The effects of exposure to environmental chemicals on child development

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CHAPTER 5

The effects of prenatal exposure to organohalogen compounds on infant's mental and motor development at 18 and 30 months of age

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ABSTRACT

Background: Organohalogen compounds (OHCs), i.e. polychlorinated biphenyls (PCBs) and brominated diphenyl ethers (BDEs), are widely spread environmental pollutants, known to be neurotoxic for the developing brain. Hydroxylated metabolites of PCBs (OH-PCBs) seem to be even more toxic. However, little is known about the toxicity of OH-PCBs in humans.

Objectives: To determine whether prenatal background exposure to OHCs has an effect on mental and motor development in children at the age of 18 and 30 months.

Methods: 181 healthy mother-infant pairs were included in this Dutch observational study. Maternal pregnancy levels of PCB-153 and three OH-PCBs were measured. In one part of the cohort another nine PCBs and three OH-PCBs were measured, in the other part five brominated diphenyl ethers (BDEs), dichloro-diphenyldichloroethylene (p,p'-DDE), pentachlorophenol (PCP) and hexabroomcyclododecane (HBCDD). Bayley's Scale of Infant Development II (BSID-II) was used to assess the infants' mental development index (MDI) en motor development index (PDI) (mean=100; 'delayed score'<85).

Results: Higher 4-OH-PCB-187 correlated with delayed MDI at 18 months. Four OH-PCBs (107, 138, 153 and 172) and ∑6 OH-PCBs correlated positively with MDI at 30 months. Higher PCB-153-levels correlated with delayed MDI at 18 months. p,p'-DDE-, PCP- and HBCDD-levels were not associated with MDI or PDI at 18 months.

Conclusions: Prenatal exposure to 4-OH-PCB-187 has an adverse effect on mental development at 18 months. At 30 months, four OH-PCBs and ∑6 OH-PCBs were positively correlated with MDI. PCB-153 and BDE-99 has adverse effects on mental development. Prenatal p,p'-DDE-, PCP- and HBCDD-levels were not associated with neurological development at 18 months.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are organohalogen compounds (OHCs). These chemical compounds were widely used in industry as for example fire retardants, hydraulic liquids and lubricants. Although these compounds have been banned by law, their properties cause them to be hard to break down and therefore they persist in the environment.[1] This raises grave concerns as studies have shown that PCBs exert adverse effects, including neurotoxicity and endocrine disruption. Animal studies showed that endocrine disruption mainly involve the reproductive and thyroid system. Developing organisms seem to be the most vulnerable.[2]

Several studies have been performed on the effects of exposure to PCBs on mental development in children. The outcomes of these studies are not consistent; some reported negative associations, whereas others reported positive or no associations (reviewed by El Majidi et al.[3]). PCBs seem to have a greater effect on motor development compared to mental development.[4-8] In the present cohort, we found a relation between several PCBs and impairment of early motor development in three-month-old children by evaluation of the movement repertoire.[9]

PCBs can be converted by the liver into hydroxylated polychlorinated biphenyls (OH-PCBs) stimulated by the P450-enzym complex. Compared with PCBs, OH-PCBs are water soluble and are transferred in a higher ratio from the mother to the fetus during pregnancy.[10] Due to these properties OH-PCBs might be more toxic than PCBs, but only a small amount of research is available on the effects of OH-PCBs in humans. In our cohort, higher prenatal exposure to 4-OH-PCB-107 was associated with less optimal early motor development in three-month-old infants, whereas 4'-OH-PCB-172 showed an opposite effect.[9] Park et al. studied the effects of six OH-PCBs on neurodevelopment at 16 months, and found that 4-OH-PCB-107 was negatively associated with mental and motor development.[11]

Other OHCs were also found to be associated with poorer mental and motor development (reviewed by Berghuis et al.[12]). Higher prenatal exposure to brominated diphenyl ethers (PBDEs) was associated with poorer mental and psychomotor development and lower IQ at preschool age. In our cohort, higher prenatal exposure to PBDEs was correlated with worse attention, worse fine manipulative abilities, better coordination, better behavior, and better visual perception in children at school age.[13] Regarding prenatal exposure to dichloro-diphenyldichloroethylene (DDE; a breakdown product of the insecticide dichlordiphenyltrichloroethane DDT), some studies reported inverse associations between exposure to DDEs and neurological development, while others found no associations. Exposure to DDEs seems to interfere more with psychomotor development than with mental development.[12]

A reliable method to assess the mental and motor development in children is the Bayley's Scale of Infant Development (BSID).[14, 15] This method is widely used as an infant assessment instrument for both clinical and research purposes.

Evidence for negative health effects of prenatal exposure to PCBs is growing, but limited knowledge exists about the effects of prenatal exposure to OH-PCBs, PBDEs and DDE.[12] Because Dutch prenatal background levels of PCBs are estimated to be three times higher than levels in the USA, and as a consequence the levels of OH-PCBs might also be higher, it is indicated to investigate whether Dutch background exposure exerts negative health effects.[16] Whether prenatal Dutch levels of OH-PCBs has an influence on neurological development at 18 and 30 months has not been studied so far. Because we assessed the development at a young age, which minimizes the effects of postnatal exposure, the findings of this study adds knowledge to the impact of prenatal background chemical exposure on child development.

The aim of this study was to determine whether prenatal Dutch background exposure to PCBs, OH-PCBs and other OHCs is associated with the mental and motor development of children at the age of 18 and 30 months. We hypothesized that exposure to higher levels of PCBs, OH-PCBs and other OHCs has a negative effect on the mental and motor development of children.

METHODS

Cohort

For this observational longitudinal cohort study, we included two cohorts composed in the northern part of the Netherlands. The first group of the study population is part of the Risk of Endocrine Contaminants on human health (RENCO) study.[10] The second group is obtained by the Groningen-Infant-COMPARE (Comparison of Exposure-Effect Pathways to Improve the Assessment of Human Health Risks of Complex Environmental Mixtures of Organohalogens) study, otherwise known as the GIC study.[17] This study was launched as part of the European COMPARE study. The RENCO cohort included pregnant women who lived in the northern part of the Netherlands. Between September 1998 and December 2000, the women were approached by their midwife or obstetrician to participate in a study on the potential effects of exposure to PCBs and OH-PCBs on the development of the child. The second group consisted of a selection of the women who were included in the GIC study. These women were invited by their midwives between October 2001 and November 2002. Both cohorts included women of western origin, who had Dutch as native language. Exclusion criteria involved women who experienced serious illness and/or complications during pregnancy and/or delivery. Only infants born at term, 37 to 42 weeks of gestation, were included.

An infant was excluded if congenital anomalies or diseases were present. If an infant had been admitted to a hospital for more than 1 day after birth, they were also excluded. The medical ethics committee of the University of Groningen approved the study.

Chemical analyses

In the RENCO and in the GIC study, a blood sample was taken from the women at the 35th week of pregnancy for the determination of PCBs in the blood.[10, 17] To determine the concentrations of PCBs and OH-PCBs, the blood was collected in a vacuum system tube (EDTA) and was centrifuged for 10 minutes at 3,600 rpm (RENCO cohort) or 5 minutes at 4,000 rpm (GIC cohort). Thereafter, the plasma was collected in separate glass tubes. These tubes were closed with a screw cap with Teflon inlayers and stored at -18 C° to -20 C° until analysis. Both studies used Hovander's clean-up and extraction procedure, which is described elsewhere.[18] The detailed description of the analyses of the blood samples have been published previously.[10, 17] To determine the effect of PCBs on the development of children, the RENCO cohort measured ten PCB congeners and six OH-PCBs. We also determined the sum of all measured PCBs and OH-PCBs. For ΣPCBs, this is the sum of all ten measured PCBs. For ΣOH-PCBs, this involves all six measured OH-PCBs. In Table 2 and 3, the measured PCB compounds and the OH-PCB metabolites are shown. The GIC cohort measured PCB-153, three hydroxylated PCB metabolites, p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE), pentachlorophenol (PCP), five different brominated diphenyl ethers (BDEs) and hexabroomcyclododecane (HBCDD). The numbering of PCBs is according Ballschmiter et al. and the OH-PCBs according to Letcher et al.[19, 20]

Assessment of neurological development

To determine the neurological development of the child, we administered the second version of Bayley's Scales of Infant Development (BSID-II) in both cohorts at 18 and in the RENCO cohort also at 30 months of age. This test is a standardized measure for the evaluation of development in children aged between 1 and 42 months. It measures both mental and motor development and is widely used as an infant assessment instrument for both clinical and research purposes.[15] The tests were conducted by trained examiners, who were unaware of the infant's levels of PCBs and OH-PCBs. The BSID-II provides scores for mental and motor development of the child. The mental development is expressed in the Mental Development Index (MDI) and consists of 178 items. The mental scale assesses the age-appropriate level of cognitive functioning, personal and social development, and language development. The motor development is expressed in the Psychomotor Development Index (PDI) and consists of 111 items. The motor scale assesses fine and gross motor skills. The test gives raw scores for mental and motor development, which can be converted into standardized index scores MDI and PDI. These index scores have a mean of 100, and a standard deviation of 15. A child

with a test score below 85 is considered to have a mildly delayed development, a score below 70 is considered to be severely delayed.

Statistical analyses

To investigate whether prenatal exposure to OHCs is associated with neurological development at 18 or 30 months of age, we used Spearman's Rank correlation test to assess correlations between levels of OHCs and MDI and PDI scores at the age of 18 and 30 months. Next, we performed univariate logistic regression analyses for those compounds that showed a statistically significant correlation with abnormal MDI or PDI (<85) and calculated odds ratios (ORs) with 95% confidence intervals (95% CI). To determine potential confounders, we used univariate logistic regression analyses. We considered gender, gestational age, birth weight, maternal smoking during pregnancy, maternal alcohol use during pregnancy, parity and maternal education as potential confounders. We adjusted for confounding using multiple logistic regression analyses, entering these variables into the model if there was an association between levels of OHCs and these variables with $p<0.15$. The tests were two-sided. We considered a *P*-value of less than 0.05 to be statistically significant. A *P*-value less than 0.1, but more than 0.05 will be mentioned as a trend, as is usual for toxicological studies. For all statistical analyses, we used 'Statistical Package for the Social Sciences (SPSS) version 22.0.

RESULTS

Study group

The study group initially consisted of 194 mother-infants pairs (MI-pairs). 104 of the MI-pairs were obtained by Soechitram et al. in the RENCO cohort.[10] Another 90 MI-pairs were obtained by Meijer et al., who were part of the GIC cohort.[17] Four of the mother-infant pairs had to be excluded of the RENCO cohort, because no values of OHCs were obtained. Of the remaining 190 MI-pairs, nine MI-pairs were excluded from the study because no BSID-II scores were available at 18 months. In total, 181 (95.3%) of the 190 MI-pairs were included at the age of 18 months. At follow-up at 30 months of age, 63 (67.0%) of the 94 invited MI-pairs of the RENCO cohort participated for assessment of BSID-II scores. Table 1 shows the characteristics of the study group. In the RENCO study a greater proportion of the mothers had been smoking during pregnancy (23% versus 9% in the GIC cohort). The mothers included in the GIC cohort had more often a higher level of education in comparison to the mothers included in the RENCO cohort (resp. 47% and 56%).

Table 1. Characteristics of the study group

Characteristic	Both cohorts (*n=181*)	RENCO (*n=94*)	GIC (*n=87*)
Infant			
Gender (male/female)	105/76 (58%)	50/44 (53%)	55/32 (63%)
Gestational age (weeks, mean ± SD)	40 ± 1	40 ± 1	40 ± 1
Apgar score 1 min [median (range)]	9 (2-10)	9 (2-10)	9 (3-10)
Apgar score 3 min [median (range)]	10 (6-10)	10 (6-10)	10 (7-10)
Birth weight (grams; mean ± SD)	-	3628 ± 499	-
Age at examination (months; mean ± SD)	18 ± 0.5	18 ± 0.5 30 ± 0.5	18 ± 0.5
Mother			
Maternal age (years; mean ± SD)	32 ± 4	32 ± 4	32 ± 4
Maternal smoking, yes/no (%)	30/150 (17%)	22/72 (23%)	8/78 (9%)
Maternal alcohol consumption, yes/no (%)	46/134 (25%)	23/71 (24%)	23/63 (26%)
Parity, first-born/second- or third-born (%)	63/118 (35%)	34/60 (36%)	29/58 (33%)
Maternal education level (%)			
Below average (≤11 years education)	16 (9%)	12 (13%)	4 (5%)
Average (12-13 years education)	72 (40%)	38 (40%)	34 (39%)
Above average (≥14 years education)	93 (51%)	44 (47%)	49 (56%)

Levels of OHCs

The measured values of PCBs and other OHCs are presented in Table 2. Table 3 shows the values of the OH-PCB congeners. The most abundant OHCs are PCB-153 (83 ng/g lipid weight) and p,p'-DDE (115 ng/g lipid weight). 4-OH-PCB-187 had the highest mean concentration for the OH-PCBs (87 ng/g fresh weight).

Table 2. Levels of organohalogens in maternal blood samples (ng/g lipid weight)

Compound	Median (IQR)	*n*
PCB-105[a]	4.2 (2.1-11.3)	94
PCB-118[a]	21.0 (14.6-33.9)	94
PCB-138[a]	68.7 (48.9-86.2)	94
PCB-146[a]	8.2 (5.0-13.7)	94
PCB-153	88.0 (68.8-144.0)	181
- RENCO	91.9 (63.2-123.2)	94
- GIC	62.9 (43.2-80.4)	87
PCB-156[a]	11.1 (7.8-14.7)	92
PCB-170[a]	19.0 (13.5-25.4)	92
PCB-180[a]	45.2 (31.7-58.5)	92
PCB-183[a]	8.2 (5.6-10.4)	94
PCB-187[a]	12.3 (8.6-17.8)	94
Σ 10 PCBs[a]	296.8 (217.5-391.1)	92
p,p'-DDE[b]	88.0 (68.8-144.0)	87
PCP[b]	972.5 (686.3-1641.2)	87
BDE-47[b]	0.85 (0.53-1.30)	60
BDE-99[b]	0.20 (0.10-0.40)	57
BDE-100[b]	0.20 (0.10-0.30)	60
BDE-153[b]	1.55 (1.20-2.20)	60
BDE-154[b]	0.50 (0.40-0.78)	60
HBCDD[b]	0.82 (0.47-1.26)	59

[a]RENCO cohort, [b]GIC cohort.

Table 3. Levels of OH-PCBs in maternal serum samples (pg/g fresh weight)

Compound	Median (IQR)	*n*
4-OH-PCB-107	42.0 (23.0-76.6)	175
- RENCO	69.0 (42.0-100.0)	91
- GIC	26.1 (17.8-38.6)	84
3'-OH-PCB-138[a]	46.0 (31.0-66.0)	91
4-OH-PCB-146	80.3 (61.0-128.4)	178
- RENCO	70.0 (53.0-100.0)	91
- GIC	102.4 (72.4-140.0)	87
3-OH-PCB-153[a]	38.0 (24.0-54.0)	91
4'-OH-PCB-172[a]	16.0 (10.0-22.0)	72
4-OH-PCB-187	105.0 (78.6-148.0)	178
- RENCO	136.0 (105.0-172.0)	91
- GIC	79.8 (59.3-100.6)	87
Σ 6 OH-PCBs[a]	391.0 (289.0-535.0)	91

[a]RENCO cohort.

Bayley's Scale of Infant Development

The BSID-II scores of the children at 18 and 30 months are presented in Table 4. The mean scores on MDI and PDI in the 181 children included at 18 months were respectively 97.1 and 90.1. At 18 months, 13 percent of the children had a delayed score (<85) on MDI and 31 percent on PDI. In the 63 infants included at 30 months of age, the mean MDI and PDI scores were 98.5 and 94.2, respectively. At 30 months, 11 percent of the children had a delayed score (<85) on MDI and 18 percent on PDI.

Table 4. Bayley's Scale of Infant Development scores in infants at 18 and 30 months of age

BSID-outcome	Both cohorts 18 months	RENCO 18 months	GIC 18 months	RENCO 30 months
MDI	*(n=181)*	*(n=94)*	*(n=87)*	*(n=63)*
Mean ± SD	97.1 ± 13.3	97.6 ± 13.8	96.7 ± 12.7	98.5 ± 10.7
Normal (≥85)	157 (87%)	84 (89%)	73 (84%)	56 (89%)
Mildly delayed (70-85)	21 (12%)	8 (9%)	13 (15%)	7 (11%)
Severely delayed (<70)	3 (2%)	2 (2%)	1 (1%)	0 (0%)
PDI	*(n=181)*	*(n=94)*	*(n=87)*	*(n=62)*
Mean ± SD	90.1 ± 10.2	89.3 ± 10.5	91.1 ± 9.9	94.2 ± 15.1
Normal (≥85)	124 (69%)	61 (65%)	63 (72%)	51 (82%)
Mildly delayed (70-85)	51 (28%)	29 (31%)	22 (25%)	7 (11%)
Severely delayed (<70)	6 (3%)	4 (4%)	2 (2%)	4 (6%)

MDI: mental development index; PDI: psychomotor development index.

Correlations prenatal exposure and OHCs and BSID scores

In Table 5 we present the Spearman's correlation coefficients of the relations between prenatal exposure to OHCs and BSID scores at the age of 18 and 30 months. Regarding exposure to OH-PCBs and mental development, higher exposure to 4-OH-PCB-187 in the GIC cohort correlated with a lower MDI score at 18 months. At 30 months of age, higher exposure to four individual OH-PCBs and the Σ6 OH-PCBs correlated with a higher MDI, a trend was seen for one OH-PCB (Table 5). Regarding exposure to OH-PCBs and psychomotor development, only higher exposure to 4'-OH-PCB-172 correlated with higher PDI at 30 months of age.

Regarding exposure to PCBs and mental development, only a trend was found between some PCB congeners and MDI scores at 18 and 30 months (Table 5). Higher exposure to PCB-153 correlated with lower MDI scores at 18 months. PCB-170 showed a positive correlation with MDI scores at 18 and 30 months, and also PCB-180 showed a positive correlation with MDI scores at 30 months of age. No correlations were found between prenatal exposure to PCBs and PDI scores at 18 or 30 months.

Regarding exposure to the other measured OHCs and mental development at 18 months of age, only higher exposure to BDE-99 correlated marginally significantly with lower MDI scores. Regarding psychomotor development at 18 months, BDE-100 correlated positively and BDE-47 showed a positive trend with higher PDI scores.

Table 5. Spearman's correlation coefficients of organohalogen compounds and Bayley's Scale of Infant Development scores at age of 18 and 30 months

	18 months					30 months					
Compound	*n*	MDI	*P*-value	PDI	*P*-value	*n*	MDI	*P*-value	*n*	PDI	*P*-value
PCB-105[a]	94	-.030	.777	-.020	.849	63	-.112	.383	62	-.138	.284
PCB-118[a]	94	.050	.635	.024	.818	63	-.029	.823	62	-.153	.234
PCB-138[a]	94	.101	.331	.139	.182	63	.129	.315	62	.055	.671
PCB-146[a]	94	.008	.941	.027	.798	63	.035	.788	62	-.028	.829
PCB-153	181	-.002	.975	.035	.640	63	.071	.578	62	.029	.825
- *RENCO*	*94*	*.092*	*.380*	*.124*	*.234*						
- *GIC*	*87*	*-.186*	*.085#*	*-.017*	*.872*		*n.a.*			*n.a.*	
PCB-156[a]	92	.073	.491	.124	.241	61	.093	.476	60	.063	.633
PCB-170[a]	92	.193	.066#	.134	.203	61	.225	.082#	60	.143	.277
PCB-180[a]	92	.169	.107	.132	.211	61	.218	.092#	60	.161	.219
PCB-183[a]	94	.028	.792	.060	.568	63	-.041	.752	62	.035	.787
PCB-187[a]	94	.072	.488	.052	.618	63	.031	.811	62	.040	.756
Σ 10 PCBs[a]	92	.092	.383	.112	.286	61	.100	.442	60	.015	.907
4-OH-PCB-107	175	.070	.355	.018	.812	61	.378	.003**	60	.172	.189
- *RENCO*	*91*	*.149*	*.159*	*.138*	*.192*						
- *GIC*	*84*	*-.076*	*.490*	*.056*	*.613*		*n.a.*			*n.a.*	
3'-OH-PCB-138[a]	91	.026	.804	.004	.969	61	.257	.046*	60	.132	.313
4-OH-PCB-146	178	-.061	.419	.072	.337	61	.243	.059#	60	.140	.285
- *RENCO*	*91*	*.071*	*.503*	*.136*	*.198*						
- *GIC*	*87*	*-.143*	*.185*	*-.072*	*.506*		*n.a.*			*n.a.*	
3-OH-PCB-153[a]	91	.144	.174	.012	.910	61	.298	.020*	60	.090	.495
4'-OH-PCB-172[a]	72	.154	.198	.024	.843	48	.371	.009**	47	.262	.075#
4-OH-PCB-187	178	-.109	.147	-.116	.122	61	.069	.595	60	-.064	.626
- *RENCO*	*91*	*-.028*	*.793*	*.002*	*.984*						
- *GIC*	*87*	*-.231*	*.032**	*-.176*	*.103*		*n.a.*			*n.a.*	
Σ 6 OH-PCBs[a]	72	.066	.580	-.050	.677	48	.382	.007**	47	.160	.281

Table 5 continued

	18 months				30 months					
Compound	***n***	**MDI**	***P*-value**	**PDI**	***P*-value**	***n***	**MDI**	***P*-value**	***n***	**PDI**	***P*-value**
p,p'-DDE[b]	87	-.051	.642	.090	.407		n.a.			n.a.	
PCP[b]	87	-.067	.537	.072	.505		n.a.			n.a.	
BDE-47[b]	60	-.182	.164	.237	.068#		n.a.			n.a.	
BDE-99[b]	57	-.253	.058#	.187	.164		n.a.			n.a.	
BDE-100[b]	60	-.082	.533	.273	.035*		n.a.			n.a.	
BDE-153[b]	60	.001	.995	.183	.161		n.a.			n.a.	
BDE-154[b]	60	-.138	.294	-.100	.448		n.a.			n.a.	
HBCDD[b]	59	-.095	.473	.003	.979		n.a.			n.a.	

[a]RENCO cohort, [b]GIC cohort; **p<0.01; *p<0.05; #p<0.10; MDI: mental development index; PDI: psychomotor development index; n.a.: not assessed

Prenatal exposure to OHCs and BSID scores of delayed development

For the compounds which were found to be significantly correlated or showed a trend with BSID scores using Spearman's correlation test (Table 5), we calculated the ORs for associations with a 'delayed' score, after dichotomizing the scores into 'normal' and 'delayed' (Table 6). Higher prenatal levels of PCB-153 and 4-OH-PCB-187 were significantly associated with a delayed MDI score at 18 months, and higher levels of BDE-99 showed a trend with a delayed score (Table 6). Next, we performed univariate regression analyses to analyze whether factors might have confound our findings. Because none of the potential confounders were associated with a delayed score on MDI at 18 months, we did not perform multivariate logistic regression analyses to adjust the associations for these factors.

Regarding PDI scores at 18 months and BSID scores at 30 months of age, none of the compounds found to be correlated with BSID scores (using Spearman's correlation test) were associated with a 'delayed' BSID score when using univariate logistic regression analyses. Regarding potential confounding factors influencing PDI scores at 18 months or BSID scores at 30 months, only lower gestational age (≤40 weeks of pregnancy; OR=2.556, p=0.005) and lower maternal education level (<14 years of education; OR=1.725, p=0.092) were associated with delayed PDI scores at 18 months, and included as potential confounders in the multivariate logistic regression models (Table 6).

Table 6. Odds ratios for associations between prenatal organohalogen compound levels and delayed mental or motor development at 18 and 30 months of age

Compound	Outcome	Age in months	OR (95% CI)	*P*-value	Adjusted OR[c] (95% CI)	*P*-value
PCB-153[b,d]	MDI	18	1.02 (1.00-1.04)	**0.030***		
PCB-170[a,d]	MDI	18	0.98 (0.91-1.07)	0.671		
	MDI	30	0.97 (0.88-1.07)	0.529		
PCB-180[a,d]	MDI	30	0.98 (0.94-1.02)	0.362		
4-OH-PCB-107[a,f]	MDI	30	0.98 (0.95-1.01)	0.110		
3'-OH-PCB-138[a,f]	MDI	30	0.99 (0.96-1.02)	0.443		
4-OH-PCB-146[a,f]	MDI	30	0.99 (0.96-1.01)	0.270		
3-OH-PCB-153[a,f]	MDI	30	0.98 (0.94-1.02)	0.264		
4'-OH-PCB-172 [a,f]	MDI	30	0.87 (0.72-1.05)	0.153		
	PDI	30	0.97 (0.87-1.07)	0.497		
4-OH-PCB-187[a,f]	MDI	18	1.39 (1.15-1.69)	**0.001****		
Σ 6 OH-PCBs[a,f]	MDI	30	0.99 (0.99-1.00)	0.153		
BDE-47[b,d]	PDI	18	0.80 (0.44-1.46)	0.464	0.82 (0.46-1.48)	0.517
BDE-99[b,e]	MDI	18	1.14 (0.98-1.33)	**0.089#**		
BDE-100[b,e]	PDI	18	0.88 (0.67-1.17)	0.379	0.90 (0.68-1.18)	0.432

[a]RENCO cohort, [b]GIC cohort; [c]adjusted for gestational age and maternal education level; [d]per ng/g lipid weight, [e]per 0.1 ng/g lipid weight, [f]per pg/g fresh weight; **p<0.01; *p<0.05; #p<0.10 OR: odds ratio, CI: Confidence Interval; MDI: mental development index; PDI: psychomotor development index.

DISCUSSION

In the present study, we found that higher prenatal Dutch background exposure to several OHCs is associated with the mental or motor development at 18 or 30 months of age. We found both positive and negative associations. Our most important finding was that OH-PCBs were negatively associated with mental development at 18 months, and positively associated with mental development at 30 months. Regarding motor development, a positive correlation was found for 4'-OH-PCB-172 at 30 months. Moreover, PCB-153 and BDE-99 had an adverse effect on mental development at 18 months, and two PCBs were positively associated with mental development at 30 months. Two PBDEs were positively associated with motor development at 18 months.

Prenatal exposure to OH-PCBs and neurodevelopment

Our most important finding is that prenatal exposure to OH-PCBs is associated with mental development. Higher exposure to one metabolite, 4-OH-PCB-187, was associated with delayed mental development at the age of 18 months, whereas positive associations were found between four OH-PCBs (4-OH-PCB-107, 3'-OH-PCB-138, 3-OH-PCB-153 and 4'-OH-PCB-172) and the sum of the six measured OH-PCBs and mental development at 30 months. Regarding motor development, only an effect was seen for 4'-OH-PCB-172, which was positively associated with motor development at 30 months.

The finding that 4'-OH-PCB-172 was positively associated with motor development at 30 months is consistent with our previous finding that the compound was positively associated with motor development at 3 months of age.[9] In contrast to previous studies and findings in our cohort at the age of 3 months, we did not find negative effects of the metabolite 4-OH-PCB-107 on motor development. An animal study in rats indicated that exposure to 4-OH-PCB-107 had a long term effect on development and behavior.[21] In the infants included in the RENCO cohort, background exposure to 4-OH-PCB-107 was associated with impairment of the motor development at the age of three months[9] and a negative association between 4-OH-PCB-107 and neurological function in boys at three months of age.[22] A Slovakian study found a negative association between prenatal exposure to 4-OH-PCB-107 and motor development in 16-month-old children, assessed by BSID-II.[11] A possible explanation for the fact that we did not find associations with levels of 4-OH-PCB-107 might be differences in levels of exposure to OH-PCB. In our study we found higher levels of 4-OH-PCB-107 than Park and collegues; 52 versus 37 pg/g wet weight.[11] We found lower levels of the other OH-PCB congeners in comparison to the Slovakian study; for 4-OH-PCB-146 99 pg/g versus 147 pg/g fresh weight, and for 4-OH-PCB-187 117 pg/g versus 273 pg/g fresh weight. However, the mean PDI found by Park et al. was much higher than in our study; 99.8 versus 90.1. In both studies, multiple examiners performed the BSID-II test. Because in the Slovakian study a Slovakian modification of the American Version was used, and in our study a standardized Dutch version was used, comparisons between the populations can be unreliable.

The Slovakian study also investigated other OH-PCB congeners, but these congeners did not show any significant associations with mental or motor development at 16 months of age.[11] This is partly in line with our results, finding only one OH-PCB metabolite associated with the mental development at the age of 18 months. We mainly found associations between prenatal levels of OH-PCBs and mental development at 30 months of age, whereas these OH-PCBs were not found to be associated with development at the age of 18 months. A possible explanation for the fact that we did not observe effects at 18 months of age could be that the effects might appear more subtle at younger ages, and therefore might not result in significant changes on MDI scores. We speculate that in later life the effects of prenatal exposure on developmental outcomes might become more obviously present, which might explain our finding of effects of four OH-PCBs on mental scores at 30 months.

Prenatal exposure to PCBs and neurodevelopment

Higher exposure to PCB-153 was found to be associated with a higher risk for an abnormal MDI score (<85) at the age of 18 months. In addition, trends were seen between PCB-170 and PCB-180 and neurological development: PCB-170 was positively associated with mental development at 18 and 30 months, and PCB-180 only at 30 months.

Other studies on the effects of PCBs showed different outcomes for the mental than for motor development, as reviewed by El Majidi et al. and Faroon et al.[3, 23] The results of the

previous studies indicate a relation between prenatal exposure to PCBs and a decrease in motor skills during first months of life. Effects later on seem to involve mainly cognitive areas.[24] Exposure to especially PCB-153 is often found to be associated with developmental outcomes in children.[25-27] PCB-153 is the most abundant congener in humans, which can be an explanation for our finding that we only found an effect of PCB-153. An animal study found that PCB-153 affects regulation of intracellular signalling systems in the brain.[28] Moreover, PCB-153 alters neurotransmitter function in rats, causing a decrease in brain serotonin and dopamine, which are essential for proper development of the brain.[29]

Prenatal exposure to PBDEs and neurodevelopment

We found a negative trend between prenatal levels of BDE-99 and mental development at 18 months of age. Regarding motor development, BDE-100 and BDE-47 showed a positive trend with outcomes at 18 months. Regarding exposure to PBDEs, most studies demonstrated strong inverse effects on mental development. For motor development, inconsistent effects were found (reviewed by Roth and Wilks[30]). Animal studies seem to confirm the impact on mental development. They revealed exposure to PBDEs alter spontaneous behavior, resulting in increased impulsivity, hyperactivity and disrupted habituation. Moreover, attention, learning and memory function were impaired (reviewed by Dingemans et al.[31]). Our results are in contrast with previously reported results obtained at five years of age. Higher prenatal exposure to PBDEs was correlated with worse attention, worse fine manipulative abilities, better coordination, better behavior, and better visual perception in children at school age.[13] It might well be that effects of prenatal exposure to PBDEs are better detectable at a later age. More studies on the potential effects of prenatal exposure to PBDEs on psychomotor development are needed.

Prenatal exposure to DDE, PCP and HBCDD and neurological development

We did not find associations between prenatal exposure to p,p'-DDE, PCP or HBCDD and mental or motor development at 18 months of age. Several studies on the effects of p,p'-DDE showed impairment of mental and motor development in children aged between 3 and 24 months (reviewed by Eskenazi et al.[32]). In later life, studies predominantly reported no associations between exposure to p,p'-DDE and neurodevelopment. A possible explanation for the fact that we did not find associations might be that the levels measured in our study were too low to exert effects in the infants.

Effect of OHCs on endocrine functioning

According to an often mentioned theory, neurotoxicity is the result of the interactions of OHCs with the endocrine system, particularly involving the thyroid and sex hormone systems.[33] Disruption of the endocrine system can be the result of an agonistic or antagonistic action of the chemicals. Several OHCs show high similarities with chemical structures of thyroid hormones and sex steroids.[1, 34] The thyroid hormone system is of great importance for normal maturation of the brain; it influences neuronal proliferation and migration in the brain, as well as synapse formation.[35] The thyroid hormone system also is essential for the timing of these processes. Studies in animals and humans showed that exposure to PCBs can lead to disturbance of the thyroid hormone system (for review see Brouwer et al.[33]).[36, 37] In vitro studies showed that both PCBs and OH-PCBs bind to TTR, but only the affinity of OH-PCBs is high enough to compete with T4 binding.[38, 39] A study in rats, showed an affinity of 4-OH-PCB-107 with the thyroid transport protein TTR.[21] Binding of 4-OH-PCB-107 to TTR could be a reason for lower circulating T4 levels in both fetal plasma and brain of animals, as seen by Morse et al.[40] The presence of OH-PCBs in the fetus may result in a decrease in circulating plasmalevels of T4 and in levels of T4 in brains of fetal animals. A similar mechanism is seen for metabolites of PBDEs.[33] In humans, however, most of T4 transport depends on the thyroxine binding globulin (TBG).[33] Binding of T4 to TTR might be important in humans for transport over the blood-brain barrier. PCBs and OH-PCBs therefore might influence brain levels of thyroid hormones in humans.

Regarding prenatal exposure to OHCs and neurological development, we mainly found associations for OH-PCBs, which suggest OH-PCBs to be more toxic than PCBs and other OHCs, which is similar to the findings in animal studies.[41, 42] According to an animal study, OH-PCBs exhibit agonistic thyroid hormonal activity as a result of interaction with thyroid hormone receptors (TR).[42] Interaction with these TRs disrupts normal thyroid homeostasis, potentially resulting in abnormalities in brain development. The study demonstrated that a 4-hydroxyl group and 3,5-chlorine substituents on the phenyl group were required for this interaction with TR. This is a possible explanation for our findings that OH-PCBs with a 4-hydroxyl group were associated with neurodevelopment.

The strengths and limitations

A strength of our study is the measurement of background exposure of individual OHCs in healthy children, in which we also investigated the effects of hydroxylated PCBs. Only limited studies have been performed on the impact of OH-PCBs on neurological development. The levels of exposure to OH-PCBs are generally lower in comparison to other studies, but nevertheless we did find associations. This suggests that even lower levels seem to affect the child's development. A second strength is that we assessed the development in a part of the cohort at two different ages. Although the sample size is smaller for the analyses at

30 months of age, we were able to get more insight into consistency between the outcomes at 18 and 30 months.

Our study also has limitations. First, we included children from two cohorts. This might have led to differences in inclusion and methods used. Although the GIC cohort included more higher educated women, other characteristics of the two cohorts were comparable, e.g. regarding demographics and methods used. Moreover, our results remained significant after adjustment for maternal education level in the multiple regression models. Secondly, we found relatively low scores for both MDI and PDI. Meta-analyses already showed that findings of the BSID-II might underestimate mental and motor development, resulting in lower scores more often.[43] Another explanation for the relatively low scores could be the relatively young age at examination. The infants were seen at the age of 18 months. At this age, a great range exists in development, regarding the achievement of various milestones in both mental and motor development. Additionally, a rapid development is present at this age, making it hard to obtain a good impression of the development at that specific moment. Testing a few weeks later can make essential differences in scores, if the infant has obtained some milestones in the meantime. Moreover, especially the evaluation of the motor development strongly depends on child's behavior and their willingness to perform the tests, which inquires experience of the examiner. Even so, the BSID-II is a standardized test, taken by experienced testers, and we kept close to 18 months of the age of assessment, resulting in a standard deviation for age of assessment of only 2 weeks. A third limitation is a potential selection bias due to voluntary participation of the women to our study. Women were asked by their midwife to participate. Women who agreed to participate, might be interested in the effects of environmental compounds and therefore more aware of their life style and eating habits. However, this awareness does not mean these women indeed changed their habits. They could not possess the means or could lack knowledge on how to avoid exposure to these pollutants. Regarding the inclusion, we did include many highly educated women. This could affect the general development of the infants. Nevertheless, we did correct for maternal education level, which had no significant effect on our outcome.

Implications

Our findings suggest that prenatal background exposure to OHCs has effects on the children's development. Less is known about the effects of prenatal exposure to particularly OH-PCBs. Our study seems to suggest that it has subtle effects early in life. The effects found could be temporarily or evolve over years. Although the effects might be subtle, this can still have great impact at a global level. Behavioural problems seem to be increasing in the population, with the exposure to environmental pollutants as a potential explanation. Research is required to determine the consequences of prenatal exposure in later life. Furthermore, knowledge is needed on the biological and biochemical actions of these compounds to prevent the production of chemical compounds with similar effects.

CONCLUSIONS

Higher prenatal Dutch background exposure to OH-PCBs seems to influence neurological development at 18 and 30 months of age, both adversely and positively. Our data suggest that OH-PCBs exert more effects on mental development compared with motor development, and that OH-PCBs exert more effects than PCBs. Prenatal exposure to 4-OH-PCB-187 adversely affects mental development at 18 months, and four OH-PCBs and ∑6 OH-PCBs positively influences mental development at 30 months. Our data also suggest that prenatal exposure to PCB-153 and BDE-99 adversely affects mental development at 18 months. Prenatal levels of p,p'-DDE-, PCP- or HBCDD were not associated with mental or motor development at 18 months of age. Further research is needed to investigate if these effects remain in later life.

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