The effects of exposure to environmental chemicals on child development

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CHAPTER 8

The effects of prenatal exposure to persistent organic pollutants on pubertal development

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ABSTRACT

Background: Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), are environmental chemicals which may interfere with hormonal processes. Knowledge about the effects of prenatal PCB-exposure and their hydroxylated metabolites (OH-PCBs) on pubertal development is limited.

Objective: To determine whether prenatal background exposure to POPs, especially PCBs and OH-PCBs, is associated with pubertal development in 13- to 15-year-old children.

Methods: Between 1998 and 2002, 194 mother-infant pairs were included in this observational longitudinal cohort study for assessment of levels of POPs. During the third trimester of pregnancy, in all mothers PCB-153 and three OH-PCBs levels were measured, in part of the mothers also nine other PCBs and three OH-PCBs, and in the other mothers, five polybrominated diphenyl ethers, dichloroethene, pentachlorophenol and hexabroomcyclododecane. Follow-up for assessment of pubertal development in the children was performed between 2014 and 2016. We assessed the Tanner stages and testicular volume (assessed by clinician or standardized self-assessment) at the clinic, and the children completed a questionnaire on the onset of pubertal characteristics.

Results: Of the 188 adolescents invited at follow-up, 101 (53.7 %) volunteered to participate. The mean age was 14.4 years ± 0.8. Regarding Tanner stage for pubic hair, positive associations were found for 6 PCBs and ΣPCBs in boys, and for PCB-153 in girls. 3 PCBs correlated negatively with age at boys' voice change. Regarding OH-PCBs, only a few positive and/or inverse associations were found with pubertal outcomes. None of the other POPs were associated with pubertal outcomes.

Conclusion: Higher prenatal exposure to Dutch background PCB-levels was associated with more advanced pubertal development in 13- to 15-year-old children. Our findings raise concern towards effects of man-made compounds on pubertal development in children.

INTRODUCTION

There is growing evidence that background exposure to environmental chemicals affect child development. There are many chemicals in the environment because of their extensive use, their resistance to biological and chemical degradation, and bio-accumulation in the food chain. Exposure to these persistent organic pollutants (POPs) continues for long periods after the production and use had been banned by law. Humans are exposed to environmental chemicals via food, drinking-water and air. POPs include polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dichloroethene (DDE), pentachlorophenol (PCP), hexabroomcyclododecane (HBCDD), and others.

PCBs are chemicals produced between 1929 and 1985 for application in a variety of products including coolants in heat-transfer systems and lubricants in plastics[1]. PCBs are metabolized in the liver to hydroxy-PCBs (OH-PCBs). Both PCBs and OH-PCBs can be transferred over the placenta from the mother to the fetus[2]. Because OH-PCBs are transferred in a higher ratio compared to PCBs, there is potentially greater toxicity of OH-PCBs for the fetus. The prenatal period is a vulnerable period because many developmental processes are initiated, and disruption of these processes might influence outcomes in later life[3]. Prenatal PCB-exposure has shown to interfere with neurological, immunological, metabolic and endocrine development in children[4-8]. PCBs can interfere with hormonal pathways, including exerting estrogenic or anti-estrogenic effects[8, 9]. Because pubertal development is a multifaceted process under control of several hormonal mechanisms, PCB-exposure might interfere with pubertal development. Evidence is accumulating that exogenous hormone disruptors may advance or delay puberty[8, 10].

Knowledge about the impact of prenatal background (OH-)PCB-exposure on pubertal development is limited. The aim of this study was to determine whether prenatal background POP-exposure is associated with pubertal development.

METHODS

Cohort and study design

This prospective longitudinal cohort study is part of the Development at Adolescence and Chemical Exposure (DACE)-study, in which we followed-up two Dutch cohorts. Between 1998 and 2000, 104 mother-infant pairs were included in the Risk of Endocrine Contaminants on human health (RENCO)-study[2]. Between 2001 and 2002, 90 mother-infant pairs were included in the Groningen-Infant-COMPARE(Comparison of Exposure-Effect Pathways to Improve the Assessment of Human Health Risks of Complex Environmental Mixtures of Organohalogens)-study, also known as GIC-study[11]. Children of both cohorts were invited

for the current study. Six children were not invited: four had no available prenatal POP-levels, one had been diagnosed with a congenital syndrome after inclusion in the cohort, and one had moved abroad. A reminder was sent in case of no response. The children were all singletons and born at term (37-42 weeks' gestation) without congenital anomalies or diseases. Their mothers are of Western European origin, and had no serious illnesses or complications during pregnancy or delivery. At time of follow-up, all children were between 13-15 years (inclusion periods April 2014-December 2014, and October 2015-August 2016). All adolescents and their parents provided their written informed consent before participation in the follow-up program. The follow-up and the original study were approved by the University Medical Center Groningen medical ethics committee.

Measurement of prenatal levels of POPs

Maternal blood samples were taken during the second and/or third trimester of pregnancy. Detailed descriptions of the analyses have been published previously[2, 11]. Levels of PCB-153, 4-OH-PCB-107, 4-OH-PCB-146, and 4-OH-PCB-187 were measured in both cohorts. In the RENCO-study, also nine other PCBs (105; 118; 138; 146; 156; 170; 180; 183; 187) and three other OH-PCBs (3-OH-PCB-153; 3'-OH-PCB-138; 4'-OH-PCB-172) were measured, and the sum of all (OH-)PCBs was calculated. The following POPs were also measured in the GIC-study: 2,2′-bis-(4 chlorophenyl)-1,1′-dichloroethene (p,p'-DDE), pentachlorophenol (PCP), five different 2,2',4,4'-tetrabromodiphenyl ethers (BDEs) and hexabroomcyclododecane (HBCDD). PCBs and OH-PCBs were numbered respectively according to Ballschmiter et al. and to Letcher et al.[12, 13]. PCB-levels are given in ng/g lipid, and OH-PCB-levels in pg/g fresh weight.

Outcome measures pubertal development

Pubertal development was staged according to Marshall and Tanner[14, 15]. Testicular volume was assessed using a Prader orchidometer. Pubertal development was assessed at the clinic by author SAB, or by the participants themselves, after instructions and looking in a mirror, using realistic colored pictures according to Carel et al.[16]. This is a valid method for self-assessment of pubertal stages[17]. A questionnaire on pubertal characteristics was filled in at the clinic. Height and weight were measured, and BMI z-scores calculated using Growth Analyzer version 3 (http://www.growthanalyser.org/), which contains age-specific and sex-specific data from the Fourth Dutch Growth Study[18]. Parents reported maternal menarche and paternal timing of growth spurt. The investigators were blinded to prenatal POP-levels.

Statistical analyses of data

T-test was used to compare POP-levels between groups. Spearman's rank correlation test was used for correlations between outcome measures. Pearson's and partial correlation test were used for continuous outcome measures, Kruskal Wallis test (KW) for categorical outcome measures. Odds ratios (ORs) were calculated using logistic regression models. The following factors were considered as potential confounders: age at examination (<173 versus ≥173 months); z-score of body mass index (BMI; <0 versus ≥0); maternal age at menarche (<13 versus ≥13 years); timing growth spurt father (early versus average/ late compared to peers); and assessor of Tanner stages (SAB versus participant). These characteristics were included in multivariate logistic regression analyses (method: enter) if they had a *P*-value below .20 in univariate logistic regression analyses. A *P*-value below .05 was considered statistically significant, and between .05 and .10 was considered a trend towards significance. Statistical Package for the Social Sciences, version 23 (SPSS) was used.

RESULTS

Study group

Of the 188 children invited, 101 (53.7 %) participated. 44 (23.4%) declined the invitation, and 43 (22.9%) did not respond. The final study group consisted of 55 boys and 46 girls. Almost all children, except one boy and girl, lived in the northern part of the Netherlands at time of follow-up. Characteristics of the study group are presented in Table 1.

Prenatal POP-levels

The POP-levels of all mother-infant pairs included initially in the cohorts have been reported previously[11, 19]. The POP-levels did not differ between the in- and excluded children, except for PBDE-154, which was lower in included children (0.497±0.241 versus 0.837±0.733 ng/g lipid; t=-2.573; *P*=.028).

Table 1. Characteristics of the study group (N=101)

Characteristic			Value
Gender, boy/girl			55/46 (54.5/45.5%)
Gestational age (weeks)			40 (37-42)
Apgar at 3 min [median (range)] (n=85)			10 (7-10)
Age at examination (years)			14.4 ± 0.8
BMI at examination			20.0 ± 3.6
Assessment Tanner stage by clinician [yes/no](n=97)			33/64 (34/66%)
Maternal education level			
Below average (≤11 years education)			9
Average (12-13 years education)			41
Above average (≥14 years education)			51
Maternal smoking [yes/no]			13/88 (13/87%)
Maternal alcohol consumption [yes/no]			21/80 (21/79%)
Maternal age at menarche (n=97; years)			12.8 ± 1.5
Paternal timing growth spurt (n=75) [early-average/late]			47/28 (63/37%)
Assessment of pubertal stage			
Boys:	-	Tanner genital stage	2.96 ± 0.88
	-	Tanner pubic hair stage	3.25 ± 1.04
	-	Testicular volume (mL)	9.98 ± 3.84
Girls:	-	Tanner breast stage	3.88 ± 0.77
	-	Tanner pubic hair stage	3.66 ± 0.62
Questionnaire on pubertal development			
Boys:	-	Onset pubic hair (n=49, 89%; years)	12.4 ± 0.92
	-	Onset growth spurt (n=44, 80%; years)	12.4 ± 1.10
	-	Age at first ejaculation (n=29, 53%; years)	13.0 ± 0.69
	-	Age at mutation of voice (n=35, 64%; years)	13.2 ± 0.76
Girls:	-	Breast growth (years)	11.7 ± 1.20
	-	Onset growth spurt (n=37, 80%; years)	11.7 ± 1.37
	-	Onset pubic hair (years)	12.0 ± 1.06
	-	Age at menarche (n=39, 85%; years)	12.4 ± 1.16

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

Pubertal development

Outcomes on pubertal development are shown in Table 1. For two boys stages were not written down after self-assessment, and for one the self-assessed testicular volume was excluded from analyses due to discrepancy with other self-reported outcomes. 4-OH-PCB-107-levels were higher in boys reporting onset of growth of pubic hair versus those reporting no onset (53.82 versus 22.07 pg/g serum; t=-4.260; *P*=.001), which might be related to two factors, five of the six reporting no onset were included in the GIC cohort and were 13-14 years at follow-up and secondly, boys in the RENCO cohort had, on average, lower 4-OH-PCB-107-levels compared to the RENCO cohort (14-15 years at follow-up). PCB-105 and PCB-118-levels were higher in boys reporting first ejaculation than in boys who did not (respectively 7.30 versus 3.14 ng/g lipid; t=-2.391; *P*=.027 and 27.91 versus 14.78 ng/g lipid;

t=-3.298; *P*=.003). Tanner stages correlated strongly with each other (R=.764; *P*<.001), and Tanner genital and pubic hair stages correlated with testicular volume (respectively R=.514; *P*<.001; and R=.515; *P*<0.001).Tanner stages and testicular volume correlated not with self-reported onset of pubertal characteristics in our study.

Regarding girls, two refused pubertal assessment, and for two girls stages were not written down after self-assessment. For one girl, assessment of pubic hair stage was not possible due to shaving. PCB-153-levels were higher in girls who reached menarche (n=39) than in those who did not (n=7) (97.41±42.70 versus 68.53±16.64 ng/g lipid; t=-3.108; *P*=.005), which can be related to the fact that six of the seven girls reporting no menarche were included in the GIC cohort (13-14 years at follow-up) with lower PCB-153-levels compared to the RENCO cohort (14-15 years at follow-up). BDE-47-levels were higher in girls who reached menarche (n=11) than in girls who did not (n=5) (0.50±0.16 versus 0.91±0.38 ng/g lipid; t=-3.020; *P*=.009). Tanner stages in girls correlated with each other (R=.458; *P*=.003). Tanner pubic hair stage correlated negatively with ages at onset of menarche (R=-.459; *P*=.006), breast growth (R=-.489; *P*=.001), growth of pubic hair (R=-.452; *P*=.003), and showed a negative trend with age at growth spurt (R=-.300; *P*=.090). Tanner breast stage did not correlate with self-reported ages at onset of pubertal characteristics.

(OH-)PCBs and pubertal development in boys

Mainly positive associations were found between prenatal (OH-)PCB-levels and pubertal development in boys, as reflected by higher Tanner stages, larger testicular volumes, and/or younger ages at onset of pubertal characteristics. Six PCBs and ΣPCBs were positively associated with Tanner pubic hair stage, two PCBs and 4-OH-PCB-107 showed a positive trend after adjustment for age at examination (Table 2a). Only PCB-146 showed a positive trend with Tanner genital stage, after adjustment for age and assessor (OR=1.15; 95%CI: 0.98-1.34; *P*=.079;). Three PCBs (118, 138 and 156) and ΣPCBs showed a positive trend with testicular volume, taking age at examination and BMI z-score into account (Supplementary Table 1). Three PCBs and 4-OH-PCB-146 (in one cohort) correlated negatively with age at voice change, and for three other PCBs, the ΣPCBs and 4'-OH-PCB-172 it was a negative trend (Table 3). A negative correlation was found between PCB-105 and PCB-118-levels and age at first ejaculation (respectively R=-.519;*P*=.027; and R=-.527;*P*=.025).

Inverse associations between prenatal (OH-)PCB-levels and pubertal development in boys were only found for two OH-PCBs: higher 4-OH-PCB-187-levels in one cohort (but not in the combined cohort) correlated with smaller testicular volume after adjustment for age and BMI z-score (R=-.497; *P*=.008), and higher 4-OH-PCB-107-levels correlated with older age at first ejaculation (R=.407;*P*=.035).

(OH-)PCBs and pubertal development in girls

Mainly positive associations were found between prenatal (OH-)PCB-levels and pubertal development in girls, as reflected by higher Tanner stages and/or a younger age at onset of pubertal characteristics. Seven PCBs and ΣPCBs were positively related to stage for breast development, after adjustment, there was still a trend for PCB-118, PCB-153, and ΣPCBs and Tanner breast stage 5 (Supplementary Table 2; Table 2b). PCB-118 and PCB-153 were positively related to pubic hair stage, although after adjustment, only PCB-153 was associated (OR=1.03; *P*=.046). Higher 4-OH-PCB-146 and 4-OH-PCB-187-levels correlated with younger age at onset of growth spurt in one cohort, taking maternal menarche into account (R= -.462; *P*=.047 and R= -.581; *P*=.009).

Inverse associations between prenatal (OH-)PCB-levels and pubertal development in girls were only found for 4-OH-PCB-187. In one cohort, this compound was negatively related to Tanner breast stage >4 (OR=0.93; 95% CI 0.86-1.00; *P*=.039), and a trend was seen for higher exposure and older age at menarche, taking maternal menarche and BMI z-score into account (R=.589; *P*=.073).

Table 2a. Logistic regression analyses for prenatal (OH-)PCB levels and Tanner pubic hair stage ≥4 in 13- to 15-year-old boys

Compound	*n*	OR (95% CI)	*P*-value	Adjusted OR (95% CI) [a]	*P*-value
PCB-118	26	1.26 (1.00-1.58)	.050**	1.22 (1.01-1.47)	.044**
PCB-138	26	1.09 (1.02-1.17)	.017**	1.09 (1.01-1.17)	.021**
PCB-146	26	1.67 (1.04-2.70)	.035**	1.73 (1.06-2.83)	.028**
PCB-153	53	1.02 (1.00-1.04)	.043**	1.02 (1.00-1.03)	.102
- RENCO	*26*	*1.06 (1.01-1.11)*	*.015***	*1.06 (1.01-1.11)*	*.016***
- GIC	*27*	*0.99 (0.95-1.02)*	*.457*	*0.99 (0.96-1.02)*	*.491*
PCB-156	26	1.41 (1.05-1.90)	.021**	1.43 (1.06-1.93)	.018**
PCB-170	26	1.15 (1.00-1.33)	.059*	1.17 (1.00-1.37)	.051*
PCB-180	26	1.06 (0.99-1.13)	.080*	1.07 (0.99-1.15)	.076*
PCB-187	26	1.48 (1.07-2.05)	.018**	1.56 (1.09-2.22)	.015**
Σ 10 PCBs	26	1.02 (1.00-1.03)	.014**	1.02 (1.00-1.04)	.015**
4-OH-PCB-107	52	1.02 (1.01-1.04)	.014**	1.02 (1.00-1.04)	.060*

[a] Adjusted for age at examination; **P*<.10; ***P*<.05.

Table 2b. Logistic regression analyses for prenatal PCB-levels and Tanner breast stage 5 in 13- to 15-year-old girls

Compound	*n*	OR (95% CI)	*P*-value	Adjusted OR (95% CI) [a]	*P*-value
PCB-105	27	1.09 (0.97-1.22)	.139	1.07 (0.96-1.20)	.209
PCB-118	27	1.15 (1.03-1.29)	.012**	1.21 (0.98-1.49)	.073*
PCB-138	27	1.04 (1.00-1.07)	.055*	1.05 (0.98-1.11)	.164
PCB-146	27	1.26 (1.03-1.54)	.025**	1.24 (0.95-1.61)	.115
PCB-153	42	1.05 (1.02-1.08)	.003***	1.05 (1.00-1.10)	.066*
PCB-156	26	1.32 (1.05-1.66)	.019**	1.34 (0.93-1.92)	.112
PCB-170	26	1.12 (0.98-1.27)	.086*	1.14 (0.94-1.38)	.174
Σ 10 PCBs	26	1.01 (1.00-1.02)	.028**	1.02 (1.00-1.03)	.081*

[a] Adjusted for age at examination, maternal age of menarche, and assessor of Tanner Stage; **P*<0.10; ***P*<0.05; ****P*<0.01.

Table 3. Partial correlation coefficients of prenatal PCBs and OH-PCBs and age at voice mutation in 13- to 15-year-old boys

Compound	R[a]	*P*-value	*n*
PCB-138	-.447	.055*	17
PCB-153			
- RENCO	-.416	.077*	17
- GIC			
PCB-156	-.416	.077*	17
PCB-170	-.568	.011**	17
PCB-180	-.562	.012**	17
PCB-187	-.556	.013**	17
Σ 10 PCBs	-.441	.059*	17
4-OH-PCB-146			
- RENCO	-.481	.037**	17
- GIC			
4'-OH-PCB-172	-.497	.071*	12

[a] Adjusted for age at examination; ***P*<.05 and **P*<.10; only correlation coefficients with *P*<.10 are shown.

Other POPs and pubertal development

Regarding other POPs, only p,p'-DDE-levels showed a trend towards an inverse correlation with testicular volume, taking age and BMI z-score into account (R=-.331; *P*=.092). No other associations were found between p,p'-DDE, PBDEs, PCP or HBCDD-levels and pubertal outcomes.

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DISCUSSION

Our study suggests that higher prenatal PCB-exposure is associated with more advanced pubertal development in both boys and girls. An important finding is that higher prenatal PCB-exposure was associated with higher pubic hair stage, especially in boys, with larger testicular volume and younger age at boys' voice change. A second finding is that OH-PCBs seem to have less effect on pubertal development than PCBs. A third finding is that PBDEs, DDE, PCP, and HBCDD were not associated with pubertal development.

Prenatal PCB-exposure and advanced pubertal development

The finding that in boys, higher prenatal PCB-exposure was associated with higher Tanner pubic hair stage, larger testicular volume and younger age at voice change has not been reported previously. Our finding that prenatal PCB-exposure is positively associated with pubertal outcomes in boys is in contrast to findings in another study reporting negative associations. A study in 438 boys in the Faroese Island, reported weak, non-significant inverse

associations between prenatal PCB-exposure and Tanner stage and testicular volume[20]. The prenatal exposure levels in the latter study on the Faroe Islands (with the traditional habit of eating pilot whale blubber) where much higher than the exposure levels in boys in our cohort: the estimation for total PCB-exposure (twice the sum PCB-congeners 138, 153 and 180) was 643.33 ng/g lipid cord blood (about 60% of maternal levels; calculated based on 3 g/L lipid in cord serum) versus 416.29 ng/g lipid in maternal serum in our study. Our findings might implicate that even relatively low prenatal PCB-exposure might interfere with pubertal development.

The finding that in girls, higher prenatal PCB-153-exposure was associated with higher pubic hair stage has not been reported previously. Two American studies reported no associations between prenatal PCB-exposure and breast or pubic hair stage[21, 22] (for review see Mouritsen et al.[23]). A possible explanation that we did find associations whereas others did not could be a differences in test method: both studies only used self-assessment at home, which might be less precise than assessment by a clinician or by self-assessment at the clinic after instructions with the possibility asking clarification. Both American studies used only total PCB measure, whereas we investigated also individual PCBs. The maternal PCB-153-levels measured in the cohort followed up by Gladen et al. are comparable to levels in our study group (80 versus 77 ng/g lipid) whereas PCB-153-levels are higher in Michigan studies[24]. Because PCB-153 is the most abundant PCB in humans, confirmed by our study, this might be a possible explanation that only this PCB was found to be associated with Tanner pubic hair stage in girls[19]. A possible explanation for the higher pubic hair stage might be an increase in production of adrenal androgens, because they are responsible for growth of pubic hair in girls. A study in a human in vitro model showed that several chemicals can disturb adrenal steroidogenesis, but effects of PCBs were not assessed in that study[25]. Whether PCB-153 might influence pubic hair development in girls by disturbing adrenal androgen levels during puberty has not been studied.

Prenatal OH-PCB-exposure and pubertal development

Regarding 4-OH-PCB-107, both positive and inverse effects were found on pubertal development in boys. Higher exposure to 4-OH-PCB-107 showed a positive trend with Tanner pubic hair stage, but was also associated with older age at first ejaculation. In 45 boys included in the GIC cohort, this compound was found to be positively associated with testosterone levels at three months of age[26]. This suggests that 4-OH-PCB-107 can interfere with hormonal processes early in life, with possible consequences for later life, for example earlier onset of puberty and faster development of pubertal characteristics. The compound 4-OH-PCB-146 was in boys associated with younger age at boys' voice mutation, and with younger age at onset of growth spurt in girls. 4-OH-PCB-146 is one of the metabolites of PCB-153, which was also found to be associated with earlier voice mutation. This finding

implicates that regarding voice mutation, the metabolite might exert a similar effect as PCB-153 itself. The compound 4-OH-PCB-187 was associated with smaller testicular volume, which might be due to an LH/FSH imbalance, because the latter stimulates the growth of the Sertolli cells, which are responsible for a large part of the testicular volume. In 41 boys included in the GIC cohort, a positive trend was found for this compound with follicle stimulating hormone (FSH) at the age of three months[26]. In girls, 4-OH-PCB-187 was associated with signs of earlier onset of puberty in the RENCO cohort (younger age at growth spurt), but with lower breast stage in the GIC cohort.

Other POPs not associated with pubertal development

In contrast to our finding that none of the other POPs were associated with pubertal development, some others did find associations. Vasiliu reported a lower age at menarche after higher prenatal DDE-exposure[27]. We only found a trend towards an inverse correlation between p,p'-DDE levels and testicular volume. In 44 boys included in the GIC cohort, p,p'-DDE showed a positive trend with luteinizing hormone (LH) at the age of three months[26]. Increased levels of LH might be the result of anti-androgenic or anti-estrogenic effects of p,p'-DDE, which can result in an estrogen/androgen imbalance. Eskenazi et al. found that prenatal DDE exposure was associated with decreases in LH concentrations in 12-year-old boys, taking Tanner stage into account[28]. Further research is needed to assess whether the observed smaller testicular volume might be caused by disturbances of hormone levels during puberty, for example an LH/FSH imbalance.

A strength of our study is that almost all children were still living in the northern part of the Netherlands, which minimizes the variability in postnatal exposure levels due to the living area. A second strength is that the time between onset of pubertal characteristics and recall is relatively short, because we included children at ages between 13-15 years. A third strength is that the pubertal questionnaires were filled in at the clinic, giving the adolescents the opportunity to ask for clarification, and thus minimizing the number of lacking or unclear answers. A final strength is that we were able to correct for a reliable BMI, because height and weight were measured at the clinic.

We notice several limitations. Firstly, there is a possibility of Type 1 errors due to the large number of comparisons, which might result in chance-findings. Nevertheless, we believe that our analyses were justified as part of a careful evaluation of a rich data set in hypothesis-driven research[29]. Secondly, there is a possibility for bias due to the way of recruitment of the pregnant women. The women who were willing to participate in a study on effects of chemical-exposure might be more aware of their lifestyle and eating habits, and possibly adapt their lifestyle, which might have lowered their POP-exposure. As a consequence, the general Dutch population might have even higher exposure levels compared to our study group, which may have an even greater impact on pubertal development.

Whether our findings of advanced pubertal development might have consequences for later life need to be studied. Associations are reported between pubertal development and cancer risk during later life. In girls, earlier onset of pubertal development was found to be related to breast cancer[30, 31]. In boys, older age at sexual maturation was found to be related to a reduced risk of later prostate cancer[32]. Whether our findings are caused by disturbances of hormone levels need to be studied to clarify possible underlying mechanisms. In a Faroese study with relatively high PCB-exposure, prenatal PCB-levels were inversely associated with testosterone and LH in 14-year-old boys[20]. Whether children with higher prenatal background exposure also have higher POP-levels during adolescence need to be investigated, as also whether the impact of POPs on pubertal development are mainly due to prenatal or postnatal exposure.

CONCLUSIONS

Higher prenatal Dutch background PCB-exposure is associated with more advanced pubertal development in 13- to 15-year-old children. OH-PCBs seem to influence pubertal development less compared to PCBs, although some positive and/or inverse associations were found for OH-PCBs. PBDEs, DDE, PCP, and HBCDD were not associated with pubertal development. Our findings raise concern towards effects of man-made compounds on pubertal development.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Prenatal exposure to PCBs and OH-PCBs and pubertal stages in 13- to 15-year-old boys

		Mean Rank Tanner genital stage							Mean Rank Tanner pubic hair stage							Testicular volume		
Compound	*n*	1 (n=2/53) (n=0/26)	2 (n=14/53) (n=4/26)	3 (n=22/53) (n=9/26)	4 (n=14/53) (n=12/26)	5 (n=1/53) (n=1/26)	KW [a]	*P*-value	1 (n=4/53) (n=0/26)	2 (n=8/53) (n=3/26)	3 (n=15/53) (n=5/26)	4 (n=23/53) (n=15/26)	5 (n=3/53) (n=3/26)	KW [a]	*P*-value	*R* [b]	*P*-value	*n*
PCB-105	26		13.25	10.78	14.58	26.00	4.057	.255		9.67	7.10	16.23	14.33	6.208	.102	.273	.208	25
PCB-118	26		10.50	11.78	14.75	26.00	4.063	.255		5.67	7.40	17.00	14.00	9.481	.024**	.360	.092*	25
PCB-138	26		9.75	11.00	15.75	24.00	4.848	.183		4.67	8.60	15.87	18.67	8.862	.031**	.370	.082*	25
PCB-146	26		9.00	10.50	16.38	24.00	6.352	.096*		3.33	9.00	15.93	19.00	10.104	.018**	.318	.139	25
PCB-153	53	11.50	21.07	25.32	36.14	50.00	11.464	.022**	29.75	14.38	22.60	31.61	43.67	12.233	.016**	.083	.558	54
- RENCO	26		10.25	11.00	15.58	24.00	4.459	.216		5.00	8.40	15.93	18.33	8.644	.034**	.334	.119	25
- GIC	27		13.60	15.38	12.50		1.453	.693		9.80	16.00	12.38		3.148	.369	-.130	.519	29
PCB-156	26		11.25	10.67	15.33	26.00	4.942	.176		6.67	8.40	15.27	20.00	7.585	.055*	.357	.095*	25
PCB-170	26		10.25	11.22	15.50	23.00	3.884	.274		6.67	9.60	14.60	21.33	7.152	.067*	.348	.104	25
PCB-180	26		10.75	10.89	15.67	22.00	3.765	.288		6.67	10.70	14.10	22.00	6.864	.076*	.332	.122	25
PCB-183	26		16.50	10.78	13.75	23.00	3.311	.346		13.33	8.40	15.07	14.33	2.889	.409	.233	.284	25
PCB-187	26		11.50	9.67	16.17	24.00	5.877	.118		6.67	7.00	15.80	19.67	9.312	.025**	.282	.193	25
Σ 10 PCBs	26		10.25	10.67	15.75	25.00	5.256	.154		5.00	8.00	15.87	19.33	9.472	.024**	.379	.074*	25

Supplementary Table 1 continued

		Mean Rank Tanner genital stage							Mean Rank Tanner pubic hair stage							Testicular volume		
Compound	*n*	1 (n=2/53) (n=0/26)	2 (n=14/53) (n=4/26)	3 (n=22/53) (n=9/26)	4 (n=14/53) (n=12/26)	5 (n=1/53) (n=1/26)	KW [a]	*P*-value	1 (n=4/53) (n=0/26)	2 (n=8/53) (n=3/26)	3 (n=15/53) (n=5/26)	4 (n=23/53) (n=15/26)	5 (n=3/53) (n=3/26)	KW [a]	*P*-value	*R* [b]	*P*-value	*n*
4-OH-PCB-107	52	2.00	21.21	26.88	35.61	14.00	12.681	.013**	10.50	24.75	22.20	31.68	36.00	9.525	.049**	-.184	.196	53
- RENCO	26	8.50	13.00	12.72	15.29	1.00	3.441	.329	17.50	16.67	10.90	13.57	14.33	1.129	.770	-.177	.419	25
- GIC	26		13.80	15.42	12.00		5.367	.147		13.20	14.10	15.14		1.486	.685	-.071	.732	28
3'-OH-PCB-138	26		10.63	12.67	14.38	22.00	2.068	.558		11.33	11.20	13.53	19.33	2.443	.486	-.149	.497	25
4-OH-PCB-146	53	40.50	25.64	27.68	26.71	8.00	3.198	.525	34.75	23.13	30.87	23.85	31.83	3.704	.448	-.175	.215	54
- RENCO	26	2.00	10.25	14.56	14.42	6.00	2.032	.566	9.50	11.00	14.00	13.17	16.83	0.942	.815	-.131	.550	25
- GIC	27		14.00	13.54	10.50		1.774	.621		11.80	16.40	10.63		3.523	.318	-.259	.192	29
3-OH-PCB-153	26		11.38	14.61	13.83	8.00	1.041	.791		9.83	11.20	14.73	14.83	1.626	.653	-.222	.308	25
4'-OH-PCB-172	20		11.33	10.64	11.00	2.50	1.967	.579		11.50	12.67	9.71	10.83	0.688	.876	.087	.731	20
4-OH-PCB-187	53	22.00	21.14	26.14	34.64	31.00	5.790	.215	20.25	21.00	26.07	29.43	38.00	4.121	.390	-.173	.220	54
- RENCO	26	20.50	11.75	15.67	13.00	7.00	1.709	.635	17.50	13.00	17.00	12.73	12.00	1.329	.722	.024	.914	25
- GIC	27		13.30	13.54	16.50		0.828	.843		11.40	14.30	14.00		0.948	.814	-.497	.008***	29
Σ 6 OH-PCBs	20		7.83	11.71	11.06	5.00	1.849	.604		8.75	13.33	10.04	10.67	0.938	.816	-.242	.333	20

[a] Calculated with the Kruskal-Wallis test; [b] Partial correlation corrected for age at examination and BMI z-score;*** *P*<.01. ** *P*<.05 and * *P*<.10

Supplementary Table 2. Prenatal exposure to PCBs and OH-PCBs and pubertal stages in 13- to 15-year-old girls

		Mean Rank Tanner breast stage					Mean Rank Tanner pubic hair stage				
Compound	n	3 (n=15/42) (n=5/27)	4 (n=17/42) (n=12/27)	5 (n=10/42) (n=10/27)	KW [a]	*P*-value	3 (n=17/41) (n=8/26)	4 (n=21/41) (n=15/26)	5 (n=3/41) (n=3/26)	KW [a]	*P*-value
PCB-105	27	10.00	11.25	19.30	7.169	0.028**	11.13	15.20	11.33	1.753	0.416
PCB-118	27	7.40	11.75	20.00	10.136	0.006***	8.38	16.07	14.33	5.317	0.070*
PCB-138	27	6.00	13.75	18.30	8.026	0.018**	9.38	15.67	13.67	3.532	0.171
PCB-146	27	8.00	12.71	18.55	6.471	0.039**	11.50	14.87	12.00	1.143	0.565
PCB-153	42	14.13	20.88	33.60	15.181	0.001***	14.41	26.19	22.00	9.106	0.011**
- RENCO	27	5.40	13.17	19.30	10.464	0.005***	9.25	16.00	12.33	4.142	0.126
- GIC	15	8.90	6.20		1.215	0.270	6.67	10.00		2.000	0.157
PCB-156	26	6.00	11.83	18.50	8.689	0.013**	9.29	15.20	10.67	3.425	0.180
PCB-170	26	6.00	12.79	17.35	6.485	0.039**	10.29	14.93	9.67	2.604	0.272
PCB-180	26	7.25	13.50	16.00	3.739	0.154	11.86	14.67	7.33	2.716	0.257
PCB-183	27	9.70	14.92	15.05	1.803	0.406	14.44	13.17	12.67	0.184	0.912
PCB-187	27	8.80	13.83	16.80	3.396	0.183	12.38	14.20	13.00	0.312	0.856
Σ 10 PCBs	26	5.50	12.17	18.30	8.679	0.013**	9.29	15.07	11.33	3.119	0.210

Supplementary Table 2 continued

Compound	n	Mean Rank Tanner breast stage 3 (n=15/42) (n=5/27)	4 (n=17/42) (n=12/27)	5 (n=10/42) (n=10/27)	KW [a]	*P*-value	Mean Rank Tanner pubic hair stage 3 (n=17/41) (n=8/26)	4 (n=21/41) (n=15/26)	5 (n=3/41) (n=3/26)	KW [a]	*P*-value
4-OH-PCB-107	39	17.60	18.83	25.94	3.270	0.195	17.47	20.06	27.67	2.233	0.327
- RENCO	25	12.80	12.32	13.94	0.247	0.884	12.88	11.85	14.33	0.336	0.845
- GIC	14	8.80	4.25		3.380	0.066*	7.67	7.20		0.040	0.841
3'-OH-PCB-138	25	9.30	14.14	13.67	1.601	0.449	14.44	11.12	13.33	1.142	0.565
4-OH-PCB-146	40	25.17	19.19	15.06	4.546	0.103	23.47	16.58	22.00	3.379	0.185
- RENCO	25	13.50	14.18	11.28	0.801	0.670	15.57	9.69	16.00	4.480	0.106
- GIC	15	9.30	5.40		2.535	0.111	7.67	8.50		0.125	0.724
3-OH-PCB-153	25	9.60	13.68	14.06	1.347	0.510	14.69	11.42	11.33	1.149	0.563
4'-OH-PCB-172	19	3.00	11.44	10.28	3.700	0.157	11.70	8.27	10.75	1.571	0.456
4-OH-PCB-187	40	19.80	20.34	21.94	0.194	0.908	19.50	19.82	24.00	0.407	0.816
- RENCO	25	14.20	14.23	10.83	1.219	0.554	15.19	10.81	12.67	1.903	0.386
- GIC	15	9.70	4.60		4.335	0.037**	7.89	8.17		0.014	0.906
Σ 6 OH-PCBs	19	2.00	11.25	10.67	4.563	0.102	11.40	8.27	11.50	1.495	0.473

[a] Calculated with the Kruskal-Wallis test; *** $P<0.01$, ** $P<0.05$ and * $P<0.10$