The effects of exposure to environmental chemicals on child development

Berghuis, Sietske Anette

CHAPTER 4

Prenatal exposure to polychlorinated biphenyls and their hydroxylated metabolites is associated with neurological functioning in 3-month-old infants

Sietske A. Berghuis, Shalini D. Soechitram, Pieter J.J. Sauer, Arend F. Bos

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ABSTRACT

Background: Polychlorinated biphenyls (PCBs) are environmental chemicals which are potentially toxic to the developing brain. Their hydroxylated metabolites (OH-PCBs) are suggested to be even more toxic. Knowledge about the health effects of prenatal OH-PCB exposure is limited.

Objective: To determine whether prenatal background exposure to PCBs and OH-PCBs is associated with neurological functioning in 3-month-old boys and girls.

Methods: In a Dutch observational cohort study, we measured 10 PCBs and 6 OH-PCBs in maternal blood samples of 98 pregnant women. We assessed their infants neurologically with Touwen examination at 3 months and calculated an Optimality Score (OS, range 0-53, low-high optimality). For boys and girls, we calculated correlation coefficients between compound levels and OS. Subsequently, we tested whether levels were associated with specific clusters and whether levels differed between infants with 'normal' (dysfunction on ≤1 cluster) and 'non-optimal' development (dysfunction on ≥2 clusters).

Results: The mean OS was 48 (range 44-52). Higher exposure to PCB-146 correlated significantly with higher OS (r=0.209;P=0.039). In boys, higher exposure to 4-OH-PCB-107 correlated with lower OS (r=-0.305;P=0.030). Higher exposure to 9 PCBs and the sum of all PCBs was associated with better visuomotor and/or better sensorimotor function. Infants classified as 'non-optimal' (n=36) had significantly lower prenatal exposure to 6 PCBs and the sum of all PCBs (P<0.05) compared with infants classified as 'normal' (n=62).

Conclusions: Higher prenatal exposure to Dutch background PCB levels is associated with better neurological functioning in 3-month-old infants. Prenatal exposure to 4-OH-PCB-107 is associated with less optimal neurological functioning in boys.

INTRODUCTION

Polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs) are chemical compounds which might interrupt neurodevelopmental processes during critical periods of development. PCBs have been widely used since the 1930s in, for example, hydraulic fluids, adhesives and lubricants. Due to adverse effects on animal and human health their production has been banned by law since 1985 in the Netherlands. The exposure is ongoing because the compounds accumulated in the food chain due to resistance to biological and chemical degradation. A possible mechanism of toxicity of PCBs is the ability to mimic thyroid hormones or other steroidal hormones, by which they can interfere with endocrine and neurological functions. They might act directly by mimicking natural hormones agonistic or antagonistic, or indirectly through alteration of receptor number or affinity of other hormones in multi hormonal regulation systems (for review see McKinney and Waller[1]).

Several studies in humans reported that prenatal exposure to PCBs might have a negative impact on mental and motor development in children (for review see El Majidi et al.[2]). In newborns, prenatal exposure to PCBs was associated with an increase of abnormal reflexes, a decrease in motor skills and deficits in the acquisition of cognitive skills (for review see Ribas-Fito et al.[3]). We recently reported negative associations between prenatal background exposure to several PCBs and alterations in spontaneous motor development at three months in our cohort.[4] A study in the Netherlands reported adverse neurological effects of exposure to PCBs in infants up to 18 months of age, whereas no adverse effects were found at 42 months of age.[5-7] Vreugdenhil et al. found that neurotoxic effects of prenatal PCB exposure might result in subtle cognitive and motor developmental delays at school age.[8]

OH-PCBs are suggested to be even more toxic than PCBs because they are more water-soluble and might pass the blood brain barrier.[9] Studies in rats showed accumulation of OH-PCBs in the fetal brain and long-term adverse effects of exposure on behavior and neurodevelopment.[10, 11] In humans, Park et al. reported a significant association between prenatal levels of 4-OH-PCB-107 and a lower mental development at 16 months.[12] In our cohort, we found that higher prenatal exposure to 4-OH-PCB-107 might impair the spontaneous motor development at three months.[4] We also found in our cohort that 4'-OH-PCB-172 was associated with a more optimal quality of motor repertoire at three months. In another cohort, at the age of 5-6 years, we found OH-PCBs to be correlated with poorer fine manipulative abilities, better attention and better visual perception.[13]

Male infants are possibly more susceptible to the effects of prenatal exposure to environmental chemicals than females. Kishi et al. reported significant gender differences in the effects of dioxins and dioxin-like PCBs on infant's birth weight, neurodevelopment at 6 months, and immune functions.[14] They reported that male infants are more susceptible

to those chemicals than female infants, which is suggested to be due to gender-specific endocrine factors.

A method to assess the neurological development in young infants is the Touwen infant neurological examination,[15] adapted by Hadders et al.[16] This is an age-specific neurological examination, which not only detects neurological syndromes, but is also fit to detect mild neurological abnormalities and non-optimal neurological functioning. Moreover, it has the ability to quantify neurological optimality of the infants into a score. This assessment method includes observation of posture and motility, and detection of neurological signs such as dysfunctional muscle tone regulation, abnormal reflexes and dysfunction of cranial nerves.[17] The findings of the Touwen examination can be classified according to criteria of Hadders-Algra, based on age-specific norms.[16]

So far, knowledge about the impact of prenatal background exposure to OH-PCBs on early development in infants is limited. By investigating the development at a young age, the effects of postnatal factors will be minimal.

The aim of this study was to determine whether prenatal background exposure to OH-PCBs and PCBs is associated with neurological functioning assessed by Touwen examination at the age of three months. A secondary aim was to determine whether gender differences exist regarding the effects of exposure to these environmental chemicals. Because several studies showed mainly negative effects of exposure to PCBs, we hypothesized that higher prenatal exposure to the hydroxylated metabolites of PCBs would be associated with less optimal neurological functioning at three months.

METHODS

Cohort and study design

This study is part of an observational prospective cohort study on exposure to PCBs and OH-PCBs and their potential effects on the development of the newborn infant.[9] Women from the northern part of the Netherlands were invited by their midwife or obstetrician in the period between September 1998 and December 2000. In total, 104 mother-infant pairs were included in the study. All women were of Western European origin, spoke Dutch as their native language and had no serious illnesses or complications during pregnancy or delivery. All infants were born at term (between 37 and 42 weeks' gestation) and had no congenital anomalies or diseases. All parents had given informed consent. The study was approved by the Medical Ethics Committee of the University of Groningen.

Measurement of PCBs and OH-PCBs

Blood samples were taken from the pregnant women in the second and/or third trimester of their pregnancy. A detailed description of the analysis of the blood samples has been published previously.[9] In brief, for extraction and clean-up, a combination of sulfuric acid treatment and silica gel/sulfuric acid column chromatography separations was used. The following 10 PCBs were measured: PCB-105, PCB-118, PCB-138, PCB-146, PCB-153, PCB-156, PCB-170, PCB-180, PCB-183, and PCB-187. The following six OH-PCBs were measured: 4-OH-PCB-107, 3-OH-PCB-153, 4-OH-PCB-146, 3'-OH-PCB-138, 4-OH-PCB-187 and 4'-OH-PCB-172. The parent PCB compounds of the OH-PCBs measured are shown in Table 3. The PCBs were numbered according to Ballschmiter et al.[18] and the OH-PCB congeners according to Letcher et al.[19] Concentrations of PCBs are given in ng/g lipid in maternal blood. Concentrations of OH-PCBs are expressed per gram fresh weight.

Touwen Infant Neurological Examination

The infants were neurologically examined with the Touwen examination at three months by author SDS. The Touwen examination consists of 53 items organized in five clusters according to the functional, neurobehavioral subsystems of the nervous system: reaching and grasping (4 items, e.g. 'playing with hands'); gross motor (17 items, e.g. 'pull-to-sit- manoeuvre, traction test'); brainstem function (9 items, e.g. 'threshold glabella reflex'); visuomotor (9 items, e.g. 'visual pursuit'); and sensorimotor function (14 items, e.g. 'regulation of tendon reflexes'). AFB classified the infants using criteria of Hadders-Algra,[16] taking the age of the infants into account. A cluster is scored as being abnormal when minimally one item is abnormal in cluster 1 (reaching and grasping), at least four items are abnormal in cluster 2 (gross motor function), and at least two items are abnormal in the clusters 3 to 5 (brain stem function, visuomotor and sensorimotor function). Next, based on the five clusters, we classified the infants as normal, non-optimal or abnormal. They were classified as neurologically normal if they show dysfunction on zero or one cluster, and non-optimal if they showed dysfunction on two or more clusters. They were classified as neurologically abnormal in the presence of a full-blown neurological syndrome, such as a hemisyndrome, a hyperexcitability syndrome, or a clear hypo- or hypertonia. We also calculated an Optimality Score. Each of the 53 items scored as normal was given a score of 1, each item scored as deviant was given a score of 0. Summing the scores of all items, an Optimality Score was derived ranging from 0 to 53, low to high optimality.

Statistical analysis of data

For statistical analyses we used the Spearman rank correlation test and the Mann–Whitney-U test (MWU) to investigate correlations and associations between prenatal exposure to PCBs and OH-PCBs and outcome variables. Regarding potentially different effects of exposure

to PCB/OH-PCBs on boys and girls, Spearman's correlation coefficients were calculated for both genders separately for the relation between PCB/OH-PCB exposure and OS.
We used the following outcome variables:
1) Optimality Score on Touwen examination;
2) Number of abnormal clusters on Touwen examination with subdivision in 'normal' development (≤1 abnormal clusters) and 'non-optimal' development (≥2 abnormal clusters).
3) Score on clusters on Touwen examination (normal/abnormal);
We used logistic regression models to calculate odds ratios (ORs). To determine whether the relation between PCBs and OH-PCBs and the quality of the motor development was confounded by other characteristics (gender, gestational age, birth weight, type of feeding, age at examination, maternal education level, and maternal smoking and alcohol consumption during pregnancy), we performed univariate logistic regression analyses. These characteristics were considered as possible confounders if they had a *P*-value of less than .20 in the univariate analysis model. We performed multivariable logistic regression analyses (method: enter) to assess whether these confounders had influenced our results.
A *P*-value of less than .05 we considered statistically significant and a *P*-value of more than .05 but less than .10, marginally significant. We used the Statistical Package for the Social Sciences, version 20 (SPSS) for the statistical analyses.

RESULTS

Study group

Of the 104 infants initially included in the study, six were later excluded for various reasons: for four infants no PCB or OH-PCB values were available, and two infants had not been examined at three months due to logistic reasons. The final study group consisted of 98 mother–infant pairs. Characteristics of the study group are presented in Table 1.

Table 1. Characteristics of the study group (N=98 mother-infant pairs)

Characteristic	Value
Gender, boy/girl	52/46
Gestational age (weeks)	40 (37-42)
Birth weight (g)	3621 ± 504
Apgar at 3 min [median (range)]	10 (6-10)
Type of feeding, breast/formula	68/30
Age at examination (weeks)	14±1
Maternal education level	
Below average (≤11 years education)	13
Average (12-13 years education)	40
Above average (≥14 years education)	45
Maternal smoking [yes/no]	23/75
Maternal alcohol consumption [yes/no]	24/74
Optimality Score Touwen	48 (44-52)
(1) Reaching and grasping (normal/abnormal)	80/18
(2) Gross motor (normal/abnormal)	96/2
(3) Brainstem function (normal/abnormal)	97/1
(4) Visuomotor function (normal/abnormal)	63/35
(5) Sensorimotor function (normal/abnormal)	40/58
Number abnormal clusters Touwen (≤1/≥2)	62/36

Data are given as frequencies (n/n), median (min-max), or mean ±SD.

Touwen examination

The mean OS was 48 (range 44-52). Table 1 shows the number of infants scoring abnormal on the five clusters of Touwen examination. Of the 98 infants, 62 infants showed dysfunction in zero (n= 26) or just one (n= 36) cluster. Thirty-six infants showed dysfunction in two (n= 30) or three (n= 6) clusters on Touwen examination. None of the infants was classified as neurologically abnormal or showed dysfunction on more than three clusters.

PCB/OH-PCB exposure and Touwen examination

Higher exposure to PCB-146 correlated significantly with higher OS (r=0.209, P=.039; Table 2). In boys, higher exposure to PCB-146 and PCB-105 correlated with marginal significance with higher OS (r=0.0259, P=0.063 respectively r=0.235, P=0.093). Higher exposure to 4-OH-PCB-107 was significantly correlated with lower OS in boys (r=-0.305; P=0.030). Prenatal exposure to PCB-105, PCB-118, PCB-138, PCB-146, PCB-153, PCB-156, PCB-187 and the sum of all the PCBs was significantly lower in infants classified as 'non-optimal' compared to infants classified as 'normal' (P<0.05; Table 3). In addition, prenatal exposure to PCB-180 was lower in girls with 'non-optimal' development (P<0.10), and prenatal exposure to 4-OH-PCB-107 was higher in boys with 'non-optimal' development (P<0.10).

Next, we investigated in more detail on which specific cluster the infants scored more or less optimal after higher exposure to PCB or OH-PCBs. Higher exposure to several PCBs was associated with more optimal scores on the fourth and fifth cluster on Touwen examination, testing visuomotor and sensorimotor function respectively (Table 4). Regarding analyses on both genders separately, we found that exposure to higher levels of several PCBs was more frequently significantly associated with more optimal scores in girls compared to boys. In boys, higher exposure to 4-OH-PCB-107 was significantly associated with an abnormal score on visuomotor function (P<0.01). We found no associations between exposure to PCB/OH-PCBs and the other clusters on Touwen.

Table 2. Spearman's correlation coefficients for prenatal exposure to PCBs and their respective metabolites and Optimality Score in three-month-old boys and girls

Compound	Both genders	♂	♀	Compound	Both genders	♂	♀
PCB-105	.137	.235*	.052	4-OH-PCB-107	-.147	-.305**	-.057
PCB-118	.160	.165	.165				
PCB-138	.136	.076	.121	4-OH-PCB-146	-.014	-.128	.119
				3'-OH-PCB-138	.116	.050	.157
PCB-153	.161	.127	.141	3-OH-PCB-153	.074	-.048	.226
				4-OH-PCB-146			
PCB-170	.047	-.055	.117	4'-OH-PCB-172	-.010	-.153	.107
PCB-180	.053	-.093	.120				
PCB-187	.142	.136	.092	4-OH-PCB-187	-.076	-.121	-.085
PCB-146	.209**	.259*	.109				
PCB-156	.163	.110	.222				
PCB-183	.038	-.020	-.033				
Sum PCBs	.132	.101	.129	Sum OH-PCBs	-.050	-.177	.037

**P<0.05; *P<0.10.

Table 3. Associations between prenatal exposure levels to PCBs and OH-PCBs and non-optimal development in three-month-old infants. [a]

Compound	'Normal'		'Non-optimal'		Optimality	MWU
	Median [b] (IQR)	*n*	Median [b] (IQR)	*n*	↑/↓	*P*-value
PCB-105	7.5 (2.5-12.0)	62	2.9 (1.7-7.7)	36	↑↑	**.035**
- *boys*	8.7 (2.1-12.1)	30	3.1 (2.2-5.6)	22		.101
- *girls*	4.8 (3.0-11.9)	32	2.0 (1.3-11.5)	14		.247
PCB-118	23.9 (16.9-33.9)	62	16.4 (10.8-22.3)	36	↑↑↑	**.007**
- *boys*	26.3 (15.9-38.0)	30	17.1 (12.3-22.0)	22	↑	**.056**
- *girls*	22.6 (18.4-30.7)	32	15.2 (9.4-43.1)	14	↑	**.081**
PCB-138	72.9 (54.8-86.4)	62	54.0 (38.6-82.9)	36	↑↑	**.028**
- *boys*	67.1 (50.8-86.8)	30	54.7 (38.3-71.1)	22		.192
- *girls*	77.0 (59.4-86.4)	32	49.4 (38.0-90.0)	14		.189
PCB-146	9.0 (6.8-15.7)	62	5.2 (3.9-12.5)	36	↑↑↑	**.003**
- *boys*	9.5 (6.1-16.6)	30	5.0 (4.0-9.6)	22	↑↑	**.016**
- *girls*	8.6 (7.0-11.0)	32	6.1 (3.6-12.9)	14		.170
PCB-153	96.3 (77.7-126.1)	62	71.0 (47.9-110.0)	36	↑↑	**.012**
- *boys*	93.0 (63.8-123.2)	30	72.7 (46.3-99.4)	22	↑	**.092**
- *girls*	96.8 (86.0-133.2)	32	63.1 (49.5-131.0)	14	↑	**.088**
PCB-156	11.7 (8.2-14.9)	60	9.2 (5.7-13.5)	36	↑	**.065**
- *boys*	12.6 (7.5-15.2)	30	9.1 (5.6-10.7)	22		.259
- *girls*	11.7 (9.6-14.3)	30	9.3 (5.7-13.9)	14		.182
PCB-170	20.0 (15.0-24.8)	60	17.8 (11.9-25.1)	36		.286
- *boys*	18.4 (12.0-24.8)	30	17.2 (12.3-28.9)	22		.941
- *girls*	21.4 (17.7-26.8)	30	19.1 (10.6-24.0)	14		.199
PCB-180	44.7 (34.5-58.5)	60	41.1 (29.0-57.2)	36		.204
- *boys*	42.6 (28.4-57.1)	30	40.2 (30.8-60.8)	22		.890
- *girls*	48.2 (36.6-68.9)	30	41.1 (26.4-55.0)	14	↑	**.091**
PCB-183	8.2 (5.6-9.8)	62	7.4 (5.5-10.8)	36		.845
- *boys*	7.5 (5.0-9.2)	30	6.7 (5.6-10.7)	22		.487
- *girls*	8.9 (6.4-10.3)	32	8.9 (4.9-12.3)	14		.625
PCB-187	12.7 (10.6-17.0)	62	9.2 (7.2-17.7)	36	↑↑	**.047**
- *boys*	12.8 (8.6-17.8)	30	9.9 (7.4-18.0)	22		.295
- *girls*	12.6 (10.9-16.2)	32	8.3 (6.6-17.6)	14		.127
Sum PCBs	318.8 (253.8-403.6)	60	244.0 (170.1-366.1)	36	↑↑	**.025**
- *boys*	312.8 (209.3-400.0)	30	244.0 (186.4-347.2)	22		.185
- *girls*	324.3 (277.9-412.2)	30	240.8 (167.5-391.7)	14		.101
4-OH-PCB-107	0.058 (0.037-0.094)	60	0.079 (0.042-0.116)	35		.422
- *boys*	0.050 (0.030-0.085)	30	0.079 (0.045-0.113)	21	↓	**.098**
- *girls*	0.059 (0.045-0.101)	30	0.077 (0.020-0.117)	14		.830
3'-OH-PCB-138	0.050 (0.033-0.069)	60	0.042 (0.030-0.060)	35		.178
- *boys*	0.046 (0.032-0.064)	30	0.039 (0.028-0.058)	21		.271
- *girls*	0.056 (0.036-0.072)	30	0.047 (0.039-0.062)	14		.537
4-OH-PCB-146	0.070 (0.052-0.111)	60	0.072 (0.054-0.101)	35		.935
- *boys*	0.070 (0.052-0.104)	30	0.072 (0.057-0.103)	21		.625
- *girls*	0.071 (0.052-0.117)	30	0.068 (0.042-0.097)	14		.435

Table 3 continued

Compound	'Normal'		'Non-optimal'		Optimality	MWU
	Median [b] (IQR)	*n*	Median [b] (IQR)	*n*	↑/↓	*P*-value
3-OH-PCB-153	0.040 (0.025-0.059)	60	0.036 (0.023-0.054)	35		.346
- *boys*	0.035 (0.023-0.057)	30	0.033 (0.019-0.055)	21		.781
- *girls*	0.043 (0.031-0.060)	30	0.037 (0.027-0.052)	14		.222
4'-OH-PCB-172	0.017 (0.010-0.022)	48	0.015 (0.100-0.027)	27		.682
- *boys*	0.016 (0.009-0.022)	26	0.014 (0.010-0.029)	16		.281
- *girls*	0.018 (0.010-0.022)	22	0.017 (0.010-0.020)	11		.566
4-OH-PCB-187	0.134 (0.103-0.180)	60	0.143 (0.105-0.178)	35		.705
- *boys*	0.123 (0.107-0.163)	30	0.147 (0.104-0.181)	21		.438
- *girls*	0.150 (0.102-0.192)	30	0.135 (0.110-0.182)	14		.830
Sum OH-PCBs	0.384 (0.285-0.559)	60	0.397 (0.265-0.513)	35		.829
- *boys*	0.353 (0.243-0.575)	30	0.379 (0.288-0.549)	21		.416
- *girls*	0.417 (0.302-0.553)	30	0.413 (0.241-0.514)	14		.734

[a] Calculated by the Mann Witney U-test; [b] PCBs are given in ng/g lipid weight, OH-PCBs in ng/g fresh weight; ↑↑↑ *P*<0.01, ↑↑ *P*<0.05 and ↑ *P*<0.10, indicating that exposure to higher levels is associated with more optimal functioning; ↓ *P*<0.10, indicating that exposure to higher levels is associated with less optimal functioning; IQR: Interquartile range.

Table 4. Associations between prenatal exposure to PCBs and OH-PCBs and abnormal scores on the clusters visuomotor and sensorimotor function in three-month-old infants. [a]

Compound	Visuomotor function			Sensorimotor function		
	Both genders (N/A=63/35)	♂ (N/A=29/23)	♀ (N/A=34/12)	Both genders (N/A=40/58)	♂ (N/A=17/35)	♀ (N/A=23/23)
PCB-105	↑↑	↑↑	↑↑↑			
PCB-118	↑↑	↑↑	↑↑			
PCB-138	↑↑		↑↑	↑↑		↑
PCB-146	↑↑	↑↑	↑↑	↑↑		↑↑
PCB-153	↑↑		↑↑	↑↑		↑↑
PCB-156	↑↑		↑↑	↑↑		↑↑
PCB-170				↑↑		↑↑
PCB-180				↑↑		↑↑
PCB-183						
PCB-187	↑↑		↑↑	↑↑		↑↑
Sum PCBs	↑↑		↑↑	↑↑		↑↑
4-OH-PCB-107	↓	↓↓↓				
3'-OH-PCB-138				↑	↑	
4-OH-PCB-146						
3-OH-PCB-153						
4'-OH-PCB-172						
4-OH-PCB-187						
Sum OH-PCBs						

[a] Calculated by the Mann Witney U-test; ↑↑↑ *P*<0.01, ↑↑ *P*<0.05 and ↑ *P*<0.10, indicating that exposure to higher levels is associated with more optimal functioning; ↓↓↓ *P*<0.01 and ↓ *P*<0.10, indicating that exposure to higher levels is associated with less optimal functioning; N/A = number of infants scoring normal/abnormal on the cluster.

Multivariable analyses

We investigated whether other factors might have confounded our findings by determining the odds ratios (ORs) for their effect on motor development. Univariate logistic regression analyses revealed that only a higher birth weight (>3660 grams; OR=2.037, *P*=0.096) and a younger age at examination (≤14 weeks; OR=2.545, *P*=0.043) were associated with ≥2 abnormal clusters on Touwen examination at P< .20. We performed univariate logistic regression analyses to determine the ORs for the effect of exposure to PCBs and OH-PCB compounds on motor development. For those compounds which were found to be related to ≥2 abnormal clusters by using MWU, we present the ORs in Table 5. Finally, we repeated the logistic regression analyses adjusting for these potential confounders yielding the adjusted ORs shown in Table 5. After correction, higher exposure to PCB-146 remained significantly associated with a more optimal ('normal') development. The association between higher exposure to PCB-153 and more optimal ('normal') development was no longer significant.

Table 5. Logistic regression analyses for associations between prenatal PCB and OH-PCB levels and non-optimal development in three-month-old infants

Compound	Gender	OR (95% CI)	*P*-value	OR after correction (95% CI) [a]	*P*-value
PCB-105	Both genders	0.826 (0.538, 1.269)[b]	0.382	0.833 (0.550, 1.262)[b]	0.388
PCB-118	Both genders	0.909 (0.702, 1.178)[b]	0.471	0.932 (0.728, 1.191)[b]	0.573
	Boys	0.987 (0.956, 1.019)[b]	0.421	0.987 (0.956, 1.019)[b]	0.415
	Girls	0.997 (0.952, 1.043)[b]	0.883	1.009 (0.960, 1.059)[b]	0.734
PCB-138	Both genders	0.904 (0.781, 1.045)[b]	0.172	0.931 (0.805, 1.077)[b]	0.337
PCB-146	Both genders	0.395 (0.172, 0.905)[b]	**0.028****	0.394 (0.170, 0.913)[b]	**0.030****
	Boys	0.896 (0.806, 0.998)[b]	**0.045****	0.895 (0.803, 0.997)[b]	**0.043****
PCB-153	Both genders	0.884 (0.790, 0.990)[b]	**0.032****	0.910 (0.831, 1.019)[b]	0.104
	Boys	0.989 (0.974, 1.004)[b]	0.148	0.989 (0.974, 1.005)[b]	0.164
	Girls	0.988 (0.971, 1.004)[b]	0.147	0.994 (0.977, 1.010)[b]	0.459
PCB-156	Both genders	0.455 (0.184, 1.126)[b]	**0.089***	0.586 (0229, 1.501)[b]	0.265
PCB-180	Girls	0.962 (0.924, 1.003)[b]	**0.068***	0.971 (0.928, 1.016)[b]	0.204
PCB-187	Both genders	0.526 (0.248, 1.116)[b]	**0.094***	0.561 (0.262, 1.203)[b]	0.137
Sum PCBs	Both genders	0.970 (0.936, 1.006)[b]	0.103	0.979 (0.944, 1.015)[b]	0.254
4-OH-PCB-107	Boys	1.130 (0.971, 1.315)[c]	0.115	1.130 (0.971, 1.315)[c]	0.114

[a] Confounders: birth weight and age at examination; [b] Per 1 ng/g lipid weight; [c] Per 0.01 ng/g fresh weight; *P<0.10; **P<0.05; OR: Odds ratio; CI: Confidence Interval

DISCUSSION

Prenatal background exposure to several PCBs was positively associated with neurological functioning assessed by Touwen examination at the age of three months. In boys, 4-OH-PCB-107 was negatively associated with neurological functioning.

Our first finding is that higher prenatal exposure to several PCBs was positively associated with neurological functioning at three months. Regarding the total number of abnormal clusters on Touwen examination, prenatal exposure to several PCBs was significantly lower in infants classified as 'non-optimal' (≥2 abnormal clusters) compared to infants classified as 'normal'(≤1 abnormal cluster). Most previous studies on early human development, investigating similar but also slightly higher levels of several PCBs, found negative associations after higher prenatal exposure to PCBs (for review see El Majidi et al.[2]). To the best of our knowledge, this is the first study reporting positive associations between prenatal PCB exposure and neurological functioning. Studying higher *post*natal PCB exposure through breast milk, Boersma et al. reported a beneficial effect of breastfeeding on the quality of movements, in terms of fluency, and on a cognitive development test in infants.[20] In our study, type of feeding was not associated with ≥2 abnormal clusters on Touwen examination. A possible explanation for the difference in the direction of the associations, compared to other studies, could be the difference in test measures, testing other activities of the brain. The mechanisms by which these compounds might have an impact on the brain are still not clear, but may have an impact on developmental processes by extra stimulation or by suppression. A faster development might possibly occur at the expense of the formation of stable neural networks. It is not clear whether faster development at a young age could have implications for developmental outcomes during later life. Another explanation for the differences in direction of association, compared to other studies, might be the level of exposure. The exposure levels in the Netherlands are relatively low compared to levels measured in the USA.[2] We suggest that higher exposure levels might exert negative effects, whereas lower levels might possibly have positive effects by incitement of neuronal and/or hormonal processes.

A second finding is that, regarding OH-PCB exposure, only the congener 4-OH-PCB-107 was significantly associated with a less optimal neurological functioning in boys. We found no other associations between prenatal exposure to OH-PCBs and neurological functioning at three months. The finding that 4-OH-PCB-107 might impair neurological development is in agreement with our previous finding in the same cohort that prenatal exposure to 4-OH-PCB-107 might impair spontaneous motor development at three months.[4] In other studies, this specific metabolite was found to be associated with a less optimal mental development in 16-month-old infants, and with long-term adverse effects on behavior and neurodevelopment in rats.[10, 12]

A third finding is that exposure to PCBs might have an impact on specific functions of the brain. Regarding the scores on the five clusters on Touwen examination, higher exposure to several PCBs was associated with more optimal visuomotor and sensorimotor function. Exposure to PCBs or OH-PCBs was not associated with functions assessed by the other clusters on Touwen examination. Several studies reported effects of prenatal exposure to environmental chemicals on visual function in infants.[21-23] In four-month-old infants, prenatal exposure to PCB-118 was related to fixation pattern.[22] They observed biological motion as a hallmark of socio-cognitive development, and suggest that prenatal PCB-exposure may have an adverse effect on early social development. Boucher et al. found that higher prenatal PCB exposure was associated with impairment of visual recognition memory at the age of 6.5 and 11 months.[21] White et al. observed differences in brain activation after prenatal exposure to methylmercury (MeHg) and PCB on tasks requiring visual processing and manual motor movement at the age of 15 years.[23] Using functional magnetic resonance imaging (fMRI) techniques, they observed greater and more widespread brain activation in the highly exposed group, suggesting that adolescents with high prenatal exposure will require more brain resources to complete tasks, and that differential specialization of brain areas may have occurred after prenatal neurotoxicants exposure.

A fourth finding is that there are some differences between boys and girls regarding the effects of prenatal exposure to PCBs and OH-PCBs. The congener 4-OH-PCB-107 was associated only in boys with a less optimal neurological functioning, and PCB-180 was associated only in girls with a more optimal neurological functioning (P<0.10). Regarding scores on visuomotor and sensorimotor functions, especially in girls higher exposure to PCBs was associated with more optimal scores, whereas higher exposure to 4-OH-PCB-107 was associated with less optimal scores on visuomotor function (P<0.10). Several studies suggested that male infants are more susceptible to environmental chemicals than female infants.[14, 24] Regarding psychomotor development at 6 months, Kishi et al. reported that higher exposure to five of the measured PCB congeners was associated with a lower developmental score in boys, compared to two PCB congeners in girls.[14] Regarding motor development in 2-month-old rats, exposure to PCB-126 impairs motor coordination in males and reduces spontaneous locomotor activity in females.[24] Cauli et al. also found that vertical activity was reduced after higher exposure to PCB-126 and PCB-153 in two-month-old female rats, whereas exposure to PCB-153 showed hyperactivity in five-month-old male rats. These results suggest that the different compounds might exert different effects in males and females.

We can think on the underlying brain processes that would be responsible for our findings. The two clusters that were particularly related to neurological functioning (clusters 4 and 5, visuomotor and sensorimotor function) both concerned the integration of sensory and motor functions. At this young age, most functions are still located in the brain stem and

in subcortical areas, although they are modulated by higher brain structures. At around three months, cortical involvement increases, as was shown by PET experiments.[25] Other experiments, particularly involving preterm infants, suggest that myelination of white matter is also related to motor behaviour at around three months.[26] This suggests that myelination of subcortical and cortical brain networks, involved in the integration of sensory and motor tasks, may be at the basis of our findings. Interestingly, some of the congeners enhance these developmental processes, whereas others inhibit them. Even so, it remains highly speculative which brain areas are involved in which way.

A strength of our study is that we measured OH-PCB levels in addition to the PCB levels in maternal blood during pregnancy. Studies on the impact of prenatal background exposure to OH-PCBs are scarce. Second, in our study we used a very sensitive method to detect subtle effects of prenatal exposure to the compounds studied. We used the Touwen neurological examination, which can detect mild neurological abnormalities and non-optimal neurological functioning. A third strong point is that the development was examined at a very young age. Because of the limited time frame at three months of age, effects of postnatal factors will be minimal in this study. A fourth strong point is that we also investigated whether gender differences were involved in the effects of exposure to the environmental chemicals. There is growing evidence that there might be differences between men and women regarding the effects of environmental chemicals on health outcomes. Studies of both genders separately are sparse.

We recognize several limitations. A first limitation of our study is the likelihood of Type I error. Because we performed a large number of comparisons, we cannot rule out the possibility of chance-findings. Even so, we believe that our analyses were justified as part of a careful evaluation of a rich data set in a hypothesis-driven research.[27] The sample size might also be a limitation. In this study, we included 104 mother-infant pairs, of which data of 98 mother-infant pairs could be analyzed. Nevertheless, we think that this is a good size for these complicated studies.

The results of this study should be interpreted with caution. We found positive associations between higher exposure to PCBs and neurological functioning at a very young age. We do not know whether these effects will persist in the children at an older age. As outcome measure we used Touwen neurological examination at three months, which has not been used previously for observing effects of environmental chemicals. Neurological condition during infancy is prone to change, although the results of the Touwen neurological examination at three to four months are related to the neurological condition at 18 months.[16] Therefore, we think that this test is an adequate method for detecting subtle effects on development at such a young age.

This study indicates that higher prenatal background exposure to background levels of PCBs and OH-PCBs might influence neurological functioning at three months both positively

and negatively. Further study is needed to explore whether this more or less optimal development at young age is consistent during later life.

CONCLUSIONS

Higher prenatal exposure to PCB-105, PCB-118, PCB-138, PCB-146, PCB-153, PCB-156, PCB-187 and the sum of the PCBs is positively associated with neurological functioning using Touwen infant neurological examination in three-month-old infants. In addition, higher exposure to PCB-180 is positively associated with neurological functioning in girls, and higher exposure to 4-OH-PCB-107 is negatively associated with neurological functioning in boys. Higher exposure to nine PCBs and the sum of all PCBs was associated with better visuomotor function and/or better sensorimotor function.

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