The effects of exposure to environmental chemicals on child development

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CHAPTER 9

General discussion and future perspectives

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This thesis comprises studies on the effects of prenatal exposure to environmental chemicals on child development up to and including adolescence, with the emphasis on neurological development and endocrine functions. Our general aim was to determine whether prenatal exposure to polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs) is associated with neurological and behavioral outcomes up to and including adolescence. Our secondary aim was to determine whether prenatal exposure to PCBs and OH-PCBs is associated with hormonal development, including thyroid hormone metabolism, and pubertal development. We found that prenatal exposure to PCBs and/or OH-PCBs can affect neurological development at different ages, that it can interfere with thyroid hormone metabolism and that it can advance pubertal development. In this chapter we summarize and discuss the main findings of the thesis and provide directions for future research.

MAIN FINDINGS

PART 1- The neurotoxic effects of exposure to environmental chemicals during childhood: an overview

In Part 1, **Chapter 2**, we present an overview of recent literature on the relationship between various persistent organic pollutants (POPs) and childhood neurodevelopmental outcomes. The findings demonstrate that exposure to environmental chemicals can affect neurological development and behavior outcomes in children in several domains, including attention, motor development and mental development. Regarding the exposure to PCBs and OH-PCBs, most studies reported inverse associations with neurodevelopmental outcomes. Regarding exposure to brominated flame retardants and polybrominated diphenyl ethers (PBDEs), poorer mental and psychomotor development and lower IQs were found at preschool age and poorer attention at school age. Regarding exposure to dichlorodiphenyldichloroethylene (DDE), a degradation product of the insecticide dichlorodiphenyltrichloroethane (DDT), poorer psychomotor development was found in early infancy, as well as poorer attention and an increase of ADHD-like behaviors. A suggested potential mechanism through which POPs may have an adverse effect on neurological development is through interference with thyroid hormone signaling in the developing brain. The overview also shows that several studies found that boys are more vulnerable to the harmful effects of exposure to environmental chemicals than girls. A possible explanation for this sex-related difference could be that these chemical compounds act as androgen or estrogen receptor antagonists or agonists and thereby affect the development of boys differently.

PART 2- Exposure to environmental chemicals and neurological functioning from birth up to and including adolescence

In Part 2 we present the effects of prenatal exposure to environmental chemicals on neurodevelopmental outcomes in two Dutch birth cohorts. These cohorts were initiated to investigate the effects of exposure to environmental chemicals on child development.[1, 2] An overview of the effects of prenatal exposure to PCBs and OH-PCBs on neurodevelopmental outcomes is shown in Table 1. In **Chapter 3** we present our study that established that prenatal exposure to PCBs and OH-PCBs is associated with the quality of the spontaneous motor repertoire in three-month-old infants. The most important finding is that exposure to high levels of 4-OH-PCB-107 is associated with less than optimal motor development. This finding, which suggests that 4-OH-PCB-107 might be more toxic than other OH-PCB compounds, is consistent with the findings in human and animal studies reported by other researchers. A second finding is that higher exposure to some PCBs is associated with reduced age-adequate movements in infants, such as fewer midline movements, less manipulations with their hands and/or feet, and fewer antigravity movements. An exception is 4'-OH-PCB-172, which we found to be associated with more age-adequate movements. A third finding is that higher exposure to PCB-118 is associated more frequently with a cramped movement character. A cramped movement character might be predictive of outcomes later in life - in children with cerebral palsy a cramped movement character at three months was found to be associated with lower levels of self-mobility at school-age.[3]

In **Chapter 4** we present our study in which we determined that prenatal exposure to PCBs and OH-PCBs is also associated with the neurological functioning of three-month-old infants. We assessed these infants with an age-adequate neurological examination based on Touwen's method of neurological examination.[4] The most important finding presented in this chapter is that higher prenatal exposure to several PCBs is positively associated with neurological functioning. These findings seem to contradict the results presented in Chapter 3 and with the findings in other studies on early neurological development that reported negative associations with neurological functioning.[5] This difference in the direction of associations may be explained by differences in testing measures and/or that other areas and functions of the brain were tested. The assessment of neurological development in Chapter 3 included the observation of the spontaneous motor repertoire, whereas the assessment in Chapter 4 included the observation of posture and motility, muscle tone regulation, reflexes and assessment of function of cranial nerves.[4] Another explanation for the differences in the direction of associations compared with other studies may be differences in the levels of exposure. In the Netherlands the exposure levels to PCBs are lower than in populations with a tradition of eating pilot whale blubber as, for instance, the people of the Faroe Islands.[6] We speculated that higher exposure levels might have negative effects, whereas lower levels might possibly have positive effects by stimulating neuronal

and/or hormonal processes. A more rapid development might possibly occur at the expense of the formation of stable neural networks. Whether a rapid development at a young age has implications for developmental outcomes later in life is not clear. A second finding is that 4-OH-PCB-107 is associated with less than optimal neurological functioning in boys. These findings are in line with our findings in Chapter 3 that also show less optimal outcomes after higher exposures to this compound. In another study, this specific metabolite is also associated with a less optimal mental development in 16-month-old infants.[7] A third finding is that higher exposure to several PCBs is associated with more optimal visuomotor and sensorimotor functioning in three-month-old children. This may suggest that exposure to PCBs possibly has an impact on specific functions of the brain. Studies by other researchers also found relations between prenatal exposure to PCBs and visual functions in children. For example, differences in brain activation were observed on tasks requiring visual processing and manual motor movement in 15-year-old children after prenatal exposure to PCB and methylmercury.[8] Functional magnetic resonance imaging (fMRI) techniques revealed greater and more widespread brain activation in the highly exposed group. This suggests that adolescents with high prenatal exposure require more brain resources to complete tasks and that differential specialization of brain areas may have occurred after prenatal exposure to neurotoxicants. A final, important finding, presented in **Chapter 4**, is that there are sex-specific differences regarding the effects of prenatal exposure to PCBs and OH-PCBs, which suggest that boys are more susceptible than girls. This finding is in line with other studies in animals and humans that also reported that male animals/boys are more vulnerable to the effects of exposure to environmental chemical than female animals/girls.[9, 10]

In **Chapter 5** we present the study in which we demonstrated that prenatal exposure to several organohalogen compounds (OHCs) is associated with mental and motor development at the age of 18 and/or 30 months. Our most important finding is that OH-PCBs seem to have more effects on neurological development compared with PCBs. Higher exposure to 4-OH-PCB-187 is associated with delayed mental development at 18 months and four OH-PCB congeners and the sum of the measured OH-PCBs correlated positively with mental development at 30 months. A possible explanation for the fact that we did not observe effects of exposure to these OH-PCB congeners at 18 months could be that the effects might be more subtle at younger ages and that their presence becomes more obvious at later ages. The compound 4'-OH-PCB-172 is positively associated with motor development at 30 months, a finding that is consistent with the finding in Chapter 3 that this compound is positively associated with motor development at the age of three months. Regarding the compound 4-OH-PCB-107, which was found to be negatively associated with neurodevelopmental outcomes in Chapters 3 and 4, we did not find negative effects on neurological development at 18 months or 30 months. This possibly implies that the negative effects of 4-OH-PCB-107 do not persist into later life, or at least, the effects were not observed with the BSID-II at

18 months or 30 months. In Chapter 5 we also found that higher exposure to PCB-153 is negatively associated with mental development at 18 months. This finding is in line with other studies that also showed that PCB-153 is more often associated with developmental outcomes in children.[11, 12] A possible explanation for the fact that PCB-153 in particular is found to be associated with neurological development is that it is the most abundant PCB-congener. Another explanation could be that PCB-153 can alter neurotransmitter functions that are essential for proper development of the brain, as shown by a decrease in brain serotonin and dopamine in rats[13] In Chapter 5, we also investigate the effects of prenatal exposure to five brominated diphenyl ethers (PBDEs), dichlorodiphenyldichloroethylene (DDE), pentachlorophenol (PCP) and hexabroomcyclododecane (HBCDD) on neurological development at 18 months. Regarding the exposure to PBDE, the most important finding presented in this chapter is that higher levels of BDE-99 are associated marginally significantly with a delayed mental score at 18 months, whereas no other PBDEs were found to be associated with neurological development at this age. A final, important finding in Chapter 5 is that DDE, PCP and HBCDD are not associated with mental or motor development at 18 months. This is in contrast to findings reported by other studies on the effects of p,p'-DDE, a degradation product of the insecticide DDT, which showed impairment of mental or motor development in children aged between 3 months and 24 months (reviewed by Eskenazi and colleagues). [14] Possible explanations for the fact that we found no associations between prenatal exposure to DDE and neurological development could be the smaller sample size of our study, differences in testing methods and lower levels of exposure.

In **Chapter 6** we present our observations that prenatal exposure to some OHCs are both positively and adversely associated with cognitive and motor outcome in 13 to 15-year-old adolescents. Our most important finding is that regarding OH-PCBs, higher prenatal exposure to OH-PCB is associated with more optimal sustained attention and more optimal balance, and that higher exposure to 4-OH-PCB-107 is not associated with motor outcome at adolescence. Previously, in our cohort, the latter compound was found to be associated with less optimal motor development and poorer visuomotor function at three months (Chapters 3 and 4) and poorer fine manipulative abilities at the age of five to six years.[15] This suggests that the negative effects of 4-OH-PCB-107 on motor outcomes observed at preschool and school age do not have clinically relevant consequences at adolescence. Our second finding is that regarding PCB-exposure, only a trend is seen for higher exposure to PCB-183 with lower total intelligence and that none of the other PCB compounds are associated with borderline/abnormal outcomes on cognitive or motor tasks. Although memory scores are within the range for normal development, higher exposure to PCBs is associated with less optimal verbal memory. This finding is in line with a study on 271 adolescents, aged 11 years to 16 years, that reported that higher prenatal exposure to PCBs is associated with less optimal long-term memory.[16] Our not finding significant associations between prenatal exposure to

PCBs and attention is in line with most other studies that reported on prenatal exposure to PCBs and attention problems in adolescents.[17-19] Our third finding is that PBDEs, DDE, PCP and HBCDD are not associated with borderline or abnormal cognitive or motor outcomes; only a negative trend is seen for HBCDD and performance intelligence. Previously, in our cohort, HBCDD was found to be associated with higher total and verbal intelligence at early school age, but not with less optimal performance intelligence.[15] This might suggest that negative effects of prenatal exposure to HBCDD develops or progresses over time. Regarding exposure to PBDEs, we did not observe effects on motor and cognitive outcomes during adolescence, whereas in our cohort effects were observed on fine manipulative abilities or attention at early school age.[15] A possible explanation for our not finding associations at time of follow-up during adolescence could be the smaller size of our sample. Overall, we conclude from Chapter 6 that the negative effects of prenatal exposure to OHCs on cognitive and motor outcomes observed at preschool and early school age in our studies (Chapter 3, 4 and 5; Table 1) do not persist up to and including adolescence, and that prenatal Dutch background levels of OHC, measured between 1998 and 2002, do not have clinically relevant consequences for cognitive and motor outcomes at adolescence.

PART 3- Endocrine disrupting effects of environmental chemicals

In Part 3 we present the effects of prenatal exposure to environmental chemicals on endocrine functions, including thyroid hormone metabolism and pubertal development. An overview of the results in both birth cohorts are shown in Table 2. In **Chapter 7** we present the study in which we found that PCBs can interfere with thyroid hormone metabolism during fetal life. Prenatal exposure to PCBs correlates positively with cord serum triiodothyronine (T3), negatively with reverse T3 (rT3) and positively with the T3/rT3 ratio. These findings suggest that prenatal exposure to PCBs can reduce activity of iodothyronine deiodinase Type 3 (D3), because this thyroid hormone metabolizing enzyme is responsible for converting thyroxine (T4) into rT3 and T3 into 3,3'-T2.[20] In particular, D3 is present in placental and fetal tissues, which might be an explanation for our observation that we did not find effects on D3 activity in serum samples at the age of 3 months or 18 months. In placenta and fetal tissues, D3 may protect developing tissue from exposure to unduly high levels of active thyroid hormone.[21] On account of the fact that D3 is suggested to play a role in regulating intracellular T3 levels in tissues, such as brain tissue, our findings in Chapter 7 might suggest that reduction of D3 activity is a potential mechanism of PCB toxicity in humans. Exposure to background levels of PCBs or OH-PCBs is not associated with T4, T4 sulfate (T4S), thyroid stimulating hormone (TSH) or thyroid-binding globulin (TBG) levels in cord blood. A second, noteworthy finding in Chapter 7 is that 4-OH-PCB-107 is positively correlated with T4, T4S and T3 at 18 months, which implies that this compound might interfere with thyroid hormone metabolism during later life. This compound is also associated with less optimal neurological

development in three-month-old children, whom we assessed using two different testing methods, as presented in Chapter 3 and 4. We did not find 4-OH-PCB-107 to be associated with neurological development at 18 months or 30 months. Further study should clarify whether the findings regarding the effects of exposure to 4-OH-PCB-107 on thyroid hormone metabolism and neurological development might have implications for later life.

In **Chapter 8** we present the study in which we demonstrated that prenatal exposure to OHCs can advance pubertal development in both boys and girls. The most important finding of this study is that in boys, higher prenatal exposure to PCBs is associated with higher Tanner stages for pubic hair, larger testicular volume and change of voice at a younger age. This finding is in contrast to another study, with even higher exposure levels, reporting weak, non-significant inverse associations between prenatal exposure to PCBs and Tanner stage for testicular volume.[22] Our findings imply that even relatively low prenatal exposure to PCBs might interfere with pubertal development. In girls, higher prenatal exposure to PCB-153 is associated with a higher Tanner stage for pubic hair. A possible explanation for this finding might be an increase in production of adrenal androgens, because they are mainly responsible for growth of pubic hair in girls. A study in a human in vitro model showed that several chemicals disturb adrenal steroid genesis, but the effects of PCBs were not assessed in that study.[23] What needs to be clarified is whether the effects of PCB-153 on the development of pubic hair, as we found in our study, could be explained by disturbances of adrenal androgen levels during puberty. Another finding presented in Chapter 8 is that OH-PCBs seem to have less effect on pubertal development than PCBs, although associations were found for some OH-PCBs, as summarized in Table 2. Higher exposure to 4-OH-PCB-107 showed a trend toward a higher Tanner stage for pubic hair in boys and was associated with first ejaculation at an older age. As shown in Table 2, in boys included in the GIC cohort, this compound was found to be positively associated with testosterone levels at the age of three months.[24] 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 or faster development of pubertal characteristics. The compound 4-OH-PCB-187 was associated with smaller testicular volume in 13 to 15-old boys, which might be due to an LH/FSH imbalance, because the latter stimulates the Sertoli cells that are responsible for a large part of the testicular volume. As shown in Table 2, a positive trend was found for this compound with follicle stimulating hormone (FSH) at the age of three months.[24] Clarification is needed as to whether the effects of prenatal exposure to OH-PCBs found in our study can be explained by the disturbance of sex hormone levels during puberty. A final finding presented in Chapter 8 is that PBDEs, DDE, PCP and HBCDD are not associated with pubertal development. Only DDE shows a negative trend with testicular volume, which also showed a positive trend with luteinizing hormone (LH) at the age of three months.[24] Increased levels of LH might be the result of anti-androgenic or anti-estrogenic effects

of p, p'-DDE that can result in an estrogen/androgen imbalance. Eskenazi and colleagues found that prenatal exposure to DDE was associated with decreases in LH concentrations in 12-year-old boys, taking into account the Tanner stages.[25] Whether the observed smaller testicular volume in our study might have been caused by disturbances in hormone levels during puberty needs to be clarified. The findings presented in Chapter 8 underline that exposure to environmental chemicals can play a role in the secular trend towards earlier pubertal timing that has been observed during the last decades.[26]

Measurement of prenatal exposure to POPs

To assess prenatal exposure to POPs we used maternal serum samples taken during the third trimester of pregnancy. The levels of PCBs and OH-PCBs measured during pregnancy correlated highly with the levels measured in cord blood samples,[27] which is a reflection of the levels to which the fetus is exposed. Because PCBs accumulate in fatty tissue, PCB-levels in the human body increase over time. Degradation products of PCBs, such as hydroxylated metabolites, are excreted via urine. On account of the fact that PCBs are stable compounds that are not readily excreted, we used serum samples rather than urine samples to assess the levels of PCBs in the mothers during pregnancy. In our studies we also measured the hydroxylated metabolites of PCBs in maternal serum samples. Only recently has a technique become available that measures OH-PCBs in human urine samples.[28] With this new technique, urinary OH-PCBs might be a suitable biomarker for lower chlorinated PCBs. Because we also aimed to determine levels of more stable, higher chlorinated PCBs, we believe that measurement in serum samples was the most suitable available method for our studies.

Differences in levels of prenatal exposure to POPs

Chemical exposure levels differ around the world on account of, for example, accidents, eating habits and the time span between the production and banning of the chemicals. For this reason it is important to compare the Dutch background levels measured in our study with levels measured in other countries. Because the quantification of exposure levels differs between studies, it is difficult to compare the PCB exposure levels. Longnecker and colleagues attempted to compare the exposure levels of ten studies after expressing PCB-153-levels in maternal pregnancy serum in a uniform manner.[6] They concluded that the exposure levels found in recent US studies were about one-third of those in recent studies in the Netherlands, Germany, and in northern Québec, Canada. Compared with most other studies, the exposure levels in the Faroe Islands study were about three-fold to four-fold higher on account of the traditional habit of eating pilot whale blubber that contains PCBs.

The strengths and limitations

A major strength of the studies contained in this thesis is that we assessed neurological development up to 13 years to 15 years after birth, which provided us with the opportunity to properly assess long-term effects of prenatal exposure to environmental chemicals. A second strength of our studies is that the children came to our clinic for assessment of neurological outcomes. We used standardized tests administered by trained examiners, thus providing a more robust insight into the performances of the children than the impression of their performances if assessed by parents or teachers.

The studies described in this thesis encountered several potential limitations. The first limitation we address is the possibility of Type 1 errors due the explorative nature of the studies. Nevertheless, we believe that our analyses were justified as part of a careful evaluation of a rich data set in hypothesis-driven research.[29] A second limitation concerns sample sizes. Despite the relatively small sizes of our samples, we are, of the opinion that the sizes are appropriate for such complicated studies that involve the assessment of several OHCs. A third limitation is the possibility of bias on account of the way we recruited the pregnant women. Women who were willing to participate in a study on the effects of exposure to chemical substances, might be more conscious of their lifestyle and eating habits and might possibly adapt their lifestyle, which might have lowered their exposure to POPs. As a consequence, the true effects on neurological developmental and endocrine development in our study group might have been underestimated in comparison to the general Dutch population that might have had even higher levels of exposure levels. A final limitation is that we cannot exclude the possibility that exposure to other POPs confounded our findings. Nevertheless, it seems implausible that these pollutants were associated with some specific PCB metabolites, for example, 4-OH-PCB-107, but not with other compounds in our study. On account of these limitations our studies should be considered explorative and our results should be interpreted with caution. We call for larger studies to confirm our findings.

CONCLUSION, IMPLICATIONS AND FUTURE PERSPECTIVES

Altogether, the studies presented in this thesis provide insight into the effects of prenatal exposure to PCBs and OH-PCBs on neurological development and endocrine functions from birth up to and including adolescence.

Neurotoxic effects of environmental chemicals

In Parts 1 and 2 we demonstrate that prenatal exposure to environmental chemicals can have neurotoxic effects in children. Prenatal exposure to Dutch background levels of PCB and OH-PCB can interfere with children's neurological development. Although the observed effects fall within the range for normal development, we did find associations between higher prenatal chemical exposure and less than optimal development at different ages, using different testing methods. Our findings imply that even relatively low prenatal exposure to PCBs might interfere with neurological development. This raises grave concern about the effects of man-made compounds on child development and it underlines the importance of exercising extreme caution when it comes to using existing chemicals and to introducing new ones. Besides the insights our studies provide into the prenatal effects of chemical exposure on neurodevelopmental outcomes, new questions have also arisen. For example, whether postnatal chemical exposure continues to interfere with development during adolescence. Future studies should therefore also focus on levels of chemical exposure during adolescence and whether such postnatal exposure interferes with neurological development and pubertal development. The studies described in this thesis comprise mainly studies on the effects of exposure to POPs that were already banned by law, but there is growing evidence that new chemicals, such as bisphenol A, may also have endocrine disrupting effects. Future studies should therefore also focus on the effects of these newly produced chemicals.

Mechanisms of PCB toxicity

The studies presented in Part 3 demonstrate that PCBs can interfere with thyroid hormone metabolism during fetal life, and we suggest that reduction of D3 activity might play a role in PCB toxicity in humans. Because thyroid hormones are essential for the developing brain, disturbances of thyroid hormone metabolism system might be an underlying mechanism for the neurotoxic effects of PCBs. Besides interference with endocrine mechanisms, there is growing evidence for interference of environmental chemicals with epigenetic mechanisms.[30] 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 and colleagues). Experimental animal studies have shown that these adverse effects can be

transmitted to unexposed generations. Jacobs and colleagues conclude from their extensive review that epigenetic changes have great potential to become useful for chemical risk assessment as early markers of adversities later on in life or in subsequent generations.[30] Further study is needed to explain whether the effects of prenatal chemical exposure on developmental outcomes we found in our studies can be explained by underlying changes in epigenetic mechanisms.

Environmental chemicals and advanced pubertal development

In Part 3 we also demonstrated that prenatal exposure to environmental chemicals can advance pubertal development in both boys and girls. This is an important finding with possible consequences for later life, because relations were found between pubertal development and risk of cancer. In girls, an earlier onset of pubertal development was found to be related to breast cancer.[31, 32] In boys, late sexual maturation was found to be related to a reduced risk of prostate cancer in adulthood.[33] Future studies should focus on whether our findings of advanced pubertal development after higher prenatal chemical exposure might have consequences later in life, including the risk of cancer and infertility. Another question raised by the effects of chemical exposure on pubertal outcomes, is whether these effects were caused by disturbances of hormone levels. Addressing this question might be valuable in understanding the underlying mechanisms of the effects of exposure to environmental chemicals on pubertal outcomes.

To conclude, the findings presented in this thesis demonstrate that prenatal exposure to POPs can interfere with normal child development, not only during the perinatal period, but up to and including adolescence. These findings raise grave concern regarding the effects of man-made chemical compounds on child development, and further study is needed to determine whether the effects of prenatal exposure persist into adulthood.

Table 1. Prenatal exposure to persistent organic pollutants and neurodevelopmental outcomes

Compound	3 months		18 months	30 months	5 to 6 years	13 to 15 years
	Motor development (Chapter 3)[34]	Neurological functioning (Chapter 4)[35]	Mental and motor development (Chapter 5)	Mental and motor development (Chapter 5)	Motor, cognitive and behavioral development[15]	Motor and cognitive development (Chapter 6)
PCB-105	-	↑ (better visuomotor function)	-	-	0	-
PCB-118	↓ (less antigravity movements; cramped movement character)	↑ (better visuomotor function)	-	-	0	-
PCB-138	↓[t] (cramped movement character)	↑ (better visuomotor and sensorimotor functions)	-	-	0	-
PCB-146	-	↑ (higher optimality score; better visuomotor and sensorimotor functions)	-	-	0	-
PCB-153	0	0	-	0	0	-
- RENCO	-	↑ (better visuomotor and sensorimotor functions)	-	-	0	-
- GIC	0	0	↓ (delayed mental development)	0	↑ and ↓ (less choreiform dyskinesia; better coordination[t]; more total behavioral problems[t]; more externalizing behavioral problems[t])	-
PCB-156	-	↑ (better visuomotor function)	-	-	0	-

Table 1 continued

Compound	3 months		18 months	30 months	5 to 6 years	13 to 15 years
	Motor development (Chapter 3)[34]	Neurological functioning (Chapter 4)[35]	Mental and motor development (Chapter 5)	Mental and motor development (Chapter 5)	Motor, cognitive and behavioral development[15]	Motor and cognitive development (Chapter 6)
PCB-170	-	↑ (better sensorimotor function)	-	↑[t] (more optimal mental development)	0	-
PCB-180	-	↑ (better sensorimotor function)	-	↑[t] (more optimal mental development)	0	-
PCB-183	-	-	-	-	0	↓[t] (lower total intelligence)
PCB-187	↓[t] (fewer midline leg movements)	↑ (better visuomotor and sensorimotor functions)	↓ (delayed mental development)	-	0	-
Σ 10 PCBs	↓[t] (cramped movement character)	↑ (better visuomotor and sensorimotor functions)	-	-	0	-
4-OH-PCB-107	0	0	-	0	0	↑[t] (better sustained auditory attention)
- RENCO	↓[t] (lower motor optimality score; reduced repertoire of coexistent movements)	↓ in boys (lower optimality score; worse visuomotor function)	-	↑ (more optimal mental development)	0	-
- GIC	0	0	-	0	↓ (worse fine manipulative abilities; worse inhibition[t])	-
3'-OH-PCB-138	-	-	-	↑ (more optimal mental development)	0	↑[t] (better static and dynamic balance)

Table 1 continued

Compound	3 months		18 months	30 months	5 to 6 years	13 to 15 years
	Motor development (Chapter 3)[34]	Neurological functioning (Chapter 4)[35]	Mental and motor development (Chapter 5)	Mental and motor development (Chapter 5)	Motor, cognitive and behavioral development[15]	Motor and cognitive development (Chapter 6)
4-OH-PCB-146	0	0	-	0	0	-
- RENCO	-	-	-	↑[t] (more optimal mental development)	0	-
- GIC	0	0	-	0	↑ and ↓ (less choreiform dyskinesia[t]; worse inhibition; more total behavioral problems; more externalizing and internalizing[t] behavioral problems)	-
3'-OH-PCB-153	-	-	-	↑ (more optimal mental development)	0	↑ (better static and dynamic balance)
4'-OH-PCB-172	↑ (more manipulation)	-	-	↑ (more optimal mental and motor[t] development)	0	-
4-OH-PCB-187	0	0	-	0	0	↑ (better sustained auditory attention)
- RENCO	-	-	-	-	0	-
- GIC	0	0	-	0	↓ (worse inhibition)	-
Σ 6 OH-PCBs	-	-	-	↑ (more optimal mental development)	0	-

[t] Trend to significance: $P < .10$; '0'= not assessed; '-'= no associations; '↓' indicates poorer outcomes and '↑' indicates better outcomes after higher exposure to POPs.

Table 2. Prenatal exposure to persistent organic pollutants and endocrine functions

Compound	Cord blood	3 months		18 months		13 to 15 years	
	Thyroid hormone (Chapter 7)[27]	Sexual development boys[24, 36]	Thyroid hormone (Chapter 7)[27]	Thyroid hormone (Chapter 7)[27]	Sexual development boys[24, 36]	Pubertal development (Chapter 8)	
PCB-105	-	-	-	-	-	♂	↓ age first ejaculation
PCB-118	↑T3; ↓rT3; ↑T3/rT3	-	-	-	-	♂	↑ stage pubic hair ↑ testicular volume[t] ↓ age first ejaculation
						♀	↑ stage breast[t]
PCB-138	↓rT3; ↑T3/rT3	-	-	-	-	♂	↑ stage pubic hair ↑ testicular volume[t] ↓ age voice mutation[t]
PCB-146	↑T3; ↓rT3; ↑T3/rT3[t]	-	-	-	-	♂	↑ stage pubic hair ↑ stage genitals[t]
PCB-153	0	0	0	0	0	♂	↓ age voice mutation [t]
						♀	↑ stage breast[t] ↑ stage pubic hair
- RENCO	↑T3[t]; ↑T3/rT3[t]	-	-	-	-	♂	↑ stage pubic hair
- GIC	0	-	0	0	-	-	
PCB-156	-	-	-	-	-	♂	↑ stage pubic hair ↑ testicular volume[t] ↓ age voice mutation[t]
PCB-170	-	-	-	-	-	♂	↑ stage pubic hair[t] ↓ age voice mutation[t]
PCB-180	-	-	-	-	-	♂	↑ stage pubic hair
PCB-183	-	-	-	-	-	♂	↓ age voice mutation
PCB-187	↑T3; ↓rT3	-	-	-	-	♂	↑ stage pubic hair ↓ age voice mutation
Σ 10 PCBs	↑T3[t]; ↓rT3; ↑T3/rT3	-	-	-	-	♂	↑ stage pubic hair ↑ testicular volume[t] ↓ age voice mutation[t]
						♀	↑ stage breast[t]

4-OH-PCB-107	0	0	0	0	0	♂	↑ stage pubic hair[t] ↑ age first ejaculation
- RENCO	-	-	↑T4	↑T4[t]; ↑T4S; ↑T3	-	-	
- GIC	0	↑ free testosterone[t] ↑ testosterone	0	0	-	-	
3'-OH-PCB-138	-	-	-	-	-	-	
4-OH-PCB-146	0	0	0	0	0	♀	↓ age growth spurt
- RENCO	-	-	-	↑T4S	-	♂	↓ age voice mutation
- GIC	0	-	0	0	-	-	
3-OH-PCB-153	-	↑ testes volume	-	-	-	-	
4'-OH-PCB-172	-	↑ FSH[t]	↓TSH	↓TSH	-	♂	↓ age voice mutation[t]
4-OH-PCB-187	0	0	0	0	0	-	
- RENCO	-	-	↑T4; ↑T3	-	-	♀	↓ age growth spurt
- GIC	0	-	0	0	-	♂	↓ testicular volume
						♀	↑ age menarche[t]
Σ 6 OH-PCBs	-	-	-	↑T4S; ↑T3	-	-	

[t] Trend to significance: $P < .10$; '0'= not assessed; '-'= no associations; '↓' indicates a decreasing value and '↑' an increasing value after higher exposure to POPs.

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