Prenatal exposure to persistent organic pollutants and cognition and motor performance in adolescence

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ABSTRACT

Background: Prenatal exposure to persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), was found to be associated with poorer neurological development in children. Knowledge about the effects on outcomes until adolescence is limited.

Objectives: To determine whether prenatal exposure to POPs, particularly hydroxylated PCBs (OH-PCBs), is associated with cognitive and motor development in 13- to 15-year-old children.

Methods: This prospective observational cohort study is part of the Development at Adolescence and Chemical Exposure (DACE)-study, a follow-up of two Dutch birth cohorts. Maternal pregnancy serum levels of PCB-153 and three OH-PCBs were measured, in part of the cohort also nine other PCBs and three OH-PCBs, and in another part five polybrominated diphenyl ethers (PBDEs), dichloroethene (DDE), pentachlorophenol (PCP) and hexabromocyclododecane (HBCDD). Of the 188 invited adolescents, 101 (53.7%) participated, 55 were boys. Cognition (intelligence, attention, verbal memory) and motor performance (fine motor, ball skills, balance) were assessed. Scores were classified into ‘normal’ (IQ > 85; scores > P15) and ‘(sub)clinical’ (IQ ≤ 85; scores ≤ P15). We used linear and logistic regression analyses, and adjusted for maternal education, maternal smoking, maternal alcohol use, breast feeding, and age at examination.

Results: Several OH-PCBs were associated with more optimal sustained attention and balance. PCB-183 was associated with lower total intelligence (OR: 1.29; 95%CI: 0.99–1.68; P = .060), and HBCDD with lower performance intelligence (OR: 3.62; 95%CI: 0.97–13.49; P = .056). PCBs, OH-PCBs and PBDEs were negatively associated with verbal memory.

Conclusions: Prenatal background exposure to several POPs can influence neuropsychological outcomes in 13- to 15-year-old Dutch adolescents, although exposure to most compounds does not have clinically relevant consequences at adolescence.

1. Introduction

Persistent organic pollutants (POPs) are man-made chemicals, used for application in a variety of products like flame-retardants, solvents, and pesticides. Despite the fact that the production and use of these chemicals are banned by law, there is still exposure to these compounds. Because POPs, for example polychlorinated biphenyls (PCBs), can be transferred from the mother to the fetus during pregnancy, fetuses are exposed to these chemicals during a critical time of development of the brain (Soechitram et al., 2004). Follow-up studies showed conflicting results, with some studies indicating associations between prenatal exposure to POPs and neurodevelopmental outcome in children whereas other studies found no associations (Berghuis et al., 2015).

Prenatal exposure to PCBs was found to be associated with an increase of attention-deficit/hyperactivity disorder (ADHD)-like behavior in children (Neugebauer et al., 2015; Polańska et al., 2013), less optimal long-term memory in adolescents (Newman et al., 2009), and lower intelligence levels in children (Chen et al., 1992; Lai et al., 2002). In contrast, some other studies found no associations between prenatal exposure to PCBs and attention in adolescents (Lee et al., 2007; Newman et al., 2014; Strom et al., 2014), memory in children at school

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; AVLT, Auditory Verbal Learning Test; DDE, dichloroethene; HBCDD, hexabromocyclododecane; Movement-ABC, Movement Assessment Battery for Children; OH-PCB, hydroxylated polychlorinated biphenyl; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl; PCP, pentachlorophenol; POP, persistent organic pollutant; TEA-Ch, Test of Everyday Attention for Children; WISC, Wechsler Intelligence Scale for Children

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age (Orenstein et al., 2014), and learning in 12- to 15-year-old adolescents (Lee et al., 2007).

Prenatal exposure to polybrominated diphenyl ethers (PBDEs) was found to be associated with lower intelligence levels (Zhang et al., 2017) and reduced motor speed (Kicinski et al., 2012). Prenatal exposure to dichlorodiphenyldichloroethene (DDE) was found to be associated with ADHD-like behaviors in 7- to 11-year-old children (Sagiv et al., 2010), and several other studies found no association with intelligence after higher exposure to DDE (Gaspar et al., 2015; Lee et al., 2007).

Previously, in children included in the cohort of this study, we observed less optimal motor development and poorer visuomotor function at three months of age, and poorer fine manipulative skills at the age of five to six years after higher prenatal exposure to the hydroxylated metabolites of PCBs (OH-PCBs) (Berghuis et al., 2013; Berghuis et al., 2014; Roze et al., 2009). Because we observed negative effects of prenatal exposure to OH-PCBs, we aimed to determine whether the observed effects persist until adolescence. To our knowledge, no study has been published on the associations between prenatal exposure to OH-PCBs and cognitive and motor outcome in adolescence. Studies on the long-term effects into adolescence of prenatal exposure to POPs, particularly on motor outcome, are sparse. Therefore, the aim of this exploratory study was to investigate whether prenatal exposure to POPs, including OH-PCBs, is associated with cognitive and motor outcome in 13- to 15- year-old adolescents.

2. Materials and methods

2.1. Cohort and study design

This longitudinal cohort study is part of the Development at Adolescence and Chemical Exposure (DACE)-study, in which we followed up two Dutch cohorts. In the cohort of the Risk of Endocrine Contaminants on human health (RENCO)-study, 104 mother-infant pairs were included between 1998 and 2000 (Soechitram et al., 2004). In the cohort of 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, 90 mother-infant pairs were included between 2001 and 2002 (Meijer et al., 2008). Children of both cohorts were invited for participation in the DACE-study during adolescence. Six children were not invited: four had no available prenatal POP-levels, one had been diagnosed with a congenital syndrome after initial inclusion in the cohort, and one had moved abroad. A reminder was sent in case of no response. The children were all singleton children, 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 and 15 years (inclusion periods were between April 2014 and December 2014, and between October 2015 and 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 Medical Ethics Committee of the University Medical Center Groningen.

2.2. Measurement of prenatal POP-levels

Levels of several POPs were measured in maternal serum samples collected during the second and/or third trimester of pregnancy. Detailed descriptions of the analyses have been published previously (Meijer et al., 2008; Soechitram et al., 2004). In both cohorts, levels of PCB-153, 4-OH-PCB-107, 4-OH-PCB-146, and 4-OH-PCB-187 were measured. 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 sums of all measured 10 PCBs and 6 OH-PCBs were calculated. In the GIC-study, in addition, the following POPs were measured: five different 2,2′,4,4′-tetrabromodiphenyl ethers (BDEs), 2,2′-bis-(4 chlorophenyl)-1,1′-di-chloroethene (p,p′-DDE), pentachlorophenol (PCP), and hexabromocyclododecane (HBCDD). PCBs and OH-PCBs were numbered according to Ballschmiter et al. (1993) and to Letcher et al. (2000) respectively. PCB-levels are given in nanograms per gram lipid, and OH-PCB-levels in picograms per gram fresh weight.

2.3. Cognitive and motor outcomes

Total, verbal and performance intelligence were assessed using a shortened form of the Wechsler Intelligence Scale for Children, third edition, Dutch version (WISC-III-NL) (Kort et al., 2002). Verbal intelligence quotients (IQ) were calculated based on subtests on vocabulary and analogies; performance IQs were calculated based on subtests on organizing pictures and block design assembly. Auditory-verbal memory was assessed using a standardized Dutch version of the Rey's Auditory Verbal Learning Test (AVLT) (van den Burg and Kingma, 1999). This test consists of five learning trials with immediate recall (learning capacity), a delayed recall trial (long-term retrieval) and a delayed recognition trial (long-term recognition). Sustained auditory attention and selective visual attention were measured using the subtests ‘Score!’ and ‘Sky Search’ of the Test of Everyday Attention for Children, Dutch Version (TEA-Ch-NL), respectively (Schittekatte et al., 2007). Sustained attention involves maintaining attention over an extended period of time. Selective attention refers to the ability to select target information from an array of distractors. Motor outcome was assessed using the Movement Assessment Battery for Children (Movement-ABC), a standardized test of motor skills for children 4 to 12 years of age (Smits-Engelsman and Niemeijer, 1998). This test yields a score for total movement performance based on separate scores for fine motor skills (manual dexterity), ball skills (object control), and static and dynamic balance (postural control). The cognitive and motor tests were administered by author SAB, trained by author KNJAVB (child neuropsychologist), or administered by a trained research assistant under supervision of author SAB. All children were seen at the same research site. The total duration of the cognitive and motor assessment was typically approximately 3 h, including breaks. One hour before the behavior assessment part of the follow-up program of the DACE-study, the children were seen at the clinic for physical examination (including assessment of pubertal stage), venipuncture and breakfast.

2.4. Statistical analyses of data

To compare POP-levels between the included and excluded children, we used the independent samples Student t-test. Regarding outcomes on the WISC-III-NL, we converted the raw scores into scaled scores using age-specific norms according to the instructions in the manual. IQs were calculated by taking the mean of the scores on the verbal and performance subtests. We classified the scores into ‘normal’ (IQ > 85), ‘subclinical’ (IQ 70–85) and ‘clinical’ (IQ < 70). Regarding outcomes on the TEA-Ch-NL, we converted the raw scores on the subtest ‘Score!’ into age-specific percentiles using the instruction manual. For the subtest ‘Sky Search’ we chose to use the raw scores, since no normed percentiles are available in the instruction manual. Regarding outcomes on the AVLT and Movement ABC, we used the Dutch norms for children of 12 years of age, because these tests were not normed for children older than 12 years. However, the study in which the norms of the AVLT were published indicated a ceiling effect between 10 and 12 years of age indicating no further improvement is expected after 12 years of age. Despite no norms for children of 13 to 15 years were available, we chose to use these tests because the same tests were used previously in our cohort at early school age, which provides us with the opportunity to compare the results on verbal memory and motor performance. We classified the scores on cognitive
and motor tests into ‘normal’ (> 15th percentile), ‘subclinical’ (5–15th percentile) and ‘clinical’ (< 5th percentile). First, we performed univariate linear regression analyses for prenatal POP-levels and the cognitive and motor outcomes. Next, we performed univariate logistic regression analyses for those associations that had a P-value < .10 in the univariate linear regression analyses to calculate odds ratios (ORs) for having a (sub)clinical outcome. The following factors were considered as potential confounders: maternal education (< 14 versus ≥ 14 years), maternal smoking and alcohol consumption during pregnancy, breast feeding and age at examination (< median age of 173 months versus ≥ 173 months). Maternal education was included in each model, because this may exert an influence on the cognition and behavior of the children (Tong et al., 2007; Whitley et al., 2011). The other characteristics were included in multivariate logistic regression analyses (method: enter) if they had a P-value of < .20 in the univariate analysis model. We also performed the analyses for boys and girls separately. In case a participant had missing data on a subtest, the total score on the test was excluded from the analyses, but the scores of this participant on other subtests were included in the analyses. A P-value below .05 was considered statistically significant, and a P-value between .05 and .10 was considered a trend towards significance. Statistical Package for the Social Sciences, version 23 (SPSS) was used.

3. Results

3.1. Study group

In total 101 (53.7%) of the 188 invited children participated in this follow-up study. 44 (23.4%) adolescents declined the invitation, and 43 (22.9%) did not respond. The participating adolescents of the GIC cohort had significantly higher total intelligence at 5–6 years of age compared to the children who were not participating at follow-up (mean ± SD: 103.7 ± 9.0 versus 99.1 ± 8.1; t = −2.289; P = .025). There was no difference between the participating and non-participating adolescents regarding total motor performance at the age of 5–6 years, and also no difference regarding maternal education level. The final study group consisted of 55 boys and 46 girls. Almost all children, except one boy and one girl, lived in the northern part of the Netherlands at time of follow-up. Characteristics of the study group are presented in Table 1.

3.2. Prenatal POP-levels

The median prenatal POP-levels of the children participating in the follow-up program at adolescence are presented in Table 2, as well as the median levels for the children who did not participate in the follow-up program. There were no differences in POP-levels between the participating and non-participating children, except for PBDE-154-levels, which were lower in the participating children (mean ± SD: 0.497 ± 0.241 versus 0.837 ± 0.733 ng/g lipid; t = −2.573; P = .028). Comparing the mean prenatal levels of both sexes, for girls in the GIC cohort (but not in the combined or RENCO cohort) the levels of 4-OH-PCB-146 were significantly higher compared with the prenatal levels for boys (103.6 versus 89.2 pg/g serum; t = 2.239; P = .030), PCB-153 and the sum of the PCBs were marginally significantly higher (PCB-153: 93.0 versus 77.7 ng/g lipid; t = 1.852; P = .067; ΣPCBs: 357.1 versus 301.1 ng/g lipid; t = 1.684; P = .098) whereas the levels of BDE-100 were marginally significantly lower for girls (0.175 versus 0.305 ng/g lipid; t = −1.997; P = .054). Although not all statistically significant, the mean prenatal levels of all 10 individual PCBs were higher for girls, whereas prenatal levels of 4 of the 5 PBDEs were higher for boys (data not shown).

3.3. Cognitive and motor outcomes

The results on cognitive and motor outcome are presented in Table 3. The mean (± SD) total IQ of the children was 102 ± 9.8 (range: 76–125); mean verbal IQ was 103 ± 11.9 (range: 70–133) and the mean performance IQ was 100 ± 10.8 (range: 63–125). Using reference values for 12-year-old children, almost all 13- to 15-year-old children in our cohort scored within the normal range for the verbal memory trials, except for three children scoring abnormal on only one trial. The scores of the children on the attention tasks were similar to the reference values. The scores of the children on the Movement-ABC were poorer than the scores of the Dutch reference group of 12-year-old children. Boys had poorer outcomes on fine motor skills compared to girls (3.7 ± 2.5 versus 1.7 ± 3.0; t = −3.6; P < .01), and better outcomes on ball skills (1.4 ± 2.0 versus 2.8 ± 2.6; t = 3.2; P < .01).

Regarding results on the intelligence test, for seven children no performance and total IQs were included in our analyses due to the following reasons: four children performed elsewhere (a subtest of) the WISC < 12 months before current testing; two children had a very low score on a performance subtest most likely due to too much emphasis on speed and insufficient on accuracy as observed during the assessment; and for one child an error in testing procedure occurred. No verbal IQ was included for one of the previously mentioned children due to poor sustained attention resulting in a low score on a verbal subtest. Regarding the memory test, for one child the test scores on immediate recall and for another child on recognition were excluded due to errors in testing procedure. Regarding the attention tests, test scores on selective visual attention were excluded for one child because of an error in testing procedure, and scores on sustained auditory attention were excluded for five children due to an error in testing procedure and suboptimal testing circumstances. Regarding testing of motor skills, scores on all subtests were missing for one child because of a muscle disease, and scores on fine motor skills were missing for two other children because of an error in testing procedure. In 75 of the 101 children, the tests were administered by author SAB, in the other 26 children the tests were administered by one of the two research assistants who were trained and supervised by author SAB. All assessors were blind to prenatal POP levels.

3.4. Linear regression POPs and outcome

In Table 4, the POPs that were significantly or marginally significantly related to cognitive and motor outcome using linear regression analyses, unadjusted for possible confounders, are presented. Higher exposure to several POPs was found to be related with both better and poorer cognitive and motor outcomes.

Regarding intelligence, higher levels of PCB-105, PCB-183 and HBCDD were related to less optimal intelligence (Table 4; P < .10). Higher levels of two OH-PCBs (P < .05), the sum of the six measured OH-PCBs (P < .10) and PCB-180 (P < .05) were associated with more optimal scores on the intelligence test. Regarding scores on memory tasks, higher levels of some OH-PCBs, PCBs and BDE-154 were related to...
Regarding attention, higher levels of some PCBs and OH-PCBs were related to more optimal scores on attention tasks, whereas higher levels of the compounds BDE-153, HBCDD and PCP were related to lower scores.

Regarding motor performance, higher levels of two PCBs were related to more optimal scores on motor tasks, only PCB-183 was associated with less optimal scores for ball skills. Higher levels of three OH-PCBs were related to more optimal scores on motor tasks, only PCP was associated with less optimal scores for ball skills. Regarding exposure to the other measured POPs, none was related to scores on motor tasks.
3.5. Prenatal POPs and (sub)clinical outcome

For the associations with a P-value < .10 in linear regression models, ORs were calculated for maternal pregnancy levels of POPs and a (sub)clinical outcome (Table 1). In Table 5, we show only the significant or marginally significant results of the univariate logistic regression analyses. Concerning confounding factors, the following factors were associated with a (sub)clinical outcome with a P < .20 in univariate logistic regression analyses: maternal smoking during pregnancy (OR = 3.42; P = .108) and lower maternal education (< 14 years; OR = 2.67; P = .174) were associated with (sub)clinical verbal intelligence; a lower maternal education was also associated with (sub)clinical total intelligence (< 14 years; OR = 5.23; P = .138); no breastfeeding (OR = 1.91; P = .131) and no maternal alcohol use during pregnancy (OR = 2.39; P = .085) were associated with a (sub)clinical motor balance; no breastfeeding (OR = 2.24; P = .067) was also associated with a (sub)clinical score on ball skills. We corrected for these factors (maternal education was included in each model) using multivariate logistic regression analyses, and reported the adjusted ORs in Table 5. As presented in Table 5, after adjustment and dichotomizing
The scores into ‘normal’ and ‘(sub)clinical’, higher levels of PCB-183 were near- significantly associated with lower intelligence (P < .10). The compounds 3-OH-PCB-153 (P < .05) and 3’-OH-PCB-138 (P < .10) were positively associated with static and dynamic balance and 4-OH-PCB-107 (P < .10) positively with fine motor skills. The compounds 4-OH-PCB-187 (P < .05) and 4-OH-PCB-107 (P < .10) were positively associated with sustained auditory attention (P < .05). Regarding maternal pregnancy levels of PBDEs, DDE, HBCDD and PCP, none of the compounds were associated with (sub)clinical cognitive or motor outcomes (Table 5); only HBCDD was near-significantly associated with lower intelligence (P < .10).

3.6. Sex-specific associations

Besides the associations found in the whole cohort, fourteen other associations were found for girls between prenatal POP levels and outcome measures using linear regression analyses, and three associations for boys (P < .05; Table 2). Multivariate logistic regression analyses revealed that in girls, after correction for maternal education, 3’-OH-PCB-138 and 3-OH-PCB-153 were marginally significantly associated with a more optimal verbal intelligence (Table 6). A positive trend was also found for 4-OH-PCB-172 and motor balance, whereas a negative trend was found for BDE-153 and fine motor skills. Regarding outcomes in boys, prenatal DDE exposure was associated with a (sub) clinical motor performance (Table 6). Trends were found between 4-OH-PCB-107 and more optimal fine motor skills, and between 3-OH-PCB-153 and more optimal balance.

4. Discussion

Prenatal exposure to POPs was found to be associated both positively and adversely with cognitive and motor outcomes in Dutch 13- to 15-year-old adolescents. PCB-183 and HBCDD were near-significantly associated with lower intelligence levels. Higher exposure to PCBs, OH-PCBs and PBDEs were associated with less optimal verbal memory, but within the range for normal development. Several OH-PCBs were associated with more optimal sustained auditory attention and more optimal motor balance.

4.1. Prenatal POP exposure and intelligence and memory

Regarding intelligence, several OH-PCBs were positively associated with intelligence levels, although not negatively associated with a (sub) clinical score. Since no other studies evaluated the effects of prenatal levels of OH-PCBs on intelligence in adolescence, we cannot compare our results with other studies. The finding that higher prenatal levels of chemical substances are related to more optimal intelligence has also been found for per- and polyfluoroalkyl substances (PFASs) in younger children. Harris et al. reported that higher prenatal levels of some PFASs were associated with a better non-verbal IQ in children with a mean age of 7.7 years (Harris et al., 2018). These findings suggest that environmental chemicals can also have neuroprotective effects. As an underlying mechanism for the neuroprotective effects of PFASs, Harris et al. suggested that PFASs can exert agonistic effects on peroxisome proliferator-activated receptor gamma (PPAR-y), which may result in inducing anti-inflammatory effects in the central nervous system. Pěnická et al. reported that three OH-PCBs (not measured in our study) did not induce or inhibit PPAR-γ-mediated activities in vitro (Pěnická et al., 2018), making it less likely to act in a similar way as suggested for PFASs. The underlying mechanism for the positive effects of OH-PCBs on intelligence is not clear, but may be related to interference with steroid or thyroid hormone metabolism. OH-PCBs have shown to act as both agonist and antagonist of steroid and thyroid hormones, which we suggested can exert opposite effects on neurodevelopment (Meerts et al., 2002; Takeuchi et al., 2011). Regarding PCBs and cognitive outcome, only a negative trend was seen for PCB-183 and intelligence. Regarding prenatal levels of PBDEs, DDE, PCB and HBCDD, none of the compounds was associated with (sub)clinical intelligence; only a negative trend was seen for HBCDD and performance intelligence. Regarding prenatal levels of HBCDD, we found in our cohort at the age of 5 to 6 years a positive correlation with total and verbal intelligence, but no correlation was found with intelligence (Rozé et al., 2009). The finding that prenatal exposure to HBCDD is near-significantly associated with lower performance intelligence at adolescence but not at early school age suggests that negative effects can develop or progress over time. Our finding of no associations between prenatal levels of DDE and intelligence is in line with previous studies (Gaspar et al., 2015; Lee et al., 2007). In contrast to the findings in our study in adolescents, Zhang et al. observed lower full-scale intelligence levels in 231 8-year-old children after increased prenatal levels of PBDEs (Zhang et al., 2017). A possible explanation for not finding associations between levels of PBDEs and cognitive outcome could be the smaller sample size in our study. Another explanation could be higher prenatal levels in the study by Zhang et al. in the US compared to levels in the Netherlands.
measured in our study (median sum of BDE-47, −99, −100, and −153 was 35.65 ng/g lipid in the study by Zhang et al., compared to 3.15 ng/g lipid in our study).

Although all outcomes on verbal memory were within the range for normal development, some PCBs were found to be associated with less optimal verbal memory, including long-term memory. This finding is in line with the finding by Newman et al. in 271 11- to 16-year-old adolescents that higher prenatal levels of PCBs were associated with less optimal long-term memory (Newman et al., 2009). The compound 4-OH-PCB-107 was negatively associated with long-term memory, and 4-OH-PCB-146 showed a negative trend with learning capacity and long-term memory. Regarding PBDEs, DDE, HBCDD and PCB, only BDE-154 was associated with less optimal memory. Consistent with our results, Cowel et al. reported that prenatal levels of PBDEs were associated with poorer performance on memory tasks (Cowell et al., 2018). They found that higher cord levels of BDE-47 and -99, but not -154, were associated with poorer performance on auditory working memory in 9- to 14-year-old girls. The negative effects of perinatal levels of POPs on long term memory can be due to alterations in the hippocampus making neurons more susceptible to cytotoxic conditions as amyloid stress as suggested by an animal study. Elnar et al. reported that perinatal exposure to non-dioxinlike PCBs (including PCB-138, −153 and −180) was associated with deficits in long-term memory in adult mice, and that the expression of pre- and postsynaptic proteins was decreased in the hippocampus of these mice, suggesting an increased susceptibility to amyloid stress (Elnar et al., 2016). Another explanation for an effect of exposure to PCBs and OH-PCBs on working memory may be the ability to exert estrogenic activities (Kikumoto et al., 2005). Prenatal PCB exposure has been found to be associated with estradiol levels in boys at the age of puberty (Hsu et al., 2005), and estrogen action in the prefrontal cortex is suggested to be associated with working memory (Galea et al., 2017). Whether the association between prenatal (OH-) PCB exposure and memory is related to estrogen levels at adolescence in our study remains to be determined.

4.2. Prenatal POP exposure and attention

Higher exposure to 4-OH-PCB-187 was less frequently associated with a (sub)clinical outcome on sustained auditory attention, and for 4-OH-PCB-107 there was a similar trend. We are the first reporting on the effects of prenatal levels of OH-PCBs on attention in adolescents, and therefore the first reporting positive effects on attention. A possible explanation for positive effects of 4-OH-PCB-187 on higher cognitive functions such as attention may be promoting neuronal development. In Purkinje cells in mouse cerebellar cultures, the metabolite 4-OH-PCB-187 (and two other OH-PCBs, not measured in our study) has shown to promote dendritic extention, whereas two other OH-PCBs (not measured in our study) showed inhibitory effects on dendritic development (Kimura-Kuroda et al., 2007). An in vitro study on steroid hormone receptor activities of one hundred OH-PCBs showed that the metabolites 4-OH-PCB-187 and 4-OH-PCB-107 both showed antagonistic activity on estrogen receptor α (with 7 other OH-PCBs) whereas 45 of the 100 OH-PCBs showed agonistic effects on that receptor (Takeuchi et al., 2011). These findings suggest that both compounds can interfere with activities of estrogen, important for cognitive functions (Villa et al., 2016), and that both compounds may exert effects in a similar manner, although the precise mechanism remains unclear.

The finding that prenatal exposure to PCBs was not associated with attention is in line with most other studies reporting on the effects of prenatal levels of PCBs on attention in adolescents (Lee et al., 2007; Newman et al., 2014; Strøm et al., 2014). In contrast to our findings, Sagiv et al. found in North-American children aged 7 to 11 years a relation between prenatal PCB-levels and a higher risk for ADHD-associated behavior as reported by teachers (Sagiv et al., 2010). A possible explanation that Sagiv et al. found such an association whereas we did not, can be a difference in sample size. Sagiv et al. included 607 children, whereas we included 101 children. Another explanation may be a difference in method of measurement; we assessed the performance of the children directly, whereas Sagiv et al. assessed the development indirectly through a teacher report form. The prenatal exposure to PCBs in the study by Sagiv et al. is likely to be lower compared to our study. Sagiv et al. measured the levels of PCBs in cord blood, whereas we measured the levels in maternal serum. The levels in cord blood measured by Sagiv et al. between 1993 and 1998 in Massachusetts are lower compared to levels measured in a study in the Netherlands between 1990 and 1991 (median sum of PCBs −118, −138, −153, and −180: 0.19 ng/g compared to 0.27 and 0.36 ng/g in a low and high exposed group, assuming that 11 cord serum weights 1060 g: 290 ng/1060 g = 0.27 ng/g and 380 ng/1060 g = 0.36 ng/g) (Vreugdenhil et al., 2004). Because a strong correlation exists between levels of PCBs measured in maternal serum and levels measured in cord blood (Soechitram et al., 2004), and because maternal serum levels measured

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### Table 6

Logistic regression analyses for prenatal POP levels and (sub)clinical cognitive and motor outcome in 13- to 15-year-old girls and boys.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Outcome</th>
<th>Optimality</th>
<th>Number N/(S)C</th>
<th>OR</th>
<th>(95% CI)</th>
<th>P-value</th>
<th>Adjusted OR</th>
<th>(95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3′-OH-PCB-138</td>
<td>Verbal intelligence</td>
<td>+</td>
<td>21/5</td>
<td></td>
<td>0.950 (0.895, 0.993)</td>
<td>.093</td>
<td>0.937</td>
<td>(0.874, 1.005)</td>
<td>.067</td>
</tr>
<tr>
<td>3-OH-PCB-153</td>
<td>Verbal intelligence</td>
<td>+</td>
<td>21/5</td>
<td></td>
<td>0.914 (0.831, 0.967)</td>
<td>.067</td>
<td>0.898</td>
<td>(0.805, 1.002)</td>
<td>.054</td>
</tr>
<tr>
<td>4′-OH-PCB-172</td>
<td>Static and dynamic balance</td>
<td></td>
<td>12/8</td>
<td></td>
<td>0.771 (0.611, 0.929)</td>
<td>.029</td>
<td>0.790</td>
<td>(0.614, 1.016)</td>
<td>.066</td>
</tr>
<tr>
<td>2, 6 OH-PCBs</td>
<td>Static and dynamic balance</td>
<td>+</td>
<td>12/8</td>
<td></td>
<td>0.992 (0.983, 0.987)</td>
<td>.087</td>
<td>0.993</td>
<td>(0.983, 1.003)</td>
<td>.147</td>
</tr>
<tr>
<td>BDE-153</td>
<td>Fine motor skills</td>
<td>−</td>
<td>11/5</td>
<td></td>
<td>1.657 (1.005, 2.732)</td>
<td>.048</td>
<td>1.865</td>
<td>(0.919, 3.782)</td>
<td>.084</td>
</tr>
<tr>
<td>Boys</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-OH-PCB-107</td>
<td>RENCO Fine motor skills</td>
<td>+</td>
<td>16/8</td>
<td></td>
<td>0.972 (0.942, 0.976)</td>
<td>.076</td>
<td>0.970</td>
<td>(0.939, 1.002)</td>
<td>.064</td>
</tr>
<tr>
<td>3-OH-PCB-153</td>
<td>Static and dynamic balance</td>
<td>+</td>
<td>11/14</td>
<td></td>
<td>0.968 (0.934, 0.985)</td>
<td>.085</td>
<td>0.967</td>
<td>(0.932, 1.003)</td>
<td>.072</td>
</tr>
<tr>
<td>DDE</td>
<td>Motor skills: total score</td>
<td>−</td>
<td>12/16</td>
<td></td>
<td>1.043 (1.002, 1.042)</td>
<td>.042</td>
<td>1.045</td>
<td>(1.002, 1.090)</td>
<td>.044</td>
</tr>
</tbody>
</table>

Only associations with a P-value < .10 in both linear and univariate logistic regression models were included; data are given as odds ratios (95% confidence interval) for (sub)clinical outcomes: normal was defined as > P15, (sub)clinical as ≤P15; regarding intelligence, normal was defined as IQ > 85, borderline and abnormal as IQ ≤85; in case of no or only one borderline or abnormal scoring child, ORs were not calculated.

* Per pg/g fresh weight.

* Per .10 ng/g lipid.

* Adjusted for maternal education and maternal smoking during pregnancy.

* Adjusted for maternal education, maternal smoking during pregnancy and breastfeeding.

* Adjusted for maternal education.

* Adjusted for maternal education.

* Adjusted for maternal education, maternal smoking during pregnancy and breastfeeding.

* Adjusted for maternal education.

* Adjusted for maternal education.

* Adjusted for maternal education.

* Adjusted for maternal education.

* Adjusted for maternal education.

* Adjusted for maternal education.

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* Adjusted for maternal education.

* Adjusted for maternal education.

* Adjusted for maternal education.

* Adjusted for maternal education.

* Adjusted for maternal education.
in our study (sum 4 PCBs: 246.6 ng/g lipid weight) are similar to the levels in the study of Vreugdenhil et al. (sum 4 PCBs: 166.7 and 383.3 ng/g lipid in a respectively low and high exposed group, and assuming that one liter maternal serum contains 8.4 g lipid (Longnecker et al., 2003), that is 1400 ng/8.4 g = 166.7 ng/g lipid and 3220 ng/8.4 g = 383.3 ng/g lipid), it is likely that the prenatal levels in our study are higher compared to the exposure in the study of Sagiv et al.

Regarding prenatal levels of PBDEs, DDE, HBCDD and DDE, none of the compounds was associated with a (sub)clinical outcome on attention, although a negative trend was observed for BDE-153, HBCDD and PCP and sustained auditory attention. Previously, in our group of children at early school age, BDE-153 was not found to be associated with poorer scores on sustained auditory attention, although BDE-47, BDE-99 and BDE-100 were associated with poorer scores at that age (Roze et al., 2009). This difference in significant associations between the two time points regarding negative effects of brominated flame retardants suggests that the effects seen at early school age did not have lasting effects in adolescence, but we cannot exclude the possibility that this difference is caused by a smaller sample size at follow-up (n = 69 compared to n = 35). Therefore, larger studies are needed to confirm our results.

4.3. Prenatal POP exposure and motor performance

Regarding OH-PCBs and motor outcome, 3-OH-PCB-153, one of the metabolites of PCB-153, which is the most abundant PCB, was found to be positively associated with balance, and for 3′-OH-PCB-138 there was a positive trend. Regarding exposure to 4-OH-PCB-107, we found a, marginally significant, positive association with fine motor skills at 13 to 15 years, whereas this compound was found to be associated with less optimal motor development and poorer visuomotor function at three months and poorer fine manipulative abilities at the age of five to six years previously in our cohort (Berghuis et al., 2013; Berghuis et al., 2014; Roze et al., 2009). This finding suggests that the negative effects of 4-OH-PCB-107 on motor outcomes observed at a preschool and school age did not have clinically relevant lasting consequences at adolescence. Because this is the first study on the associations between prenatal levels of OH-PCBs and outcomes at adolescence, we cannot compare our results with other studies. Prenatal levels of PCBs, PBDEs, DDE, HBCDD and PCP were not associated with (sub)clinical motor outcome. The positive effects found for some OH-PCBs on motor performance may be explained by a similar mechanism as suggested for the positive effects found on attention, that is by promoting dendritic development of neurons in the cerebellum (Kimura-Kuroda et al., 2007). OH-PCBs have shown to act as both agonists and/or antagonist on receptors for estrogen, androgen, glucocorticoid (Takeuchi et al., 2011) and thyroid hormones (Meerts et al., 2002), which can be underlying mechanisms for the effects of OH-PCBs, including positive effects on neurodevelopment.

4.4. Sex-specific findings

The mean prenatal levels of PCB-153, the most abundant PCB congener, and one of its metabolites OH-PCB-146 and the sum of all measured PCBs were higher in girls than boys. Although not all significant, all 10 individual PCBs were higher in serum of women pregnant with female fetuses than in serum of women pregnant with male fetuses. Differences in maternal serum levels of lipophilic compounds between women pregnant with female and women pregnant with male fetuses are also reported by Helland et al. (1998). They found that women pregnant with female fetuses had a significant increase in plasma leptin, a highly lipophilic protein positively correlated with body fat mass, between the 18th and 35th week of pregnancy, whereas in mothers pregnant with male fetuses this increase was not significant. They also found that the leptin levels in umbilical cord plasma were significantly higher for girls than for boys. Ferrante et al. reported that PCB-153 can increase leptin transcription (Ferrante et al., 2014). Taken together, our finding that the lipophilic PCBs are higher in mothers pregnant with girls suggests that a possibly similar underlying mechanism or interaction with leptin metabolism may explain this sex difference.

The difference in prenatal exposure levels of PCBs may be an explanation for the finding that more associations were found for girls than boys. Another explanation for sex-specific effects can be differences in interference with expression of genes. An animal study showed differences in the effects of perinatal PCB exposure in expression of hypothalamic genes between male and female rats (Bell et al., 2018). For example, in female rats PCBs increased the expression of the gene estrogen receptor 2 when they were treated with an inflammatory challenge (lipopolysaccharide), whereas no alteration in expression of this gene was observed in male rats. Such differences suggest that neural gene expression may be a possible underlying mechanism for sex-specific effects of PCBs. An explanation for the sex-specific effects found for DDE may be different effects of this chemical compound on DNA methylation. Leung et al. reported that, only in boys, changes were seen for DDE and specific sites in cord blood of which some sites were enriched in cytobands of the X-chromosome associated with neurological disorders (Leung et al., 2018). This suggests that underlying epigenetic mechanisms might explain our finding that prenatal DDE exposure interferes with outcome in boys but not in girls.

4.5. Mechanisms

PCBs are metabolized to OH-PCBs by the cytochrome P450 system in the liver. Within a population, differences exist in the activity of isoenzymes in this system, resulting in differences in speed of metabolizing compounds, such as for example caffeine (Koch et al., 1999). Differences in the metabolism of PCBs, and underlying genetic variations, can therefore be an explanation for individual differences in sensitivity to PCB and OH-PCB toxicity.

Besides interference with endocrine mechanisms as mentioned previously, there is growing evidence for the interference of environmental chemicals with epigenetic mechanisms (Jacobs et al., 2017). Epigenetic changes (changes in gene expression, not involving changes in gene sequence or structure, like DNA methylation, histone modifications, and microRNAs) could underlie long-lasting adverse effects of endocrine disrupting chemicals (as reviewed by Jacobs et al.). Further study is needed to explain whether the effects of chemical exposure on developmental outcomes found in our study can be explained by underlying changes in epigenetic mechanisms.

4.6. Motor performance

The motor performance participating adolescents was rather poor. This is in line with previous studies on motor performance in Dutch children. Runhaar et al. showed a decrease in motor fitness in 9–12 year-old children between 1980 and 2006 (Runhaar et al., 2010), and recently the Dutch education inspection published a report showing reduced motor performance in 11–12 year-old Dutch children between 2006 and 2016, including poorer performance on ball skills (n = 975) and balance (n = 1288) (Inspectie van het onderwijs, 2018).

4.7. Strengths and limitations

A strength of our study is the use of standardized tests with trained examiners at the clinic to assess cognitive and motor outcome, instead of indirect measures, such as questionnaires. This provides us with the opportunity to gain a more robust insight into the cognitive and motor outcome, instead of the impression of the development as rated by parents or teachers. A second strength is that 74% of the children were seen by the same examiner, and the other children were seen under supervision of this examiner by one of the two research assistants. The
limited number of examiners minimizes the risk for bias due to inter-observer variation. A third strength is that we followed the children with known prenatal POP-levels until adolescence, because longitudinal follow-up studies until adolescence are sparse. To the best of our knowledge, this is the first study investigating the associations between prenatal exposure to OH-PCBs and cognitive and motor outcomes in children aged 13 to 15 years, and also the first study investigating associations between prenatal exposure to POPs and motor outcome into adolescence. A final strength is that we used a set of tests assessing different domains, including cognition, attention and motor performance. This provides us with the opportunity to gain more insight on which specific developmental domain may be influenced by prenatal levels of POPs.

There are also some limitations. First, due to the exploratory nature of the study, there is an increased risk for a Type I error due to the large number of tests performed. We assessed whether there was a relation between 26 POPs or sum of a group POPs and 12 different outcome measures, resulting in 312 comparisons. Considering a P-value of .10 as significant or marginally significant, we will expect to find 31.2 significant associations by chance. As presented in Table 3, we found a total of 35 associations with a P-value below .10 (excluding sub analyses of separate cohorts for compounds measured in both cohorts). The number of significant associations found in our explorative study is only slightly higher than the number of expected significant associations due to chance, and the results of our explorative study should therefore be interpreted with caution. However, all associations between (OH-)PCB exposure and attention, and between POP exposure and verbal memory were in the same direction, which is rather unlikely to be explained only by chance. We believe that our analyses are justified as part of a careful evaluation of a rich data set in hypothesis-driven research (Rothman, 1990). A second limitation is the small sample size. In the children of both cohorts (n = 101) prenatal exposure to four POPs were measured, the other POPs were only measured in one of the cohorts (n = 54 or n = 47). Since there were significant differences in exposure levels for the four compounds measured in both cohorts, we additionally performed statistical analyses for these compounds for both cohorts separately. The prenatal levels of the brominated compounds were only measured in 35 of the included children. Nevertheless, we believe that this sample size is appropriate for such complex studies, with regard to the measured environmental chemicals and assessed outcome measures. A third limitation is the possibility for selection bias. The finding that the mean intelligence level at the age of 5–6 years of the participating adolescents was significantly higher compared with the non-participating adolescents may be explained by several reasons. A first reason may be that the adolescents with a higher intelligence level were more motivated to take part in the follow-up program. Another reason may be that we excluded results on the intelligence test for four adolescents because they performed an intelligence test < 12 months before the assessment. Suggesting that there was a possible clinical indication for this previous assessment, the intelligence levels of these participating adolescents may be lower. Excluding these expected lower scores may have increased the mean intelligence level of the participating adolescents. Because the mean intelligence levels of our study group at adolescence are around the mean of the Dutch reference group for the intelligence test (100), we believe that our study group is a valid representation of the population in our region. A fourth limitation is that not all tests we used were normed for children aged 13 to 15 years. Particularly for the verbal memory test, this was a limitation because almost all 13- to 15-year-old children in our study group scored within the normal range for all trials when using the reference values for 12-year-old children. Nevertheless, we believe that the tests we used were appropriate to gain insight into whether the attention, verbal memory and motor outcomes were within the range for normal development, and to relate these performances to prenatal exposure to POPs. A final limitation is that we cannot rule out the effects of co-exposure to other environmental chemicals such as methyl mercury, and the effects of postnatal exposure to POPs. Further study on levels of POPs during adolescence is needed to identify whether the current exposure affects cognitive and motor outcomes.

5. Conclusions

Higher prenatal levels of OH-PCBs were associated with more optimal sustained attention and more optimal motor balance in 13- to 15-year-old adolescents. PCB-183 and HBCTDD showed a trend with respectively lower total and lower performance intelligence. Some sex-specific associations were found. In girls, positive trends were found between OH-PCBs and verbal intelligence, and a negative trend between BDE-153 and fine motor skills. In boys, prenatal DDE levels were associated with (sub)clinical motor performance, and a positive trend was seen between 4-OH-PCB-107 and fine motor skills. Prenatal levels of other PBDEs and PCP were not associated with (sub)clinical cognitive and motor outcomes at adolescence. Although memory scores were within the range for normal development, higher levels of PCBs, OH-PCBs and PBDE-154 were associated with less optimal verbal memory. Overall, the results of our study suggest that prenatal Dutch background exposure to POPs, measured between 1998 and 2002, has an effect, albeit not clinically relevant, on cognitive and motor development in adolescence.

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Conflicts of interest

The authors declare they have no competing financial interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.08.030.

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