The effects of exposure to environmental chemicals on child development

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PART 3

Endocrine disrupting effects of environmental chemicals

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CHAPTER 7

Polychlorinated biphenyl exposure and deiodinase activity in young infants

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ABSTRACT

Background: Several studies have shown effects of polychlorinated biphenyls (PCBs) on serum thyroid hormone levels in pregnant woman and their infants, while other studies did not find such effects. How PCBs might affect thyroid hormone metabolism, is still unclear. Potential mechanisms are direct influence on the thyroid gland, binding to thyroid-binding proteins, increased excretion or metabolism of thyroid hormones by deiodinases or sulfatases. It is also not well known whether the effect on thyroid hormone levels is caused by PCBs themselves, or by their hydroxylated metabolites (OH-PCBs).

Objective: To determine the effects of perinatal exposure to PCBs and OH-PCBs on thyroid hormone levels in cord blood and in serum of newborn infants.

Methods: In a Dutch cohort of 100 mother-infant pairs, exposed to background PCB levels, correlations were assessed between 10 PCBs and 6 OH-PCBs in maternal blood during pregnancy and serum thyroxine (T4), T4 sulfate (T4S), triiodothyronine (T3), reverse T3 (rT3), thyroid-stimulating hormone (TSH) and thyroid-binding globulin (TBG) levels in cord blood and in serum of three- and 18-month-old infants.

Results: Prenatal levels of six of 10 measured PCBs showed a positive correlation with cord serum T3, and 4 PCBs showed a negative correlation with cord serum rT3. Five of these PCBs were positively correlated with the cord serum T3/rT3 ratio, an indicator of deiodinase 3 activity. No correlations were found between PCBs and T4, TSH and TBG in cord blood. 4-OH-PCB-107 was correlated with T4 at 3 months and T4, T4S and T3 at 18 months.

Conclusion: Our results suggest that PCBs have a negative effect on deiodinase type 3 activity, as reflected by a positive correlation with the T3/rT3 ratio. We identified a potential mechanism by which PCBs may affect thyroid hormone metabolism during human development.

INTRODUCTION

Despite the ban on their production decades ago, polychlorinated biphenyls (PCBs) are still present in the environment and in human tissues.[1, 2] Levels of PCBs are higher in more densely populated countries such as the Netherlands and Belgium compared to countries such as the USA and Canada.[2-5] Levels in the Netherlands seem to decrease, when comparing cohorts from 1990-1992 and 2011-2013 with mean levels of PCB 153 of 91 ng/L and 35 ng/L, respectively, in the first and second period.[6, 7] A study from Belgium from 2007 showed a PCB-153 level in cord blood of 70 ng/L.[8] Levels are decreasing due to the ban on the production of these compounds. Different studies found negative effects of prenatal exposure to these compounds on thyroid hormone levels (THs), and on neurodevelopment of infants. In a cohort in The Netherlands in the early nineties, negative effects of PCBs on THs were found.[6] In contrast, a later study in Germany did not show an effect of background PCB levels on TH status.[9] Studies have shown lower levels of thyroxine (T4) and tri-iodothyronine (T3) in pregnant women in relation to PCB exposure.[6, 10, 11] Maternal T4 is an important source of TH for the fetus. Decreased maternal levels of T4 can have negative effects on the fetal neurodevelopment. The development of the central nervous system depends on the concentration of THs, and therefore may be at risk for PCB-induced alterations in brain TH concentrations.[12] In a Dutch cohort in the early nineties, negative effects of exposure to PCBs on neurodevelopment were found.[4, 13] Also in the cohort of the current study, associations were found between prenatal exposure to PCBs and neurodevelopment at the age of three months.[14, 15]

Studies in humans mainly investigated the effect of PCBs themselves on human development. PCBs are metabolized by hydroxylation to OH-PCBs, which are more water-soluble and therefore more rapidly excreted from the body.[2] Some OH-PCBs have a higher affinity for transthyretin (TTR) than T4 itself.[16] Animal studies showed that OH-PCBs can accumulate in the brain of the fetal rat, thereby reducing fetal brain T4 levels. This reduction in brain T4 levels is partially compensated by increased type 2 deiodinase (D2) activity, a well-known response of the rat brain to maintain brain T3 levels when circulating T4 concentrations are decreased.[12, 17, 18]

A study showed a negative correlation between cord blood levels of 4-OH-PCB-107 and mental and psychomotor development in children at 16 months of age.[10] In the cohort of the current study, prenatal exposure to 4-OH-PCB-107 was found to be negatively associated with neurodevelopment at three months.[14, 15]

Not much is known on how PCBs might affect thyroid function in the human fetus and newborn infant. In animals the most likely mechanism is binding of PCBs to transthyretin (TTR). In humans, thyroid-binding globulin (TBG) is the most prominent TH-binding protein.[17] TTR plays a role in mediating the delivery of T4 across the blood-brain barrier

and transferring T4 from the mother to the fetus. Thus, it may still be possible that binding of PCBs to TTR may play an important role on thyroid function in the human fetus. Other potential mechanisms influencing TH levels in humans are changes in the activities of TH metabolizing enzymes as deiodinases and sulfotransferases. Schuur et al. studied different iodothyronine sulfotransferases catalyzing the sulfation of THs in humans and in rats.[19] They found that TH sulfation is potently inhibited by hydroxylated metabolites of various PCBs. Whether PCBs have effects on the TH deiodinase or sulfatase activity in newborn infants is still not clear.

The aim of this study was to measure the thyroid hormone status of newborn infants in cord blood and at 3 and 18 months and to evaluate the effect of PCBs and OH-PCBs on these levels as well as on deiodinase and sulfotransferase activities as indicated by TH metabolites.

METHODS

Study group

This study is part of a prospective study on the exposure to PCBs and OH-PCBs and their potential effect on the development of the newborn infant.[2] From September 1998 to December 2000, pregnant women from the Northern part of The Netherlands were invited by their midwife or obstetrician to participate in a study on the exposure to PCBs and OH-PCBs and their potential effect on the development of the newborn infant. Mothers had to be of Western European origin, and Dutch had to be their native language. Pregnancy and delivery had to be without complications. Only infants born at term (37-42 weeks of gestation) with a birth weight above -2SD and without congenital abnormalities were included Admission of an infant to the hospital for more than one day after birth was an exclusion criterion. In total, 104 mother-infant pairs were included in the cohort. The Medical Ethical Committee of the University Medical Centre Groningen approved the study.

Measurements of PCBs and OH-PCBs

Results of the measurements of the prenatal levels of PCBs and OH-PCBs in a part of the cohort have been published previously.[2] In short, blood samples were taken from the pregnant women in the third trimester of pregnancy for measurement of the levels of 10 PCB congeners and 6 OH-PCBs, as shown in Table 2. PCBs and OH-PCBs were measured using a gas chromatograph (Varian 3400 GC) equipped with an electron capture detector, a Varian 822\00 autosampler and a split/splitless injector operated in a splitless mode. The fused silica-capillary column used was a non-polar column, CP-SIL 8CB (25 m x 0.15 mm x 0.12 µm) from Chrompack (Middelburg, The Netherlands). The chemical activated luciferase

gene expression (Calux) essay was used for measuring total Toxic Equivalent Quotient (TEQ) levels in maternal serum. Calux can be used to detect certain planar halogenated aromatic hydrocarbons (PHAH), including PCBs. The binding of the compounds to the aryl hydrocarbon receptor (AhR) is followed by transportation of the PHAH-AhR complex into the nucleus of the cell, and subsequent binding to specific sequences in the DNA (BioDetection Systems).

Thyroid hormone parameters at three and 18 months of age

Cord blood, as well as blood taken from the infants by vena puncture at the age of 3 and 18 months, was collected for the determination of T4, T4 sulfate (T4S), thyroid stimulating hormone (TSH), T3, reverse T3 (rT3) and TBG. All blood samples were analyzed within one year after collection. Serum T4, T3 and rT3 were measured by in-house radioimmunoassay; TSH by Dynotest IRMA; and TBG by Dynotest RIA (Brahms, Berlin, Germany). The measurements of serum T4S were performed by radioimmunoassay (Murphy et al., 2004). Within-assay coefficients of variation were calculated as 2-8% for T4, 2-6% for T3, 3-4% for rT3, 6-17% for T4S, 2-5% for TSH and 2-4% for TBG. Between-assay coefficients of variation were 5-10% for T4, 8% for T3, 9-16% for rT3, 4-19% for T4S, 2-14% for TSH, and 2-3% for TBG. Thyroid hormones were normally distributed.

Statistic analyses

Results are presented as mean and standard deviation where appropriate. PCB levels are expressed on lipid weight basis (ng/g lipid) and OH-PCB levels on fresh weight basis (ng/g serum). The PCB and OH-PCB levels are presented as median and range. Bivariate non-parametric correlations were calculated using Spearman rank correlation test. To determine whether the association between PCBs and OH-PCBs and the TH parameters was confounded by other characteristics (age of the mother, gender, gestational age, type of feeding), 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 multivariate logistic regression analyses (method: enter) to assess whether these confounders had influenced our results. Differences were considered statistically significant at $P<0.05$ level. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS), version 22.

RESULTS

Study group

Of the 104 mother-infant pairs initially included in the cohort, PCB or OH-PCB and thyroid hormone metabolism parameters were measured in 100 mother-infant pairs. Clinical details of the study group are presented in Table 1.

Table 1. Characteristics of the study group (N=100 mother-infant pairs)

Characteristic	Value
Gender, boy/girl	53/47
Gestational age (weeks)	40 (37-42)
Birth weight (grams)	3629 ± 499
Type of feeding, breast/formula	71/29
Maternal age (years)	32 ± 4
Maternal smoking [yes/no]	23/77

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

Levels of PCBs, OH-PCBs and thyroid hormone parameters

Levels of both dioxin-like and non-dioxin-like PCBs and OH-PCBs and Calux measured in maternal serum during the third trimester of pregnancy are shown in Table 2. PCB-153 was the most prevalent congener, followed by PCB-138. The median sum of 10 PCBs (ng/g lipid weight) was 293.4 with IQR 216.5-390.4. For six OH-PCBs, the median sum was 19.55 ng/g (fresh weight) with IQR 14.43-59.53 ng/g. Calux results were positively correlated with the individual dioxin-like PCBs and the sum of the measured dioxin-like PCBs (Supplementary Table 1). Serum TH levels are shown in Table 3. All values of T4, T3 and TSH were within the normal range for our laboratory. Normal values for T4S, rT3 and TBG are not available for newborns in our laboratory.

Table 2. PCB- and OH-PCB-levels in maternal serum during pregnancy

Compound	Median (IQR)	*n*
PCB-105	4.2 (2.1-11.1)	100
PCB-118	21.0 (14.7-33.3)	100
PCB-138	68.4 (48.0-85.8)	100
PCB-146	8.1 (4.7-13.0)	100
PCB-153	90.5 (63.5-121.1)	100
PCB-156	11.1 (7.7-14.1)	98
PCB-170	19.0 (13.3-25.0)	98
PCB-180	44.7 (31.4-58.1)	98
PCB-183	8.2 (5.6-10.4)	100
PCB-187	12.0 (8.7-17.1)	100
Sum PCBs[a]	293.4 (216.5-390.4)	98
Sum dl-PCBs[b]	38.7 (24.6-60.7)	98
Sum non-dl-PCBs[c]	252.7 (180.3-334.3)	98
4-OH-PCB-107	0.065 (0.041-0.101)	97
3'-OH-PCB-138	0.045 (0.031-0.065)	97
4-OH-PCB-146	0.070 (0.052-0.101)	97
3-OH-PCB-153	0.037 (0.024-0.054)	97
4'-OH-PCB-172	0.016 (0.010-0.022)	77
4-OH-PCB-187	0.136 (0.105-0.175)	97
Sum OH-PCBs[d]	0.386 (0.281-0.531)	97
Calux	19.55 (14.43-59.53)	98

PCBs in ng/g lipid weight; OH-PCBs in ng/g fresh weight; Calux levels in pg TEQ/g lipid; [a] sum of all 10 PCBs; [b] sum of dioxin-like PCBs (105; 118; 156); [c]sum of non-dioxin-like PCBs (138; 146; 153; 170; 180; 183; 187); [d] sum of all measured 6 OH-PCBs.

Table 3. Plasma thyroid hormone parameter levels in cord and serum of infants at 3 and 18 months of age

Thyroid hormone parameter	Umbilical cord		Serum 3 months		Serum 18 months	
	Mean±SD	*n*	Mean±SD	*n*	Mean±SD	*n*
T4 (nmol/L)	126.3±26.7	87	132.2±21.7	90	114.9± 18.1	85
T4S (pmol/L)	485.0±177.1	86	50.3±20.3	88	21.7±12.6	83
TSH (mU/L)	13,9±8.19	86	3.32±1.52	90	3.25±1.39	84
T3 (nmol/L)	0.71±0.24	85	2.78±0.38	89	2.42±0.32	84
rT3 (nmol/L)	3.7±1.22	85	0.56±0.14	87	0.32±0.06	84
TBG (mg/L)	30.3±6.10	86	30.0±6.16	90	25.7±4.31	84
T4-TBG ratio	4.20±0.54	86	4.50±1.02	90	4.52±0.57	84
T3-rT3 ratio	0.22±0.14	85	5.25±1.54	87	7.73±1.66	84

T4:thyroxine; T4S:T4 sulfate; TSH:thyroid stimulating hormone; T3:tri-iodothyronine; rT3:reverse T3; TBG:thyroid-binding globulin

Correlations between PCBs, OH-PCBs and thyroid hormone parameters

A significant positive correlation was found between PCB 118, 146, 153, 138, 187, 183, as well as the sum of PCB levels (sum of 10 PCBs measured, see Table 2) with cord serum T3 levels. A significant negative correlation was found between 4 of these 6 PCBs and the sum of all measured PCBs with cord serum rT3 (Table 4a). Exposure to PCBs 118, 146, 153, 138 and 187 showed a positive correlation with the T3/rT3 ratio (all $p < 0.01$). The sum of both the dioxin-like as well as the non-dioxin-like PCBs showed a significant correlation with the T3/rT3 ratio. No correlation between any of the PCBs or OH-PCBs and cord T4, TSH, T4S or TBG was found. Also no correlation was found between the Calux results and any of the cord TH levels.

At three months, 4-OH-PCB-107 and 4-OH-PCB-187 showed a positive correlation with T4. The results of the Calux showed a positive correlation with T4S and rT3 (Table 4b). At 18 months, 4-OH-PCB-107 showed a positive correlation with T4S, T4 and T3. The sum of OH-PCBs also showed a positive correlation with T4S and T3 (Table 4c).

Table 4a. Spearman's rho correlations between prenatal PCB and OH-PCB levels and T3, rT3 in cord blood

Compound	T3 (nmol/L)		rT3 (nmol/L)		Ratio T3-rT3	
	Rho	*P*-value	Rho	*P*-value	Rho	*P*-value
PCB-118	0.279	0.010**	-0.237	0.029*	0.307	0.004**
PCB-138	0.259	0.017*	-0.235	0.030*	0.301	0.005**
PCB-146	0.343	0.001**	-0.271	0.012*	0.356	0.001**
PCB-153	0.263	0.015*	-0.210	0.054	0.284	0.008**
PCB-183	0.224	0.040*	-0.037	0.734	0.156	0.153
PCB-187	0.320	0.003**	-0.223	0.040*	0.321	0.003**
Sum PCBs[a]	0.279	0.011*	-0.224	0.042*	0.300	0.006**
Sum dl-PCBs[b]	0.253	0.021*	-0.215	0.051	0.270	0.014*
Sum non-dl-PCBs[c]	0.274	0.012*	-0.217	0.049*	0.297	0.006**
Calux	-0.075	0.502	0.053	0.633	-0.100	0.367

[a] sum of all 10 measured PCBs; [b]sum of dioxin-like PCBs (105; 118; 156); sum of non-dioxin-like PCBs (138; 146; 153; 170; 180; 183; 187); T3:tri-iodothyronine; rT3:reverse T3;*P<0.05;**P< 0.01

Correction for confounders

We investigated whether factors might have confounded our findings by determining the odds ratios (ORs) for their effect on TH parameters. Univariate logistic regression analyses in cord blood revealed that higher age of the mother (OR=1.127; P=0.037) and female gender (OR=2.745; P=0.024) were associated with higher T3 (above the 50th percentile), and higher gestational age (OR=1.980; P=0.005) was associated with higher T3/rT3 ratio. Table 5 shows univariate logistic regression analyses, and multivariate logistic regression analyses to correct for confounders.

Univariate logistic regression analyses revealed that at the age of three months, female gender (OR=2.047; P=0.095) was associated with higher T4 (above the 50th percentile), and lower age of the mother (OR=0.931; P=0.176) and breastfeeding (OR=4.615; P=0.004) with higher T4S (above the 50th percentile). Formula feeding (OR=2.667; P=0.041) was associated with higher TSH at 3 months (above the 50th percentile). At 18 months, higher gestational age (OR=1.321; P=0.162) was associated with higher T4 and higher TSH (above the 50th percentile).

After correction for confounders, the Calux result remained significantly associated with T4S at three months (OR=1.019; P=0.039). Using univariate logistic regression models, the relations between OH-PCBs and TH parameters at three months were not significant. After correction for gestational age, 4-OH-PCB-107 was marginally significantly associated with T4 at 18 months (OR=1.112; P=0.052), and lower 4'-OH-PCB-172 remained associated with higher TSH at 18 months (OR=0.470; P= 0.030; ORs given per 0.01 pg/g fresh weight).

Table 4b. Spearman's rho correlations between prenatal PCB and OH-PCB levels and thyroid hormone parameters at three months

Compound	T4 (nmol/L)		T4S (pmol/L)		TSH (mU/L)		T3 (nmol/L)		rT3 (nmol/L)		T4-TBG ratio		T3-rT3 ratio	
	Rho	*P*-value	Rho	*P*-value	Rho	*P*-value	Rho	*P*-value	Rho	*P*-value	Rho	*P*-value	Rho	*P*-value
PCB-146											0.246	0.020*		
4-OH-PCB-107	0.210	0.050*												
3'-OH-PCB-138											0.228	0.033*		
3-OH-PCB-153											0.238	0.026*		
4'-OH-PCB-172					-0.243	0.044*								
4-OH-PCB-187	0.235	0.027*					0.269	0.012*						
Calux			0.235	0.029*					0.214	0.048*			-0.234	0.030*

T4: thyroxine; T4S: T4 sulfate; TSH: thyroid stimulating hormone; T3:tri-iodothyronine; rT3:reverse T3; TBG: thyroid-binding globulin;*P<0.05

Table 4c. Spearman's rho correlations between prenatal PCB and OH-PCB levels and thyroid hormone parameters at 18 months

Compound	T4 (nmol/L)		T4S (pmol/L)		TSH (mU/L)		T3(nmol/L)		T4-TBG ratio	
	Rho	*P*-value	Rho	*P*-value	Rho	*P*-value	Rho	*P*-value	Rho	*P*-value
PCB-156									0.217	0.050*
4-OH-PCB-107	0.226	0.041*	0.291	0.009**			0.275	0.013*		
4-OH-PCB-146			0.228	0.042*						
4'-OH-PCB-172					-0.298	0.015*				
Sum OH-PCBs[a]			0.242	0.030*			0.237	0.033*		

[a] sum of all 6 measured OH-PCBs; T4: thyroxine; T4S: T4 sulfate; TSH: thyroid stimulating hormone; T3: tri-iodothyronine; TBG: thyroid-binding globulin; *P<0.05;**P< 0.01

Table 5. Logistic regression analyses for associations between prenatal PCB levels and thyroid hormone parameters in cord blood

Compound	TH parameter	OR (95% CI)[c]	*P*-value	OR after correction (95% CI)[c]	*P*-value
PCB-118	T3[a]	1.032 (0.997, 1.067)	0.071	1.041 (1.003, 1.080)	0.034*
	T3/rT3 ratio[b]	1.036 (1.001, 1.073)	0.045*	1.039 (1.002, 1.076)	0.036*
PCB-138	T3[a]	1.016 (0.999, 1.033)	0.062	1.014 (0.997, 1.032)	0.108
	T3/rT3 ratio[b]	1.020 (1.003, 1.038)	0.024*	1.019 (1.002, 1.037)	0.032*
PCB-146	T3[a]	1.142 (1.042, 1.250)	0.004**	1.146 (1.038, 1.265)	0.007**
	T3/rT3 ratio[b]	1.098 (1.010, 1.194)	0.028*	1.086 (0.999, 1.180)	0.052
PCB-153	T3[a]	1.014 (1.002, 1.026)	0.025*	1.012 (0.999, 1.025)	0.073
	T3/rT3 ratio[b]	1.012 (1.000, 1.024)	0.045*	1.011 (1.000, 1.023)	0.060
PCB-183	T3[a]	1.031 (0.959, 1.109)	0.411	1.038 (0.955, 1.129)	0.381
	T3/rT3 ratio[b]	0.977 (0.922, 1.034)	0.419	0.962 (0.884, 1.047)	0.372
PCB-187	T3[a]	1.118 (1.028, 1.215)	0.009*	1.121 (1.020, 1.232)	0.018*
	T3/rT3 ratio[b]	1.063 (0.989, 1.142)	0.097	1.053 (0.980, 1.131)	0.162
Sum PCBs[d]	T3[a]	1.004 (1.000, 1.008)	0.040*	1.004 (1.000, 1.008)	0.076
	T3/rT3 ratio[b]	1.005 (1.001, 1.009)	0.024*	1.005 (1.001, 1.009)	0.027*
Sum dl-PCBs[e]	T3[a]	1.017 (0.997, 1.038)	0.105	1.020 (0.998, 1.042)	0.073
	T3/rT3 ratio[b]	1.017 (0.996, 1.038)	0.107	1.019 (0.997, 1.041)	0.085
Sum non-dl-PCBs[f]	T3[a]	1.005 (1.000, 1.010)	0.043*	1.004 (0.999, 1.009)	0.097
	T3/rT3 ratio[b]	1.006 (1.001, 1.011)	0.025*	1.006 (1.001, 1.011)	0.031*

[a] Confounders: age of mother and female gender; [b] Confounder: gestational age; [c] ORs are given per 1 ng/g lipid weight; [d] sum of all 10 measured PCBs; [e] sum of dioxin-like PCBs (105; 118; 156); [f] sum of non-dioxin-like PCBs (138; 146; 153; 170; 180; 183; 187); *P<0.05; **P<0.01; OR: Odds ratio

DISCUSSION

In this study we observed a positive correlation between prenatal background exposure to six PCBs and cord serum T3, as well as a negative correlation between exposure to four of these six PCBs and cord serum rT3 levels. Five PCBs and the sum of the measured PCBs were positively correlated with the T3/rT3 ratio. No effect was found of exposure background levels of PCBs or OH-PCBs on T4, T4S, TSH and TBG in cord blood. 4-OH-PCB-107 was correlated with T4 at 3 months and T4, T4S and T3 at 18 months. The findings of this study suggest that background exposure to PCBs might influence thyroid hormone metabolizing enzymes such as iodothyronine deiodinases during fetal life. 4-OH-PCB-107 might have an effect after birth.

Possible mechanisms of PCBs influencing thyroid hormone levels

Studies on animal models have shown effects of PCBs on thyroid function. A number of mechanisms have been identified in animals that might play a part: direct negative effects on the thyroid gland, competition with TH binding to TTR (the main TH-binding protein in rodents), increased biliary excretion of THs, interaction with T3 receptors, interaction with the pituitary-thyroid axis, and, finally, changes in the activities of deiodinases (enzymes regulating the levels of active THs).[17, 20-22] It is unclear which of these mechanisms may play a role in the human fetus and newborn. Although the main TH binding protein in humans is not TTR, but TBG, TTR still plays a role in mediating the delivery of T4 across the blood-brain barrier, transporting T4 into the cerebrospinal fluid, and transferring T4 from the mother to the fetus over the placenta.[23, 24] Most (>85%) of the hydroxylated PCB metabolites in human plasma were found to be associated with human TTR.[16] Therefore, it is reasonable to suggest that fetal accumulation of hydroxylated PCBs may also occur in human and wildlife species.[17] During the first half of gestation, fetal TH levels are dependent on maternal T4 supply. After the onset of fetal TH production, maternal T4 supply to the fetus continues to represent an important proportion of TH available to the fetus.[25] Different studies have shown a negative correlation between PCBs and TH levels in pregnant women.[6, 11, 26] Lower levels of maternal TH might result in lower TH levels in the fetus. Lower thyroid levels in the fetus might negatively influence brain growth and development, as is seen in infants with increased levels of TSH at birth.[27] Julvez et al. found that low fT4 levels during pregnancy were associated with a moderate delay in child development indicating the crucial role of perinatal thyroid hormone status for infant development.[28] These findings indicate that disturbances in neonatal thyroid hormone levels might have an influence on neuromotor development.

Effects of PCBs on T3 and T3/rT3 ratio

In the present study we showed a positive correlation between a number of PCBs with T3 in umbilical cord blood, and a negative correlation with rT3. Also, the serum T3/rT3 ratio showed a significant positive correlation with PCBs. We did not find a relation between the serum lipid concentration and thyroid hormones (data not shown). This suggests that the correlations found between PCBs and serum thyroid hormones are not influenced by lipid concentration.[29] TH homeostasis in humans is regulated by the activities of the iodothyronine deiodinases D1, D2 and D3, glucuronidation and sulfation.[25] D1 is present in the liver, kidney and thyroid gland, and removes iodine from the inner and outer ring. D1 in adults is considered to be an important source of T3 and responsible for the clearance of rT3. D2 is present in the brain, anterior pituitary and thyroid gland. It plays an important role in the production of T3 in the brain. D3 is especially present in the fetal brain, placental and fetal tissues. D3 catalyzes the inner ring deiodination, resulting in the conversion of T4 into rT3 and T3 into 3,3'-T2.[25] D3 is suggested to play a role in the regulation of intracellular T3 levels in tissues such as brain tissue. In placenta and fetal tissues, D3 may protect developing tissues against exposure to unduly high levels of active TH.[30]

The pattern of activity of the deiodinases is different in the fetus and the adult. D2 activity is relatively low in the fetus.[31, 32] D3, however, is found to be very active during fetal life, while this activity rapidly decreases after birth.[31-34] Due to the high D3 activity, especially in placental tissues, fetal rT3 levels are much higher than in the postnatal period, whereas the opposite is true for T3. A high D3 activity is not only found in placental tissue, but also in human fetal liver and fetal brain tissue.[35] The high D3 activities in fetal and placental tissues are thought to protect growing tissues against exposure to unduly high T3 levels. Our results showing a positive correlation between PCB-118, PCB-146, PCB-153, PCB-138 or PCB-187 and the sum of the measured PCBs and the T3/rT3 ratio, suggest a negative effect of these compounds on D3 activity. This correlation was seen both for dioxin-like and non-dioxin-like PCBs. The found effects on the T3/rT3 ratio do not seem to be dioxin-related effects, because the correlation was not seen for the Calux results, while the dioxin-like PCBs were highly significantly correlated with the Calux results. The possible effect of this lower D3 activity on the human fetus is still unclear.

A number of studies, summarized in Table 6, investigated the potential effect of PCBs on thyroid hormone levels. It is difficult to compare the results of these studies due to differences in methodology. Most studies used maternal serum taken during pregnancy to estimate PCB levels, but some studies measured this in cord blood, and single studies used placenta tissue and breast milk. The type and number of PCBs varied between studies, with some studies expressing results per unit serum, others per g lipid weight. Finally, some studies expressed the levels as Toxic Equivalents, TEQ. Thyroid hormones were measured either in cord blood, or at 2-21 days after birth. A number of studies, but certainly not

all, found a positive correlation between PCBs and TSH. Only a few studies measured T3. Koopman-Esseboom et al. found no effect, but measured two weeks after birth.[6] Sandau et al. found no effect in cord blood in relation to PCBs, but lower levels of T3 when PCBs and OH-PCBs were combined.[36] Takser et al. found no effect in cord blood.[11] Wang et al. found a higher level of T3 in girls, not in boys.[37] Darnerud et al. found lower levels of T3, but measured three weeks after birth.[26] Dallaire et al. found no effect in cord blood but only measured PCB 153.[38] Maervoet et al. found lower levels of fT3 in cord blood.[8] Leijs et al. reported a positive correlation between PCBs and T3 at the age of 14 to 19 years.[39] This is in contrast to studies by Koopman-Esseboom et al. and Takser et al. who found lower levels of T3 in pregnant woman in relation to PCBs.[6, 11] That the effect we found in the present study on T3 and rT3 is related to the level of PCBs is unclear. The median level of PCB 153 was 90.5 ng/g lipid in our study compared to 31.7 in the study of Maervoet et al.[8] In the studies by Koopman et al., levels are unfortunately only expressed as TEQs and therefore not comparable to the results of either Maervoet et al. or the present study. More studies are needed to resolve whether the associations found by us in this study are causally related to PCB levels.

Table 6. Overview of studies on PCB-exposure and thyroid hormone metabolism

Reference	Location	Number of participants	PCB-exposure			Thyroid hormone parameters						Additional results
			Sample	Compound	Level	Sample	T3	fT3	T4	fT4	TSH	
Koopman-Esseboom et al.[6]	The Netherlands	78	Breast milk	ΣPCB-TEQ	42.7 ng/g fat	2 wks	0	0	↓	0	↑	
Steuerwald et al.[40]	Faroe Islands	173	Maternal serum	ΣPCB-138, 153, 180	0.56 µg/g fat	Cord	0	→	→	→	→	Neg. corr. ΣPCB and fT3 uptake
Sandau et al.[36]	Canada	30	Maternal serum	Σ 49 PCBs	1.69 pg/g plasma	Cord	→	0	0	→	→	Neg.corr. ΣOH-PCB and T3
Ribas-Fito et al.[41]	Spain	98	Maternal serum	Σ PCB-28,52,101, 118,138,153,189	0.27 ng/mL	3 days	0	0	0	0	↑	
Takser et al.[11]	Canada	92	Maternal serum	Σ 14 PCBs-	0.39 µg/L	Cord	→	0	0	→	↑	
Wang et al.[37]	Taiwan	119	Cord	Σ PCB dioxin-TEQ	15 µg/g fat	Cord	→	0	→	→	↑	Higher T3 and T4 in girls
Maervoet et al.[8]	Belgium	198	Cord	Σ PCB-118,138,153,170,180	91.7 ng/g fat	Cord	0	↓	0	↓	→	
Otake et al.[42]	Japan	23	Day 1	Σ209 PCBs	130 ng/g fat	4-6 days	0	0	0	→	→	Pos. corr. OH-PCB-187 and T4
Álvarez-Pedrerol et al.[43]	Spain	387	Cord	Σ PCB-28,52,101, 118,138,153,189	0.70 and 0.87 ng/mL	3 days	0	0	0	0	↑	
Dallaire et al.[38]	Canada	670	Cord	PCB-153	95.0 and 63.6 µg/kg	Cord	→	0	0	→	↑	
Wilhelm et al.[9]	Germany	84	Maternal serum	ΣPCB-TEQ	5.71 ng/g fat	Cord	→	→	→	→	→	
Dallaire et al.[44]	Canada	120	Maternal plasma	PCB-153	107.7 µg/kg	Cord	→	0	0	→	→	Pos. corr. OH-PCB and maternal T3
Darnerud et al.[26]	Sweden	150	Maternal serum	Σ 10 PCBs	208 ng/g fat	3 wks	↓	0	0	0	0	
Lopez-Espinosa et al.[45]	Spain	453	Cord	Σ PCB-118,138,153,180	131 ng/g fat	Cord	0	0	0	0	→	
Brucker-Davis et al.[46]	France	84	Cord	Σ PCB-28,52,101, 118, 138,153,180	0.4 ng/mL	Cord	0	↑	0	→	↑	
Hisada et al.[47]	Japan	79	Maternal serum	Σ 29 PCBs	69 ng/g lipid	5 days	0	0	0	→	→	Pos. corr. OH-PCB and TSH

↓ indicates negatively associated;↑ indicates positively associated; → indicates no effect on thyroid hormone parameters; 0 indicates not reported

Effects of PCBs on T4, T4S, TSH and TBG

In the present study we did not find an effect of any of the PCBs or OH-PCBs on either T4, T4S, TSH or TBG in cord blood, which might be due to the lower levels of PCBs currently found in pregnant mothers compared with the levels in our first cohort published in 1994.[6] A number of studies have evaluated the effect of PCBs on TH and TSH levels in newborns (Table 6). Mostly cord samples were used to measure the TH status and exposure levels. Two European studies with a relatively high background exposure found a negative correlation between T4, fT4 and PCBs.[6, 8] A number of studies found a positive correlation between PCBs and TSH. No effect was seen in studies from Canada and the Faroe Islands. This latter finding might be related to high fish consumption in these locations, an important source of both PCBs and iodine. For T4, T3 and TSH reference values were available from our laboratory. All levels were within the normal range. This, however, does not exclude that PCBs might have affected thyroid hormone levels. Reference levels are obtained from infants from the Netherlands who were probably exposed to the same background levels of PCBs as individuals in our cohort. A lower level within the reference range might have clinical implications. Infants with congenital hypothyroidism have poorer neurodevelopmental outcomes, even when thyroid hormone levels are within the reference range after early treatment.[48] We did not find an effect of PCBs on levels of T4S in cord blood, but a positive correlation was found between the Calux results and T4S at 3 months and the sum OH-PCB and T4S at 18 months. This might indicate that although the prevailing PCB concentrations do not influence the sulfation of T4 during fetal life, it may still exert an effect in early childhood.

Effects of OH-PCBs on TH metabolism

We did not observe a correlation between any of the OH-PCBs and TH levels in cord blood. Sandau et al. reported a negative correlation between sum OH-PCBs and fT4, while Otake et al. observed a positive correlation.[36, 42] We found that 4-OH-PCB-107 showed a positive effect on T4 at both three and 18 months, while 4'-OH-PCB-172 showed a negative effect on TSH at both three and 18 months. Whether OH-PCBs have an effect on human TH levels, or are currently present at levels too low to have an effect, is not clear. It is interesting to note that different studies found an effect of 4-OH-PCB-107 on neurodevelopment.[10, 14, 15] Whether this effect on neurodevelopment is related to effects on the thyroid hormones, needs further study.

A strength of our study is that we evaluated the effect of PCBs on the activity of deiodinases and sulfotransferase in human infants. To our knowledge, this is the first study evaluating whether PCBs might have an effect on the activity of these thyroid hormone metabolizing enzymes in human infants. A study in rats shows that PCBs competitively inhibit T4 binding to TTR and type I deiodinase (D1) activity.[19] Another study by Morse et al. shows that reductions in brain T4 levels were most severe in the fetus, and that the

induction of D2 activity in the fetal forebrain can account for the maintenance of forebrain T3 levels in the fetus.[12] It has been suggested that type III iodothyronine 5-deiodinase (D3) regulates cerebellar T3 levels by regulating the deiodination of T3 to T2. A second strength is that in addition to the effects of PCBs, we also investigated whether prenatal exposure to OH-PCBs might have effects on thyroid hormone metabolism. A third strong point is that we measured thyroid hormone parameters at several time points. We measured thyroid hormone parameters in cord blood, and in samples of infants at the age of three and 18 months.

We also recognize limitations to this study. A first limitation is that we have a relatively small study group. We evaluated the effects of PCBs on thyroid hormone parameters in 100 mother-infant pairs. Because of the relatively small study group, the results need to be interpreted with caution, and larger studies are needed to confirm our results. A second limitation is that we only investigated the effects of PCBs until the age of 18 months. In our study, we did not find effects of prenatal exposure to PCBs or OH-PCBs on thyroid hormone parameters at the age of three and 18 months. It is not clear whether the associations found between PCBs and OH-PCBs and thyroid hormone parameters in cord blood might have consequences for developmental processes after the age of 18 months. Leijs et al. found a positive correlation between dioxin-like PCBs and T3 levels in infants at the age of 14 to 18 years old.[39] To assess whether our findings have consequences for hormone metabolism and developmental outcomes at later age, we are currently working on a follow-up study, in which we will measure levels of several PCBs and thyroid hormone parameters during adolescence. A further limitation is that other environmental pollutants, like brominated compounds, may have the same effects as PCBs. We found no correlation between the Calux results in cord blood and thyroid hormones. The Calux is an overall measure of dioxin-like compounds. Still, we found a correlation between the T3/rT3 ratio and the dioxin-like PCBs. This suggests that the effect of the dioxin-like PCBs on the T3/rT3 ratio is not mediated by the AhR receptor. This is also supported by the finding that non-dioxin-like PCBs also influenced the T3/rT3 ratio. It might also be that different dioxin-like compounds might have different, sometimes opposing, effects. However, it is difficult to study this in humans, as all humans are subjected to combinations of compounds. Studies in animals might not reflect the metabolism of these compounds in humans.

CONCLUSIONS

In conclusion, prenatal background exposure to several PCBs influences cord T3 and rT3 levels, giving indications for reduced D3 activity. No effect was found on T4, T4S, T3, TSH and TBG in infants at three and 18 months. 4-OH-PCB-107 was related to thyroid hormones at the age of 3 and 18 months. Our findings suggest that prenatal background exposure to PCBs might influence the regulation of T3 levels, and therefore affect early development.

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SUPPLEMENTARY MATERIAL

Table S1. Spearman's rho correlations between prenatal PCBs and Calux

Compound	Calux (nmol/l) Rho	*P*-value
PCB-105	0.376	0.000**
PCB-118	0.310	0.002**
PCB-138	0.186	0.066
PCB-146	0.242	0.016*
PCB-153	0.197	0.052
PCB-156	0.271	0.007**
PCB-170	0.151	0.143
PCB-180	0.109	0.291
PCB-183	0.218	0.031*
PCB-187	0.213	0.035*
Sum PCBs[a]	0.227	0.026*
dl-PCBs[b]	0.327	0.001**
non-dl-PCBs[c]	0.190	0.063

Calux: Chemical-activated luciferase gene expression; [a] Sum PCBs: sum of all 10 measured PCBs; [b] Sum dl-PCBs: sum of dioxin-like PCBs (105; 118; 156); [c] Sum non-dl-PCBs: sum of non-dioxin-like PCBs (138; 146; 153; 170; 180; 183; 187);*P<.05;**P<.01