Effects of prenatal exposure to hydoxylated PCB metabolites and some brominated flame retardants on the development of rats.

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Introduction

Possible human health effects from low-level exposure to environmental chemicals are an issue that has attracted much attention. Environmental compounds that may play a role are those that may disrupt endocrine function. Organohalogen compounds, and in particular their hydroxylated metabolites, show a striking resemblance to steroid hormones.The main objective of this research is to investigate comparative pathways of early life-stage exposure and long-term effects for several classes of organohalogens, including polychlorinated biphenyls (PCBs) and flame retardants, polybrominated bisphenols and –diphenylethers, and their hydroxylated metabolites.

Due to their prevalence in human plasma, the hydroxylated PCB metabolites 4-OH-CB107 and 4-OH-CB187, as well as 6-OH-BDE47 and 2,4,6- tribromophenol, were selected as test compounds. BDE 47 has been included as a test compound due to its relatively high levels in the environment and biota, whereas tetrabromobisphenol A (TBBPA) was selected because of its high volume production.

The *in vivo* studies involved prenatal exposure of rats to test compounds during critical stages of gonadal development and were focussed on low dose effects. Several endpoints were investigated, including endocrine (thyroid and sex steroid hormones) effects, developmental landmarks, sexual and neurobehavioural development. Blood plasma and tissue levels of test compounds were analysed to determine transplacental transfer of (hydroxylated) organohalogens.

Experimental design

Pregnant Wistar rats were orally dosed with test compounds during gestation day 10-16 as described previously[1,2]. The test compounds were: Aroclor 1254 (positive control, 25 mg/kg/day); 4-OH-CB107 (0.1, 0.5 and 5 mg/kg/bw/day); 4-OH-CB187 (0.5 and 5 mg/kg/bw/ day); TBBPA (25 mg/kg/day); 2,4,6-tribromophenol (25 mg/kg/day); BDE 47 (20 mg/kg/day); and 6-OH-BDE 47 (5 mg/kg/day). Starting on postnatal day (PND) 1, offspring were scored for developmental landmarks including onset of hair grow, pinna detachment, bilateral eye opening, vaginal opening and balanopreputial separation. Thyroid hormone- and estrogen levels were measured at various time points. Offspring were subjected to several different neurobehavioural tests, like open field, catalepsy test, sweet preference test and brain auditory evoked potentials. Female offspring was monitored daily for estrous cycle length during three different periods in the experiment. The first directly after vaginal opening (PND 30-68), the second between PND 150-170 and the last between PND 210-230. Plasma concentrations of test compounds were measured in pooled plasma samples of F1 pups at PND4, and of individual F0 mothers following weaning at PND24, according to methods described elsewhere[2].

Results and Discussion

Measurement of test compounds in prenatally exposed rats showed clear placental transfer of hydroxylated PCBs and BFRs from mother to fetus (Figure 1). Placental transfer of 4-OH-CB 107 was shown in an earlier experiment[4]. F0 females still contained detectable levels of test compounds in plasma on PND 24.

Evaluation of the datasets and analysis of the effects of prenatal exposure to the PCB metabolites and BFRs are still ongoing. No effects were observed on developmental landmarks following exposure to (metabolites of) PCBs (Table 1B) and BFR (Table 1A), indicating low, non-teratogenic exposure doses. Exposure to hydroxylated PCBs resulted in a marked reduction in plasma total thyroxin in offspring at PND 4. Behavioural studies with the PCB metabolite-exposed offspring have indicated some neurodevelopmental effects, such as impaired habituation in the open field test.

Analysis of the estrous cycle of female offspring showed a prolongation of the estrous cycle length in all three 4-OH-CB107 groups and the 0.5 mg/kg/day 4-OH-CB187 group, between PND 210-230 (Figure 2). This was not observed during earlier periods. This suggests an aging effect of prenatal exposure to the hydroxylated PCB metabolite. Previous research has also shown that prolongation of the estrous cycle occured between PND 210-230[5].

We are currently determining if this phenomenon is also present in the BFR-exposed groups, and investigating possible mechanisms underlying this aging effect.

Table 1: Effects of prenatal exposure to A) BFRs and B) PCB metabolites, from GD10-16, on some developmental landmarks in rat.

Table 1: A

Parameters		Control	Aroclor 1254 (25 mg/kg)	BDE-47 (20 mg/kg)	6-OH-BDE47 (5 mg/kg)	2,4,6-TBPh (25 mg/kg)	TBBPA (25 mg/kg)
nr litters		12	7	9	12	10	10
litter size		12.9 ± 1.3	10.6 ± 3.4	12.8 ± 1.6	10.9 ± 3.1	13.0 ± 1.3	10.8 ± 2.3
Gestational period		20.8 ± 0.5	21.4 ± 0.5	21.0 ± 0.5	21.3 ± 0.5	20.9 ± 0.3	21.0 ± 0.7
male to female ratio		1.2 ± 0.4	1.3 ± 1.8	1.5 ± 1.2	1.2 ± 0.9	1.7 ± 1.1	1.1± 1.0
body weight PND 4	male	10.94 ± 1.06	10.46 ± 1.19	10.72 ± 0.71	11.91 ± 0.71	11.07 ± 0.97	11.59 ± 0.61
	female	10.55 ± 1.01	9.70 ± 0.73	10.16 ± 0.56	11.38 ± 0.62	10.55 ± 0.78	11.03 ± 0.72
AGD PND 4	male	4.27 ± 0.23	4.32 ± 0.24	4.21 ± 0.23	4.22 ± 0.16	4.28 ± 0.26	4.38 ± 0.19
	female	1.87 ± 0.17	1.88 ± 0.12	1.78 ± 0.11	1.78 ± 0.16	1.77 ± 0.17	1.78 ± 0.14
CRL PND 4	male	61.17 ± 2.11	60.50 ± 2.07	61.71 ± 1.14	63.79 ± 1.32	61.68 ± 1.99	63.23 ± 1.29
	female	60.71 ± 2.53	58.97 ± 2.14	60.20 ± 1.57	61.99 ± 1.61	60.09 ± 2.12	61.73 ± 1.43
AGD/CRL PND 4	male	0.070 ± 0.0035	0.072 ±0.004	0.068 ±0.004	0.066 ± 0.002	0.069 ±0.004	0.069 ± 0.003
	female	0.031 ± 0.003	0.032 ± 0.002	0.030 ± 0.002	0.029 ± 0.002	0.030 ± 0.003	0.029 ± 0.002
Age at bilateral eye opening	male	15.5 ± 0.5	14.9 ± 0.7	15.2 ± 0.5	15.1 ± 0.4	15.6 ± 0.4	15.4 ± 0.5
	female	15.4 ± 0.5	14.8 ± 0.7	15.1 ± 0.3	15.1 ± 0.5	15.6 ± 0.3	15.2 ± 0.6
Age at vaginal opening		32.7 ± 1.3	33.0 ± 1.0	33.2 ± 1.6	32.0 ± 1.7	32.7 ± 1.1	32.4 ± 1.1
Age at preputial separation		43.2 ± 1.6	43.3 ± 1.2	42.2 ± 2.0	41.8 ± 0.8	42.2 ± 1.4	42.41 ± 1.26

AGD = anogenital distance (in mm)
CRL = crown-rump length (in mm)
Data are given as mean ± standard deviation

Table 1: B

Parameters		Control	4-OH-CB 107 (5 mg/kg)	4-OH-CB 187 (0.5 mg/kg)
nr litters		7	9	7
litter size		12.7 ± 1.8	11.7 ± 2.1	12.0 ± 1.0
Gestational period		21.9 ± 0.4	22 ± 0.0	21.9 ± 0.7
male to female ratio		1.0 ± 0.4	0.7 ± 0.5	2.2 ± 1.4
body weight PND 10	male	23.2 ± 1.3	24.2 ± 1.5	23.6 ± 2.1
	female	22.4 ± 1.4	23.5 ± 1.5	23.0 ± 1.7
AGD PND 10	male	5.82 ± 0.40	5.65 ± 0.64	5.97 ± 0.44
	female	3.43 ± 0.11	3.35 ± 0.48	3.4 ± 0.26
CRL PND 10	male	71.48 ± 0.88	72.13 ± 0.90	71.85 ± 1.17
	female	71.03 ± 0.91	71.26 ± 0.88	71.32 ± 1.24
AGD/CRL PND 10	male	0.081 ± 0.006	0.078 ± 0.009	0.083 ± 0.006
	female	0.048 ± 0.002	0.047 ± 0.007	0.048 ± 0.004
Age at bilateral eye opening	male	15.68 ± 0.28	15.3 ± 0.4	15.7 ± 0.8
	female	15.36 ± 0.45	15.1 ± 0.3	15.6 ± 0.8
Age at vaginal opening		33.43 ± 0.88	32.6 ± 0.8	33.0 ± 1.5
Age at preputial separation		43.21 ± 1.15	41.8 ± 0.8	43.2 ± 1.2

AGD = anogenital distance (in mm)
CRL = crown-rump length (in mm)
Data are given as mean ± standard deviation

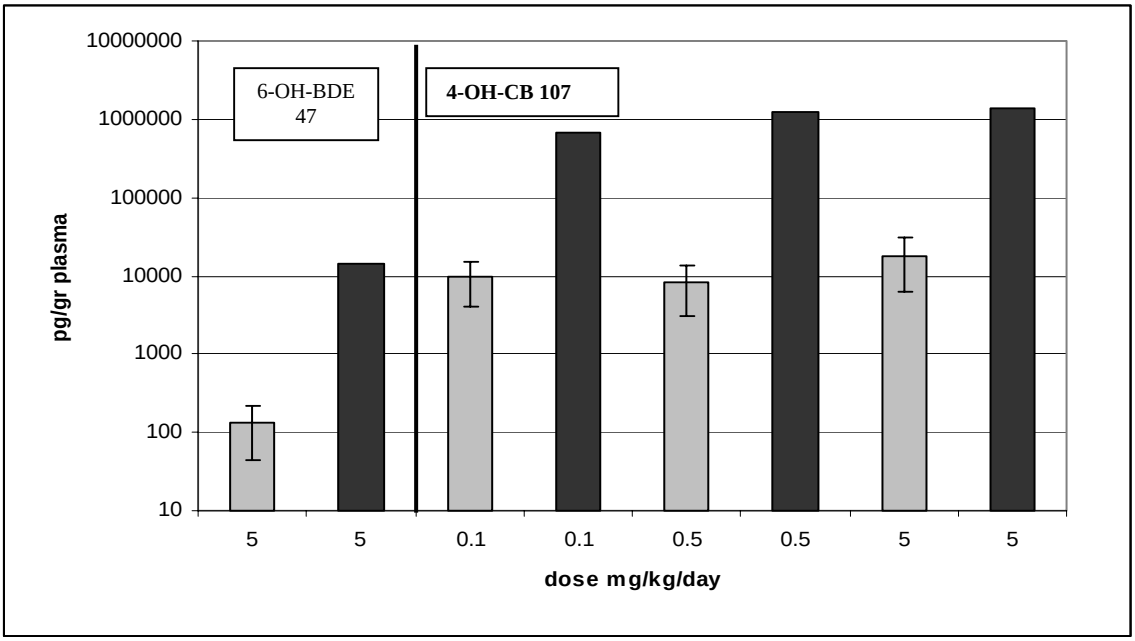

Figure 1. Plasma concentrations of 6-OH-BDE 47 and 4-OH-CB107 in rats following prenatal exposure (gestation day 10-16). Compounds were measured in pooled plasma samples of F1 pups at PND 4 (right bars) and in individual F0 mothers at PND 24 (n=3-4) (left bars). Data are given as mean ± S.E.M.

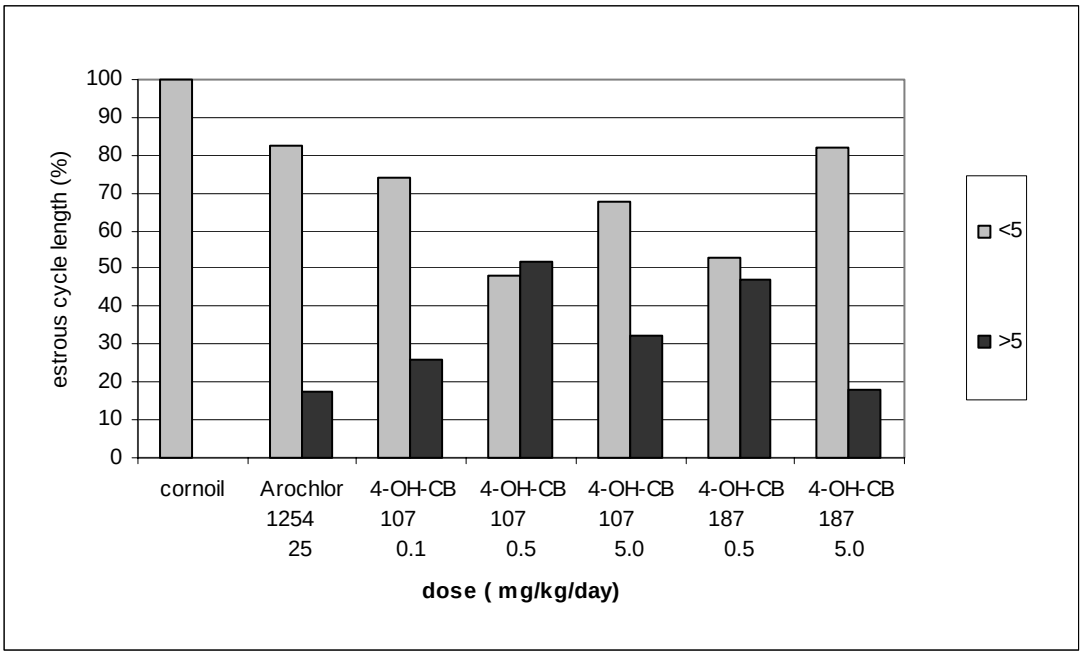

Figure 2. Effects of prenatal exposure, from GD10-16 to hydroxylated PCB metabolites on the estrous cycle of female rats between PND 210-230. Shown is the percentage of animals with an average estrous cycle equal to or shother than 5 days (gray bars) or an estrous cycle longer than 5 days (black bars)

Conclusions:

The results indicate that both the hydroylated PCB metabolites and the BFRs are capable of placental transfer. The concentrations used in the experiments had no effect on the developmental landmarks, indicating that non teratogenic concentrations were used. The results of prenatal exposure to 4-OH-CB 107 and 4-OH-CB 187 indicate an aging effect on the estrous cycle of the female rats.

Acknowledgement

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