# The effects of exposure to environmental chemicals on child development

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# CHAPTER 6

## The effects of prenatal exposure to persistent organic pollutants on neurological development during adolescence

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*Submitted*

**ABSTRACT**

**Background:** Exposure to persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), was associated with poorer neurological development in children. Knowledge about the effects of prenatal exposure to PCBs and their hydroxylated metabolites (OH-PCBs) on neurological development 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 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 hexabroomcyclododecane (HBCDD). Of the 188 invited 13- to 15-year-old adolescents, 101 (53.7%) participated, 55 boys and 46 girls. Cognition (intelligence, attention, verbal memory) and motor performance (fine motor skills, ball skills, balance) were assessed. Scores were classified into 'normal' (IQ>85; scores>P15) and 'borderline/abnormal' (IQ≤85; scores≤P15). We used linear and logistic regression analyses, adjusted for maternal education.

**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:** Higher prenatal exposure to OH-PCBs was positively associated with sustained attention and balance in 13- to 15-year-old Dutch adolescents. PCB-183 and HBCDD showed a negative trend with IQ. The other POPs were not associated with abnormal outcomes at adolescence. Despite limitations, such as increased risk of chance findings and small sample size, our study indicates that prenatal Dutch background exposure to several POPs can influence neuropsychological outcomes into adolescence, although exposure to most compounds does not have clinically relevant consequences for outcomes at adolescence.

## INTRODUCTION

Persistent organic pollutants 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.[1] Follow-up studies showed conflicting results with some studies indicating an effect of prenatal exposure to POPs on neurodevelopmental outcome in children and other studies not.[2]

Prenatal exposure to PCBs was found to be associated with an increase of attention-deficit/hyperactivity disorder (ADHD)-like behaviors in children,[3, 4] less optimal long-term memory in adolescents,[5] and lower intelligence levels in children.[6, 7] In contrast, some other studies did not find an effect of prenatal exposure to PCBs on attention problems in adolescents,[8-10] on memory in children at school age,[11] and on learning in 12- to 15-year-old adolescents.[10]



Prenatal exposure to polybrominated diphenyl ethers (PBDEs) was found to be associated with lower intelligence levels[12] and reduced motor speed.[13] Prenatal exposure to dichlorodiphenyldichloroethylene (DDE) was found to be associated with ADHD-like behaviors in 7- to 11-year-old children,[14] but several other studies did not find a relation with intelligence levels after higher DDE-exposure.[10, 15]

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 abilities at the age of five to six years after higher prenatal exposure to OH-PCBs.[16-18] Because we observed negative effects of prenatal exposure to OH-PCBs, we aimed to determine whether the observed effects persist until adolescence. To the best of our knowledge, there are no studies investigating the effects of prenatal exposure to OH-PCBs on cognitive and motor outcome at adolescence. Studies on the long-term effects into adolescence of prenatal POP-exposure, particularly on motor outcome, are sparse. Therefore, the aim of this exploratory study was to investigate the effects of prenatal POP-exposure, including OH-PCBs, on cognitive and motor outcome in 13- to 15- year-old adolescents.

## 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. In the cohort of the Risk of Endocrine Contaminants on human health (RENCO)-study, 104 mother-infant pairs were included between 1998 and 2000.[1] 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.[19] 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.

### Measurement of prenatal levels of POPs

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.[1, 19] 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′-dichloroethene (p,p'-DDE), pentachlorophenol (PCP), and hexabroomcyclododecane (HBCDD). PCBs and OH-PCBs were numbered according to Ballschmiter et al.[20] and to Letcher et al.,[21] respectively. Levels PCBs are given in ng/g lipid, and levels of OH-PCBs in pg/g fresh weight.

### 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).[22] Verbal

intelligence quotients (IQ) were calculated based on subtests on vocabulary and analogies; performance IQ scores were calculated based on subtests on organizing pictures and block design assembly. 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).[23] 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 distracters. Auditory-verbal memory was assessed using a standardized Dutch version of the Rey's Auditory Verbal Learning Test (AVLT).[24] 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). 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.[25] 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 total duration of the neuropsychological part of the follow-up was approximately three hours, including breaks. One hour before the neuropsychological part of the follow-up program of the DACE-study, the children came to the clinic for physical examination (including assessment of pubertal stage), venipuncture and breakfast.



**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. IQ scores were calculated by taking the mean of the scores on the verbal and performance subtests. We classified the scores into 'normal' (IQ>85), 'borderline' (IQ 70-85) and 'abnormal' (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, because we were not able to convert them into percentiles using the norm-table in the instruction manual. Regarding outcomes on the AVLT, we used the Dutch norms for children of 12 years of age, because the test was not normed for children older than 12 years. However, the study in which the norms were published indicated a ceiling effect was attained between 10 and 12 years of age suggesting no further improvement can be expected after 12 years of age. Despite the fact that the test is not normed for children of 13 to 15 years, we chose to use this test because the same test was used previously in our cohort in the children at early school age, which provides us with the opportunity to compare the results on verbal memory. Regarding outcomes on the Movement-ABC, we converted the raw scores into scaled scores based on reference values for Dutch children of 12 years of age, because the test was not normed

for children older than 12 years. Despite the fact that the test is not normed for children of 13 to 15 years, we again chose to use this test because it was also used in our cohort at early school age, thereby providing us with the opportunity to compare the results on motor skills. We classified the scores on cognitive and motor tests into 'normal' (>15$^{th}$ percentile), 'borderline' (5-15$^{th}$ percentile) and 'abnormal' (<5$^{th}$ percentile). First, we performed univariate linear regression analyses for prenatal POP-levels and the cognitive and motor outcomes. Second, we performed univariate logistic regression analyses to calculate odds ratios (ORs) for those associations that had a *P*-value <0.1 in the univariate linear regression analyses. Third, we performed multivariate logistic regression analyses (method: enter) including maternal education (<14 versus ≥14 years of education) as a potential confounder. 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.

## RESULTS

### 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 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.

**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 |
| 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%) |

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

**Prenatal levels of POPs**

The POP-levels of all mother-infant pairs included initially in the two cohorts have been reported previously.[19, 26] There were no differences in POP-levels between the in- and excluded children, except for PBDE-154-levels, which were lower in the included children (mean±SD: 0.497±0.241 versus 0.837±0.733 ng/g lipid; t=-2.573; *P*=.028).

**Cognitive and motor outcomes**

The results on cognitive and motor outcome are presented in Table 2. 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). The scores of the children on the attention tasks were comparable to the reference values. 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 Movement-ABC were poorer than the scores of the Dutch reference group of 12-year-old children.



**Table 2. Cognitive and motor outcomes in 13- to 15-year-old children (N=101)**

| Domain | Outcome | *n* | Normal | Borderline | Abnormal |
|---|---|---|---|---|---|
| Intelligence | - total intelligence | 94 | 88 | 6 | 0 |
| | - verbal intelligence | 100 | 90 | 10 | 0 |
| | - performance intelligence | 94 | 84 | 9 | 1 |
| Attention | - selective visual attention | 100 | 91 | 6 | 3 |
| | - sustained auditory attention | 96 | 86 | 5 | 5 |
| Verbal memory[a] | - immediate recall | 100 | 100 | 0 | 0 |
| | - delayed recall | 101 | 98 | 0 | 3 |
| | - delayed recognition | 100 | 100 | 0 | 0 |
| Motor skills[a] | - total motor skills | 98 | 54 | 30 | 14 |
| | - fine motor skills | 98 | 72 | 17 | 9 |
| | - ball skills | 100 | 68 | 23 | 9 |
| | - static and dynamic balance | 100 | 45 | 31 | 24 |

Data are given as number. Normal was defined as >P15, borderline as P5-P15 and abnormal as <P5; regarding intelligence, normal was defined as IQ>85, borderline as IQ 70-85 and abnormal as IQ <70.
[a]Based on norm-percentiles for 12-year-old children.

Regarding results on the intelligence tests, for seven children no performance and total IQ scores were included in our analyses due to the following reasons: four children performed elsewhere (a subtest of) the WISC less than 12 months before current testing; two children had a very low score on a performance subtest most likely explained by 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 a low score on a verbal subtest, which was most

likely related to poor attention. Regarding 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 or suboptimal testing circumstances. Regarding the AVLT, for one child the test scores on immediate recall and for another child on recognition were excluded due to errors in testing procedure. Regarding testing on 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 the examiner forgot to administer one subtest.

**Exposure to POPs and cognitive and motor outcome**

In Table 3, 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. As presented in Table 3, higher exposure to OH-PCBs was related to more optimal scores on attention and intelligence tests and less optimal scores on memory tasks. Higher exposure to OH-PCBs was related to more optimal scores on motor tasks, only 4-OH-PCB-187 was associated with less optimal scores for ball skills. Similar to the effects of higher exposure to several OH-PCBs, higher levels of PCBs were related to more optimal attention, and to less optimal scores on memory tasks. Higher exposure to PCB-180 was related to more optimal intelligence ($P<.05$), whereas higher exposure to PCB-105 and PCB-183 was related to less optimal intelligence ($P<.10$). Higher exposure to PCBs was related to more optimal scores on motor tasks, only PCB-183 was associated with less optimal scores for ball skills. Regarding exposure to the other measured POPs, higher exposure to several compounds was related to lower scores on cognitive tasks, and none of them was related to scores on motor tasks.

**Table 3. Linear regression analyses for prenatal exposure to persistent organic pollutants, and cognitive and motor outcomes in 13 to 15-year-old children**

| Compound | Cognitive outcome | Optimality[g] | Motor outcome | Optimality[g] | ß coefficient (95% CI) | | | *P*-value |
|---|---|---|---|---|---|---|---|---|
| PCB-105 | Verbal intelligence[a] | - | | | -0.234 | (-0.870 | 0.063) | .089* |
| | Verbal memory- Delayed recognition[b] | - | | | -0.258 | (-0.033 | 0.001) | .062* |
| PCB-138 | Verbal memory- Immediate recall[c] | - | | | -0.298 | (-0.309 | -0.016) | .030** |
| | Verbal memory- Delayed recall[c] | - | | | -0.302 | (-0.393 | -0.026) | .026** |
| PCB-153 | Selective visual attention[d] | + | | | -0.282 | (-0.011 | -0.002) | .005# |
| - RENCO | Selective visual attention[d] | + | | | -0.258 | (-0.009 | 0.000) | .062* |
| PCB-156 | | | Static and dynamic balance[e] | + | -0.247 | (-0.331 | 0.018) | .077* |
| PCB-170 | Selective visual attention[d] | + | | | -0.243 | (-0.047 | 0.003) | .083* |
| | | | Motor skills- Total score[e] | + | -0.244 | (-0.391 | 0.026) | .084* |
| | | | Static and dynamic balance[e] | + | -0.247 | (-0.213 | 0.012) | .078* |
| PCB-180 | Verbal intelligence[a] | + | | | 0.310 | (0.031 | 0.413) | .024** |
| PCB-183 | Total intelligence[a] | - | | | -0.243 | (-0.265 | 0.015) | .080* |
| | Verbal memory- Delayed recognition[b] | - | | | -0.264 | (-0.012 | 0.000) | .056* |
| | | | Ball skills[e] | - | 0.265 | (0.000 | 0.067) | .044** |
| PCB-187 | Selective visual attention[d] | + | | | -0.319 | (-0.068 | -0.006) | .020** |
| Σ 10 PCBs | Selective visual attention[d] | + | | | -0.267 | (-0.003 | 0.000) | .056* |
| 4-OH-PCB-107 | Selective visual attention[d] | + | | | -0.246 | (-0.001 | -0.009) | .016** |
| | Sustained auditory attention[f] | + | | | 0.193 | (-0.006 | 0.186) | .065* |
| | | | Fine motor skills[e] | + | -0.173 | (-0.024 | 0.002) | .097* |
| - GIC | Verbal memory- Delayed recall[c] | - | | | -0.311 | (-0.890 | -0.027) | .038** |
| 3'-OH-PCB-138 | | | Static and dynamic balance[e] | + | -0.249 | (-0.042 | 0.002) | .078* |
| 4-OH-PCB-146 | Verbal intelligence[a] | + | | | 0.223 | (0.006 | 0.103) | .027** |
| | Total intelligence[a] | + | | | 0.203 | (0.000 | 0.081) | .052* |
| - RENCO | Sustained auditory attention[f] | + | | | 0.239 | (-0.021 | 0.240) | .098* |
| | Verbal memory- Immediate recall[c] | - | | | -0.241 | (-0.237 | 0.016) | .085* |
| | Verbal memory- Delayed recall[c] | - | | | -0.267 | (-0.311 | 0.004) | .056* |
| - GIC | Performance intelligence[a] | + | | | 0.280 | (-0.005 | 0.103) | .076* |
| | Total intelligence[a] | + | | | 0.343 | (0.006 | 0.095) | .028** |
| 3-OH-PCB-153 | | | Motor skills- Total score[e] | + | -0.265 | (-0.106 | 0.003) | .063* |
| | | | Static and dynamic balance[e] | + | -0.340 | (-0.064 | -0.007) | .015** |

**Table 3** continued

| Compound | Cognitive outcome | Optimality[g] | Motor outcome | Optimality[g] | ß coefficient (95% CI) | | | *P*-value |
|---|---|---|---|---|---|---|---|---|
| 4'-OH-PCB-172 | Verbal intelligence[a] | + | | | 0.461 | (0.264 | 1.171) | .003# |
| | Total intelligence[a] | + | | | 0.382 | (0.098 | 0.856) | .015** |
| 4-OH-PCB-187 | Sustained auditory attention[f] | + | | | 0.250 | (0.020 | 0.177) | .015** |
| | Selective visual attention[d] | + | | | -0.225 | (-0.008 | 0.000) | .026** |
| - RENCO | Sustained auditory attention[f] | + | | | 0.303 | (0.008 | 0.195) | .034** |
| - GIC | | | Ball skills[e] | - | 0.330 | (0.003 | 0.044) | .023** |
| Σ 6 OH-PCBs | Verbal intelligence[a] | + | | | 0.289 | (-0.002 | 0.055) | .071* |
| BDE-153 | Sustained auditory attention[f] | - | | | -0.379 | (-23.407 | -1.302) | .030** |
| BDE-154 | Verbal memory- Immediate recall[c] | - | | | -0.303 | (-58.161 | 3.172) | .077* |
| | Verbal memory- Delayed recognition[b] | - | | | -0.348 | (-1.617 | -0.037) | .041** |
| HBCDD | Performance intelligence[a] | - | | | -0.352 | (-6.525 | 0.029) | .052* |
| | Total intelligence[a] | - | | | -0.355 | (-5.854 | -0.001) | .050* |
| | Sustained auditory attention[f] | - | | | -0.301 | (-13.739 | 0.913) | .084* |
| PCP | Sustained auditory attention[f] | - | | | -0.253 | (-0.008 | 0.001) | .094* |

Only associations with a *P*-value<0.10 were included; **P*<.10; ***P*<.05; *#P*<.01; [a]Intelligence quotients; [b]Raw scores (higher scores indicate better outcome; 12-years norm); [c]Percentiles (higher percentiles indicate better outcome; 12-years norm);[d]Raw scores (higher scores indicate poorer outcome); [e]Raw scores (higher scores indicate poorer outcome; 12-years norm);[f]Percentiles (higher percentiles indicate better outcome; based on age-specific norms);[g]'-' indicates poorer optimality and '+' indicates better optimality after higher POP-exposure.

For the associations with a *P*-value <.10 in linear regression models, ORs were calculated for prenatal POP-exposure and a 'borderline/abnormal' outcome (Table S1). In Table 4, we only show the significant or marginally significant results of the univariate logistic regression analyses. We corrected for maternal education using multivariate logistic regression analyses, and reported the adjusted ORs in Table 4. As presented in Table 4, after adjustment and dichotomizing the scores into 'normal' and 'borderline/abnormal', only 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-187 (*P*<.05) and 4-OH-PCB-107 (*P*<.10) were positively associated with sustained auditory attention (*P*<.05). Higher exposure to PCB-183 and HBCDD were near- significantly associated with lower intelligence (*P*<.10). Regarding prenatal exposure to PBDEs, DDE, PCP and HBCDD, none of the compounds were associated with borderline or abnormal cognitive or motor outcomes.

**Table 4. Logistic regression analyses for prenatal POP-exposure, and borderline and abnormal cognitive and motor outcome in 13- to 15-year-old children**

| Compound | Outcome | Optimality[a] | *n* | OR (95% CI) | | | *P*-value | Adjusted OR (95% CI)[b] | | | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PCB-105 | Verbal intelligence | - | 54 | 1.090 | (0.994 | 1.194) | .066* | 1.087 | (0.975 | 1.213) | .132 |
| PCB-138 | Verbal memory- Delayed recall | - | 54 | 1.038 | (0.999 | 1.079) | .057* | 1.031 | (0.994 | 1.069) | .102 |
| PCB-183 | Total intelligence | - | 53 | 1.369 | (1.041 | 1.801) | .024** | 1.291 | (0.989 | 1.684) | .060* |
| 4-OH-PCB-107 | Sustained auditory attention | + | 92 | 0.966 | (0.933 | 1.000) | .052* | 0.967 | (0.934 | 1.001) | .055* |
| 3'-OH-PCB-138 | Motor skills- Static and dynamic balance | + | 51 | 0.982 | (0.962 | 1.003) | .086* | 0.982 | (0.962 | 1.002) | .084* |
| 3-OH-PCB-153 | Motor skills- Static and dynamic balance | + | 51 | 0.964 | (0.935 | 0.993) | .015** | 0.963 | (0.933 | 0.993) | .015** |
| 4-OH-PCB-187 | Sustained auditory attention | + | 94 | 0.978 | (0.958 | 0.998) | .034** | 0.978 | (0.958 | 0.998) | .031** |
| HBCDD | Performance intelligence | - | 31 | 3.323 | (0.950 | 11.625) | .060* | 3.617 | (0.969 | 13.492) | .056* |

Only associations with a *P*-value<0.10 in linear and univariate logistic regression models were included. Data are given as odds ratios (95% confidence interval) for borderline and abnormal outcomes: Normal was defined as >P15, borderline and abnormal as ≤P15; regarding intelligence, normal was defined as IQ>85, borderline and abnormal as IQ ≤85; [a]'-' indicates poorer optimality and '+' indicates better optimality after higher POP-exposure; [b]Adjustment for maternal education level; *$P$<.10; **$P$<.05.

## 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. Several OH-PCBs were associated with more optimal sustained auditory attention and more optimal balance. 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.

### Prenatal exposure to OH-PCBs and cognitive and motor outcome

Regarding OH-PCBs and cognitive outcome, some positive effects on optimality of attention, intelligence and some negative effects on optimality of memory were found after higher prenatal exposure to some OH-PCBs. Higher exposure to 4-OH-PCB-187 was less frequently associated with a borderline or abnormal outcome on sustained auditory attention, and for 4-OH-PCB-107 this effect was a trend. Regarding intelligence, several OH-PCBs were positively associated with intelligence levels, although not negatively associated with a borderline or abnormal score. Although all outcomes on verbal memory were within the range for normal development, 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 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 this effect was a positive trend. Regarding exposure to 4-OH-PCB-107, we did not find associations with motor development or 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.[16-18] 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 consequences at adolescence. Because this is the first study on the effects of prenatal exposure to OH-PCBs on outcomes at adolescence, we cannot compare our results with other studies.

### Prenatal exposure to PCBs and cognitive and motor outcome

Regarding PCBs and cognitive outcome, none of the PCBs were associated with borderline or abnormal intelligence, attention or verbal memory, only a negative trend was seen for PCB-183 and intelligence. Prenatal exposure to PCBs was also not associated with borderline or abnormal motor outcome. Although the neuropsychological scores of the children in our cohort 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 exposure to PCBs was associated with less optimal long-term memory.[5] The finding that prenatal exposure to PCBs was not associated with attention is in line with most other studies reporting on prenatal exposure to PCBs and attention problems in adolescents.[8-10] In contrast to our findings, Sagiv et al. found in American children aged 7 to 11 years a relation between prenatal levels of PCBs and a higher risk for ADHD-associated behavior as reported by teachers.[14] A possible explanation for their finding of associations whereas we did not, could be differences in levels of exposure, because the mothers of the children in the mentioned study were living near a PCB-contaminated harbor. However, comparison between the exposure levels is challenging, because Sagiv et al. measured levels of PCBs in cord serum (median level of the sum of PCB 118, 138, 153, and 180 was 0.19 ng/g), whereas we measured the levels in maternal serum based on lipid weight (median level of the same summation of PCBs: 246.6 ng/g).

**Prenatal exposure to other POPs and outcome**

Regarding prenatal exposure to PBDEs, DDE, PCP and HBCDD, none of the compounds was associated with borderline or abnormal cognitive or motor outcomes; only a negative trend was seen for HBCDD and performance intelligence. Regarding prenatal exposure to 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 performance intelligence.[18] 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 exposure to DDE and intelligence is in line with previous studies.[10, 15] 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 exposure to PBDEs.[12] A possible explanation for the fact that we did not find associations between exposure to PBDEs and cognitive outcome could be the smaller sample size of our study.

A strength of our study is the fact that the children performed standardized tests with trained examiners at the clinic to assess cognitive and motor outcome, instead of using questionnaires. This provides us with the opportunity to gain a more robust insight into the performance on cognitive and motor tasks, instead of the impression of the performance as rated by parents or teachers. A second strength is that we followed the children with known prenatal levels of POPs until adolescence, because longitudinal follow-up studies until adolescence are sparse. To the best of our knowledge, this is the first study investigating the effects of prenatal exposure to OH-PCBs on cognitive and motor outcomes in children aged 13 to 15 years, and also the first study investigating the effects of prenatal POP-exposure on motor outcome into adolescence.

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. Nevertheless, we believe that such testing is justified as part of a careful evaluation of a rich data set in hypothesis-driven research.[27] A second 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 third 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.



## CONCLUSIONS

Higher prenatal exposure to OH-PCBs was associated with more optimal sustained attention and more optimal balance in 13- to 15-year-old adolescents. PCB-183 and HBCDD showed a trend with respectively lower total and lower performance intelligence. Prenatal exposure to PBDEs, DDE and PCP was not associated with borderline or abnormal cognitive and motor outcomes at adolescence. Although memory scores were within the range for normal development, higher exposure to PCBs, OH-PCBs and PBDE-154 was 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, does not have clinically relevant consequences for cognitive and motor outcomes at adolescence.

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## SUPPLEMENTARY MATERIAL

**Table S1. Logistic regression analyses for prenatal POP-exposure, and borderline and abnormal cognitive and motor outcome in 13- to 15-year-old children**

| Compound | Outcome | Optimality | *n* | OR (95% CI) | | | *P*-value | Adjusted OR (95% CI) [a] | | | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PCB-105 | Verbal intelligence | - | 54 | 1.090 | (0.994 | 1.194) | .066* | 1.087 | (0.975 | 1.213) | .132 |
| PCB-138 | Verbal memory- Delayed recall | - | 54 | 1.038 | (0.999 | 1.079) | .057* | 1.031 | (0.994 | 1.069) | .102 |
| PCB-153 | Selective visual attention | + | 100 | 0.991 | (0.971 | 1.011) | .363 | 0.990 | (0.970 | 1.011) | .348 |
| - RENCO | Selective visual attention | + | 53 | 0.986 | (0.961 | 1.013) | .308 | 0.986 | (0.961 | 1.013) | .313 |
| PCB-156 | Motor skills- Static and dynamic balance | + | 52 | 0.950 | (0.848 | 1.065) | .379 | 0.951 | (0.847 | 1.067) | .390 |
| PCB-170 | Selective visual attention | + | 52 | 0.920 | (0.801 | 1.057) | .238 | 0.920 | (0.800 | 1.058) | .242 |
| | Motor skills- Total score | + | 51 | 0.979 | (0.907 | 1.057) | .591 | 0.976 | (0.903 | 1.055) | .541 |
| | Motor skills- Static and dynamic balance | + | 52 | 0.953 | (0.885 | 1.027) | .208 | 0.959 | (0.889 | 1.035) | .281 |
| PCB-180 | Verbal intelligence | + | 53 | 0.976 | (0.925 | 1.030) | .376 | 0.990 | (0.930 | 1.054) | .750 |
| PCB-183 | Total intelligence | - | 53 | 1.369 | (1.041 | 1.801) | .024** | 1.291 | (0.989 | 1.684) | .060* |
| | Motor skills- Ball skills | - | 54 | 1.022 | (0.979 | 1.067) | .324 | 1.022 | (0.978 | 1.067) | .335 |
| PCB-187 | Selective visual attention | + | 53 | 0.830 | (0.651 | 1.060) | .135 | 0.832 | (0.652 | 1.061) | .138 |
| Σ 10 PCBs | Selective visual attention | + | 52 | 0.995 | (0.986 | 1.004) | .274 | 0.995 | (0.986 | 1.004) | .279 |
| 4-OH-PCB-107 | Selective visual attention | - | 96 | 1.001 | (0.986 | 1.016) | .891 | 1.001 | (0.987 | 1.016) | .886 |
| | Sustained auditory attention | + | 92 | 0.966 | (0.933 | 1.000) | .052* | 0.967 | (0.934 | 1.001) | .055* |
| | Motor skills- Fine motor skills | + | 95 | 0.988 | (0.974 | 1.001) | .078 | 0.988 | (0.975 | 1.001) | .079 |
| 3'-OH-PCB-138 | Motor skills- Static and dynamic balance | + | 51 | 0.982 | (0.962 | 1.003) | .086* | 0.982 | (0.962 | 1.002) | .084* |
| 4-OH-PCB-146 | Verbal intelligence | + | 98 | 0.997 | (0.983 | 1.012) | .726 | 0.999 | (0.985 | 1.013) | .853 |
| | Total intelligence | - | 92 | 1.002 | (0.987 | 1.018) | .779 | 1.004 | (0.988 | 1.020) | .618 |
| - RENCO | Verbal memory- Delayed recall | - | 52 | 1.024 | (0.991 | 1.058) | .160 | 1.022 | (0.992 | 1.053) | .160 |
| - GIC | Performance intelligence | + | 41 | 0.995 | (0.966 | 1.025) | .734 | 0.995 | (0.966 | 1.025) | .744 |

**Table S1** continued

| Compound | Outcome | Optimality | *n* | OR (95% CI) | | | *P*-value | Adjusted OR (95% CI) [a] | | | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-OH-PCB-153 | Motor skills- Total score | + | 50 | 0.990 | (0.969 | 1.012) | .375 | 0.990 | (0.969 | 1.012) | .378 |
| | Motor skills- Static and dynamic balance | + | 51 | 0.964 | (0.935 | 0.993) | .015** | 0.963 | (0.933 | 0.993) | .015** |
| 4'-OH-PCB-172 | Verbal intelligence | + | 40 | 0.877 | (0.742 | 1.037) | .126 | 0.900 | (0.748 | 1.083) | .265 |
| | Total intelligence | + | 40 | 0.950 | (0.820 | 1.101) | .496 | 0.993 | (0.840 | 1.174) | .933 |
| 4-OH-PCB-187 | Sustained auditory attention | + | 94 | 0.978 | (0.958 | 0.998) | .034** | 0.978 | (0.958 | 0.998) | .031** |
| | Selective visual attention | - | 98 | 1.004 | (0.991 | 1.016) | .555 | 1.004 | (0.991 | 1.016) | .552 |
| - GIC | Motor skills- Ball skills | - | 47 | 1.016 | (0.996 | 1.036) | .123 | 1.014 | (0.994 | 1.035) | .162 |
| Σ 6 OH-PCBs | Verbal intelligence | + | 40 | 0.998 | (0.991 | 1.004) | .483 | 0.998 | (0.991 | 1.005) | .666 |
| BDE-153 | Sustained auditory attention | - | 33 | 2.146 | (0.490 | 9.411) | .311 | 1.851 | (0.467 | 7.342) | .381 |
| HBCDD | Performance intelligence | - | 31 | 3.323 | (0.950 | 11.625) | .060* | 3.617 | (0.969 | 13.492) | .056* |
| | Sustained auditory attention | - | 34 | 1.686 | (0.777 | 3.661) | .186 | 1.518 | (0.692 | 3.332) | .298 |
| PCP | Sustained auditory attention | - | 45 | 1.000 | (0.999 | 1.000) | .614 | 1.000 | (0.999 | 1.000) | .581 |

Only associations with a *P*-value<0.10 in linear regression models were included. Data are given as odds ratios (95% confidence interval) for borderline and abnormal outcomes: Normal was defined as >P15, borderline and abnormal 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; [a] Adjustment for maternal education level; *$P$<.10; **$P$<.05.