlex-M T4 RIA Kit (Kodak Clinical Diagnostics).
Determination of ethoxyresorufin O-deethylase (EROD) and methoxyresorufin-O-demethylase activity (MROD): Liver samples were collected from treated dams and fetuses with 50 µmol/kg b.wt. of 4-OH-TCB, 4-OH-$PeCB_A$, or 4-OH-$PeCB_B$. The microsomes were isolated from the homogenized liver by a modified method of Fouts and Pohl[12]. The microsome pellet was suspended in 0.5-1.0 ml of buffer A and kept frozen at -70 °C. The EROD and MROD activity were determined fluorometrically essentially as described by Pohl and Fouts[13], but with use of buffer A. The protein concentration was assayed by the method of Lowry et al[14].

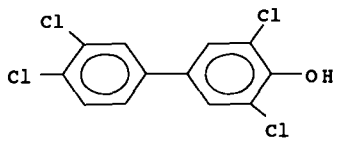
Results and Discussion

Effect on fetal and maternal TTA in plasma:
Fetal TT4 levels were moderately, although significantly, reduced after 4-OH-TCB and 4-OH-$PeCB_A$ treatment (20 and 50 µmol/kg b.wt.), whereas 4-OH-$PeCB_B$ gave less effect (table 1). The TT4 levels of the exposed dams showed, in spite of decreased values, low or no significant difference compared to control values. The values from 4-OH-TCB exposed dams showed a dose response relationship, whereas this was not obvious in case of the fetal values.
The decrease in fetal TT4 plasma levels (81-82% of controls) at day 17 of age (one day after maternal exposure to 20 and 50 µmol of 4-OH-TCB/kg b.wt), may be explained by binding of 4-OH-TCB to fetal TTR in correspondence with conclusions from earlier studies[6]. However, fetal T4 effects could also result from impairment of transplacental transport of T4, as 4-OH-TCB and T4 have similar chemical structures and could compete also at other mutual binding sites than TTR. Some present evidence could support this later idea: The relatively small decrease in fetal TT4 levels may be explained by the fact that only a minor part of the T4 in fetus at late gestation is of maternal source (18 % in fetal rat at day 21 of gestation[15]), and only this part may be affect by 4-OH-TCB. Moreover, the lack of fetal effects from the 5 µmol/kg dose could be explained by the "non-saturation state" of maternal plasma (at higher doses fetal and maternal plasma levels of radioactivity are in equilibrium). The TTR-binding theory may also have difficulties in explaining of the observed decreases in fetal T4, as degradation/glucuronidation of free T4 released from TTR would expected to be slow.
The differences in effects in fetal mouse TT4 level in the present study and in an earlier one using TCB (34 µmol/kg b.wt.; 50% decrease in TT4 concentration at late gestation, four days after administration[6]), may be a consequence of the differences in experimental time schedule. However, differences may also be explained with TCB affecting T4 levels also in some other way than via 4-OH-TCB binding to TTR. Also the actual concentration of the metabolite in fetal compartment has to be taken in consideration.
The effect of 4-OH-$PeCB_A$ on fetal and maternal plasma TT4 was similar that of 4-OH-TCB and it is known that 4-hydroxylated metabolites of pentachlorobiphenyl (PeCB) have higher affinity than T4 to binding TTR in vitro[16].

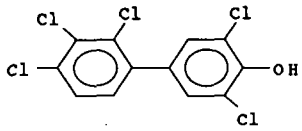
Fig. 1. Thyroxine (T4) and hydroxylated polychlorinated biphenyl structures.



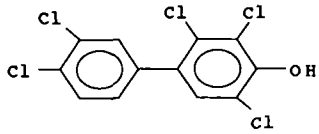
Thyroxine (T4)



4-OH-3,5,3′,4′-tetrachlorobiphenyl (4-OH-TCB)



4-OH-3,5,2′,3′,4′-pentachlorobiphenyl (4-OH-PeCB$_A$)



4-OH-2,3,5,3′,4′-pentachlorobiphenyl (4-OH-PeCB$_B$)

Table 1. Plasma levels (nmol/l) of total thyroxine (TT4) and ^{14}C-4-OH-TCB (nmol /ml) in fetal and pregnant mice (day 17), one day after iv prenatal exposure to PCB metabolites. *,** and *** indicates P< 0.05, 0.005 and 0.001 levels of significant difference between control and exposed animals. Pregnant = P, Pooled Fetus = P F, # = nmol ^{14}C-4-OH-TCB/ml plasma.

Compound	Dose	P/PF	n	Metab. conc.#	TT4 conc.	% of contr
4-OH-TCB	0	P	2		22.5	
	"	P F	2		11.9	
	5	P	4	1.78±0.21	22.7±0.4	101
	"	P F	4	4.44±0.37	11.5±0.9	97
	0	P	6		20.6±2.0	
	"	P F	6		8.3±0.2	
	20	P	6	4.34±0.81	18.6±1.4	90
	"	P F	6	5.00±0.38	6.8±0.1***	82
	0	P	8		30.8±8.0	
	"	P F	6		9.5±0.2	
	50	P	5	8.18±1.26	23.3±4.1	76
	"	P F	4	8.40±0.56	7.7±0.3***	81
4-OH-PeCB$_A$	0	P	8		30.8±8.0	
	"	P F	6		9.5±0.2	
	50	P	4		23.8±2.4*	77
	"	P F	4		8.1±<0.1***	86
4-OH-PeCB$_B$	0	P	2		21.2	
	"	P F	2		7.2	
	50	P	5		15.6±1.4**	72
	"	P F	5		7.0±0.2*	97

Effect on EROD and MROD activity:
EROD acitvity in hepatic microsomes from dams treated with hydroxylated PCB metabolites was higher than 1.5-fold of control animals, whereas the MROD activity in liver to 4-OH-TCB treated dams measured up to 2 - 2.5-fold of control in animals treated with 4-OH-$PeCB_A$ or with 4-OH-$PeCB_B$ (Table 2). EROD and MROD activities in fetal liver were not detectable neither in control nor in intrauterine exposed fetuses. Earlier we showed that induction of hepatic EROD activity in pregnant and fetal mouse was dose-dependent[17] at day 17 of gestation, 2 days after treatment. The potency of EROD induction for the parent compound at the dose 10 mg/kg body wt. is 16 times higher than that for the metabolite 4-OH-TCB. This difference could be explain by the difference in the affinity of the parent compound and its metabolites to bind cytosolic aryl hydrocarbon (Ah) receptor[9].

Table 2. Activity of CYP 1A1/2 - measured by 7-ethoxyresorufin-O-deethylase (EROD) and methoxyresorufin-O-demethylase (MROD) in hepatic microsomes of pregnant mice exposed to PCB metabolites at day 16 -17. (iv adm. of 50 μmol/kg body wt.), n=5-6.

	Enzyme activity (formed nmol resorufin/mg protein/min)	
	EROD	MROD
Control	0.059±0.013	0.087±0.013
4-OH-TCB	0.112±0.031	0.223±0.067
4-OH-$PeCB_A$	0.095±0.019	0.111±0.026
4-OH-$PeCB_B$	0.097±0.008	0.093±0.015

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