Neurodevelopmental effects of perinatal exposure to environmental levels of PCBs and dioxins in children at school age

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Ontwikkelingseffecten van perinatale blootstelling
aan achtergrondniveaus van PCB's en dioxinen
bij schoolgaande kinderen

Proefschrift

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Contents

Preface & Structure of the thesis

PCBs (polychlorinated biphenyls) and dioxins are persistent environmental pollutants which are known for their (neuro)toxic potential, especially in children. These compounds are lipid-soluble and poorly eliminated, features that make them accumulate in the food chain when they are emitted in the environment. Humans are exposed to these compounds predominantly through food, causing accumulation of PCBs and dioxins in human's body fat. A fetus is exposed to maternal body burdens of these compounds through transplacental transport. Moreover, PCBs and dioxins are excreted in breast milk causing additional exposure of the infant to relatively large amounts of PCBs and dioxins (1).

In the late 80's, breast milk PCB and dioxin concentrations in The Netherlands were amongst the highest worldwide (2). Prenatal exposure to *high* maternal levels of these compounds was known to cause neurodevelopmental abnormalities in children (3, 4). To explore effects of prenatal and postnatal (lactational) exposure to *environmental* levels of PCBs and dioxins as measured in The Netherlands, a prospective cohort study was initiated in 1989. This Dutch PCB/dioxin cohort was recruited by two study centers, in Rotterdam (Sophia Children's Hospital Rotterdam), a highly industrialized area, and in Groningen (Academic Hospital Groningen), a more rural area. In the Dutch PCB/dioxin cohort, effects of prenatal and postnatal (i.e. perinatal) exposure on growth, health, neurodevelopment and behavior have been studied from birth to school age. The Dutch PCB/dioxin study started as a cooperative effort between animal and human studies and was initially supported by the Dutch Toxicology Research Promotion Program and the Health Research Stimulation Program. Effects on end-points from birth to 18 months of age were described in the doctoral theses of C. Koopman-Esseboom (Rotterdam) (5) and M. Huisman (Groningen) (6). At 42 months of age, a follow-up study was initiated in the Dutch cohort as part of a multi-center cohort study in which additionally a German and Danish cohort, both recruited between 1994 and 1995, participated. The European Commission financed this multi-center study. The cohort studies applied the same inclusion and exclusion criteria, however the German and Danish studies held no restrictions in the inclusion of the number of breast-feeding mothers, in contrast to the Dutch cohort in which half of the infants was breast-fed for at least six weeks and the other half formula-fed. The major developmental assessments were similar in the cohorts, although study centers included additional outcome variables of their own particular interest. The results of the study at 42 months of age in the Dutch cohort are described in the doctoral theses of S. Patandin (7) (Rotterdam) and C.I. Lanting (8) (Groningen).

This thesis describes results of a follow-up study in the Dutch PCB/dioxin cohort at school age. Associations between perinatal exposure to PCBs and dioxins and several neurodevelopmental outcomes, assessed at 6/7 and 9 years of age, are evaluated in these studies. This study was also carried out in cooperation with the German and Danish cohorts

and was financed by the European Commission. Moreover, the supplemental studies that were performed in the Rotterdam cohort were sponsored by the American Environmental Protection Agency (EPA).

In Chapter 1, a general introduction is presented on PCBs and dioxins as well as a discussion of the results of several cohort studies that address perinatal exposure to PCBs and dioxins, including the Dutch PCB/dioxin study. The studies in this thesis are structured in two parts. Part I (Chapter 2, 3, and 4) describes relations between perinatal exposure to environmental levels of PCBs and dioxins and cognitive and motor abilities at school age. Moreover, relations between perinatal exposure and the development of these abilities from 3 to 84 months of age are described. Additionally, in this part of the thesis, modification of neurodevelopmental effects of perinatal exposure by parental and home environmental conditions is addressed. In Part II (Chapter 5, 6, and 7) mechanisms of neurotoxic action of perinatal exposure to PCBs and dioxins are explored by means of studying relations between perinatal exposure to PCBs and dioxins and behavioral, neuropsychological, and neurophysiological endpoints. Chapter 8 summarizes the results of the studies in this thesis. In addition, neurotoxic mechanisms of neurodevelopmental effects of perinatal exposure to PCBs and dioxins are discussed as well as whether breast-feeding is still preferred over formula-feeding given the PCB and dioxin contamination. Moreover, the magnitude of the effects is discussed and future perspectives are presented as well as the overall conclusion.

References

1. Patandin S, Weisglas-Kuperus N, de Ridder MA, Koopman-Esseboom C, van Staveren WA, van der Paauw CG, et al. Plasma polychlorinated biphenyl levels in Dutch preschool children either breast-fed or formula-fed during infancy. Am J Public Health 1997;87(10):1711-4.
2. World Health Organisation. Levels of PCBs, PCDDs, and PCDFs in breast milk: results of the WHO-coordinated interlaboratory quality control studies and analytical field studies. Environmental Health Series 1989;34.
3. Chen YC, Guo YL, Hsu CC, Rogan WJ. Cognitive development of Yu-Cheng ("oil disease") children prenatally exposed to heat-degraded PCBs. Jama 1992;268(22):3213-8.
4. Harada M. Intrauterine poisoning: Clinical and epidemiological studies and significance of the problem. Bull Inst Const Med Kumamoto Univ. 1976;25:1-69.
5. Koopman-Esseboom C. Effects of perinatal exposure to PCBs and dioxins on early human development. Rotterdam, The Netherlands: Erasmus University Rotterdam; 1995.
6. Huisman M. Effects of early infant nutrition and perinatal exposure to PCBs and dioxins on neurological development. Groningen, The Netherlands: University of Groningen; 1996.
7. Patandin S. Effect of environmental exposure to polychlorinated biphenyls and dioxins on growth and development in young children. Rotterdam, The Netherlands: Erasmus University Rotterdam; 1999.
8. Lanting CI. Effects of perinatal PCB and dioxin exposure and early feeding mode on child development. Groningen, The Netherlands: University of Groningen; 1998.

1 General Introduction

1.1 PCBs and dioxins

PCBs and polychlorinated dibenzo-*para*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (the latter two are summarized as dioxins) are polyhalogenated aromatic hydrocarbons with comparable molecular structures. They consist of a biphenyl ring and, depending on the number and position of chlorine atoms on the two rings, there are 209 theoretically possible discrete PCB compounds, called congeners, and 210 different dioxin congeners (75 PCDDs and 135 PCDFs).
Their basic structure is presented in Figure 1.

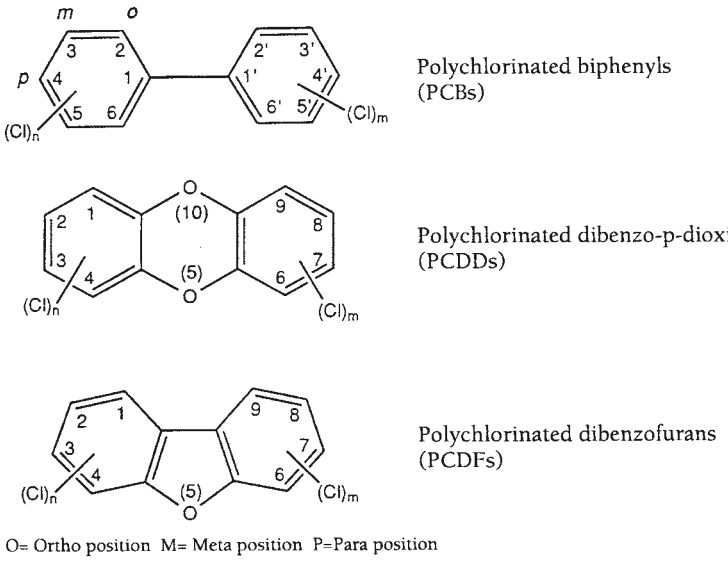

Figure 1.1 Molecular structures of PCBs, PCDDs and PCDFs

PCBs were commercially produced as complex mixtures (under trade names such as Aroclor, Clophen, Phenoclor) for a variety of applications such as dielectric fluids for capacitors and transformers, heat transfer fluids, hydraulic fluids, lubricating and cutting oils, and as additives in pesticides, paints, adhesives sealants, carbonless copy paper, flame retardants, organic dilutents, and plastics. Their commercial utility was based largely on their chemical and physical stability, including low flammability and their miscibility with organic compounds. The total amount of PCBs produced worldwide from 1929 to the 1980s, when most countries reduced or stopped the production, has been estimated at

approximately 1.5 million metric tons (1, 2). In 1982, it was estimated that 31 % had been released to the environment and 65 % was still in use or in storage, or deposited in landfills (3). Moreover, PCBs can be formed unintentionally as byproducts in a variety of chemical processes that contain chlorine and hydrocarbon sources.

Dioxins are generally formed as unwanted and often unavoidable byproducts during the synthesis of a wide array of commercial chemical products, especially those based on chlorinated aromatics, precursors, and intermediates. Moreover, they are formed during various combustion processes, such as burning of solid waste from municipal incinerators.

1.1.1 Molecular structure and mechanisms of (neuro)toxicity

The first mechanism that was described for toxic effects of PCBs and dioxins was, after entering cells, their interaction with a cytoplasmic receptor protein, the aryl-hydrocarbon (Ah) receptor (4). Depending on the positions of the chlorine atoms on the biphenyl ring structure (*ortho*, *meta*, or *para* position), and consequently the planar shape, the different compounds bind to a certain extent to this receptor. Dioxins as well as dioxin-like PCBs (coplanar PCBs; having no chlorine atom on the *ortho* position) are recognized as potent compounds to interact with the Ah receptor (5). Furthermore, two other groups of PCBs can be distinguished based on the ability to interact with the Ah-receptor, *mono-ortho*-substituted PCBs (weak dioxin-like) and *ortho*-substituted PCBs (nondioxin-like PCBs). *Mono-ortho*-substituted congeners have one chlorine atom on the ortho position and are intermediate in their ability to interact with Ah receptors. *Ortho*-substituted have more than one *ortho*-substitution on the biphenyl ring, which reduces the planarity of the molecule and reduces the ability to interact with the Ah-receptor (6).

Both non-*ortho*-substituted (coplanar compounds) and *ortho*-substituted PCBs are toxic. Their mechanism of toxicity however is likely to be different. As described previously, toxicity of coplanar compounds appears to be mediated by the Ah receptor (5). The toxic potency of a coplanar PCB congener is reflected in a toxic equivalent factor (TEF), based on its ability to bind the Ah receptor relative to the binding ability of the most potent dioxin, TCDD (7, 8). For noncoplanar PCBs the ability of the TEF to predict their neurotoxic potency is low (9, 10). In the last decade there is growing evidence that especially nondioxin-like PCBs and weak dioxin-like PCBs and their metabolites such as hydroxilated PCBs may produce a wide spectrum of neurotoxic effects, while dioxin-like PCBs may have less activity in the central nervous system (CNS) (10-12).

Neurochemical studies have shown that many elements of the CNS, and especially of the developing CNS, are susceptible to exposure to PCBs and dioxins, including cellular and synaptic processes, and endocrine systems (13-16). These aspects will be further discussed below.

At the cellular level, PCBs induced alteration in markers for neuronal and glial cell development have been reported in several brain areas in rats that were perinatally exposed to a PCB mixture (17). The levels of these markers for structural and functional brain development were altered in a complex manner, depending on age, sex, or brain region of the animal. The changes were suggestive of neuronal damage or death and were reported in several areas of the brain including the lateral olfactory tract, striatum, prefrontal cortex, and in the cerebellum and brain stem (17). Perturbations were also reported on intracellular calcium homeostatic mechanisms and second messenger systems that play a role in neuronal growth and normal physiology of cells (18-21).

Effects of exposure to PCBs on synaptic processes included an inhibition of the synaptic transmission assessed in the dendate gyrus of the cerebral cortex in adult rats (22) as well as in the hippocampus (23) and in the visual cortex in prenatally exposed rats (24). Synaptic transmission can be measured by means of long term potentiation, which is a model of synaptic plasticity that is suggested to be related to learning and memory at the synaptic level (25). Several brain neurotransmitter systems have been shown to be affected by exposure to PCBs and dioxin including dopamine, serotonine, glutamate, GABA, and cholinergic systems (15, 26-29). Effects of perinatal exposure on dopaminergic systems have been documented most thoroughly. It appeared that in rats, developmental exposure to PCBs can result in opposite alterations in brain dopamine concentrations depending on the type of the congener. For example, perinatal exposure to *ortho*-substituted PCBs led to decreases in brain dopamine whereas perinatal exposure to a coplanar PCB congener resulted in elevated concentrations of dopamine (13, 28).

PCBs and dioxins, and especially one type of PCB metabolites, the hydroxylated PCB metabolites, are presently known as endocrine disrupters (3). Multiple PCB congeners may impact upon multiple endocrine systems that may communicate with each other and are involved in fetal CNS development. Much of these complex mechanisms of actions have not been studied, and their role in developmental neurotoxic PCB and dioxin effects remains largely unknown. Most information is available on thyroid hormone changes, generally including decreases in plasma thyroid hormone levels in fetal and neonatal rats as well as in plasma of the women of the Dutch cohort and their children, two weeks after birth (13, 30, 31). Moreover, interactions with the steroid hormone system are suggested, due to PCB and dioxin induced changes in steroid hormone homeostasis or to endocrine-like actions of these contaminants, particularly during development (32). Estrogenic (12, 33, 34), anti-estrogenic (35-38) and anti-androgenic (39) effects have been described in *in vivo* and *in vitro* studies, possibly depending on congener type and/or metabolite.

1.2 Human exposure to PCBs and dioxins

Human exposure to PCBs and dioxins occurs for 90 % through the diet, with food of animal origin being the predominant source (i.e. background exposure) (40). Contamination of food is primarily caused by deposition of emissions of various sources on farmland and water (e.g waste incineration, production of chemicals) followed by bioaccumulation in the food chains in which they are particularly related with fat. Other sources may include contaminated feed for cattle, chicken and farmed fish, improper application of sewage sludge, flooding of pastures, and waste effluents (40).

Since PCBs and dioxins are lipid-soluble and are only slowly degraded, with half-life times in humans ranging from 1.8 years to 9.9 years (41, 42), these compounds accumulate in adipose tissue. During pregnancy, PCBs and dioxins are transferred through the placenta and are able to cross the blood-brain barrier, exposing the fetus during a vulnerable time of CNS development (43). PCBs have been detected in brain tissue of still born babies, exposed to environmental levels of PCBs, from 17 weeks of gestational age onwards (44). A breast-fed infant is additionally exposed to relative large amounts of PCBs and dioxins, since these compounds are excreted in breast milk. For example, PCB levels were still approximately four times higher in 42 month old children that were breast-fed during infancy than in their formula-fed counterparts that were predominantly prenatally exposed to PCBs and dioxins (45).

Since these neurotoxic compounds are able to interact with many processes of the CNS, including neurotransmitters and hormones that mediate brain development, the developing CNS is considered to be especially vulnerable to exposure to these neurotoxic compounds. Hence prenatally, the CNS may be most vulnerable to harmful effects of exposure to these compounds. Prenatal exposure can be regarded as chronic exposure of the developing brain. So far, neurochemical studies do not provide evidence of specific brain areas to be especially vulnerable. Postnatally, the CNS continues to develop rapidly doubling in weight in the first year of life, reaching 90% of its adult size by 5 years of age. Much of this increase is due to an increase in neuronal maturation, production of glial cells, outgrow of dendrites and axons, formation of synapses and myelination of axons (46). Moreover, extensive cell death and synapse elimination takes place postnatally. These postnatal maturation processes may be especially vulnerable to adverse effects of lactational exposure to PCBs and dioxins. The maturation rates vary for different brain structures. Therefore, lactational exposure to PCBs and dioxins can be hypothesized to cause structure related functional differences depending on the time window of exposure. For example, during the first two years of life in humans, functional cortical activity increases earliest in the sensorimotor and occipital cortices, before 3 to 6 months, the auditory and visual association cortices from 4 to 7 months, and latest in the frontal cortex, after 6 to 12 months (47, 48). Moreover, timing

of maximum brain growth, maximum synaptic density, dendritic arborizations, myelination, all occur first in primary motor and sensory areas, and later in the frontal cortex (49-53).

1.3 Perinatal exposure to PCBs and dioxins and neurodevelopmental outcome

1.3.1 Accidental exposure

Two accidents ('Yusho', Japan, 1968 and 'Yu Cheng', Taiwan, 1979) clearly showed the neurotoxic potential of prenatal exposure to these compounds. Large populations were accidentally exposed for relatively short periods to rice oil that was contaminated during the manufacturing process with heat transfer fluids containing PCBs, PCDFs, and polychlorinated quarterphenyls (PCQs). Children born to exposed 'Yusho' mothers were described as dull and inactive at 6 years of age and had IQs averaging 70 (54). Cognitive functions were more thoroughly addressed in the Yu Cheng cohort (n=118), showing consistent cognitive delays of 5 points from 4 to 7 years of age compared to a matched control group (55, 56). In children born up to 6 years after the incident, cognitive abilities were comparably affected (55, 56). Moreover, in 7 to 12-year-old Yu Cheng children, latencies and amplitudes of the P300 peak of an auditory event related potential, reflecting CNS mechanisms that evaluate and process relevant stimuli, were respectively longer and decreased in the exposed offspring compared to their matched controls (57). The measured P300 latencies in that study were inversely correlated with IQs. In the Yu Cheng cohort at 6, 7, 8, and 9 years of age, more spatially related cognitive abilities were differently affected in boys and girls. Only the exposed boys scored lower than their nonexposed matched controls (58). These results, therefore, may have provided the first evidence of sex steroid hormone modulating effects of PCBs and dioxins on cognitive development in humans.

1.3.2 Environmental exposure

The neurodevelopmental effects described in the Yusho and Yu Cheng cohorts leave little doubt that high levels of prenatal exposure to mixtures of PCBs and dioxins result in neurotoxic effects of these compounds in humans. Subtle neurodevelopmental effects of perinatal exposure to PCBs and dioxins have also been described in several cohorts of children that were perinatally exposed to environmental levels of PCBs and dioxins (59-64). In these cohort studies, neurological, cognitive and psychomotor aspects have been studied prospectively. The largest PCB cohorts include two cohorts that were selected based on maternal consumption of PCB-contaminated fish from the North American Great Lakes: the Lake Michigan cohort (n=313) that was recruited between 1980 and 1981 (65, 66) and the more recently (1991-1994) recruited Oswego cohort (n=293) (67). Another large cohort study has been executed in North Carolina, consisting of 912 mother-infant pairs that were

recruited from a general population between 1978 and 1982 (68). In Europe, the main cohort studies include cohorts in Denmark, The Netherlands, and Germany. The two Danish cohorts were recruited in the Faroer Islands, the first cohort consists of 435 children born between 1986 and 1987 (69), the second cohort was recruited from 1994 to 1995 (n=192), as part of a multi-center cohort study in which the Dutch PCB/dioxin study and a German study participated as well. The Danish cohorts are different from other Northern European cohorts, mainly due to local dietary habits that include consumption of pilot whale blubber and whale meat. In these children, PCB levels were higher compared to levels in Northern Europe, whereas dioxin levels were comparable (70). The Dutch cohort (n=418) (71) and German cohort (n=171) (72), respectively recruited between 1990-1992 and 1994-1995 both consist of mother-infant pairs that were drawn from the general population. The cohorts had similar inclusion criteria and used similar neurodevelopmental tests. In the Dutch cohort, however, restrictions were applied on the number of included breast-fed children to study lactational exposure to PCBs and dioxins more thoroughly. Half of the recruited population has been breast-fed for at least six weeks during infancy and the other half was fed with formula milk in which PCBs and dioxins were not detectable. The formula-fed children represent children that were exposed mainly prenatally to PCBs and dioxins. The study design, inclusion and exclusion criteria and PCB and dioxin measurements applied in the Dutch PCB/dioxin study are presented in more detail in paragraphs 1.5.2 and 1.5.3.

Neonatal neurological effects of prenatal exposure to PCBs include deficits such as poorer autonomic regulation and more abnormal reflexes (66, 67), hypotonia (73, 74) and hyporeflexia (73). At 18 months of age, prenatal exposure to PCBs was negatively associated with the neurological condition in the Dutch PCB/dioxin cohort (75), however this adverse effect was not seen on the neurological condition in these children at 42 months of age (76).

Assessment of standardized developmental tests, measuring general cognitive and psychomotor abilities, showed negative effects of prenatal exposure to PCBs on psychomotor abilities until 2 years of age in the North Carolina (63, 77) and in the Dutch cohort at 3 months of age (61). Cognitive effects of prenatal exposure to PCBs were seen at 7 months of age (72) and more pronounced negative effects were seen on more matured general cognitive abilities measured at 42 months (59, 60) and at 11 years of age (62). In the North Carolina study, however, prenatal exposure to PCBs was not related to cognitive and psychomotor abilities at 3, 4, and 5 years of age (78).

Negative effects of prenatal exposure to PCBs have also been described on more specific cognitive domains such as, processing time, attention, and memory skills (both verbal and numerical auditory memory) in children at 4 years of age (79, 80). Moreover, negative relations between prenatal PCB exposure and verbal comprehension skills at 42 months of age (60) and 11 years of age (62) have been described, in addition to verbal IQs and concentration skills (62).

Table 1.1 Significant associations between perinatal exposure to PCBs and dioxins and neurodevelopmental outcomes that were assessed in the Dutch cohort at 2 weeks, 3, 7, 18 and 42 months of age.

Outcome variable	Age	Cohort[a]	Exposure variable	Effect description
Neurological condition	2 wk	R&G	$\Sigma PCB_{breast\ milk}$, TTEQ	PCB and dioxin breast milk levels were associated with lower NOS[b] and a higher incidence of hypotonia (74)
Psychomotor development PDI, Bayley Scales of Infant Abilities	3 m	R	$\Sigma PCB_{maternal}$	Prenatal exposure was negatively associated with PDI scores (61)
Psychomotor development PDI, Bayley Scales of Infant Abilities	7 m	R	Dioxin TEQ	The highest exposed BF children (33%) scored lower than the less exposed BF children and comparable to FF children (61)
Neurological condition	18 m	R&G	$\Sigma PCB_{maternal/cord}$	Prenatal exposure was negatively associated with NOS[b] (75)
General cognitive abilities K-ABC[c]: Cognitive, Sequential & Simultaneous processing scales	42 m	R&G	$\Sigma PCB_{maternal/cord}$	Prenatal exposure was negatively associated with scores on the three scales. Effects were more pronounced in the FF group, lacking significance in the BF group (60)
Verbal comprehension Reynell Developmental Language Scales	42 m	R	$\Sigma PCB_{maternal/cord}$	Prenatal exposure was negatively associated with verbal comprehension skills in the FF group, not in the BF group (60)
Attentional processes Free play observation	42 m	R	$\Sigma PCB_{maternal/cord}$	Prenatal exposure was negatively associated with the number of episodes of high level play, suggestive of less attentional abilities (122)
Reaction time and sustained attention Computerized vigilance task	42 m	R	ΣPCB_{cord} $\Sigma PCB_{42\ months}$	Prenatal exposure was associated with more errors in the beginning of the task, suggestive of less focussed attention ΣPCB levels at 42 months were associated with longer reaction times (RT) and increasing RTs in course of time, suggestive of less sustained attention (122)
Problem Behavior Teacher CBCL[d]	42 m	R	$\Sigma PCB_{maternal/cord}$ $\Sigma PCB_{breast\ milk}$, TTEQ	Prenatal PCB and dioxin exposure was associated with a higher prevalence of Withdrawn/Depressed behavior (122)
Behavior GBO[e]	42 m	R	$\Sigma PCB_{42\ months}$	ΣPCB levels at 42 months were associated with a higher score on the GBO questionnaire, indicating more hyperactive behavior (122)

[a] R=Rotterdam, G=Groningen cohort; [b] NOS=Neurological optimality score; [c] K-ABC=Dutch Kaufman Assessment Battery for Children; [d] CBCL=Child Behavior Checklist; [e] GBO=Groninger Behavior Observation Scale. ΣPCBs=sum of PCBs, IUPAC nos. 118, 138, 153, and 180 in maternal plasma measured during pregnancy, cord plasma, breast milk, plasma of 42-months-old children. $\Sigma PCB_{42months}$ represents mainly lactational transfer of maternal PCBs (the BF group) and in part during gestation (the FF group) (45). TEQ: Toxic Equivalent; TTEQ: total TEQ, sum of the TEQs of 6 dioxin-like PCBs (IUPAC nos. 77, 105, 118, 126, 156, and 169) and 17 dioxin congeners measured in breast milk. Shaded cells are results that give evidence of negative effects of lactational exposure.

Effects of lactational or postnatal exposure to PCBs and dioxins have been detected in a few studies. In the Dutch cohort, psychomotor abilities at 7 months of age were decreased in children that were breast-fed with relatively high concentrations of PCBs and dioxins (61). At 42 months of age, in the German cohort, negative effects of postnatal exposure have been described on general cognitive abilities (59).

The results of the neurodevelopmental studies from birth to 42 months of age in the Dutch PCB/dioxin cohort are summarized in Table I.

1.3.3 Behavioral animal studies

The potential of subtle neurodevelopmental effects of perinatal exposure to environmental levels of PCBs and dioxins seen in human studies is supported by the results of behavioral animal studies. Perinatal exposure to PCBs and dioxins has been related to several motor deficits, including impaired development of the righting reflex in rats and in mice with impaired ability to remain on a rotating rod (81, 82). Moreover, in mice, perinatal exposure to a dioxin-like PCB congener was related with 'spinning' behavior, diminished grip strength, and ability to traverse a wire rod (83).

Perinatal exposure to a PCB mixture resulted in impairment on several tasks that involve acquisition or recollection of spatial information, including impaired performance on spatial (based on the location of an object) discrimination reversal tasks (84-86) and decreased accuracy on a spatial delayed alteration task in monkeys (85, 87). In both tasks, memory and attentional processes are involved. Since the accuracy deficit did not worsen with increasing delay, the effect was interpreted not as a memory impairment but rather as failure of attentional processes (85). Monkeys that were perinatally exposed to a mixture of PCBs also performed differently on a fixed interval scale (88). In this task, a range of functions is assessed including inhibitory processes, maximal response rates and temporal organization of behavior (89). The exposed monkeys showed disruptions in the temporal pattern of responding and slight elevations in their response rate (88).

It has been suggested that in some of these behavioral deficits processes related to the prefrontal cortex are involved in the mechanism of neurotoxic action of PCBs, potentially including mesocortical dopaminergic projections that terminate in the prefrontal cortex (84, 85). The deficit patterns on the discrimination reversal learning task (84, 85) and on the delayed spatial alteration showed similarities with deficits of monkeys with lesions to the dorsolateral area of the prefrontal cortex (90). However, the current knowledge on brain structure related effects of perinatal exposure to PCBs is too limited to support the hypothesis of prefrontal cortex involvement in the mechanism of effect.

1.4. Topics of interest to human neurodevelopmental PCB and dioxin risk assessment studies

1.4.1 Prenatal versus postnatal exposure to PCBs and dioxins

First, it is important to know whether it is still safe to breast-feed a child considering the contamination by PCBs and dioxins. A second object of interest is to differentiate the potential effects of prenatal and lactational exposure to PCBs and dioxins. Due to the long half-life times of PCBs and dioxins in humans, maternal levels of these compounds and consequently fetal exposure levels are difficult to reduce. The amount of lactational exposure, however, could be controlled by breast-feeding a child for a limited period or giving formula milk that contains no PCBs and dioxins. It is therefore important to distinguish the extent and effect of prenatal and postnatal exposure to PCBs and dioxins and not only consider the negative (e.g. the contamination), but also the positive effects of breast-feeding.

Although much larger quantities of PCBs and dioxins are transferred to the child postnatally through lactation than prenatally, human epidemiological studies suggest more pronounced neurodevelopmental effects of prenatal exposure to PCBs and dioxins compared to postnatal exposure to these compounds. However, several animal studies have shown profound behavioral impairments induced by postnatal exposure to low levels of a mixture of ortho-substituted PCB mixtures that is representative of the PCB mixture found in human milk. In these monkeys impaired performance was seen on spatial learning tasks, including impairment in learning a delayed spatial alteration task (91, 92), and more perseverative responding (91, 93). Moreover, slower acquisition of a fixed interval task and an inability to inhibit inappropriate responding have been associated with postnatal exposure to PCB mixtures (91, 94). These impairments suggest a discrimination learning deficit and difficulty in adaptively changing response patterns; deficits that are suggestive of involvement of prefrontal cortex processes in the neurotoxic mechanism of PCBs and dioxins (91). Effects of lactational exposure on these functions need to be addressed more thoroughly in the human studies.

In human studies, addressing neurodevelopmental effects of lactational exposure to PCBs and dioxins is complex. Breast milk contains several substances, such as several long-chain polyunsaturated fatty acids, that are not available in formula milk. These acids are important constituents of the structural lipids of nonmyelinated cell membranes in the developing nervous system and essential for growth, function and integrity (95), and may therefore be important for optimal brain development. A meta-analysis of studies that addressed neurodevelopmental benefits of breast-feeding provided evidence for enhanced early cognitive development that sustained through childhood and adolescence (96), taking in account a number of studies that suggested that differences in cognitive development were attributable to the generally associated differences in social economic conditions. The

latter aspect forms another complicating feature of assessing neurodevelopmental effects of lactational exposure: in Western societies, parents who choose to breast-feed their child are likely to be different in several parental and home environmental conditions. These aspects may influence the susceptibility to harmful effects of perinatal exposure to PCBs and dioxins. The results of prenatal exposure to PCBs and dioxins on general cognitive abilities at 42 months of age in the Dutch cohort may illustrate the complexity of exploring effects of exposure to PCBs and dioxins in breast-fed children. At 42 months of age, negative effects of prenatal exposure to these compounds were more pronounced in the formula-fed group compared to the breast-fed group of children (60).

1.4.2 Sex steroid related behavioral PCB and dioxins effects

Neurotoxic effects of perinatal exposure to PCBs and dioxins that cause developmental deficits may be mediated by endocrine-disrupting properties of PCBs and dioxins. For example, steroid hormones play a mediating role in CNS development and influence not only reproductive but also nonreproductive behaviors that show sex differences (97, 98). In animals, some effects of perinatal exposure to PCBs and dioxins on nonreproductive behaviors have been reported. For example, a feminizing effect on sweet preference was found in male rats that were perinatally exposed to a PCB mixture representative to PCBs found in human milk. In their female counterparts, sweet preference was not affected (39). In contrast, prenatal exposure to a dioxin (TCDD) and coplanar (dioxin-like) PCBs decreased sweet preference in female rats, which can be interpreted as a masculinizing effect in females. In the exposed males no change in sweet preference was seen (38). The animal studies suggest both feminizing and masculinizing effects of perinatal exposure to PCBs and dioxins on sex-specific behavior, which may suggest steroid hormone mediated effects of PCB and dioxin exposure.

In human studies, effects of perinatal exposure to PCBs and dioxins on nonreproductive sex-specific behavior have hardly been addressed. The only study that provided some evidence for steroid hormone mediated behavioral effects of prenatal exposure to PCBs and dioxins is the study in the highly exposed children of the Yu Cheng cohort. In this cohort, more spatially related cognitive abilities, which generally show some sex differences, were differently affected in boys and girls. Only the exposed boys scored lower than their nonexposed matched controls (58).

1.4.3 Neurodevelopmental interstudy differences

The results of epidemiological studies that address neurodevelopmental effects of perinatal exposure to environmental levels of PCBs show inconsistencies both between cohorts as well as within cohorts at different ages. These differences in outcome do not necessarily

undermine conclusions that prenatal exposure to environmental levels of PCBs is related to subtle harmful effects on child neurodevelopment. The differences could be related to a number of factors including differences in exposure assessment techniques, differences in composure of environmental PCB mixtures, and differences in exposure levels. Moreover, differences in parental and home environmental conditions or the occurrence of other neurotoxic agents, which may confound relationships between exposure and neurodevelopmental outcome, may have lead to differences in results. Additionally, different neurodevelopmental outcome variables have been used in the cohort studies. Furthermore, addressing neurodevelopmental effects of perinatal exposure to PCBs and dioxins as well as comparison of effects assessed by different cohorts at different ages is complicated by the fact that the outcome variables are developmental qualities. Effects of perinatal exposure to PCBs and dioxins may not become evident until further maturation of the child. Some of these issues will be discussed below.

1.4.3.a Exposure levels

Exposure levels in the cohorts are difficult to compare since in the earlier American studies different assessment techniques have been used compared to the later initiated studies. In a recent effort to compare exposure levels of several cohorts, median levels of PCB153 in maternal blood were used for comparison (99). This congener is always among the PCB congeners present at the highest concentration and constitutes a large proportion of the PCBs mixtures in all studies. That study showed that the median Dutch PCB153 level (0.10 µg/g lipid) was comparable to the median level in the Lake Michigan cohort (0.12 µg/g lipid), the North Carolina cohort (0.08 µg/g lipid) and the German median levels (0.14 µg/g lipid). The median exposure level in the Faroer Islands cohort (recruited between 1994 and 1995) was 3 to 4 times higher than in these studies (0.45 µg/g lipid).

1.4.3.b Confounding variables and potential differences in susceptibility to effects of PCB and dioxin exposure

It is a common feature of the epidemiological studies that subjects could not be randomly assigned to predetermined levels of exposure or type of feeding during infancy. Samples were based on volunteer mother-infant pairs and parents were free in choosing the type of infant feeding they preferred because of acceptable ethical concern. Therefore, all cohort studies have made efforts to assess potential confounding variables, to adjust for these variables when studying the relation between perinatal exposure to PCBs and dioxins and neurodevelopment.

In Western societies, the relation between perinatal exposure to PCBs and dioxins and neurodevelopment is often confounded by parental and home environmental conditions. Due to the physical stability and accumulation of PCBs and dioxins in human tissues, PCB

and dioxin body burdens are strongly related to maternal age at birth. Women at older age that give birth to a child are often higher educated and have higher IQs than women at younger age that give birth to a child. Maternal age may also reflect other aspects of social economic conditions as well as psychosocial age-related attributes (100).

Child development is a process in which structural changes and environmental experiences influence each other mutually. For example, many cognitive skills, including IQs, verbal and spatial abilities, perceptual speed (101-103), have been shown to be under genetic influences. Environmental or psychosocial aspects, such as intellectual stimulation, organization of the home environment, verbal responsivity of the parents, variability of daily experience, and parental involvement also influence cognitive development (104). In animal studies, environmental aspects influenced cortical differentiation and dendritic formation, thereby changing the functional connectivity of the nervous system. Several lines of evidence point towards the relationship between dendritic and synaptic changes and experiences and more specifically learning (105-108). Moreover, numerous animal studies showed that environmental enrichment can compensate for and possibly even reverse some of the adverse effects of developmental insults (109-111). These studies suggest potential for structural and functional recovery throughout the cortical maturation period in animals. In humans, evidence of neural plasticity throughout the maturation period may be supported by results of studies in low birth weight children. These studies reported that in children at high biological risk, favorable early parental and home characteristics could compensate for or mask developmental delays (112-114). Hence, these genetic and environmental conditions are important predictors of cognitive development and can additionally be important in determining the vulnerability of an individual child or a given population to the effects of neurotoxicants. It can be hypothesized that favorable parental and home environmental conditions may protect some groups against negative neurodevelopmental effects of perinatal PCB and dioxin exposure. Evidence for this hypothesis is seen in the Dutch study showing less pronounced effects of prenatal exposure to PCBs in breast-fed children compared to formula-fed children at 42 months of age (60). A reanalysis in the Lake Michigan cohort similarly showed that prenatal exposure to PCBs was related with lower IQs at 11 years of age in predominantly the group of formula-fed children (n=56, 31%) (115). In the North Carolina cohort, prenatal exposure to PCBs was not related to later cognitive and motor development from 3 to 5 years of age, in contrast to the Lake Michigan and Dutch study. In the North Carolina cohort, a relatively high proportion of the population was breast-fed during infancy (88%). Moreover, the average years of college education in the North Carolina cohort was 3 years and in the Lake Michigan cohort 1 year. In the Dutch cohort, 40% of the mothers have finished high school and 30% of them finished professional and university training. Some of the differences in neurodevelopmental effects of PCB exposure between study centers can therefore be

hypothesized to be related to cohort differences in the levels of these conditions that are important to child development.

1.4.3.c Confounding by other neurotoxic compounds

Due to the correlational feature of the epidemiological studies addressing effects of perinatal exposure to PCBs and dioxins, relations between these compounds and outcome are potentially related to exposure to other neurotoxic compounds, such as methyl mercury and lead. For example, in the Faroer study, in which the local diet consists predominantly of fish and fish products, PCB and dioxin levels were seen to be relatively high compared to other European studies as were the levels of methyl mercury. Significant relations between prenatal exposure to PCBs and reaction time and (semantic) memory skills appeared to be mainly attributable to prenatal exposure to methyl mercury compounds (116). However, in children exposed to high levels of methyl mercury, effects of prenatal exposure to PCBs on these outcome variables were more pronounced than in children exposed to lower levels of methyl mercury, suggesting a potential interaction between these neurotoxic compounds in their neurodevelopmental effects. In the Lake Michigan cohort, mothers were selected based on their diet history on Lake Michigan PCB-contaminated fish. Fish and other aquatic species form often the source of exposure to PCBs as well as other neurotoxic compounds, such as methyl-mercury. The relations between neurodevelopment and prenatal PCB exposure as described in the Lake Michigan studies, therefore, may have been confounded by exposure to this compound. However, based on the congruence between the results of animal studies and several human cohort studies it has been suggested that the deficits observed in the Lake Michigan studies result at least in part from PCB exposure (117). In contrast to the Lake Michigan study, the North Carolina, Dutch and German cohort were recruited from the general public which may reduce the risk of confounding by methyl-mercury. In the Netherlands, PCB and dioxin exposure occurs mainly through dietary intake of predominantly dairy products, as well as processed food and meat and fish products (118). In the Dutch PCB/dioxin population, lead and cadmium levels in blood samples drawn from 18 months old children (n=151) were relatively low (119) and not related to cognitive outcome at 42 months of age (60).

1.4.3.d Neurodevelopmental tests and the development of cognitive abilities

The cohort studies also show differences in the neurodevelopmental testing protocols that were used to explore neurotoxic effects of perinatal exposure to PCBs and dioxins. Neurodevelopmental assessment has occurred at different ages using different test materials, which complicates comparison of the different cohorts, especially due to the

developmental nature of cognitive and motor abilities and since some affected functions may not become apparent at a more mature age.

Most prospective longitudinal studies have assessed general cognitive and motor abilities by means of developmental tests that were reassessed repeatedly through childhood. Performance on the developmental tests reflects, especially at a more mature age, a broad range of domains of function, including memory, visuo-spatial abilities, verbal and quantitative reasoning, and attentional aspects. Although general cognitive development seems to be the most relevant outcome variable in risk assessment studies, because of its predictive feature for later outcome, general cognitive ability indices may be too general to assess subtle effects of exposure to neurotoxic compounds. The general cognitive score can obscure important individual differences in specific cognitive profiles, since children with different cognitive profiles can have comparable scores on this outcome variable. Moreover, it can be reasoned that general cognitive scores reflect the product of learning, which is strongly related to social economic aspects, rather than processes of learning.

The development of general cognitive abilities may progress at different rates. Reported negative effects of prenatal exposure to PCBs at different assessment times within one cohort do not resolve the question whether the same children are affected in their abilities at the different assessment times. Consequently, risk assessment studies into effects of perinatal exposure to PCBs and dioxins on cognitive and motor abilities in children may benefit from addressing the level and course of the development of these abilities.

It can be hypothesized that early PCB and dioxin exposure induces changes in brain structures that continue to influence neurodevelopment during maturation resulting in delayed effects on functions that develop later in childhood. Especially when effects of lactational exposure to PCBs and dioxins are addressed, structure related functional differences, potentially depending on the time window of exposure, can be hypothesized due to differences in maturation rates of different brain structures. The exploration of neurotoxic effects of perinatal exposure to neurotoxic agents therefore should address more specific domains of cognitive functioning that can be assessed at a more mature age. These domains are not sufficiently measured by developmental or IQ tests, since most domains are indicated by too few items to provide reliable measurement of domain specific performance.

1.5 Content of this thesis

This thesis describes the results of follow-up assessment in the Dutch PCB/dioxin cohort at school age. Children enrolled in the Dutch PCB/dioxin cohort (Rotterdam and Groningen cohort) were invited to participate in follow-up assessment at 6/7 years of age and half of the Rotterdam cohort was invited at 9 years of age as well. The assessment included

(general) cognitive and motor abilities, gender role play behavior, neuropsychological functions and a neurophysiological assessment.

1.5.1 Aims of the study

The general aim of this thesis was to evaluate neurodevelopmental effects of perinatal exposure to environmental levels of PCBs and dioxins in normal Dutch children at school age, as well as to explore effects on the development of general cognitive and motor abilities from 3 to 84 months of age. In addition, the goal was to gain more insight into potential compensating effects of parental and home environmental conditions and breast-feeding, as well as into neurotoxic mechanisms of effects of perinatal exposure to these compounds.

This aim was addressed by studying the following questions:

1. Is perinatal exposure to environmental levels of PCBs and dioxins related to cognitive and motor abilities at school age and are effects of perinatal exposure to these compounds related to breast-feeding or parental and home environmental conditions (Chapter 2)?
2. Is perinatal exposure to environmental levels of PCBs related to the development of general cognitive and motor abilities from 3 to 84 months of age, and what are important determinants of these outcome variables (Chapter 3)?
3. Is the interrelationship of general cognitive and motor development and parental and home environmental conditions different for low versus high prenatally exposed children that are born to younger or older mothers (Chapter 4)?
4. Is perinatal PCB and dioxin exposure related to sex-specific play behavior at 7 years of age and are observed effects sex-specific (Chapter 5)?
5. Is perinatal PCB and dioxin exposure related to neuropsychological functions at 9 years of age (Chapter 6)?
6. Is perinatal PCB and dioxin exposure related to neurophysiological endpoints at 9 years of age (Chapter 7)?

1.5.2 Subjects and inclusion and exclusion criteria

The Dutch PCB/dioxin cohort consists of 418 healthy mother-infant pairs who were recruited from June 1990 to June 1992. Half of the study population was recruited in Rotterdam (n=207), a highly industrialized and densely populated area, and the other half in Groningen (n=211), a semiurban area in The Netherlands. Healthy pregnant women were asked by their obstetrician or midwife to participate in a prospective neurodevelopmental study. The cohort consists of Caucasian mother-infant pairs. Pregnancy and delivery had

been without complications; instrumental deliveries or caesarian sections were excluded. Only first or second at term born infants (37-42 weeks of gestation) were included who had no congenital anomalies or diseases. Because of these criteria, the cohort of children can be presumed to be at relatively low risk for neurodevelopmental deficits.

To study the effects of prenatal as well as postnatal PCB and dioxin exposure, it was aimed to include an equal number of women who intended to breast-feed their child for at least six weeks (BF) and women who intended to use formula-feeding (FF). All infants in the FF group received formula from a single batch (Almiron M2, Nutricia NV, Zoetermeer, The Netherlands) from birth until 7 months of age. In this formula, concentrations of both PCBs and dioxins were below the detection limit.

The medical ethics committee of the University Hospital Rotterdam/ Sophia Children's Hospital and the Academical Hospital Groningen approved the study design and the parents gave informed consent.

1.5.3 Exposure measurements

The exposure variables that were used in these studies included PCB levels in maternal and cord plasma. Maternal plasma samples were collected from the mothers during the last month of pregnancy and cord plasma samples were collected directly after birth. These samples were analyzed by means of gas chromatography with electron capture detection (GC-ECD) for four PCB congeners, International Union for Pure and Applied Chemistry (IUPAC) numbers 118, 138, 153 and 180 (71, 120).

Two weeks after delivery, a 24-hour representative breast milk sample was collected from the mothers who were breast-feeding their children. These samples were analyzed for 17 most abundant dioxins (PCDDs and PCDFs), and three dioxin-like PCBs (IUPAC numbers 77, 126, 169) by means of gas chromatography-high-resolution mass spectometry (GC-HRMS). In these samples, 23 nondioxin like PCBs (IUPAC numbers 28, 52, 66, 70, 99, 101, 105, 118, 128, 137, 138, 141, 151, 153, 156, 170, 177, 180, 183, 187, 194, 195, and 202) were measured by GC-ECD (71). Toxic potency of the mixture of dioxins and dioxin-like PCBs was expressed by using the toxic equivalent factor approach (121).

Prenatal exposure to PCBs is defined as the sum of the concentrations of the four PCB congeners measured in maternal plasma and in cord plasma. PCB and dioxin concentrations in breast milk were assessed shortly after birth and form an indirect measure of prenatal exposure (68). Postnatal exposure to PCBs and dioxins through lactation was estimated in the BF group by multiplying breast milk levels of PCBs, dioxin-like PCB TEQs and dioxin TEQs with the number of weeks of breast-feeding.

References

1. de Voogt P, Brinkman UAT. Production, properties and usage of polychlorinated biphenyls. In: Kimbrough RD, Jensen A, editors. Halogenated biphenyls, terphenyls, naphtalenes, dibenzodioxins and related products. 2nd ed. Amsterdam: Elsevier; 1989; 2-46.
2. WHO, Organisation. WH. Levels of PCBs, PCDDs, and PCDFs in breast milk: results of the WHO-coordinated interlaboratory quality control studies and analytical field studies. Environmental Health Series 1989;34.
3. Tanabe S. PCB poblems in he future: forsight from current knowledge. Environ. Pollut. 1988;50: 5-28.
4. Safe S, Goldstein JA. Mechanisms of action and structure-activity relationships for the chlorinated dibenzo-p-dioxins and related compounds. In: Kimbrough RD, Jensen A, editors. Halogenated biphenyls, terphenyls, naphtalenes, dibenzodioxins and related products. 2nd ed. Amsterdam: Elsevier; 1989; 239-294.
5. Safe SH. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol 1994;24(2):87-149.
6. Kafafi SA, Afeefy HY, Ali AH, Said HK, Kafafi AG. Binding of polychlorinated biphenyls to the aryl hydrocarbon receptor. Environ Health Perspect 1993;101(5):422-8.
7. Van den Berg M, Birnbaum L, Bosveld AT, Brunstrom B, Cook P, Feeley M, et al. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 1998;106(12):775-92.
8. Safe S. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit Rev Toxicol 1990;21(1):51-88.
9. Giesy JP, Kannan K. Dioxin-like and non-dioxin-like toxic effects of polychlorinated biphenyls (PCBs): implications for risk assessment. Crit Rev Toxicol 1998;28(6):511-69.
10. Shain W, Bush B, Seegal R. Neurotoxicity of polychlorinated biphenyls: structure-activity relationship of individual congeners. Toxicol Appl Pharmacol 1991;111(1):33-42.
11. Fischer LJ, Seegal RF, Ganey PE, Pessah IN, Kodavanti PR. Symposium overview: toxicity of non-coplanar PCBs. Toxicol Sci 1998;41(1):49-61.
12. Korach KS, Sarver P, Chae K, McLachlan JA, McKinney JD. Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: conformationally restricted structural probes. Mol Pharmacol 1988;33(1):120-6.
13. Brouwer A, Ahlborg UG, Van den Berg M, Birnbaum LS, Boersma ER, Bosveld B, et al. Functional aspects of developmental toxicity of polyhalogenated aromatic hydrocarbons in experimental animals and human infants. Eur J Pharmacol 1995;293(1):1-40.
14. Brouwer A, Longnecker MP, Birnbaum LS, Cogliano J, Kostyniak P, Moore J, et al. Characterization of potential endocrine-related health effects at low- dose levels of exposure to PCBs. Environ Health Perspect 1999;107 Suppl 4:639-49.
15. Mariussen E, Fonnum F. The effect of polychlorinated biphenyls on the high affinity uptake of the neurotransmitters, dopamine, serotonin, glutamate and GABA, into rat brain synaptosomes. Toxicology 2001;159(1-2):11-21.
16. Tilson HA, Kodavanti PR. The neurotoxicity of polychlorinated biphenyls. Neurotoxicology 1998;19(4-5):517-25.
17. Morse DC, Plug A, Wesseling W, van den Berg KJ, Brouwer A. Persistent alterations in regional brain glial fibrillary acidic protein and synaptophysin levels following pre- and postnatal polychlorinated biphenyl exposure. Toxicol Appl Pharmacol 1996;139(2):252-61.
18. Shafer TJ, Mundy WR, Tilson HA, Kodavanti PR. Disruption of inositol phosphate accumulation in cerebellar granule cells by polychlorinated biphenyls: a consequence of altered Ca2+ homeostasis. Toxicol Appl Pharmacol 1996;141(2):448-55.
19. Kodavanti PR, Shin DS, Tilson HA, Harry GJ. Comparative effects of two polychlorinated biphenyl congeners on calcium homeostasis in rat cerebellar granule cells. Toxicol Appl Pharmacol 1993;123(1):97-106.

20. Kodavanti PR, Shafer TJ, Ward TR, Mundy WR, Freudenrich T, Harry GJ, et al. Differential effects of polychlorinated biphenyl congeners on phosphoinositide hydrolysis and protein kinase C translocation in rat cerebellar granule cells. Brain Res 1994;662(1-2):75-82.
21. Kodavanti PR, Tilson HA. Structure-activity relationships of potentially neurotoxic PCB congeners in the rat. Neurotoxicology 1997;18(2):425-41.
22. Gilbert ES, Crowley DE. Plant compounds that induce polychlorinated biphenyl biodegradation by Arthrobacter sp. strain B1B. Appl Environ Microbiol 1997;63(5):1933-8.
23. Niemi WD, Audi J, Bush B, Carpenter DO. PCBs reduce long-term potentiation in the CA1 region of rat hippocampus. Exp Neurol 1998;151(1):26-34.
24. Altmann L, Weinand-Haerer A, Lilienthal H, Wiegand H. Maternal exposure to polychlorinated biphenyls inhibits long-term potentiation in the visual cortex of adult rats. Neurosci Lett 1995;202(1-2):53-6.
25. McNaughton BL. The mechanism of expression of long-term enhancement of hippocampal synapses: current issues and theoretical implications. Annu Rev Physiol 1993;55:375-96.
26. Mariussen E, Morch Andersen J, Fonnum F. The effect of polychlorinated biphenyls on the uptake of dopamine and other neurotransmitters into rat brain synaptic vesicles. Toxicol Appl Pharmacol 1999;161(3):274-82.
27. Eriksson P, Lundkvist U, Fredriksson A. Neonatal exposure to 3,3',4,4'-tetrachlorobiphenyl: changes in spontaneous behaviour and cholinergic muscarinic receptors in the adult mouse. Toxicology 1991;69(1):27-34.
28. Seegal RF, Brosch KO, Okoniewski RJ. Effects of in utero and lactational exposure of the laboratory rat to 2,4,2',4'- and 3,4,3',4'-tetrachlorobiphenyl on dopamine function. Toxicol Appl Pharmacol 1997;146(1):95-103.
29. Morse DC, Seegal RF, Borsch KO, Brouwer A. Long-term alterations in regional brain serotonin metabolism following maternal polychlorinated biphenyl exposure in the rat. Neurotoxicology 1996;17(3-4):631-8.
30. Brouwer A, Morse DC, Lans MC, Schuur AG, Murk AJ, Klasson-Wehler E, et al. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. Toxicol Ind Health 1998;14(1-2):59-84.
31. Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lutkeschipholt IJ, Van der Paauw CG, Tuinstra LG, et al. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatr Res 1994;36(4):468-73.
32. Golden RJ, Noller KL, Titus-Ernstoff L, Kaufman RH, Mittendorf R, Stillman R, et al. Environmental endocrine modulators and human health: an assessment of the biological evidence. Crit Rev Toxicol 1998;28(2):109-227.
33. Kester MH, Bulduk S, Tibboel D, Meinl W, Glatt H, Falany CN, et al. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. Endocrinology 2000;141(5):1897-900.
34. Bitman J, Cecil HC. Estrogenic activity of DDT analogs and polychlorinated biphenyls. J Agric Food Chem 1970;18(6):1108-12.
35. Moore M, Mustain M, Daniel K, Chen I, Safe S, Zacharewski T, et al. Antiestrogenic activity of hydroxylated polychlorinated biphenyl congeners identified in human serum. Toxicol Appl Pharmacol 1997;142(1):160-8.
36. Kramer VJ, Helferich WG, Bergman A, Klasson-Wehler E, Giesy JP. Hydroxylated polychlorinated biphenyl metabolites are anti-estrogenic in a stably transfected human breast adenocarcinoma (MCF7) cell line. Toxicol Appl Pharmacol 1997;144(2):363-76.
37. Jansen HT, Cooke PS, Porcelli J, Liu TC, Hansen LG. Estrogenic and antiestrogenic actions of PCBs in the female rat: in vitro and in vivo studies. Reprod Toxicol 1993;7(3):237-48.
38. Amin S, Moore RW, Peterson RE, Schantz SL. Gestational and lactational exposure to TCDD or coplanar PCBs alters adult expression of saccharin preference behavior in female rats. Neurotoxicol Teratol 2000;22(5):675-82.
39. Hany J, Lilienthal H, Sarasin A, Roth-Harer A, Fastabend A, Dunemann L, et al. Developmental exposure of rats to a reconstituted PCB mixture or aroclor 1254: effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. Toxicol Appl Pharmacol 1999;158(3):231-43.

40. Furst P, Beck H, Theelen R. Assessment of human intake of PCDDs and PCDFs from different environmental sources. Toxic Substances Journal 1992;12:133-150.
41. Steele G, Stehr-Green P, Welty E. Estimates of the biologic half-life of polychlorinated biphenyls in human serum. N Engl J Med 1986;314(14):926-7.
42. Taylor PR, Lawrence CE. Polychlorinated biphenyls: estimated serum half lives. Br J Ind Med 1992;49(7):527-8.
43. Masuda Y, Kagawa R, Kuroki H, Kuratsune M, Yoshimura T, Taki I, et al. Transfer of polychlorinated biphenyls from mothers to foetuses and infants. Food Cosmet Toxicol 1978;16(6):543-6.
44. Lanting CI, Huisman M, Muskiet FA, van der Paauw CG, Essed CE, Boersma ER. Polychlorinated biphenyls in adipose tissue, liver, and brain from nine stillborns of varying gestational ages. Pediatr Res 1998;44(2):222-5.
45. Patandin S, Weisglas-Kuperus N, de Ridder MA, Koopman-Esseboom C, van Staveren WA, van der Paauw CG, et al. Plasma polychlorinated biphenyl levels in Dutch preschool children either breast-fed or formula-fed during infancy. Am J Public Health 1997;87(10):1711-4.
46. Dodgson MCH. The growing brain: An essay in developmental neurology. Bristol, England: Wright; 1962.
47. Chugani HT, Phelps ME, Mazziotta JC. Positron emission tomography study of human brain functional development. Ann Neurol 1987;22(4):487-97.
48. Chiron C, Raynaud C, Maziere B, Zilbovicius M, Laflamme L, Masure MC, et al. Changes in regional cerebral blood flow during brain maturation in children and adolescents. J Nucl Med 1992;33(5):696-703.
49. Mrzljak L, Uylings HB, Van Eden CG, Judas M. Neuronal development in human prefrontal cortex in prenatal and postnatal stages. Prog Brain Res 1990;85:185-222.
50. Becker LE, Armstrong DL, Chan F, Wood MM. Dendritic development in human occipital cortical neurons. Brain Res 1984;315(1):117-24.
51. Huttenlocher PR, Dabholkar AS. Regional differences in synaptogenesis in human cerebral cortex. J Comp Neurol 1997;387(2):167-78.
52. Barkovich AJ, Kjos BO. Normal postnatal development of the corpus callosum as demonstrated by MR imaging. AJNR Am J Neuroradiol 1988;9(3):487-91.
53. Barkovich AJ, Kjos BO, Jackson DE, Jr., Norman D. Normal maturation of the neonatal and infant brain: MR imaging at 1.5 T. Radiology 1988;166(1 Pt 1):173-80.
54. Harada M. Intrauterine poisoning: Clinical and epidemiological studies and significance of the problem. Bull Inst Const Med Kumamoto Univ. 1976;25:1-69.
55. Chen YC, Guo YL, Hsu CC, Rogan WJ. Cognitive development of Yu-Cheng ("oil disease") children prenatally exposed to heat-degraded PCBs. Jama 1992;268(22):3213-8.
56. Lai TJ, Guo YL, Yu ML, Ko HC, Hsu CC. Cognitive development in Yucheng children. Chemosphere 1994;29(9-11):2405-11.
57. Chen YJ, Hsu CC. Effects of prenatal exposure to PCBs on the neurological function of children: a neuropsychological and neurophysiological study. Dev Med Child Neurol 1994;36(4):312-20.
58. Guo YL, Lai TJ, Chen SJ, Hsu CC. Gender-related decrease in Raven's progressive matrices scores in children prenatally exposed to polychlorinated biphenyls and related contaminants. Bull Environ Contam Toxicol 1995;55(1):8-13.
59. Walkowiak J, Wiener JA, Fastabend A, Heinzow B, Kramer U, Schmidt E, et al. Environmental exposure to polychlorinated biphenyls and quality of the home environment: effects on psychodevelopment in early childhood. Lancet 2001;358(9293):1602-7.
60. Patandin S, Lanting CI, Mulder PG, Boersma ER, Sauer PJ, Weisglas-Kuperus N. Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J Pediatr 1999;134(1):33-41.
61. Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MA, Van der Paauw CG, Tuinstra LG, Sauer PJ. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics 1996;97(5):700-6.
62. Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychorinated biphenyls in utero. N Engl J Med 1996;335:783-9.

63. Rogan WJ, Gladen BC. PCBs, DDE, and child development at 18 and 24 months. Ann Epidemiol 1991;1(5):407-13.
64. Darvill T, Lonky E, Reihman J, Stewart P, Pagano J. Prenatal exposure to PCBs and infant performance on the fagan test of infant intelligence. Neurotoxicology 2000;21(6):1029-38.
65. Jacobson JL, Fein GG, Jacobson SW, Schwartz PM, Dowler JK. The transfer of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) across the human placenta and into maternal milk. Am J Public Health 1984;74(4):378-9.
66. Jacobson JL, Jacobson SW, Fein G, Scwartz P, Dowler J. Prenatal exposure to environmental toxin: A test of the multiple effects model. Developmental Psychology 1984;20(4):523-532.
67. Lonky E, Reihman J, Darvill T, Mather JE, Daly H. Neonatal Behavioral Assessment Scale performance in humans influenced by maternal consumption of environmentally contaminated Lake Ontario fish. Journal of Great Lakes 1996;22(2):198-212.
68. Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, et al. Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) in human milk: effects of maternal factors and previous lactation. Am J Public Health 1986;76(2):172-7.
69. Grandjean P, Weihe P, Jorgensen PJ, Clarkson T, Cernichiari E, Videro T. Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. Arch Environ Health 1992;47(3): 185-95.
70. Grandjean P, Weihe P, Needham LL, Burse VW, Patterson DG, Jr., Sampson EJ, et al. Relation of a seafood diet to mercury, selenium, arsenic, and polychlorinated biphenyl and other organochlorine concentrations in human milk. Environ Res 1995;71(1):29-38.
71. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N, Van der Pauw CG, Tuinstra LGMT, Boersma ER, et al. PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 1994;28(9):1721-1732.
72. Winneke G, Bucholski A, Heinzow B, Kramer U, Schmidt E, Walkowiak J, et al. Developmental neurotoxicity of polychlorinated biphenyls (PCBS): cognitive and psychomotor functions in 7-month old children. Toxicol Lett 1998;102-103:423-8.
73. Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, et al. Neonatal effects of transplacental exposure to PCBs and DDE. J Pediatr 1986;109(2):335-41.
74. Huisman M, Koopman-Esseboom C, Fidler V, Hadders-Algra M, van der Paauw CG, Tuinstra LG, et al. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. Early Hum Dev 1995;41(2):111-27.
75. Huisman M, Koopman-Esseboom C, Lanting CI, van der Paauw CG, Tuinstra LG, Fidler V, et al. Neurological condition in 18-month-old children perinatally exposed to polychlorinated biphenyls and dioxins. Early Hum Dev 1995;43(2):165-76.
76. Lanting CI, Patandin S, Fidler V, Weisglas-Kuperus N, Sauer PJ, Boersma ER, et al. Neurological condition in 42-month-old children in relation to pre- and postnatal exposure to polychlorinated biphenyls and dioxins. Early Hum Dev 1998;50(3):283-92.
77. Gladen BC, Rogan WJ, Hardy P, Thullen J, Tingelstad J, Tully M. Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. J Pediatr 1988;113(6):991-5.
78. Gladen BC, Rogan WJ. Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. J Pediatr 1991;119(1):58-63.
79. Jacobson JL, Jacobson SW, Humphrey HE. Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J Pediatr 1990;116(1):38-45.
80. Jacobson JL, Jacobson SW, Padgett RT, Brumitt GA, Billings RL. Effects of Prenatal PCB Exposure on Cognitive Processing Efficiency and Sustained Attention. Developmental Psychology 1992;28(2):297-306.
81. Thiel R, Koch E, Ulbrich B, Chahoud I. Peri- and postnatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin: effects on physiological development, reflexes, locomotor activity and learning behaviour in Wistar rats. Arch Toxicol 1994;69(2):79-86.
82. Overmann SR, Kostas J, Wilson LR, Shain W, Bush B. Neurobehavioral and somatic effects of perinatal PCB exposure in rats. Environ Res 1987;44(1):56-70.

83. Tilson HA, Davis GJ, McLachlan JA, Lucier GW. The effects of polychlorinated biphenyls given prenatally on the neurobehavioral development of mice. Environ Res 1979;18(2):466-74.
84. Schantz SL, Levin ED, Bowman RE, Heironimus MP, Laughlin NK. Effects of perinatal PCB exposure on discrimination-reversal learning in monkeys. Neurotoxicol Teratol 1989;11(3):243-50.
85. Schantz SL, Levin ED, Bowman RE. Long-term neurobehavioral effects of perinatal polychlorinated biphenyl (PCB) exposure in monkeys. Environmental Toxicology and Chemistry 1991;10:747-756.
86. Bowman RE, Heironimus MP, Allen JR. Correlation of PCB body burden with behavioral toxicology in monkeys. Pharmacol Biochem Behav 1978;9(1):49-56.
87. Levin ED, Schantz SL, Bowman RE. Delayed spatial alternation deficits resulting from perinatal PCB exposure in monkeys. Arch Toxicol 1988;62(4):267-73.
88. Mele PC, Bowman RE, Levin ED. Behavioral evaluation of perinatal PCB exposure in rhesus monkeys: fixed-interval performance and reinforcement-omission. Neurobehav Toxicol Teratol 1986;8(2):131-8.
89. Rice DC. Schedule-controlled behavior in infant and juvenile monkeys exposed to lead from birth. Neurotoxicology 1988;9(1):75-87.
90. Goldman PS, Rosvold HE, Vest B, Galkin TW. Analysis of the delayed-alternation deficit produced by dorsolateral prefrontal lesions in the rhesus monkey. J Comp Physiol Psychol 1971;77(2):212-20.
91. Rice DC. Behavioral impairment produced by low-level postnatal PCB exposure in monkeys. Environ Res 1999;80: S113-S121.
92. Rice DC. Effects of postnatal exposure of monkeys to a PCB mixture on spatial discrimination reversal and DRL performance. Neurotoxicol Teratol 1998;20(4):391-400.
93. Rice DC, Hayward S. Effects of postnatal exposure to a PCB mixture in monkeys on nonspatial discrimination reversal and delayed alternation performance. Neurotoxicology 1997;18(2):479-94.
94. Rice DC. Effect of postnatal exposure to a PCB mixture in monkeys on multiple fixed interval-fixed ratio performance. Neurotoxicol Teratol 1997;19(6):429-34.
95. Innis SM. The 1993 Borden Award Lecture. Fatty acid requirements of the newborn. Can J Physiol Pharmacol 1994;72(12):1483-92.
96. Anderson JW, Johnstone BM, Remley DT. Breast-feeding and cognitive development: a meta-analysis. Am J Clin Nutr 1999;70(4):525-35.
97. Matsumoto A. Synaptogenic action of sex steroids in developing and adult neuroendocrine brain. Psychoneuroendocrinology 1991;16(1-3):25-40.
98. Fitch RH, Denenberg VH. A role for ovarian hormones in sexual differentiation of the brain. Behav Brain Sci 1998;21(3):311-27.
99. Longnecker MP, Wolff MS, Gladen BC, Brock JW, Grandjean P, Jacobson JL, et al. Comparison of polychlorinated biphenyl (PCB) levels across studies of human neurodevelopment. Environ Health Perspect in press.
100. Stein ZA. A woman's age: childbearing and child rearing. Am J Epidemiol 1985;121(3):327-42.
101. Plomin R, Loehlin JC. Direct and indirect IQ heritability estimates: a puzzle. Behav Genet 1989;19(3):331-42.
102. Alarcon M, Plomin R, Fulker DW, Corley R, DeFries JC. Multivariate path analysis of specific cognitive abilities data at 12 years of age in the Colorado Adoption Project. Behav Genet 1998;28(4):255-64.
103. Posthuma D, de Geus EJ, Boomsma DI. Perceptual speed and IQ are associated through common genetic factors. Behav Genet 2001;31(6):593-602.
104. Bradley RH, Convyn RF, Burchinal M, McAdoo HP, Coll CG. The home environments of children in the United States part II: relations with behavioral development through age thirteen. Child Dev 2001;72(6):1868-86.
105. de Voogd TJ, Nixdorf B, Nottebohm F. Synaptogenesis and changes in synaptic morphology related to acquisition of a new behavior. Brain Res 1985;329(1-2):304-8.
106. Wolff JR, Laskawi R, Spatz WB, Missler M. Structural dynamics of synapses and synaptic components. Behav Brain Res 1995;66(1-2):13-20.

107. Moser MB, Trommald M, Andersen P. An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. Proc Natl Acad Sci U S A 1994;91(26):12673-5.
108. Rosenzweig MR, Bennett EL. Psychobiology of plasticity: effects of training and experience on brain and behavior. Behavior Brain Research 1996;78:57-65.
109. Kolb B. Brain development, plasticity, and behavior. Am Psychol 1989;44(9):1203-12.
110. Diamond MC, Lindner B, Johnson R, Bennett EL, Rosenzweig MR. Differences in occipital cortical synapses from environmentally enriched, impoverished, and standard colony rats. J Neurosci Res 1975;1(2):109-19.
111. Brenner E, Mirmiran M, Uylings HB, Van der Gugten J. Growth and plasticity of rat cerebral cortex after central noradrenaline depletion. Exp Neurol 1985;89(1):264-8.
112. Landry SH, Denson SE, Swank PR. Effects of medical risk and socioeconomic status on the rate of change in cognitive and social development for low birth weight children. J Clin Exp Neuropsychol 1997;19(2):261-74.
113. Pfeiffer SI, Aylward GP. Outcome for preschoolers of very low birthweight: sociocultural and environmental influences. Percept Mot Skills 1990;70: 1367-78.
114. Weisglas-Kuperus N, Baerts W, Smrkovsky M, Sauer PJ. Effects of biological and social factors on the cognitive development of very low birth weight children. Pediatrics 1993;92(5):658-65.
115. Jacobson JL, Jacobson SW. Developmental effects of PCBs in the fish eater cohort studies. In: Robertson LW, Hansen LG, editors. PCBs: recent advances in environmental toxicology and health effects. Kentucky: The University Press of Kentucky; 2001; 127-136.
116. Grandjean P, Weihe P, Burse VW, Needham LL, Storr-Hansen E, Heinzow B, et al. Neurobehavioral deficits associated with PCB in 7-year-old children prenatally exposed to seafood neurotoxicants. Neurotoxicol Teratol 2001;23(4):305-17.
117. Rice DC. Neurotoxicity of lead, methylmercury, and PCBs in relation to the Great Lakes. Environ Health Perspect 1995;103 Suppl 9:71-87.
118. Patandin S, Dagnelie PC, Mulder PG, Op de Coul E, van der Veen JE, Weisglas-Kuperus N, et al. Dietary exposure to polychlorinated biphenyls and dioxins from infancy until adulthood: A comparison between breast-feeding, toddler, and long-term exposure. Environ Health Perspect 1999;107(1):45-51.
119. Weisglas-Kuperus N, Patandin S, Berbers GA, Sas TC, Mulder PG, Sauer PJ, et al. Immunologic Effects of Background Exposure to Polychlorinated Biphenyls and Dioxins in Dutch Preschool Children. Environ Health Perspect 2000;108(12):1203-1207.
120. Burse VW, Korver MP, Needham LL, Lapeza CR, Jr., Boozer EL, Head SL, et al. Gas chromatographic determination of polychlorinated biphenyls (as Aroclor 1254) in serum: collaborative study. J Assoc Off Anal Chem 1989;72(4):649-59.
121. Van den Berg M, Birnbaum L, Bosveld ATC, Brunstrom B, Cook P, Feeley M, et al. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 1998;106(12):775-92.
122. Patandin S. Effect of environmental exposure to polychlorinated biphenyls and dioxins on growth and development in young children. Rotterdam, The Netherlands: Erasmus University Rotterdam; 1999.

PART I

Effects of perinatal exposure to PCBs and dioxins
on general cognitive and motor development in interaction
with parental conditions and the home environment of the child

2 Effects of prenatal PCB and dioxin exposure on cognitive and motor abilities in Dutch children at school age

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Abstract

The purpose of this study was to evaluate whether effects of exposure to environmental levels of PCBs and dioxins on development in the Dutch cohort persist until school age. In the Dutch PCB/dioxin study, cognitive and motor abilities were assessed with the McCarthy Scales of Children's Abilities in children at school age. During infancy, half of this population was fully breast-fed for at least 6 weeks and the other half formula-fed. Prenatal exposure to PCBs was defined as the sum of PCB118, 138, 153, and 180 in maternal and cord plasma. In breast milk, additional measurements of 17 dioxins, 6 dioxin-like PCBs, and 20 nondioxin-like PCBs were done. Negative effects of prenatal PCB and dioxin exposure on cognitive and motor abilities were seen when parental and home characteristics were less optimal. These effects were not measurable in children raised in more optimal environments.

Conclusions: Neurotoxic effects of prenatal PCB and dioxin exposure may persist into school age, resulting in subtle cognitive and motor developmental delays. More optimal intellectual stimulation provided by a more advantageous parental and home environment may counteract these effects of prenatal exposure to PCBs and dioxins on cognitive and motor abilities.

Introduction

Negative effects of prenatal exposure to environmental levels of PCBs and dioxins on child development have been described in a number of prospective long-term follow-up studies. Animal studies addressing effects of perinatal exposure on the developing central nervous system (CNS) show direct effects on neuronal and glial cell development and disruption of neurotransmitters and several endocrine systems, such as thyroid and sex hormones, that may affect CNS development indirectly (1, 2). In humans, several epidemiological studies have addressed these neurotoxic effects with cognitive and motor abilities as neurodevelopmental outcome. In the North Carolina cohort, lower psychomotor skills from 6 to 24 months of age were associated with higher prenatal PCB exposure (3, 4). At 3, 4, and 5 years of age, cognitive and motor abilities were not related to prenatal PCB levels (5). In the Lake Michigan cohort, however, lower visual recognition memory at 7 months of age (6), lower verbal and memory scores at 4 years of age (7) and lower IQ scores at 11 years of age (8) were associated with higher prenatal PCB exposure. In the Oswego Study, negative effects of prenatal exposure on visual recognition memory were described at 6 and 12 months of age (9).

In The Netherlands, a prospective follow-up study was started in 1989. In contrast to the previously described studies, half of the study group was formula-fed (FF) during infancy, representing children with mainly in utero exposure to PCBs and dioxins. The other half of the group was breast-fed (BF) and was exposed to PCBs and dioxins also postnatally through lactation. Prenatal PCB exposure was related to poorer neurological condition at birth (10) and 18 months of age (11), lower psychomotor abilities at 3 months of age (12), and lower cognitive abilities at 42 months of age (13). Postnatal PCB and dioxin exposure was only related to lower psychomotor abilities at 7 months of age (12).

At 42 months of age, negative effects of prenatal PCB exposure on cognitive abilities were more pronounced in the FF than in the BF group (13), although BF children were exposed to higher prenatal and postnatal PCB levels (14). Children in the BF group had higher general cognitive abilities, as well as older mothers, parents with higher education levels and verbal IQs, and higher scores on the HOME questionnaire (13). It was not clear whether nutrients in breast milk, or the more optimal parental and home environment often provided by families of BF children, might have counteracted negative effects of prenatal PCB exposure.

At school age, the Dutch cohort was reassessed to examine the effects of perinatal exposure to PCBs and dioxins on cognitive and motor abilities, to explore the potential differences of these effects in FF and BF children, and to evaluate whether these effects are related to differences between the two feeding groups in parental and home environmental characteristics.

Methods

Subjects

The study population consisted of 418 healthy mother-infant pairs who were recruited from 1990 to 1992. Half of the study population was from Rotterdam, a highly industrialized and densely populated area, and the other half from Groningen, a semiurban area, in The Netherlands. The study design, recruitment process, and chemical analysis of PCBs and dioxins have been described in detail elsewhere (15). All included mother-infant pairs were white, and pregnancy and delivery had been without complications. Only first or second term-born infants were included. In addition to women who intended to use formula-feeding, women who intended to breast-feed their child for at least six weeks were also included. All infants in the FF group received formula from a single batch (Almiron M2, Nutricia NV, Zoetermeer, The Netherlands) from birth until 7 months of age. In this formula, concentrations of both PCBs and dioxins were below the detection limit. The medical ethics committee of the University Hospital Rotterdam/ Sophia Children's Hospital and the Academical Hospital Groningen approved the study design. The parents gave informed consent.

Test Material

The Dutch version of the McCarthy Scales of Children's Abilities (16, 17) was used to assess cognitive and motor abilities at 6 ½ years of age. The McCarthy Scales of Children's Abilities consists of 18 subtests from which six subscales are composed: verbal, perceptual-performal, quantitative, memory and motor subscale (mean, 50; SD, 10). An age-standardized General Cognitive Index (GCI) (mean, 100; SD, 15) is derived from the sum of the verbal, perceptual-performal and the quantitative subscales. Effects of PCB and dioxin exposure on the GCI and the memory and motor scales will be evaluated.

Assessment of exposure variables

Plasma samples were collected from the mothers during the last month of pregnancy and cord plasma samples were collected immediately after birth. These samples were analyzed for four PCB congeners, International Union for Pure and Applied Chemistry (IUPAC) numbers 118, 138, 153 and 180 (15, 18). Two weeks after delivery, a 24-hour representative breast milk sample was collected from the mothers who were breast-feeding their children. Breast milk samples were analyzed for 17 dioxins (PCDDs and PCDFs), 6 dioxin-like PCBs (IUPAC numbers 77, 105, 118, 126, 156, and 169), and 20 nondioxin-like PCBs (IUPAC numbers 28, 52, 66, 70, 99, 101, 128, 137, 138, 141, 151, 153, 170, 177, 180, 183, 187, 194, 195, and 202) (15, 19). Toxic potency of the mixture of dioxins and dioxin-like PCBs was

expressed by the toxic equivalence factor (TEF) approach (20). Toxic equivalents (TEQs) were calculated by multiplying the concentration of each congener by its TEF value.

Prenatal exposure to PCBs in the total study population is defined as the sum of the concentrations of the four PCB congeners measured in maternal plasma ($\Sigma PCB_{maternal}$) and in cord plasma (ΣPCB_{cord}). The breast milk samples were used as indirect measures of prenatal exposure to PCBs and dioxins (21). In the BF group, therefore, three additional prenatal exposure measurements were defined: the TTEQ value (the sum of the TEQ values of the 17 dioxins and the 6 dioxin-like PCBs), ΣPCB_{milk} (the sum of PCB118, 138, 153, and 180), $\Sigma PCB_{20\ nondioxin\text{-}like}$ (the sum of 20 nondioxin-like PCBs).

Postnatal exposure to PCBs and dioxins through lactation was estimated in the BF group by multiplying, respectively, breast milk levels of TTEQ, ΣPCB_{milk}, and $\Sigma PCB_{20\ nondioxin\text{-}like}$ with the number of weeks of breast-feeding.

Assessment of covariables

Variables that may influence child neurodevelopment were assessed. These variables included birth weight, duration of gestation, fetal exposure to alcohol and cigarette smoking, maternal age at birth, parental education level, and parity (items on a questionnaire addressing obstetric, social economic, and perinatal conditions (22)), type of feeding during infancy, duration of breast-feeding, and sex. The quality of intellectual stimulation and emotional support provided by the child's home environment was assessed by the Home Observation for Measurement of the Environment (HOME) (23). The verbal IQ of the parent who spent the most time with the child (usually the mother) was measured by 2 subtests, Information and Vocabulary from the Dutch version of the Wechsler Adult Intelligence Scale (WAIS) (24).

Statistical analysis

We used a Student's t-test, χ^2 test, and Mann-Whitney *U* test to compare groups for a single variable. PCB and dioxin levels were positively skewed and were, therefore, normalized by a natural logarithmic transformation (LnExposure).

Effects of PCB and dioxin exposure on cognitive and motor abilities were studied by means of multiple regression analyses. Variables that were likely to affect cognitive and motor abilities, based on literature and clinical knowledge, were included in the regression model as a fixed set of explanatory variables. These variables were [1] type of feeding during infancy (BF or FF) and [2] duration of breast-feeding (0 for FF children), [3] sex, [4] age at examination, [5] highest education level of the parents (*low*, primary school, secondary school not finished; *middle,* secondary school finished; *high*, high school finished, professional and university training), [6] parental verbal IQ, and [7] HOME score.

Because two examiners, one in each study center, carried out the cognitive and motor assessment, the variable study center was included in the fixed set to adjust for interrater variability. In addition covariables were selected by means of partial F tests. Variables with a relation ($p \leq 0.2$) with at least one of the exposure variables and at least one of the six subscales of the McCarthy Scales of Children's Abilities, adjusted for the variables in the fixed set, were included in the final regression model. These variables were considered potential confounders for effects of PCB and dioxin exposure on cognitive and motor abilities. Candidate confounders were alcohol use (yes/no) and smoking (yes/no) during pregnancy, duration of gestation (weeks), birth weight (grams), maternal age at birth, and parity (first or second born). In the final regression model, the variables included were study center, sex, parity, type of feeding, duration of breast-feeding, maternal age at birth, parental education level, parental verbal IQ, HOME score, and age at examination.

To evaluate effects of prenatal PCB exposure in the two feeding groups separately, an interaction variable (the product of feeding type and lnΣPCB) 'feeding type*lnΣPCB' was included in the regression model.

Interaction effects, first-order (linear interaction) and second-order interaction effects (parabolic interaction) of PCB and dioxin exposure and the variables in the regression model that were significantly different for the two feeding groups (maternal age, parental education and verbal IQ, and HOME scores) were explored in separate analyses. Because of the explorative nature of this study, no correction for multiple testing was made, and 2-tailed p-values ≤ 0.05 were considered significant. Nonetheless, all relevant interactions, including the nonsignificant ones, will be presented together with the actually calculated p-values.

Results

At school age, 376 children (90 %) of the original cohort of 418 children (189 from Rotterdam, 187 from Groningen) were willing to participate in the follow-up assessment. Forty-two children were lost to follow-up, 21 because of a lack of interest, 20 because of emigration, and one because of death in an accident. Four children were excluded from data analyses because of circumstances that are known to influence the score on the McCarthy scales of Children's Abilities other than PCB and dioxin exposure (Turner's syndrome, pervasive development disorder, Volkmann's contracture after a humerus fracture, and attention deficit hyperactivity disorder treated with methylphenidate hydrochloride). Five children failed to finish all subtests of the McCarthy Scales of Children's Abilities; these GCIs (n=5), memory (n=2), and motor scores (n=4) were not included in the data analyses.

Prenatal PCB and dioxin levels of the children that did not participate in the study at 6 ½ years of age and the participating children were similar. However, nonparticipating children

were significantly more likely to be FF and BF for shorter periods. Maternal age, parental education level, and verbal IQ scores were significantly lower in this group (Table 2.1). In addition, more boys than girls did not participate at school age.

Table 2.I Significant differences in characteristics of participating and nonparticipating children at school age.

Characteristics	Participants	Nonparticipants	p-value
Sex (male/female)	190/182	32/14	0.019[a]
BF/FF	194/178	15/31	0.018[a]
Breast-feeding period (wk)	24.2 (± 15.2)	13.8 (± 5.4)	0.005[b]
Maternal age (y)	29.2 (± 3.8)	27.9 (± 4.1)	0.030[b]
Parental education (low/medium/high)	37/112/223	8/24/14	0.001[a]
Parental verbal IQ	119.2 (± 15.9)	111.0 (± 17.0)	0.005[b]

Values are numbers or means (± standard deviations). Parental education: low = primary school, secondary school not finished, middle = secondary school finished, high = high school finished, professional and university training; Parental verbal IQ: score on two subtests, Information and Vocabulary, of the Wechsler Adult Intelligence Scale, assessed from one of the parents; [a]Chi-square test; [b]Mann-Whitney U test.

The mean age at examination of the total group was 6.7 years (+ 0.3; 6.1-7.3 years) (Table 2.2). The mean GCI and scores on the memory and motor scales were comparable to a normal population. Maternal age, parental education level and verbal IQ, HOME scores, and prenatal PCB exposure levels were significantly higher in the BF group than in the FF group, as were the mean (not adjusted) GCI and memory scores (Table 2.2).

Results of multiple regression analyses on GCI, memory and motor scores by using maternal PCB levels are presented in Table 2.3. Significant effects using cord PCB levels ($p \leq 0.05$) are presented in the text, and for reasons of clarity not shown in Table 2.3 or in the figures. Prenatal PCB levels were not related to GCI, memory and motor skills, after adjustment for covariables (Table 2.3A). In Table 2.3B, results of multiple regression analyses evaluating the effects of prenatal PCB exposure on the GCI, memory, and motor scores in the two feeding groups are presented. Effects of prenatal PCB exposure on the GCI, memory, and motor scores were not significantly different for BF and FF children. In the two feeding groups separately, prenatal PCB exposure was not related to GCI or memory skills. In FF children, however, higher maternal PCB levels tended to be related to lower motor scores (Table 2.3B).

Table 2.2 Characteristics of the study population.

Characteristics	Total (n=372)	Breast-fed (n=194)	Formula-fed (n=178)
Study center (Rotterdam), n (%)	186 (50.0 %)	96 (49.5 %)	90 (50.6 %)
Sex (male), n (%)	190 (51.3 %)	105 (54.1 %)	85 (47.8 %)
Parity (1st born), n (%)	179 (48.1 %)	100 (51.5 %)	79 (44.4 %)
Breast-feeding period (wk)		20 (6-78)	
Maternal age at birth (y) *	29.2 (± 3.8)	29.7 (± 3.5)	28.7 (± 4.0)
Parental education level **			
Low, n (%)	37 (9.9 %)	8 (4.1 %)	29 (16.3 %)
Middle, n (%)	112 (30.1 %)	36 (18.6 %)	76 (42.7 %)
High, n (%)	223 (59.8 %)	150 (77.3 %)	73 (41.0 %)
Parental verbal IQ **	119.2 (± 15.9)	125.2 (± 11.8)	112.55 (± 17.1)
HOME **	47.8 (± 3.2)	48.5 (± 2.9)	47.0 (± 3.3)
Exposure variables			
$\Sigma PCB_{maternal}$ (µg/l) **	2.04 (0.59-7.35)	2.22 (0.73-7.35)	1.85 (0.59-5.08)
ΣPCB_{cord} (µg/l) **	0.38 (0.08-2.08)	0.38 (0.08-2.08)	0.34 (0.08-1.98)
TTEQ (ng/kg fat)		63.30 (24.16-136.54)	
ΣPCB_{milk} (µg/kg fat)		403.66 (158.35-1226.38)	
$\Sigma PCB_{20\ nondioxin\text{-}like}$ (µg/kg fat)		451.05 (186.11-1121.02)	
School age scores on the McCarthy Scales of Children's Abilities			
GCI **	104.7 (± 12.6)	108.2 (± 11.7)	100.8 (± 12.4)
Memory **	46.5 (± 7.6)	48.2 (± 7.2)	44.7 (± 7.7)
Motor	52.2 (± 9.8)	52.3 (± 9.2)	52.06 (± 10.5)

Values are numbers (percentages), means (± SD) or medians (range).
Parental education level: low=primary school, secondary school not finished, middle=secondary school finished, high=high school finished, professional and university training; Parental verbal IQ score on two subtests, Information and Vocabulary, of the Wechsler Adult Intelligence Scale, assessed from one of the parents; ΣPCBs: sum of PCB congeners IUPAC nos. 118, 138, 153, 180; TTEQ: sum of the dioxin TEQ's and dioxin-like PCB TEQs in breast milk. *p ≤ 0.05; ** p ≤ 0.01, difference between BF and FF group.

In Table 2.3C to F, results of multiple linear regression analyses are presented, including the following interaction variables in the regression models, respectively: '$\ln\Sigma PCB_{maternal}$*maternal age', 'maternal age^2', and '$\ln\Sigma PCB_{maternal}$*maternal age^2' (because the interaction effect of $\ln\Sigma PCB_{maternal}$ and maternal age was of parabolic nature) (C), '$\ln\Sigma PCB_{maternal}$*parental education level' (D), '$\ln\Sigma PCB_{maternal}$*parental verbal IQ' (E), '$\ln\Sigma PCB_{maternal}$*HOME' (F). In the Figure (2.1A to D), these interaction effects are visualized by presenting the effects of

doubling maternal PCB exposure on GCI, memory, and motor scores in relation to maternal age (A), parental education level (B), parental verbal IQ (C) and HOME score (D).

Effects of prenatal PCB exposure on the GCI were significantly modified by maternal age (combined $p_{PCBcord,\ mat.age}$ ($p_{PCBcord*mat.\ age}$ and $p_{PCBcord*(mat\ age)^2}$) =0.003) and parental verbal IQ ($p_{PCBcord*VIQ}$=0.004). Negative effects of prenatal PCB exposure on the GCI were seen in children born to younger mothers and to parents with lower verbal IQ scores; these effects, however, were not evident with increasing maternal age and parental verbal IQ. Similar relations were seen exploring effect modification of prenatal PCB exposure by parental education level.

Table 2.3 Estimated effects of $\ln\Sigma PCB_{maternal}$ on the three outcome variables GCI, memory and motor scores on the McCarthy Scales of Children's abilities.

$\ln\Sigma PCB_{maternal}$	GCI (n=353)			Memory (n=354)			Motor (n=352)		
	Regr. coef.	SE	p	Regr. coef.	SE	p	Regr. coef.	SE	p
A [a]									
PCB	-0.14	1.58	0.929	-0.36	1.02	0.725	-2.45	1.45	0.092
B [a]									
PCB in BF	-0.01	2.00	0.996	-0.25	1.30	0.844	-1.28	1.84	0.486
PCB in FF	-0.30	2.22	0.891	-0.49	1.44	0.733	-3.92	2.04	0.055
PCB*FT (BF=0)	-0.29	2.79	0.916	-0.24	1.81	0.896	-2.64	2.56	0.305
C [a]									
PCB	-147.51	50.44	0.004	-82.58	32.89	0.013	-77.99	46.78	0.096
PCB*Mat. age	9.37	3.41	0.006	5.38	2.22	0.016	4.73	3.16	0.132
PCB*(Mat. age)^2	-0.15	0.06	0.011	-0.09	0.04	0.021	-0.07	0.05	0.166
D [a]									
PCB	-5.84	3.29	0.077	-3.25	2.14	0.129	-5.93	3.05	0.052
PCB*Education[b]	3.75	1.92	0.052	1.92	1.25	0.125	2.41	1.78	0.177
E [a]									
PCB	-26.06	10.37	0.012	-16.49	6.72	0.015	-21.24	9.58	0.027
PCB*VIQ	0.22	0.09	0.012	0.13	0.06	0.016	0.16	0.08	0.048
F [a]									
PCB	-26.92	22.79	0.238	-12.99	14.75	0.380	-46.65	20.92	0.026
PCB*HOME	0.60	0.48	0.240	0.26	0.31	0.392	0.92	0.44	0.035

PCB: $\ln\Sigma PCB_{maternal}$; GCI: General Cognitive Index; FT: Feeding type (BF or FF); MatAge: maternal age at birth; Education: parental education level; VIQ: parental verbal IQ; HOME: score on the Home Observation for the Measurement of the Environment, at school age. [a] Results of regression analysis, adjusted for study center, sex, age at examination, type of feeding, duration of breast-feeding, maternal age, parental education level, parental verbal IQ, HOME score, and parity. [b] Education is used as a linear trend variable, 0=low, 1=middle, 2=high. For each outcome variable, 6 separate regression analyses (A-F) are presented according to various effect modification of $\ln\Sigma PCB_{maternal}$. In A the regression coefficient of $\ln\Sigma PCB_{maternal}$, SE of the mean, and p-value are presented assuming no effect modification. In B the regression coefficient of $\ln\Sigma PCB_{maternal}$, SE of the mean and p-value are presented for the BF and FF group and the statistical difference in effect of $\ln\Sigma PCB_{maternal}$ between the feeding groups is indicated by the regression coefficient, SE of the mean, and p-value of the interaction variable PCB*FT. In C, D, E, F the regression coefficient, SE of the mean, and p-value of the interaction variable indicate the effect modification of $\ln\Sigma PCB_{maternal}$ by maternal age, parental education level, parental verbal IQ, and HOME score, respectively.

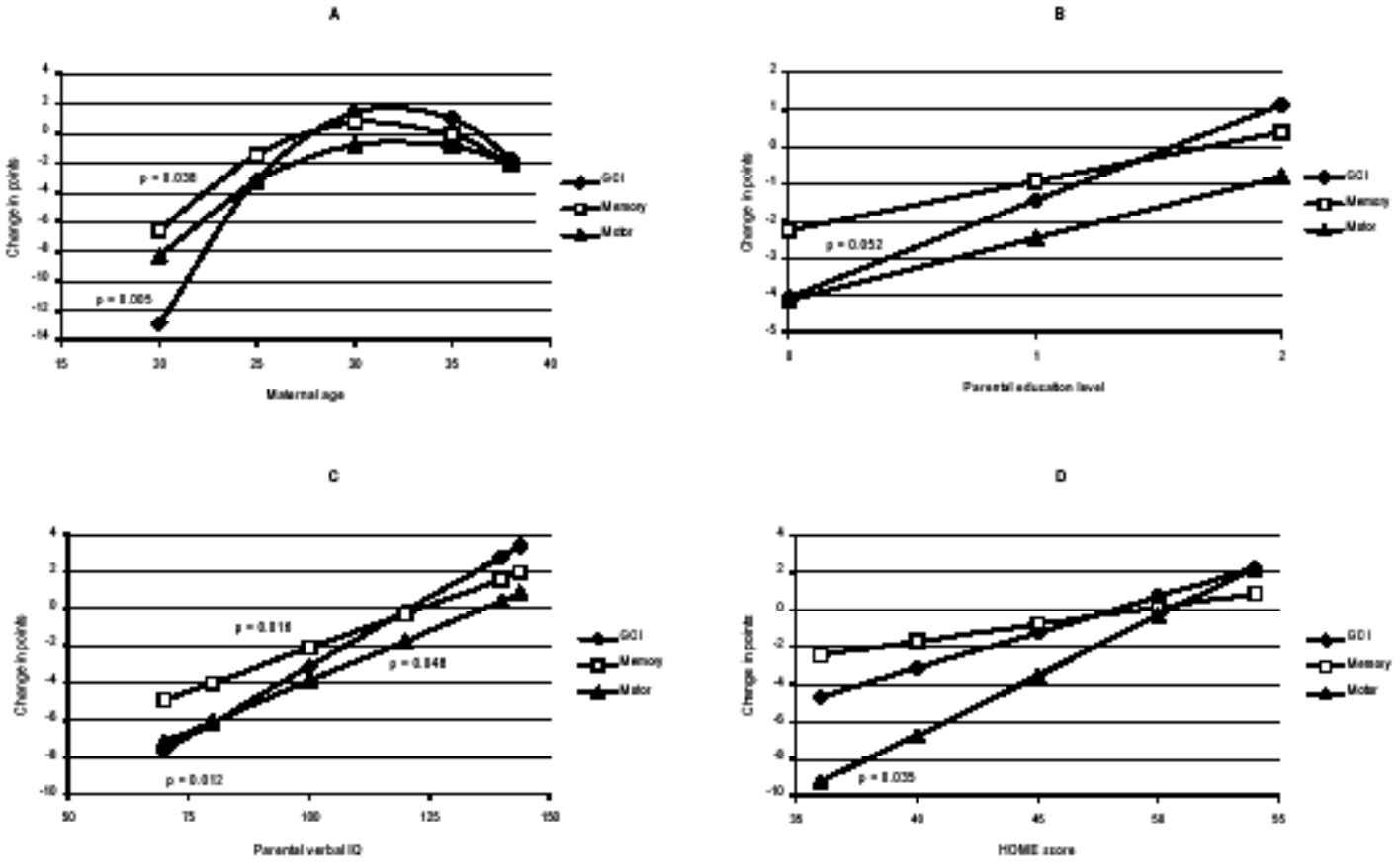

Figure 2.1 Effect of doubling prenatal PCB exposure on GCI, memory and motor skills in relation to (A) maternal age, (B) parental education level, (C) parental verbal IQ, (D) HOME score.

The Y-axis represents the effect on the GCI, memory and motor scales, of doubling maternal PCB level sa is modified by (A) maternal age, (B) parental education level, (C) parental verbal IQ, (D) Home score. The p-values indicate significance of modification of the PCB effect by maternal age (A), parental education level (B), parental verbal IQ (C), and Home score (D). In (A), the PCB effect on motor skills is not significantly different from a constant effect of -1.70 (=-2.45 ln(2)). In (B), the PCB effect on memory and motor skills is not significantly different from a constant effect of respectively -0.25 (=-0.36 ln(2)) and -1.7 (=-2.45 ln(2)). In (D), the PCB effect on GCI and memory skills is not significantly different from a constant effect of respectively -0.1 (=-0.14 ln(2)) and -0.25 (=-0.36 ln(2)).

Effects of prenatal PCB exposure on memory skills were significantly modified by maternal age (combined $p_{PCBcord,\ mat.age}$=0.027) and parental verbal IQ ($p_{PCBcord*VIQ}$=0.050). Negative effects of prenatal PCB exposure on memory skills appeared to decrease when maternal age and parental verbal IQ increased.

Effects of prenatal PCB exposure on motor skills were significantly modified by parental verbal IQ ($p_{PCBcord*VIQ}$=0.021) and HOME scores. Negative effects of prenatal PCB exposure on the motor scores were seen to decrease in children born from parents with higher verbal IQ and higher HOME scores.

In the BF group, levels of the TTEQ, ΣPCB_{milk}, and $\Sigma PCB_{20\ nondioxin\text{-}like}$ in breast milk were not significantly related to GCI, memory, and motor scores when adjusted for confounding variables. Effects of prenatal TTEQ exposure on motor skills were modified by parental verbal IQ levels (β_{TTEQ}= -48.005, p= 0.036, $\beta_{TTEQ*VIQ}$= 0.386, p= 0.036). Negative effects of prenatal TTEQ exposure were seen to decrease in children born to parents with higher verbal IQ's.

Postnatal exposure to PCBs and dioxins through lactation was not significantly related to GCI, memory, and motor scores, and effects of postnatal exposure were not significantly modified by parental and home environmental characteristics.

Discussion

In the Dutch PCB and dioxin study at school age, subtle effects of prenatal exposure to PCBs and dioxins were seen on cognitive and motor abilities. At 42 months (13) and at 6 1/2 years of age, FF children had significantly lower cognitive abilities than BF children. In the FF group in our cohort, parental and home environmental characteristics are less optimal compared with these characteristics in the BF group. Effects of prenatal PCB exposure on cognitive abilities of children at 42 months of age were more pronounced in the FF than in the BF group, although BF children were exposed to higher prenatal exposure levels and higher postnatal exposure levels of PCBs and dioxins in particular. To explore whether these differences in effect were related to nutritional benefits of breast-feeding or to more advantaged parental and home characteristics, we evaluated whether effects of PCB and dioxin exposure were modified by parental and home characteristics that were significantly different for the two feeding groups. The present results give evidence for effect modification by parental and home environmental conditions in the total cohort. The adverse effects of prenatal PCB exposure were more pronounced when parental and home characteristics were less optimal, whereas these effects were not evident when parental and home characteristics were more advantaged. At school age, differences in vulnerability to prenatal exposure to PCBs between the BF and FF group were not statistically different. Differences in vulnerability to effects, however, were related to more subtle differences

in parental and home environmental characteristics, varying across both feeding groups. These results suggest, therefore, that the differences in vulnerability of the BF and FF children, seen at 42 months of age, were more likely to be related to parental and home characteristics than to beneficial effects of breast-feeding per se.

In some neurotoxic epidemiological studies, effects of exposure to these compounds on child development were also seen to be related to socioeconomic risk factors (25-28). Children from lower social economic backgrounds were more vulnerable to negative cognitive effects of prenatal exposure to lead than children in more advantaged families. Comparable effects have also been reported in studies of low birth weight children where in children at high biological risk, favorable early parental and home characteristics could compensate for or mask developmental delays (29-33). The results of the present study give evidence for counteracting processes or for 'cumulative deficits' in respect of effects of prenatal exposure to PCBs on cognitive and motor abilities. Cognitive and motor development is influenced by many factors, and not all of them were controlled for in this study. Whether the interaction effects presented here reflect not measured variables such as subtle parent-child interaction aspects or aspects related to self-esteem and emotional development is not known. On the one hand, maternal age at birth is related with higher PCB and dioxin levels, and conversely, with higher education levels, verbal IQs and HOME scores. There is no reason to believe that maternal age itself is directly related to cognitive or motor outcome; factors associated with maternal age are more likely to explain the reported interaction effects. We suggest that these factors include among others that older mothers may more consciously choose for parenthood and may have different parental values and orientation towards child development than younger mothers. Effect modification of PCB and dioxin exposure by maternal age at birth, parental education level and verbal IQ, and HOME scores could not be analyzed in one regression analysis because these interaction variables correlated highly with each other. We therefore choose a statistical procedure in which effect modification of exposure by parental and home environmental characteristics was explored separately. Consequently, we are not able to differentiate the relative effects of the several aspects of parental and home characteristics.

It should be stressed here that the study population consists of families that were motivated to participate in this study for 7 years. Parental and home characteristics of this group are likely to be more advantaged than in the average Dutch population. These results suggest that effect of exposure to these environmental pollutants on cognitive and motor abilities might be more pronounced in less advantaged populations.

As cognitive outcome variables, we used the GCI and memory scales of the McCarthy Scales of Children's Abilities. The GCI scale is a composite scale of three subscales, verbal, perceptual-performal, and quantitative. Because of the complex nature of this study, we decided not to include these subscales in the analyses and use only the composite GCI scale, memory and motor scales as outcome variables.

In the present study, the demonstrated effects of cord plasma PCB levels on cognitive and motor abilities are generally comparable to those using maternal PCB levels. In the BF group, prenatal exposure to TTEQ was associated with lower motor scores in children born to parents with lower verbal IQ scores. Exposure to nondioxin-like PCBs and ΣPCB_{milk} showed comparable relationships, although they did not reach significance. In the environment, PCBs and dioxins are present as complex mixtures of various congeners that may vary in metabolism and toxicity. The sum of PCBs 118, 138, 153, and 180 consists of the four most abundant congeners, constituting 46% of the total PCBs (34). In our cohort TTEQ levels, the sum of the nondioxin-like PCBs, and the sum of the four PCBs in breast milk and in maternal and cord plasma, correlated highly (35). It is uncertain whether described effects of the sum of the four PCBs in plasma might also reflect effects of dioxins and other related organochlorine compounds and their metabolites.

In agreement with the results at 42 months of age in this cohort (13), and other epidemiologic studies (3-5, 7-9), our results show that postnatal exposure to PCBs and dioxins through lactation was not related to cognitive and motor abilities at school age. Prenatally, the developing CNS seems to be more susceptible to harmful effects of these compounds than during the early postnatal period.

In contrast to the examined effects at school age, negative effects at preschool age of prenatal PCB exposure on cognitive abilities were seen in the total cohort. This difference could be explained by a number of factors. At school age, children that participated in the follow-up had significantly higher parental and home characteristics compared with the nonparticipating children. The higher mean levels of these background variables might explain that no effect of prenatal PCB exposure is seen in the total cohort, adjusting for the mean population levels of the confounders. The interaction effects seen at school age between PCB and dioxin exposure and parental and home characteristics show the importance of the distribution of these variables in a cohort. Differences in the results of effects of prenatal PCB exposure could also reflect differences in the test materials used to assess cognitive abilities. At 42 months of age, the Kaufman-ABC (36) was used, and at school age the McCarthy Scales of Children's Abilities was used. These developmental tests assess different neuropsychologic functions to compose a general cognitive index. The McCarthy Scales of Children's Abilities was also used in the North Carolina cohort (5) (at 3 to 5 years) and in the Lake Michigan cohort (7) at 4 years of age. In both study populations, no relationship between prenatal PCB exposure and the GCI was seen. In the Lake Michigan cohort however, prenatal PCB exposure was related with lower memory skills and lower scores on the verbal scale of the McCarthy Scales of Children's Abilities. In comparing effects seen in different cohorts, we have to consider differences in exposure levels between these cohorts that are difficult to compare because of differences in analytic methods used to measure exposure. There is reason to believe that exposure levels in the

Dutch cohort and the Lake Michigan cohort are roughly comparable, whereas exposure levels in the North Carolina cohort are suspected to be lower (13).

We conclude that neurotoxic effects of prenatal PCB and dioxin exposure may persist into school age and may result in subtle cognitive and motor developmental delays. Parental and home environmental characteristics influenced the consequences of these neurotoxic effects for cognitive and motor abilities. When these characteristics were less optimal, negative effects of prenatal PCB exposure were seen on cognitive and motor abilities, whereas these negative effects of prenatal PCB exposure were not measurable in children raised in more optimal environments. These data indicate that children might be at risk to these neurotoxic pollutants because of prenatal exposure to PCBs and dioxins. Follow-up studies into adulthood in children exposed to different levels of these contaminants, while growing up in different environments, should be conducted to investigate the future implications of our findings.

References

1. Brouwer A, Ahlborg UG, Van den Berg M, Birnbaum LS, Boersma ER, Bosveld B, et al. Functional aspects of developmental toxicity of polyhalogenated aromatic hydrocarbons in experimental animals and human infants. Eur J Pharmacol 1995;293(1):1-40.
2. Weisglas-Kuperus N. Neurodevelopmental, immunological and endocrinological indices of perinatal human exposure to PCBs and dioxins. Chemosphere 1998;37(9-12):1845-53.
3. Gladen BC, Rogan WJ, Hardy P, Thullen J, Tingelstad J, Tully M. Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. J Pediatr 1988;113(6):991-5.
4. Rogan WJ, Gladen BC. PCBs, DDE, and child development at 18 and 24 months. Ann Epidemiol 1991;1(5):407-13.
5. Gladen BC, Rogan WJ. Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. J Pediatr 1991;119(1):58-63.
6. Jacobson SW, Fein GG, Jacobson JL, Schwartz PM, Dowler JK. The effect of intrauterine PCB exposure on visual recognition memory. Child Dev 1985;56(4):853-60.
7. Jacobson JL, Jacobson SW, Humphrey HE. Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J Pediatr 1990;116(1):38-45.
8. Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychorinated biphenyls in utero. N Engl J Med 1996;335:783-9.
9. Darvill T, Lonky E, Reihman J, Stewart P, Pagano J. Prenatal exposure to PCBs and infant performance on the fagan test of infant intelligence. Neurotoxicology 2000;21(6):1029-38.
10. Huisman M, Koopman-Esseboom C, Fidler V, Hadders-Algra M, van der Paauw CG, Tuinstra LG, et al. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. Early Hum Dev 1995;41(2):111-27.
11. Huisman M, Koopman-Esseboom C, Lanting CI, van der Paauw CG, Tuinstra LGMT, Fidler V, et al. Neurological conditions in 18 months-old children perinatally exposed to polychlorinated biphemils and dioxins. Early Human Development 1995:165-176.
12. Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MA, Van der Paauw CG, Tuinstra LG, Sauer PJ. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics 1996;97(5):700-6.
13. Patandin S, Lanting CI, Mulder PG, Boersma ER, Sauer PJ, Weisglas-Kuperus N. Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J Pediatr 1999;134(1):33-41.
14. Patandin S, Weisglas-Kuperus N, de Ridder MA, Koopman-Esseboom C, van Staveren WA, van der Paauw CG, et al. Plasma polychlorinated biphenyl levels in Dutch preschool children either breast-fed or formula-fed during infancy. Am J Public Health 1997;87(10):1711-4.
15. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N, Boersma ER, de Ridder MA, Van der Paauw CG, et al. Dioxin and PCB levels in blood and human milk in relation to living areas in The Netherlands. Chemosphere 1994;29(9-11):2327-38.
16. McCarthy D. McCarthy Scales of Children's Abilitiesm manual. New York: The Psychological Corporation; 1972.
17. Van der Meulen BF, Smorkovsky M. MOS 2 1/2- 8 1/2 McCarthy Ontwikkelingsschalen. Lisse, The Netherlands: Swets & Zeitlinger B.V.; 1985.
18. Burse VW, Korver MP, Needham LL, Lapeza CR, Jr., Boozer EL, Head SL, et al. Gas chromatographic determination of polychlorinated biphenyls (as Aroclor 1254) in serum: collaborative study. J Assoc Off Anal Chem 1989;72(4):649-59.
19. van Rhijn JA, Traag WA, van de Spreng PF, Tuinstra LG. Simultaneous determination of planar chlorobiphenyls and polychlorinated dibenzo-p-dioxins and -furans in Dutch milk using isotope dilution and gas chromatography-high-resolution mass spectrometry. J Chromatogr 1993;630(1-2):297-306.

20. Van den Berg M, Birnbaum L, Bosveld ATC, Brunstrom B, Cook P, Feeley M, et al. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 1998;106(12):775-92.
21. Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, et al. Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) in human milk: effects of maternal factors and previous lactation. Am J Public Health 1986;76(2):172-7.
22. Prechtl HF. The optimality concept. Early Hum Dev 1980;4(3):201-5.
23. Caldwell B, Bradley R. Home observation of the environment. Administration manual. Little Rock, Arkansas; 1984.
24. Stinissen J, Willems PJ, Coetsier P, Hulsman WLL. Manual, Dutch version of the Wechsler Adult Intelligence Scale (WAIS). Amsterdam: Swets & Zeitlinger NV; 1970.
25. Davidson PW, Myer GJ, Shamlaye C, Cox C, Gao P, Axtell C, et al. Association between prenatal exposure to methylmercury and developmental outcomes in Seychellois children: effect modification by social and environmental factors. Neurotoxicology 1999;20(5):833-41.
26. Bellinger D, Leviton A, Waternaux C, Needleman H, Rabinowitz M. Low-level lead exposure, social class, and infant development. Neurotoxicol Teratol 1988;10(6):497-503.
27. Dietrich KN, Succop PA, Berger OG, Hammond PB, Bornschein RL. Lead exposure and the cognitive development of urban preschool children: the Cincinnati Lead Study cohort at age 4 years. Neurotoxicol Teratol 1991;13(2):203-11.
28. Winneke G, Kraemer U. Neuropsychological effects of lead in children: interactions with social background variables. Neuropsychobiology 1984;11(3):195-202.
29. Weisglas-Kuperus N, Baerts W, Smrkovsky M, Sauer PJ. Effects of biological and social factors on the cognitive development of very low birth weight children. Pediatrics 1993;92(5):658-65.
30. Siegel LS. Reproductive, perinatal, and environmental factors as predictors of the cognitive and language development of preterm and full-term infants. Child Dev 1982;53(4):963-73.
31. Pfeiffer SI, Aylward GP. Outcome for preschoolers of very low birthweight: sociocultural and environmental influences. Percept Mot Skills 1990;7:1367-78.
32. Landry SH, Denson SE, Swank PR. Effects of medical risk and socioeconomic status on the rate of change in cognitive and social development for low birth weight children. J Clin Exp Neuropsychol 1997;19(2):261-74.
33. Aylward GP. The relationship between environmental risk and developmental outcome. J Dev Behav Pediatr 1992;13(3):222-9.
34. Jacobson JL, Humphrey HE, Jacobson SW, Schantz SL, Mullin MD, Welch R. Determinants of polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), and dichlorodiphenyl trichloroethane (DDT) levels in the sera of young children. Am J Public Health 1989;79(10): 1401-4.
35. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N, van der Paauw CG, Tuinstra LGMT, Boersma ER, et al. PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 1994;28 (9):1721-32.
36. Neutel RJ, van der Meulen BF, Lutje Spielber HJ. Groningse Ontwikkelingsschalen, Dutch version of the Kaufman Assessment Battery for Children (K-ABC). Lisse: Swets & Zeitlinger BV, The Netherlands; 1996.

3 Effects of prenatal exposure to PCBs on cognitive and motor development from 3 to 84 months of age; a longitudinal study

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Abstract

In the present study, we analyzed effects of prenatal PCB exposure on cognitive and motor development from 3 to 84 months of age by random regression modeling. Additionally, important determinants of cognitive and motor development were identified. In the Rotterdam cohort (n=207) of the Dutch PCB/dioxin study, cognitive and motor abilities were assessed at 3, 7, 18, 42, and 84 months of age. Prenatal exposure to PCBs was defined as the sum of PCB118, 126, 138, 153 in maternal plasma. Higher prenatal exposure to PCBs was associated with a lower level of cognitive and motor development. Effects of prenatal exposure to PCBs on cognitive development were significantly modified by maternal age. Important determinants of cognitive development were prenatal PCB levels, its modification by maternal age, along with parental education, parental verbal IQ and HOME scores. Motor development was efficiently predicted by prenatal PCB levels including its interaction with HOME scores along with parental education.

Conclusions: These results suggested neurotoxic effects of prenatal exposure to PCBs and related compounds on the level of cognitive and motor development; effects that may be modified by conditions that are favorable to child development. Compared to the large positive effects of more optimal parental and home environmental conditions, the negative effects of prenatal PCB exposure on cognitive development were relatively small. Effects prenatal PCB exposure on motor development were more pronounced and this outcome may serve as a sensitive tool to assess neurodevelopment risks of prenatal PCB exposure.

Introduction

PCBs and polychlorinated dibenzo-*para*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (the latter two are summarized as dioxins) are lipophilic and bioaccumulating environmental pollutants that have comparable molecular structures. These compounds are known for their potential neurotoxic effects in animals and humans. Children are prenatally exposed to maternal PCBs and dioxins and, additionally, through breast milk, thus during vulnerable periods of rapid development of the central nervous system (CNS). The neurotoxic mechanism of effects of these compounds on the CNS is complex, including direct neuronal effects and effects on neurotransmitters and endocrine systems, such as thyroid hormones and sex-steroid hormones, that may indirectly affect CNS development (1, 2).

To address neurotoxic effects of perinatal exposure to environmental levels of PCBs, most epidemiologic studies used general cognitive and motor abilities as endpoints. The results are inconsistent, showing discernable differences between study centers and within cohorts at different ages of assessment. In the North Carolina study, cognitive and motor development was studied in children from 6 months to 5 years of age (3-5). A negative effect of prenatal exposure to PCBs was only detected on psychomotor abilities until 2 years of age (3, 5). In the Lake Michigan cohort, prenatal exposure to PCBs was associated with lower verbal and memory scores but not with general cognitive abilities at 4 years of age (6) whereas at 11 years of age higher prenatal PCB exposure was related with lower IQ scores (7).

In the Dutch PCB/dioxin study, effects of prenatal and lactational exposure to PCBs on cognitive and motor development have been evaluated in a cohort of healthy born children from birth to school age. Prenatal PCB exposure was related to lower psychomotor scores at 3 months of age (8) and lower cognitive abilities at 42 months (9). Cognitive abilities at 3, 7, and 18 months and psychomotor abilities at 7 and 18 months of age were, however, not related with prenatal PCB exposure (8). At school age, negative effects of prenatal exposure to PCBs on general cognitive and motor abilities were suggested in children that were raised in lower parental and home environmental conditions, whereas in children raised in relatively more privileged environments these subtle effects of prenatal PCB exposure were not detectable (10).

Although in human studies, addressing neurotoxic effects of prenatal exposure, cognitive and motor abilities are highly relevant, these outcome variables are complex because they are developing qualities and because they are influenced by various factors such as prenatal circumstances and hormonal status, and parental and home environmental characteristics. Epidemiologic studies usually examine neurodevelopmental effects at separate assessment ages without properly capturing the course of development of these outcome variables. Therefore, the objective of this study was to evaluate effects of prenatal exposure to PCBs on the development of cognitive and motor abilities assessed from 3 to 84 months of age. Additionally we aimed to identify important (significant) determinants of cognitive and motor development.

Methods

Subjects

In 1990, a prospective follow-up study in healthy children was started in Rotterdam, The Netherlands. The study population comprised 207 Caucasian mother-infant pairs, recruited between 1990 and 1992. The study design and recruitment process, chemical analysis and PCB concentrations have been described in detail elsewhere (11). Only first or second, term born healthy children were included. One hundred and five children were exclusively breast-fed for at least six weeks and 102 children were fed with formula from a single batch (Almiron M2, Nutricia NV, Zoetermeer, The Netherlands) from birth until 7 months of age. In this formula, concentrations of PCBs were undetectable. The medical ethics committee of the University Hospital Rotterdam/ Sophia Children's Hospital approved the study design and the parents signed informed consent.

Exposure variables

Plasma samples were collected from mothers during the last month of pregnancy. These samples were analyzed for four nonplanar PCB congeners, International Union for Pure and Applied Chemistry numbers 118, 138, 153, and 180. The sum of the four PCB congeners is used as prenatal exposure variable (ΣPCB).

Outcome variables

The children were invited to participate in cognitive and motor assessments at 3, 7, 18, 42 and 84 months of age. Cognitive and motor abilities at 3, 7 and 18 months were assessed by the mental developmental index and psychomotor developmental index of the Dutch version of the Bayley Scales of Infant Development (BOS 2-30) (12, 13). At 42 months of age, cognitive abilities were assessed by the combined score of the sequential and simultaneous processing scales of the Dutch version of the Kaufman Assessment Battery for Children (Dutch K-ABC) (14). Motor development at 42 months of age was measured by the sum of the standardized scores of two subtests of the Dutch K-ABC: gross and fine motor skills. At 84 months of age, the general cognitive index and the motor score of the Dutch version of the McCarthy Scales of Children's Abilities (15) assessed cognitive and motor abilities, respectively. All developmental scores were derived from tests that have been standardized and scores were, except for the motor scores at 42 and 84 months, transformed into scores with a mean (± SD) of 100 (± 15). The motor score at 42 months of age is a sum score of two subtests after being normalized to a mean of 10 (± 3). The motor score at 84 months of age was normalized into a mean score of 50 (± 10).

Confounding variables

Variables that may influence child neurodevelopment have been assessed from birth to school age. These included prenatal circumstances such as birth weight, duration of gestation, fetal exposure to alcohol and cigarette smoking, maternal thyroid plasma levels during pregnancy (total thyroxine TT_4, free thyroxine FT_4, total triiodothyronine TT_3, and thyroid stimulating hormone TSH), child and nutritional characteristics such as gender, parity and type of feeding during infancy (breast-fed or formula-fed) and duration of breast-feeding, and demographic characteristics such as maternal age at birth of the child and parental education level. Parental verbal IQ was assessed by two subtests, Information and Vocabulary from the Dutch version of the Wechsler Adult Intelligence Scale (WAIS) (16) in the parent who spent the most time with the child, usually the mother. The quality of intellectual stimulation and emotional support provided by the child's home environment was assessed by the Dutch version of the Home Observation for Measurement of the Environment (HOME) (17) at 18, and 42 months of age (version 0-3 years), and 84 months of age (version 3-6 years).

Data analyses

Longitudinal data analysis was done on repeated measurements of cognitive and motor abilities from 3 to 84 months of age by means of Random Regression Modeling (RRM). The outcome variables, cognitive and motor development, represent the measurements of cognitive and motor abilities as a function of time both at individual and population levels and can be referred to as a personal trend or change model (18). RRM has the advantage over and beyond MANOVA that missing data on the outcome variable is allowed, assuming the data are missing at random (MAR) (19). Moreover, time-varying and invariant covariates can be included in the models (20, 21). RRM allows more general and realistic error structures. In this study, the error structure is assumed to be unstructured which implies that both correlations between the measurements and variances within the measurements are allowed to be different.

The general analysis strategy started by exploring whether the time trend was linear, and whether the linear trend should be considered as fixed or random. Next, the exposure variable as well as confounding variables and, if relevant, effect modification terms (e.g. effect modification of PCB exposure by time was explored) were added as fixed terms to the RRM model. To address the first objective of the study, the unbiased effect of perinatal exposure to PCBs was estimated taking into account all relevant confounding variables and effect modification. To address the second objective of the study, to identify relevant determinants of cognitive and motor development, the analysis strategy was similar, except that nonsignificant variables were eliminated from the model. Details on the elimination procedure are presented below.

Preparation of the data for RRM consisted of the following adaptations: Motor scores at 42 and 84 months of age were transformed for data analysis into scores with a mean of 100 (± 15). Maternal plasma ΣPCB concentrations were positively skewed and were therefore normalized by natural logarithmic transformation (lnΣPCB). The HOME score was assessed at three moments of measurement, using two age-appropriate versions of the HOME. To use these scores in the analyses as a time-varying covariate, the mean and standard deviation of the scores at 42 and 84 months were adapted to the mean and standard deviation of the HOME score at 18 months.

In order to reduce the number of observed variables and to avoid the occurrence of multicollinearity, principal components analysis was performed. The two variables, type of feeding, breast-fed or formula-fed, and duration of breast-feeding (0 for formula-fed children) were combined to distinguish three feeding groups (formula-fed, breast-fed for 6-17 weeks (< 50th percentile), and breast-fed longer than 17 weeks (>50th percentile). Two dummy variables were used to represent these groups in the models.

In the initial RRM model the following variables were included: lnΣPCB, age at assessment (3, 7, 18, 42 and 84 months of age, to control for a linear time trend in the outcome variable), alcohol use (0/1= no/yes) and smoking (0/1= no/yes) during pregnancy, birth weight, gender (0/1=boy/girl), parity (0/1= fist/second born), maternal age, breast-feeding duration, HOME, parental education level (maximum level of either parent; 0=low = primary school, secondary school not finished; 1=middle = secondary school finished; 2=high = high school finished, professional and university training, entered as a continuous variable) and parental verbal IQ.

To eliminate skewness, the cognitive and motor development outcome variables were logarithmically transformed. Effects of the prenatal thyroid hormone status were explored by analyzing the four maternal thyroid hormone measurements separately.

Since at 84 months of age, effects of prenatal exposure to PCBs on cognitive and motor abilities were modified by maternal age at birth, parental education level, HOME score, and parental verbal IQ, these interaction variables were added simultaneously to the models. To avoid multicollinearity, these variables in addition to LnΣPCB were centered and their products were included in the models (i.e. LnΣPCB*Maternal age, LnΣPCB*Education level, LnΣPCB*HOME, LnΣPCB*Verbal IQ) (22). Centering of these variables decreased their intercorrelations discernibly.

To identify important determinants of cognitive and motor development, separate random regression models were applied to cognitive and motor development (backward elimination procedure, one by one ($p>0.05$, two-tailed)), while the linear time trend was included in the models throughout. Interaction variables and their main terms were hierarchically eliminated; the main term was not eliminated from the model before the interaction term with the main effect in it. Analyses were carried out with SAS version 8 for Windows. Results were considered significant if $p\leq0.05$ (two tailed).

Results

Three children were excluded from data analysis due to circumstances that were likely to influence cognitive and motor outcome other than perinatal PCB exposure (i.e. Pervasive Development Disorder, Turner syndrome, Volkmann contracture). Subjects with missing data on the determinants were not included in the analysis. The number of subjects included in the analyses, therefore, depended on the nonoccurrence of missing data on the particular variables in the models (see Table 3.1). Data on cognitive and motor abilities assessed from 3 to 84 months of age were available for all analyzable children (n=204), the mean cognitive and motor scores are presented in Table 3.2.

Table 3.1 Characteristics of all subjects included in the analyses (n=204).

Characteristics	
Birth weight (g)	3465 (± 447)
Fetal exposure to alcohol, yes	35 (17 %)
Fetal exposure to smoking, yes	48 (24 %)
Gender, boys	108 (53 %)
Maternal age (yr)	29 (18-39)
Feeding type, breast-fed	102 (50 %)
Breast-feeding duration (wk)	17 (6-72)
Parity, 1st born	100 (49 %)
Parental education	
Low	27 (13 %)
Middle	69 (34 %)
High	108 (53 %)
HOME	
18 months *(n=204)*	40.5 (± 2.6)
42 months *(n=190)*	39.2 (± 3.6)
84 months *(n=186)*	48.0 (± 3.2)
Parental verbal IQ *(n=190)*	121.7 (± 16.0)
Maternal thyroid levels	
FT_4 (pmol/L) *(n=203)*	11.6 (± 2.0)
TT_4 (nmol/L) *(n=203)*	157.5 (± 27.6)
TT_3 (nmol/L) *(n=203)*	2.5 (± 0.4)
TSH (µIU/ml *(n=203)*	1.4 (± 1.3)
Exposure variable	
ΣPCB (µg/L) *(n=203)*	p10=1.23 / p25=1.55 / p50=2.04 / p75=2.76 / p90=3.48

Values are numbers (percentages), means (± standard deviations) or medians (range).
Parentaleducation:low=primaryschool,secondaryschoolnotfinished,middle=secondaryschoolfinished,high=highschoolfinished, professionalanduniversitytraining;ParentalverbalIQ:scoreontwosubtestoftheWechslerAdultIntelligenceScale,InformationandVocabulary,assessedfromoneoftheparents;HOME:HomeObservationfortheMeasurementoftheEnvironment,version0-3years(18and42months)andversion3-6years(84months);FT_4:freethyroxine;TT_4:totalthyroxine;TT_3:totaltriiodothyronine;TSH:thyroidstimulatinghormone; ΣPCB: sum of PCB congeners IUPAC nos. 118, 138, 153, 180 assessed from maternal plasma.

Table 3.2 Mean cognitive and motor scores at 3, 7, 18, 42 and 84 months of age.

Cognition	
3 months (n=198)[a]	126.7 (± 12.8)
7 months (n=204)[a]	113.3 (± 10.0)
18 months (n=204)[a]	109.9 (± 17.5)
42 months (n=188)[b]	115.5 (± 13.8)
84 months (n=186)[c]	104.2 (± 12.8)
Motor	
3 months (n=196)[a]	117.4 (± 12.0)
7 months (n=204)[a]	113.2 (± 13.9)
18 months (n=203)[a]	109.0 (± 15.4)
42 months (n=188)[b]	113.9 (± 14.7)[d]
84 months (n=184)[c]	106.3 (± 14.8)[d]

The values are means ± standard deviations.
[a]Bayley Scales of Infant Development; [b]Kaufman Assessment Battery for Children; [c]McCarthy Scales of Children's Abilities; [d]transformed scores.

Initially, all selected variables of potential relevance to cognitive and motor development from 3 to 84 months of age were included in the RRM models in addition to a fixed linear time trend. There was no evidence of modification of effects of PCB exposure by the age of assessment. Higher prenatal exposure to PCBs was significantly related to a lower level of cognitive (β=-0.07, p=0.048) and motor development (β=-0.07, p=0.004), both adjusted for all other variables. Maternal thyroid hormone levels TT_4, FT_4, TT_3, and TSH were not related to cognitive and motor development when analyzed in separate models.

Table 3.3 Results of RRM analysis estimating effects of prenatal exposure on cognitive and motor development from 3 to 84 months of age, including the interaction variables simultaneously in the models.

	Cognitive development			Motor development		
	Regression coefficient	SE	p	Regression coefficient	SE	p
LnΣPCB	-0.033	0.013	0.014	-0.051	0.020	0.002
LnΣPCB*Maternal age	0.008	0.003	0.008	0.006	0.003	0.109
LnΣPCB*Education	-0.008	0.021	0.719	0.020	0.026	0.446
LnΣPCB*Verbal IQ	0.001	0.001	0.621	-0.001	0.001	0.561
LnΣPCB*HOME	0.002	0.005	0.681	0.006	0.005	0.275

Results of RRM analysis on repeated measurements of cognitive and motor abilities, natural logarithmic transformed, adjusted for: time trend, alcohol and smoking during pregnancy, birth weight, gender, parity and breast-feeding duration. LnΣPCB; natural logarithmic transformation of the sum of PCB congeners IUPAC nos. 118, 138, 153, 180 assessed from maternal plasma; Education; parental education level, 0=low; 1=middle; 2=high; Verbal IQ; parental verbal IQ; HOME=Home Observation for the Measurement of the Environment, version 0-3 years (18 and 42 months) and version 3-6 years (84 months).

Inclusion of the four interaction variables (i.e. LnΣPCB*Maternal age, LnΣPCB*Education level, LnΣPCB*HOME, LnΣPCB*Verbal IQ) showed that effects of prenatal exposure to PCBs on cognitive development were significantly modified by maternal age (Table 3.3).

Effect modification by the other parental and home environmental variables was not significant in this model (p>0.60). In regard to motor development, the PCB effects were not significantly modified by either of the four parental and home environmental variables in the full model.

Table 3.4 Results of RRM analysis estimating significant determinants of cognitive and motor development from 3 to 84 months of age.

	Cognitive development			Motor development		
	Regression coefficient	SE	p	Regression coefficient	SE	p
Intercept	4.770	0.006	<0.001	4.734	0.007	<0.001
Time	-0.002	<0.001	<0.001	-0.001	<0.001	<0.001
LnΣPCB	-0.031	0.013	0.014	-0.046	0.014	0.001
Maternal age	-0.001	0.001	0.523			
Education	0.039	0.009	<0.001	0.032	0.009	<0.001
Verbal IQ	0.001	<0.001	0.014			
HOME	0.010	0.002	<0.001	0.005	0.002	0.010
LnΣPCB*Maternal age	0.008	0.002	0.001			
LnΣPCB*HOME				0.009	0.004	0.025

RRM analysis on repeated measurements of cognitive and motor abilities, natural logarithmic transformed. Time: time trend; LnΣPCB: natural logarithmic transformation of the sum of PCBs (UPAC nos. 118, 138, 153 and 180, assessed from maternal plasma; Education: parental education level, 0=low; 1=middle; 2=high; VerbalIQ: parental verbal IQ; HOME: HOME Observation for the Measurement of the Environment, version 0-3 years (18 and 42 months) and version 3-6 years (84 months).

Backward elimination, one by one, of nonsignificant variables (p>0.05) from the RRM models, resulted in different models for cognitive and motor development (Table 3.4). For cognitive development, effects of prenatal PCB exposure were significantly modified by maternal age. Additionally, parental education level, HOME scores, and parental verbal IQ were identified as important determinants of cognitive development. In regard to motor development, effects of prenatal exposure to PCBs were significantly modified by the HOME scores. Additionally, parental education was identified as an important determinant of motor development. Moreover, for both cognitive and motor development, a significant negative linear time trend was seen.

Joint effects of the important determinants are visualized in Figure 3.1, presenting the estimated cognitive (a) and motor (b) development for children with either low or high levels of the appropriate determinants (i.e. $25^{th}/75^{th}$ percentile) relative to prenatal PCB levels. Effect modification of prenatal exposure to PCBs by maternal age and HOME score, respectively, on cognitive and motor development, is integrated in these figures. The estimated level of cognitive development is presented for children born to younger and older mothers ($25^{th}/75^{th}$ percentile). The level of motor development is presented for children raised by parents that had low and high HOME scores ($25^{th}/75^{th}$ percentile).

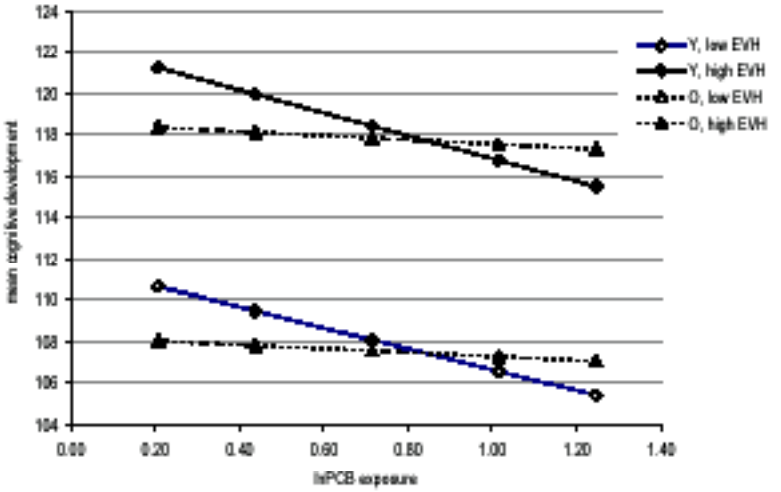

Figure 3.1a Mean cognitive development from 3 to 84 months of age in relation to prenatal PCB exposure for children born to younger (Y) or older (O) mothers with low or high levels of parental education (E), verbal IQ (V) and HOME score (H), (EVH).

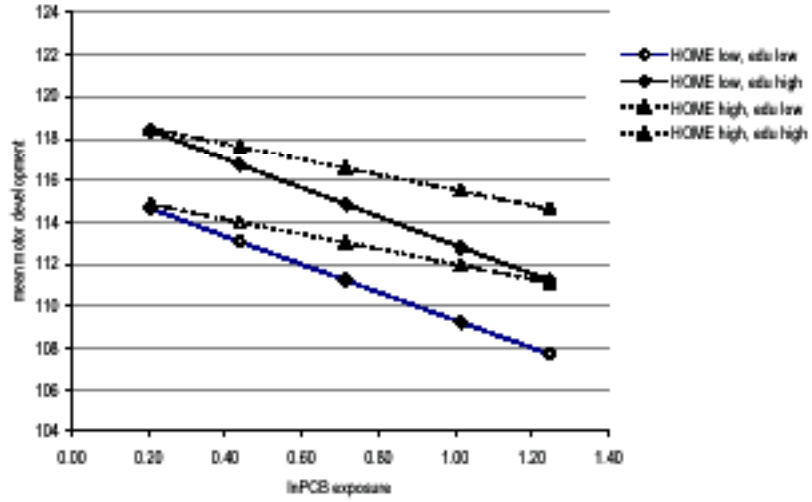

Figure 3.1b Mean motor development from 3 to 84 months of age in relation to prenatal PCB exposure for children with low or high levels of parental education (edu) and HOME score.

In children born to younger mothers, the estimated level of cognitive development of high prenatally exposed children (75th percentile) was approximately 3 points lower than in low exposed children (25th percentile), whereas in children born to older mothers, this estimated difference was approximately ½ a point. A difference of approximately 10 points was seen between children raised in low and high parental and home environmental conditions (i.e. low or high parental education level, HOME scores, and parental verbal IQ).

In regard to motor development, high exposed children with low HOME scores scored approximately 4 points lower than their low exposed counterparts, whereas a difference of 2 points was estimated for low and high exposed children with high HOME scores. In children raised by higher educated parents, the level of motor development was approximately 4 points higher than in children with lower educated parents.

Discussion

This is the first empirical investigation of neurotoxic effects of PCBs on the longitudinal development of cognitive and motor abilities. Analyses of cognitive and motor abilities at separate ages showed inconsistencies of effects of prenatal PCB exposure in the Dutch PCB/dioxin project. In contrast to the studies so far, we have analyzed effects of prenatal exposure to PCBs on the development of cognitive and motor abilities of the individual child. Higher prenatal exposure levels were related with a lower level of cognitive and motor development. Moreover, effects of prenatal exposure to PCBs on cognitive development were modified by maternal age, suggesting that higher prenatal exposure levels were related with more pronounced negative effects in children born to younger mothers than in children born to older mothers.

At 84 months of age, effects of prenatal exposure to PCBs on cognitive abilities were similarly modified by maternal age, as well as by parental education, parental verbal IQ and HOME score, when these effect modifications were studied in separate regression models. In the present study, centering of the relevant variables allowed us to include these effect modification terms simultaneously in the models. The effect modification of prenatal exposure to PCBs on cognitive development by maternal age overruled effect modification by the other parental and home environmental variables. In the Dutch cohort, older maternal age is related to higher education levels, higher verbal IQs and higher scores on the HOME environment questionnaire. Maternal age is also likely to reflect other aspects of social economic conditions as well as psychosocial age-related attributes (23).

In this study, we additionally aimed to identify important determinants of cognitive and motor development. Cognitive and motor development appeared to have different important determinants. Cognitive development was estimated more efficiently by parental and home environmental conditions that are more related to cognitive genetic or stimulating aspects, whereas motor development was predicted efficiently by generally stimulating home environments. In the predictive model for motor development from 3 to 84 months of age, effects of prenatal PCB levels were modified by the HOME scores. This is in agreement with the results of the previous study in the Dutch cohort at 84 months of age in which the HOME environment was also the most pronounced modifier of the effect prenatal exposure on motor development.

These results suggest that neurotoxic effects of prenatal exposure to PCBs can be masked or compensated by conditions that can be considered to be relatively more favorable to child development. These results are in line with animal studies that show a positive impact of an enriched environment on brain development and on effects of brain lesions (24). In human studies, comparable evidence of effect modification by parental and home environmental conditions on neurodevelopment has been reported in some of the studies that address effects of perinatal exposure to lead (25, 26) and methyl mercury (27) as well as in follow-up studies in very low birth weight children (28-30). It should be stressed here that the study population consists of families that were motivated to participate in this study for a long period. Parental and home characteristics of this group are likely to be more advantaged than in the average Dutch population. These results suggest that effects of exposure to these environmental pollutants on cognitive and motor abilities might be more pronounced in less advantaged populations.

The Figures showed that the estimated negative effects of prenatal exposure to PCBs on cognitive development were relatively small compared to the positive effects of higher parental education levels, verbal IQs and higher HOME scores. In these models, effects of prenatal exposure to PCBs on motor development were more pronounced than on cognitive development. Moreover, the magnitude of the negative effects of prenatal exposure on motor development in children with low HOME scores was equal to that of the positive effect of higher parental education levels. This may suggest that motor development is more vulnerable to prenatal exposure to PCBs than cognitive abilities. However, it may also reflect the complexity of the cognitive outcome variable that is influenced by a relatively broader spectrum of socio-parental factors compared to motor development. Motor development may, therefore, be a more sensitive outcome measurement in risk assessment studies of prenatal exposure to PCBs. Figure 3.1a. illustrated some of the complexity of the cognitive outcome. Older maternal age is likely to reflect favorable conditions to cognitive development and these results also suggest that older maternal age masks or counteracts cognitive effects of prenatal exposure to PCBs. However, based on the present model, at lower levels of prenatal PCB exposure children born to older mother had a lower estimated cognitive development than children born to younger mothers. The current knowledge of the factors affecting cognitive development as well as on the characteristics of mothers at older age that give birth to children is too limited to explain this finding.

Cognitive and motor abilities were measured with different tests at different assessment ages. The Dutch versions of the Bayley and the K-ABC have been standardized in Dutch populations in respectively 1976 (13), and 1989 (14). For the McCarthy scales of Children's Abilities only American norms were available (31). The negative linear time trend, therefore, can be attributed to standardization of the different tests, and should not be interpreted as a decrease in growth of cognitive and motor abilities over time. The linear time trend in

the model can therefore be understood as an adjustment for the different test materials that were used over the years.

Different neurotoxic mechanisms are suggested for the planar dioxin and dioxin-like PCB compounds and the weak dioxin-like or nondioxin-like PCB compounds. Toxicity of dioxins and dioxin-like PCB compounds appears to be mediated by the Ah receptor. The toxic potency of a these compounds is reflected in a toxic equivalent factor (TEF), based on its ability to bind the Ah receptor relative to the binding ability of the most potent dioxin, TCDD. For nondioxin-like, or nonplanar, PCBs the ability of the TEF to predict their neurotoxic potency is low. In the last decade there is growing evidence that especially nondioxin-like PCBs and weak dioxin-like PCBs may produce a wide spectrum of neurotoxic effects, while dioxin-like PCBs may have less activity in the CNS. The four PCB congeners (IUPAC numbers 118, 138, 153, and 180) that maternal plasma samples were analyzed for are nonplanar PCB congeners of which PCB 118 is considered to be a weak dioxin-like compound. Environmental mixtures contain mostly nonplanar PCB congeners (32). The four PCB congeners that were assessed in maternal plasma are among the four most abundant PCB congeners, constituting 46% of the total PCBs (33). For the study population, multiple prenatal exposure data on PCBs and dioxins were available. Apart from the sum of four PCBs in maternal plasma during pregnancy, the sum of the four PCBs was measured in cord blood and dioxins, dioxin-like PCBs, and 20 nondioxin-like PCBs were assessed in breast milk. PCB levels in maternal and cord blood, and total TEQ (Toxic Equivalent) levels of dioxins and dioxin-like PCBs were highly interrelated (11). Therefore, whether the observed effects are due to PCBs or other (related) contaminants, such as dioxins, is uncertain. In this study we used maternal PCB levels to estimate prenatal exposure to PCBs since this prenatal exposure variable showed the strongest relation with cognitive and motor development at different time points (8, 9).

Although a breast-fed child is exposed to considerable amounts of PCBs through lactation, negative neurodevelopmental effects of lactational exposure to PCBs have hardly been detected (3, 6, 7, 34). In the Rotterdam cohort, negative effects of lactational exposure were only detected on psychomotor abilities at 7 months of age. The results of the study at 84 months of age did not show evidence of effect modification of lactational exposure by parental and home environmental conditions. Generally, the exploration of effects of lactational exposure is complicated by a number of aspects. First, the amount of lactational exposure can only be estimated by the product of duration of breast-feeding and exposure levels in breast milk, or other maternal body burden indicators such as exposure levels in maternal blood. The duration of breast-feeding it self is likely to reflect not only the duration of exposure to PCBs but also exposure to substances that may be important for optimal brain development. In the present study, the duration of breast-feeding was not related to cognitive or motor development. We also explored whether lactational exposure, derived from the product of the PCB levels in maternal plasma and the duration of breast-

feeding, was related to cognitive and motor development. These preliminary analyses did not suggest negative effects of lactational exposure to PCBs. Secondly, in the Dutch cohort, parents that choose to breast-feed their child are often higher educated and have higher verbal IQs and higher HOME scores than parents that choose to feed their child with formula milk. Therefore, the exploration of interrelations of lactational exposure, parental and home environmental conditions and neurodevelopment may benefit from more sophisticated statistical modeling techniques.

In our cohort, higher levels of PCBs and dioxins in breast milk were related to lower maternal TT_3 and TT_4 plasma levels, although all levels were within the normal range, except for one mother who appeared to have an autoimmune hypothyroidism with high TSH levels (35). In the present study, maternal thyroid hormone status was not related to cognitive and motor development. These results, therefore, are not suggestive of thyroid hormone involvement in the neurotoxic effect of PCBs on cognitive and motor development. A different statistical procedure, however, could be more proper to explore in more depth potential mediation by thyroid hormones in effects of exposure to PCB on cognitive and motor development. Moreover, hormonal involvement in the mechanism of neurotoxic PCB effects may be more pronounced using more specific neuropsychological abilities as outcome variables.

In conclusion, the results of the present study give evidence for negative effects of prenatal exposure to environmental levels of PCBs and related compounds, such as dioxins, on cognitive and motor development in a normal population of Dutch children. RRM is a very useful method to evaluate effects of prenatal PCB exposure on cognitive and motor development. These results suggest neurotoxic effects of prenatal PCB exposure on the developing brain that can be modified by parental and home environmental conditions. To increase our knowledge of potential consequences for human development, the neurotoxic mechanism should be studied in more depth in animals as well as in humans. Compared to the positive effects of more optimal parental and home environmental conditions, the negative effect of prenatal PCB exposure on cognitive development is relatively small. Effects of prenatal PCB exposure on motor development were more pronounced and this outcome may serve as a more sensitive tool to assess neurodevelopment risks of exposure to PCBs. Considering that only one class of neurotoxic agents is addressed in this study, these results emphasize efforts to reduce environmental levels of these contaminants and other related compounds to reduce maternal body burdens and lower fetal exposure to these neurotoxic compounds.

References

1. Brouwer A, Ahlborg UG, Van den Berg M, Birnbaum LS, Boersma ER, Bosveld B, et al. Functional aspects of developmental toxicity of polyhalogenated aromatic hydrocarbons in experimental animals and human infants. Eur J Pharmacol 1995;293(1):1-40.
2. Weisglas-Kuperus N. Neurodevelopmental, immunological and endocrinological indices of perinatal human exposure to PCBs and dioxins. Chemosphere 1998;37(9-12):1845-53.
3. Rogan WJ, Gladen BC. PCBs, DDE, and child development at 18 and 24 months. Ann Epidemiol 1991;1(5):407-13.
4. Gladen BC, Rogan WJ. Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. J Pediatr 1991;119(1):58-63.
5. Gladen BC, Rogan WJ, Hardy P, Thullen J, Tingelstad J, Tully M. Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. J Pediatr 1988;113(6):991-5.
6. Jacobson JL, Jacobson SW, Humphrey HE. Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J Pediatr 1990;116(1):38-45.
7. Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychorinated biphenyls in utero. N Engl J Med 1996;335:783-9.
8. Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MA, Van der Paauw CG, Tuinstra LG, Sauer PJ. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics 1996;97(5):700-6.
9. Patandin S, Lanting CI, Mulder PG, Boersma ER, Sauer PJ, Weisglas-Kuperus N. Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J Pediatr 1999;134(1):33-41.
10. Vreugdenhil HJ, Lanting CI, Mulder PG, Boersma ER, Weisglas-Kuperus N. Effects of prenatal PCB and dioxin background exposure on cognitive and motor abilities in Dutch children at school age. J Pediatr 2002;140(1):48-56.
11. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N, Van der Pauw CG, Tuinstra LGMT, Boersma ER, et al. PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 1994;28(9):1721-1732.
12. Bayley N. Manual for the Bayley scales of infant development. New York: The psychological corporation; 1969.
13. Van der Meulen BF, Smorkovsky M. BOS 2-30: Bayley Ontwikkelings-schalen. Lisse, The Netherlands: Swets and Zeitlinger; 1983.
14. Neutel RJ, van der Meulen BF, Lutje Spielber HJ. Groningse Ontwikkelingsschalen, Dutch version of the Kaufman Assessment Battery for Children (K-ABC). Lisse: Swets & Zeitlinger BV, The Netherlands; 1996.
15. Van der Meulen BF, Smorkovsky M. MOS 2 1/5- 8 1/2 McCarthy Ontwikkelingsschalen. Lisse, The Netherlands: Swets & Zeitlinger B.V.; 1985.
16. Stinissen J, Willems PJ, Coetsier P, Hulsman WLL. Manual, Dutch version of the Wechsler Adult Intelligence Scale (WAIS). Amsterdam: Swets & Zeitlinger NV; 1970.
17. Caldwell B, Bradley R. Home observation of the environment. Administration manual. Little Rock, Arkansas; 1984.
18. Gibbons RD, Hedeker D, Elkin I, Waternaux C, Kraemer HC, Greenhouse JB, et al. Some conceptual and statistical issues in analysis of longitudinal psychiatric data. Application to the NIMH treatment of Depression Collaborative Research Program dataset. Arch Gen Psychiatry 1993;50(9):739-50.
19. Little RJA, Rubin DB. Statistical analysis with missing data. New York: Wiley; 1987.
20. Gibbons RD, Hedeker D, Waternaux C, Davis JM. Random regression models: a comprehensive approach to the analysis of longitudinal psychiatric data. Psychopharmacol Bull 1988;24(3): 438-43.

21. Hedeker D, Gibbons RD, Waternaux C, Davis JM. Investigating drug plasma levels and clinical response using random regression models. Psychopharmacol Bull 1989;25(2):227-31.
22. Cohen J, Cohen P. Applied multiple regression/correlation analysis for the behavioral sciences. Hillsdale, New Jersey: Lawrence Erlbaum Associated Publishers; 1983.
23. Stein ZA. A woman's age: childbearing and child rearing. Am J Epidemiol 1985;121(3):327-42.
24. Rosenzweig MR, Bennett EL. Psychobiology of plasticity: effects of training and experience on brain and behavior. Behav Brain Res 1996;78(1):57-65.
25. Bellinger D, Leviton A, Waternaux C, Needleman H, Rabinowitz M. Low-level lead exposure, social class, and infant development. Neurotoxicol Teratol 1988;10(6):497-503.
26. Winneke G, Kraemer U. Neuropsychological effects of lead in children: interactions with social background variables. Neuropsychobiology 1984;11(3):195-202.
27. Davidson PW, Myer GJ, Shamlaye C, Cox C, Gao P, Axtell C, et al. Association between prenatal exposure to methylmercury and developmental outcomes in Seychellois children: effect modification by social and environmental factors. Neurotoxicology 1999;20(5):833-41.
28. Weisglas-Kuperus N, Baerts W, Smrkovsky M, Sauer PJ. Effects of biological and social factors on the cognitive development of very low birth weight children. Pediatrics 1993;92(5):658-65.
29. Pfeiffer SI, Aylward GP. Outcome for preschoolers of very low birthweight: sociocultural and environmental influences. Percept Mot Skills 1990;70:1367-78.
30. Landry SH, Denson SE, Swank PR. Effects of medical risk and socioeconomic status on the rate of change in cognitive and social development for low birth weight children. J Clin Exp Neuropsychol 1997;19(2):261-74.
31. McCarthy D. McCarthy Scales of Children's Abilities, Manual: The Psychological Corporation; 1972.
32. Kodavanti PR, Ward TR. Interactive effects of environmentally relevant polychlorinated biphenyls and dioxins on [3H]phorbol ester binding in rat cerebellar granule cells. Environ Health Perspect 1998;106(8):479-86.
33. Jacobson JL, Humphrey HE, Jacobson SW, Schantz SL, Mullin MD, Welch R. Determinants of polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), and dichlorodiphenyl trichloroethane (DDT) levels in the sera of young children. Am J Public Health 1989;79(10): 1401-4.
34. Darvill T, Lonky E, Reihman J, Stewart P, Pagano J. Prenatal exposure to PCBs and infant performance on the fagan test of infant intelligence. Neurotoxicology 2000;21(6):1029-38.
35. Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lutkeschipholt IJ, Van der Paauw CG, Tuinstra LG, et al. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatr Res 1994;36(4):468-73.

4 Neurodevelopmental effects of prenatal exposure to PCBs and other important determinants of cognitive and motor development; a structural equation modeling study

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Submitted

Abstract

The aim of the study was to explore the interrelationships of parental and home environmental variables and cognitive and motor development by means of structural equation modeling (SEM) and to explore differences in this model to be attributed to prenatal exposure to PCB and maternal age. In the Rotterdam cohort (n=207) cognitive and motor abilities were assessed at 3, 7, 18, 42, and 84 months of age. Prenatal exposure to PCBs was estimated by the sum of PCB118, 126, 138, 153 in maternal plasma. The interrelationships of cognitive and motor development and their determinants, parental education level and verbal IQ and HOME scores, were modeled using SEM. Subsequently, the total population was divided in four subgroups, based on prenatal PCB levels (low/high) and maternal age (</ ≥ 29 years). These groups were compared on both the level and the interrelationships of the determinants and outcome variables. The level of cognitive/motor outcome and their determinants, as well as the relations between determinants and cognitive outcome variables were different for the groups. Higher prenatal exposure was associated with larger decrements in cognitive and motor development in children raised under lower parental and home environmental conditions. For 'early' cognitive and motor development (3, 7, 18 months of age) this difference was more pronounced in children born to *older* mothers, for 'late' cognitive and motor development (42, 84 months of age) in children born to *younger* mothers.

Conclusion: These results suggest complex effects of maternal age and other parental and home environmental conditions on the neurotoxic mechanism of PCBs and related neurotoxic compounds.

Introduction

Polychlorinated biphenyls (PCBs) and dioxins are environmental pollutants that are lipophylic and are not degraded easily. Due to these properties, they are ubiquitous in the food chain and detectable in humans in most populations that have been examined. PCBs and dioxins are known for their potential neurotoxic effects, especially when the developing central nervous system (CNS) is exposed to these compounds. PCBs and dioxins are able to cross the placenta, exposing the fetus to maternal PCB and dioxin body burdens. Moreover, a breast-fed infant is exposed to relatively large amounts of PCBs and dioxins.

Neurotoxic effects of predominantly prenatal and to a lesser extent lactational exposure to environmental levels of PCBs have been described on cognitive and motor abilities at several ages in several cohort studies (1-9). A common feature of these epidemiological studies is that subjects were not randomly assigned to predetermined levels of exposure and that samples were based on volunteer mother-infant pairs. Above-mentioned studies, therefore, have made efforts to identify potential confounding variables when studying relations between neurodevelopmental outcome and perinatal exposure to PCBs and dioxins.

In the Netherlands, neurodevelopmental effects of perinatal exposure to environmental levels of PCBs and dioxins have been prospectively evaluated in healthy children from birth to school age (1, 2, 8, 9). Half of the Dutch PCB/dioxin cohort was breast-fed during infancy, and the other half was formula-fed. In this cohort, complex interrelationships of confounding, exposure and outcome variables have been observed. At school age, effects of prenatal exposure to PCBs on cognitive and motor abilities were related to parental and home environmental conditions. In children who were born to younger mothers or who were raised by parents who were either less educated or had lower verbal IQs, or HOME scores, negative effects of prenatal exposure on cognitive and motor abilities were detected. However, if these conditions were more favorable to child development, effects of prenatal exposure to PCBs were not detectable (9). Random regression analysis of cognitive and motor development from 3 to 84 months of age showed similar modification of the effects of prenatal exposure to PCBs by parental and home environmental levels (8). In that study we aimed to explore effects of prenatal exposure to PCBs on the development of cognitive and motor abilities as well as to identify important determinants of these outcomes. For cognitive development, prenatal PCB levels, and its modification by maternal age, along with parental education level and verbal IQ and HOME scores were important determinants. Motor development was efficiently estimated by prenatal PCB levels including its modification by HOME scores and by parental education levels (8).

Maternal age plays a highly complex role in neurodevelopmental risk assessment of perinatal exposure to PCBs since it is positively associated with prenatal PCB body burden as well as with parental education level, parental verbal IQ and the HOME score. In the

random regression analysis study, the modification of cognitive effects of prenatal exposure to PCBs by maternal age overruled effect modification by the other candidate effect modifiers (i.e. parental education level, parental verbal IQ, and HOME score) (8). Structural modeling of these variables may contribute to disentangle the complex interrelationships of these determinants of cognitive and motor development when studying PCB related neurodevelopmental effects. Therefore, the first objective of this study was to identify the interrelationships of parental education level, parental verbal IQ, and HOME score and cognitive and motor development, assessed from 3 to 84 months of age, by the method of structural equation modeling. The second objective was to explore, once the most plausible model was identified, whether high level of prenatal PCB exposure along with maternal age can be differentiated from low level of exposure on this model.

Methods

Subjects

In 1990, a prospective follow-up study in healthy children was started in Rotterdam, The Netherlands. The study population comprised 207 Caucasian mother-infant pairs, recruited between 1990 and 1992. The study design and recruitment process, chemical analysis and PCB concentrations have been described in detail elsewhere (10). Only first or second, term born healthy children were included. One hundred and five children were exclusively breast-fed for at least 6 weeks and 102 children were fed with formula milk from a single batch (Almiron M2, Nutricia NV, Zoetermeer, The Netherlands) from birth until 7 months of age. In this formula, concentrations of PCBs were undetectable. The medical ethics committee of the University Hospital Rotterdam/ Sophia Children's Hospital approved the study design and the parents signed informed consent.

Assessment of exposure variables

Plasma samples were collected from the mothers during the last month of pregnancy and cord plasma samples were collected directly after birth. These samples were analyzed for four PCB congeners, International Union for Pure and Applied Chemistry (IUPAC) numbers 118, 138, 153 and 180 (10, 11). Two weeks after delivery, a 24-hour representative breast milk sample was collected from the mothers who were breast-feeding their children. Breast milk samples were analyzed for 17 dioxins (PCDDs and PCDFs), 6 dioxin-like PCBs (IUPAC numbers 77, 105, 118, 126, 156, and 169), and 20 nondioxin like PCBs (IUPAC numbers 28, 52, 66, 70, 99, 101, 128, 137, 138, 141, 151, 153, 170, 177, 180, 183, 187, 194, 195, and 202) (10).

In the present study, we compared four groups distinguished by prenatal exposure level and maternal age at birth of the child. Assignment to these groups was based on the sum of the concentrations of the four PCB congeners measured in maternal plasma ($\Sigma PCB_{maternal}$). Children in which $\Sigma PCB_{maternal}$ levels were < median level (2.04µg/L) were assigned to the low exposed group (PCB_{low}), and children in which $\Sigma PCB_{maternal}$ levels were ≥ median to the high exposed group (PCB_{high}). Subsequently, these exposure groups were dichotomized at the median of maternal age at birth (< 29 years = M_{young}; ≥ 29 years = M_{old}) resulting in the following four groups: PCB_{low}/M_{young}; PCB_{low}/M_{old}; PCB_{high}/M_{young}; PCB_{high}/M_{old}.

Outcome variables

The children were invited to participate in cognitive and motor assessments at 3, 7, 18, 42 and 84 months of age. Cognitive and motor abilities at 3, 7 and 18 months were assessed by the mental developmental index and psychomotor developmental index of the Dutch version of the Bayley Scales of Infant Development (12) (BOS 2-30) (13). At 42 months of age, cognitive abilities were assessed by the combined score of the sequential and simultaneous processing scales of the Dutch version of the Kaufman Assessment Battery for Children (Dutch K-ABC) (14). Motor abilities at 42 months of age were measured by the sum of the standardized scores of two subtests of the Dutch K-ABC: gross and fine motor skills. At 84 months of age, the general cognitive index and the motor score of the Dutch version of the McCarthy Scales of Children's Abilities (15) assessed cognitive and motor abilities, respectively. All developmental scores were derived from tests that have been standardized and scores were, except for the motor scores at 42 and 84 months, transformed into scores with a mean (± SD) of 100 (± 15). The motor score at 42 months of age is a sum score of two subtests after being normalized to a mean of 10 (± 3). The motor score at 84 months of age was normalized into a mean score of 50 (± 10).

Determinants

Parental education was estimated as the highest education level of the parents (low = primary school, secondary school not finished; middle = secondary school finished; high = high school finished, professional and university training). Parental verbal IQ was assessed by two subtests, Information and Vocabulary from the Dutch version of the Wechsler Adult Intelligence Scale (WAIS) (16) in the parent that spent the most time with the child, usually the mother. The quality of intellectual stimulation and emotional support provided by the child's home environment was assessed by the Dutch version of the Home Observation for Measurement of the Environment (HOME) (17) at 18, and 42 months of age (version 0-3 years), and 84 months of age (version 3-6 years).

Data analysis

The analyses for constructing structural equation models (SEM) were conducted with the M-Plus program for Windows (version 2.01). Parameters were estimated by using the method of maximum likelihood, based on the covariance matrix of the observed variables. Any SEM may be expressed as a set of relations between independent and dependent exogenous and endogenous variables, and as (co)variances of independent variables. SEM involves identification and estimation of parameters representing relations, variances and covariances for variables in a postulated or implied model. The aim was that the postulated or implied model was the most plausible in representing the observed covariances of all included variables. Plausibility was based on statistical criteria and theoretical and clinical knowledge on cognitive and motor development. Statistical plausibility comprised the following performance measures: (1) χ^2 (including degrees of freedom, and p-value): a nonsignificant value indicates that the model at issue is not rejected; (2) χ^2 /df: a value ≤ 1.5 indicates a good fit; (3) Comparative Fit Index (CFI) (18) (range 0-1): a value greater than 0.95 is indicative of a good fitting model; (4) Tucker Lewis Index (TLI) (19): a value approximating 1 indicates a good model fit (5) root mean square error of approximation (RMSEA) (20, 21): a value of 0.05 indicates a close fit and (6) standardized root mean square residual (SRMR): a value <0.05 suggests adequate fit. Moreover, we applied the principle of 'parsimonious' modeling: if two models, a simple and a more complicated, are equally plausible, the more simple model is preferred.

Modeling procedure

Motor scores at 42 and 84 months of age were transformed for data analysis into scores with a mean of 100 (±15). The HOME score was assessed at three measurement moments, using two age appropriate versions of the HOME. The mean and standard deviation of the scores at 42 and 84 months of age were adapted to the mean and standard deviation of the HOME18 score.

Preceding the modeling procedure, missing data analysis and replacement was performed, based on multiple regression analysis in which a random component (i.e. a residual from a randomly selected complete subject) was added (22).

Cognitive abilities, assessed at 3,7,18, 42, and 84 months of age, were combined in two scores: $C_{3, 7, 18}$ (the mean of the scores at 3, 7, and 18 months of age) and $C_{42, 84}$ (the mean of the scores at 42 and 84 months of age). The motor scores, assessed at 3, 7, 18, 42, and 84 months of age, were similarly grouped: $M_{3, 7, 18}$ and $M_{42, 84}$. The HOME scores at 18, 42, and 84 months of age were combined in two scores, comparable to the developmental data: $HOME_{18}$ and $HOME_{42, 84}$ (the mean score on the HOME at 42 and 84 months of age).

Based on clinical knowledge we developed a baseline model in which the paths from $C_{3, 7, 18}$ to $C_{42, 84}$ and from $M_{3, 7, 18}$ to $M_{42, 84}$ were included as well as paths from $C_{3, 7, 18}$ to $M_{3, 7, 18}$

and from $C_{42, 84}$ to $M_{42, 84}$ and paths for both cognitive variables from the age-appropriate HOME variable ($HOME_{18}$ and $C_{3, 7, 18}$; and $HOME_{42,84}$ and $C_{42, 84}$). The covariances between the determinants parental education and parental verbal IQ were permitted to be free, and for simplification of modeling we decided to model these two variables simultaneously. In Table 4.3 the process of modeling is described in the total study population. The most plausible model was used for the next step in the modeling procedure: model fit comparison of the four groups (PCB_{low}/M_{young}; PCB_{low}/M_{old}; PCB_{high}/M_{young}; PCB_{high}/M_{old}).

Group comparison was done by applying constraints of equality across the four groups on three parameters: (a) the level of the developmental outcome variables ($C_{3, 7, 18}$ and $C_{42, 84}$ and of $M_{3, 7, 18}$ and $M_{42, 84}$), (b) the level of the determinants (parental education and verbal IQ, and HOME), and (c) the regression coefficients between the determinants and outcome variables. Eight combinations of constraints were possible, reflecting eight models. By comparing the fit of these models we explored group differences in the three parameters.

Results

Three children of the total population of 207 children were excluded from data analysis due to circumstances that were known to influence cognitive and motor outcome (Pervasive Development Disorder, Turner syndrome, Volkmann contracture).

The characteristics of the four subgroups are presented in Table 4.1. In Table 4.2, the Pearson intercorrelation matrix of the variables that were used in the models are presented.

Table 4.1 Characteristics of the four groups.

Characteristics	PCB_{low}/M_{young} (n=63)	PCB_{low}/M_{old} (n=35)	PCB_{high}/M_{young} (n=30)	PCB_{high}/M_{old} (n=75)
$C_{3, 7, 18}$[a]	116.4 (± 8.5)	115.8 (± 8.2)	116.7 (± 8.2)	117.5 (± 9.6)
$C_{42, 84}$[a]	106.2 (± 10.6)	110.8 (± 10.6)	107.4 (± 11.5)	112.7 (± 12.8)
$M_{3, 7, 18}$[b]	114.6 (± 9.2)	112.7 (± 8.2)	116.1 (± 7.6)	111.1 (± 10.7)
$M_{42, 84}$[b]	109.6 (± 11.0)	110.6 (± 12.4)	107.6 (± 11.5)	111.4 (± 12.6)
$HOME_{18}$	39.8 (± 2.7)	40.3 (± 2.5)	40.1 (± 3.1)	41.3 (± 2.2)
$HOME_{42, 84}$	39.7 (± 2.3)	40.8 (± 1.7)	39.9 (± 2.6)	41.0 (± 2.0)
Education	1.1 (± 0.8)	1.4 (± 0.7)	1.4 (± 0.6)	1.7 (± 0.6)
Verbal IQ	115.5 (± 17.1)	119.6 (± 15.1)	124.1 (± 13.2)	126.7 (± 14.2)

Numbers are means (± standard deviations). [a]C=Mean cognitive scores at 3,7,18 months of age ($C_{3,7,18}$) and at 42 and 84 months of age ($C_{42,84}$); [b]M=Mean motor scores at 3,7,18 months of age ($M_{3,7,18}$) and at 42 and 84 months of age ($M_{42,84}$). $HOME_{18}$ and $HOME_{42,84}$=HomeObservation for the Measurement of the Environment assessed at 18 months of age ($HOME_{18}$) and the mean of the transformed scores (to $HOME_{18}$ mean (SD)) on the HOME assessed at 42 and 84 months of age; Education: parental education level, 0=low=primary school, secondary school not finished, 1=middle=secondary school finished, 2=high=high school finished, professional and university training; VerbalIQ: parental verbal IQ, score on two subtests of the Wechsler Adult Intelligence Scale, Information and Vocabulary, assessed from one of the parents.

Table 4.2 Pearson intercorrelations of predictor and outcome variables.

4.2a Low prenatally PCB exposed group, maternal age < 29 years (M_{young})

		1	2	3	4	5	6	7
1	$C_{3, 7, 18}$[a]	1.00						
2	$C_{42, 84}$[a]	0.50	1.00					
3	$M_{3, 7, 18}$[b]	0.44	0.37	1.00				
4	$M_{42, 84}$[b]	0.23	0.61	0.30	1.00			
5	$HOME_{18}$	0.43	0.28	0.06	0.06	1.00		
6	$HOME_{42, 84}$	0.36	0.44	0.09	0.16	0.62	1.00	
7	Education	0.39	0.49	0.11	0.21	0.37	0.66	1.00
8	Verbal IQ	0.30	0.32	0.22	0.06	0.50	0.66	0.71

4.2b Low prenatally PCB exposed group, maternal age ≥ 29 years (M_{old})

		1	2	3	4	5	6	7
1	$C_{3, 7, 18}$[a]	1.00						
2	$C_{42, 84}$[a]	0.13	1.00					
3	$M_{3, 7, 18}$[b]	0.13	-0.23	1.00				
4	$M_{42, 84}$[b]	0.27	0.66	0.05	1.00			
5	$HOME_{18}$	0.14	0.19	-0.09	0.19	1.00		
6	$HOME_{42, 84}$	0.05	0.49	-0.15	0.13	0.20	1.00	
7	Education	0.10	0.50	0.01	0.36	0.40	0.55	1.00
8	Verbal IQ	0.14	0.42	0.05	0.37	0.41	0.23	0.49

4.2c High prenatally PCB exposed group, maternal age < 29 years (M_{young})

		1	2	3	4	5	6	7
1	$C_{3, 7, 18}$[a]	1.00						
2	$C_{42, 84}$[a]	0.48	1.00					
3	$M_{3, 7, 18}$[b]	0.02	0.14	1.00				
4	$M_{42, 84}$[b]	0.35	0.66	0.49	1.00			
5	$HOME_{18}$	0.17	0.05	0.01	0.01	1.00		
6	$HOME_{42, 84}$	0.28	0.43	-0.07	0.31	0.70	1.00	
7	Education	0.23	0.50	-0.06	0.24	0.03	0.30	1.00
8	Verbal IQ	0.11	0.65	0.20	0.43	-0.13	0.30	0.59

4.2d High prenatally PCB exposed group, maternal age ≥ 29 years (M_{old})

		1	2	3	4	5	6	7
1	$C_{3,7,18}$[a]	1.00						
2	$C_{42,84}$[a]	0.54	1.00					
3	$M_{3,7,18}$[b]	0.52	0.41	1.00				
4	$M_{42,84}$[b]	0.62	0.66	0.55	1.00			
5	$HOME_{18}$	0.36	0.38	0.26	0.25	1.00		
6	$HOME_{42,84}$	0.24	0.49	0.15	0.26	0.54	1.00	
7	Education	0.37	0.52	0.27	0.29	0.19	0.32	1.00
8	Verbal IQ	0.25	0.59	0.14	0.11	0.37	0.53	0.54

[a]C=Meancognitivescoresat3,7,18monthsofage($C_{3,7,18}$)andat42and84monthsofage($C_{42,84}$),[b]M=Meanmotorscoresat3,7,18months ofage($M_{3,7,18}$)andat42and84monthsofage($M_{42,84}$),HOME$_{18}$andHOME$_{42,84}$=HomeObservationfortheMeasurementoftheEnvironment assessedat18monthsofage(HOME$_{18}$)andthemeanofthetransformedscores(toHOME$_{18}$mean(SD))ontheHOMEassessedat42and84 monthsofage;Education:parentaleducationlevel,0=low=primaryschool,secondaryschoolnotfinished,1=middle=secondaryschool finished,2=high=highschoolfinished,professionalanduniversitytraining;VerbalIQ:parentalverbalIQ,scoreontwosubtestsoftheWechsler Adult Intelligence Scale, Information and Vocabulary, assessed from one of the parents.

The modeling in the total group is presented in Table 4.3. The model building procedure showed that Models 5 and 6 (Table 4.3) were most plausible. These models are essentially similar. For group comparison we decided to use Model 6 since this model includes paths between the variables parental education level and verbal IQ and both HOME variables, which is more consistent than permitting one of these relations to be free as in Model 5. The interrelationships of the variables in Model 6 (Table 4.3) are presented as standardized regression coefficients in a path diagram (Figure 4.1). All interrelations were significant, except for the relation between parental verbal IQ and $C_{3,7,18}$, which was only indirectly related to $C_{3,7,18}$, via $HOME_{18}$.

Subsequently, Model 6 (Table 4.3) was applied when four groups were distinguished (i.e. PCB_{low}/M_{young}; PCB_{low}/M_{old}; PCB_{high}/M_{young}; PCB_{high}/M_{old}). In Table 4.4, the model performance is presented when no constraints of equality across the four groups were applied as well as when different combinations of constraints were applied on the three parameters (i.e. the mean level of outcome and determinants and relations between the determinants and the outcome variables). When constraints of equality across the groups were applied on all the parameters, the model did not fit at all (Table 4.4, Model 8). In contrast, the model without equality constraints fitted adequately (Table 4.4, Model 1). Moreover, Model 1 appeared to have the best fit compared to the other models in which different combinations of constraints were applied (Table 4.4, Models 2 to 7). These findings suggested that there are discernable differences between the four groups in the mean levels of cognitive and/or motor development, and in the mean levels of parental education and/or parental verbal IQ, and/or HOME scores. Additionally, the relations between either parental education, and/or parental verbal IQ, and/or HOME and cognitive (and indirectly motor) development were significantly different for the four groups.

Table 4.3 Modeling in total group (n=203).

		χ^2	df	p	χ^2/df	CFI	TLI	RMSEA	SRMR
	Baseline model (B): $C_{3,7,18} \Rightarrow C_{42,84}$ & $M_{3,7,18}$ $C_{42,84}$ & $M_{3,7,18} \Rightarrow M_{42,84}$ $HOME_{18} \Rightarrow C_{3,7,18}$ & $HOME_{42,84}$ $HOME_{42,84} \Rightarrow C_{42,84}$ VIQ ≈ Education								
1	(B) + VIQ & Education $\Rightarrow$ $HOME_{18}$&$HOME_{42,84}$ VIQ & Education /$\Rightarrow$ $C_{3,7,18}$&$C_{42,84}$	56.87	16	<0.001	3.55	0.91	0.85	0.11	0.08
2	(B) + VIQ & Education $\Rightarrow$ $HOME_{18}$&$HOME_{42,84}$ VIQ & Education $\Rightarrow$ $C_{3,7,18}$ VIQ & Education /$\Rightarrow$ $C_{42,84}$	45.71	14	<0.001	3.27	0.93	0.87	0.11	0.05
3	(B) + VIQ & Education $\Rightarrow$ $HOME_{18}$&$HOME_{42,84}$ VIQ & Education /$\Rightarrow$ $C_{3,7,18}$ VIQ & Education $\Rightarrow$ $C_{42,84}$	29.19	14	0.01	2.09	0.97	0.94	0.07	0.06
4	(B) + VIQ & Education $\Rightarrow$ $HOME_{18}$ VIQ & Education /$\Rightarrow$ $HOME_{42,84}$ VIQ & Education $\Rightarrow$ $C_{3,7,18}$&$C_{42,84}$	65.50	14	<0.001	4.68	0.89	0.79	0.14	0.08
5	(B) + VIQ & Education ≈ $HOME_{18}$ VIQ & Education $\Rightarrow$ $HOME_{42,84}$ VIQ & Education $\Rightarrow$ $C_{3,7,18}$&$C_{42,84}$	18.03	12	0.11	1.50	0.99	0.97	0.05	0.04
6	(B) + VIQ & Education $\Rightarrow$ $HOME_{18}$&$HOME_{42,84}$ VIQ & Education $\Rightarrow$ $C_{3,7,18}$&$C_{42,84}$	18.03	12	0.11	1.50	0.99	0.97	0.05	0.04
7	(B) + Model 6 + $HOME_{18} \Rightarrow M_{3,7,18}$ HOME42,84$\Rightarrow$ $M_{42,84}$	15.53	10	0.11	1.55	0.99	0.97	0.05	0.03

/$\Rightarrow$: no path; ≈: free correlation.
$HOME_{18}$ and $HOME_{42,84}$, HomeObservationfortheMeasurementoftheEnvironmentassessedat18monthsofage($HOME_{18}$)andthemeanofthe transformedscores(to$HOME_{18}$mean(SD))ontheHOMEassessedat42and84monthsofage;Education:parentaleducationlevel;MQ:parental verballQ;χ^2,df,p:anonsignificantvalueindicatesthatthemodelisnotrejected;χ^2/df:avalue≤1.5indicatesagoodfit;CFI:ComparativeFit Index(range0-1),avalue>0.95isindicativeofagoodfit;TLI:Tucker-LewisIndex,avalueapproximating1indicatesagoodmodelfit;RMSEA: rootmeansquareerrorofapproximation,avalueof0.05indicatesaclosefit;SRMR:standardizedrootmeansquareresidual,avalue<0.05 suggests adequate fit.

To explore whether group differences were more pronounced in relation to either maternal age or prenatal exposure level, changes in model fit were compared applying constraints of equality when two groups were distinguished (i.e. either dichotomized by maternal age or by prenatal PCB levels). The model fit decreased more when constraints of

equality were applied across groups that were different in prenatal PCB levels than across groups that were different in regard to maternal age (Table 4.5). This suggested that the differences between the two prenatal PCB exposure groups are larger than the differences between the two maternal age groups.

Table 4.4 Model performance distinguished by prenatal PCB level and maternal age (PCB_{low}/M_{young}, PCB_{low}/M_{old}, PCB_{high}/M_{young}, PCB_{low}/M_{old}).

	Across groups constraints +/- [a]			χ^2	df	p	χ^2/df	CFI	TLI	RMSEA	SRMR
	Means[b]	Intercept[c]	Regr. coef.								
1	-	-	-	59.88	48	0.12	1.25	0.98	0.95	0.07	0.06
2	-	-	+	89.89	66	0.03	1.36	0.95	0.92	0.08	0.11
3	-	+	-	83.15	60	0.03	1.39	0.95	0.91	0.09	0.10
4	-	+	+	114.63	78	<0.01	1.47	0.92	0.90	0.10	0.06
5	+	-	-	116.90	62	<0.01	1.89	0.89	0.80	0.13	0.14
6	+	-	+	146.76	80	<0.01	1.83	0.86	0.81	0.13	0.16
7	+	+	-	140.10	74	<0.01	1.89	0.86	0.80	0.13	0.16
8	+	+	+	171.48	92	<0.01	1.86	0.84	0.81	0.13	0.19

[a] + = constrained to be equal across the four groups
- = not constrained to be equal across the four groups
[b] Applies to the variables parental education level, parental verbal IQ, and $HOME_{18}$ and $HOME_{42,84}$
[c] Applies to the variables $C_{3,7,18}$, $C_{42,84}$ and $M_{3,7,18}$, $M_{42,84}$; χ^2, df, p: a non significant value indicates that the model is not rejected; χ^2/df: a value ≤1.5 indicates a good fit; CFI: Comparative Fit Index (range 0-1), a value >0.95 is indicative of a good fit; TLI: Tucker-Lewis Index, a value approximating 1 indicates a good model fit; RMSEA: root mean square error of approximation, a value of 0.05 indicates a close fit; SRMR: standardized root mean square residual, a value <0.05 suggests adequate fit.

Table 4.5 Constraints or no constraints of equality across maternal age groups and across PCB exposure groups.

Group comparison	Constraints +/-[a]	χ^2	df	p	χ^2/df	CFI	TLI	RMSEA	SRMR
1 Mat. Age[b]	-	32.77	24	0.11	1.37	0.98	0.96	0.06	0.04
2 Mat. Age[b]	+	87.32	38	<0.01	2.30	0.89	0.84	0.11	0.14
3 PCB[c]	-	31.70	24	0.13	1.32	0.98	0.96	0.06	0.04
4 PCB[c]	+	115.22	38	<0.01	3.03	0.84	0.77	0.14	0.18

[a] + = constrained to be equal (means, intercepts, and regression coefficients) across the two groups
- = not constrained to be equal (means, intercepts, and regression coefficients) across the two groups
[b] comparison of the two maternal age groups, dichotomized at the total population median of maternal age (<29=M_{young} and ≥29 years=M_{old}); [c] comparison of the two prenatal PCB exposure groups, dichotomized at the total population median of Σ$PCB_{maternal}$ (<2.04 μg/L=PCB_{low} and ≥2.04 μg/L=PCB_{high}); χ^2, df, p: a non significant value indicates that the model is not rejected; χ^2/df: a value ≤1.5 indicates a good fit; CFI: Comparative Fit Index (range 0-1), a value >0.95 is indicative of a good fit; TLI: Tucker-Lewis Index, a value approximating 1 indicates a good model fit; RMSEA: root mean square error of approximation, a value of 0.05 indicates a close fit; SRMR: standardized root mean square residual, a value <0.05 suggests adequate fit.

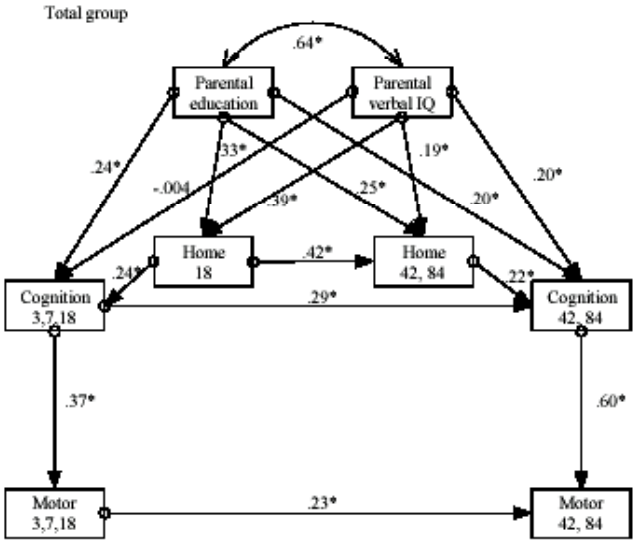
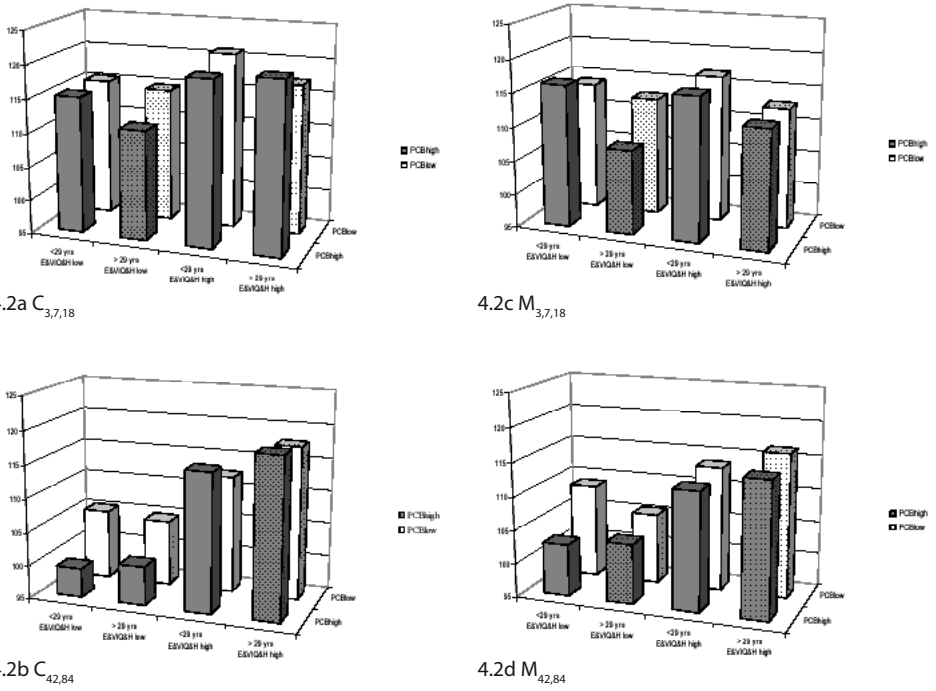


Figure 4.1 Path diagram for total group, Model 6 (Table 4.3).
Estimates on the paths are standardized regression coefficients; * p<0.05.

In Figures 4.2a to d, estimated mean cognitive and motor scores of the four groups are presented based on Model 1 (Table 4.4). Since the influences of the determinants on the outcome variables were different for the four groups, mean cognitive and motor scores were estimated for two levels of parental education, parental verbal IQ and HOME scores: the 25% and 75% scores on these variables (parental education level: 1=low, 2=high; parental verbal IQ: 111=low, 135 =high; $HOME_{18}$: 39.1=low, 42=high; $HOME_{42,84}$: 39.0=low, 42=high). Differences in estimated $C_{42,84}$ and $M_{42,84}$ scores were seen between children raised in low and high levels of parental and home environmental conditions, across the maternal age groups as well as across the exposure groups. Comparisons of differences in cognitive and motor outcome between low and high exposure within the parental and home environmental condition groups and maternal age groups showed the largest decrements in scores ($C_{3,7,18}$, $M_{3,7,18}$, $C_{42,84}$, $M_{42,84}$) in the children raised in lower levels of parental and home environmental conditions. For $C_{3,7,18}$ and $M_{3,7,18}$ the discrepancy was more pronounced in children born to older mothers whereas for $C_{42,84}$ and $M_{42,84}$ the discrepancy was more pronounced in children born to younger mothers.



4.2a $C_{3,7,18}$ 4.2c $M_{3,7,18}$

4.2b $C_{42,84}$ 4.2d $M_{42,84}$

Figure 4.2 Estimated mean cognitive and motor development for low and high PCB exposed children born to young and old mothers relative to parental education level (E) and verbal IQ (VIQ) and HOME score (H).

Discussion

Structural equation models were compared for different subgroups of the Rotterdam PCB/dioxin cohort. The advantage of group comparison by means of SEM over multiple regression modeling is that interrelationships of outcome variables and determinants can be more properly modeled. Group differences can be estimated in the levels of the variables in the model as well as in their interrelationships. In addition, SEM enables to analyze more than one outcome variable simultaneously.

The results of this study suggest that the four groups, distinguished by prenatal PCB exposure and maternal age, are significantly different not only in levels of cognitive and/or motor development and in the levels of parental education and/or parental verbal IQ, and/or HOME scores, but also in the relations between either of these determinants and cognitive (and indirectly motor) development. The differences in these aspects were larger when the total group was dichotomized according to prenatal PCB levels than when dichotomization by maternal age was done.

Previously, we have reported that effects of prenatal exposure to PCBs on cognitive and motor abilities at school age were modified by maternal age, parental education level, parental verbal IQ and HOME score (9). Moreover, amongst the four candidate effect modifiers, maternal age was the most pronounced modifier of effects of prenatal exposure to PCBs on the level of cognitive development from 3 to 84 months of age (8). For motor development, effect modification by HOME scores was suggested (8). The presently used SEM method suggested that, apart from effect modification by maternal age, at least one of the other parental and home environmental predictors modified the prenatal PCB effect on cognitive (and indirectly motor) development. Consequently, we estimated the difference in cognitive and motor development between low and high exposed groups for children born to relatively young and old mothers, and raised in relatively low and high parental and home environmental conditions. Compared to the difference in scores between the low and high exposed children who were raised in relatively high parental and home environmental conditions, larger cognitive and motor decrements were seen in children who were raised in lower parental and home environmental conditions. For early cognitive and motor development this was more pronounced in children born to older mothers whereas for later development this effect was more pronounced in children born to younger mothers. In higher parental and home environmental conditions, no evidence of negative effects of PCB exposure on cognitive and motor development is seen. Figures 4.2a and b even suggest that in this group of children early and late cognitive scores, in respectively children born to older and younger mothers, were higher in high exposed children than in their low exposed counterparts. We explored whether these differences in cognitive outcome between low and high exposed children were significant. Applying equality constraints (on the level of cognitive outcome variables) across the low and high exposed children raised under these conditions did not decrease the model fit. Consequently, these additional tests gave no evidence that the cognitive scores were significantly different in high and low exposed children raised under high parental and home environmental conditions. In The Netherlands, as reflected in our cohort, relatively higher educated women generally give birth at older age to children, which is related to a higher PCB body burden due to the physical stability of PCBs and their accumulation in human tissues. Consequently, the group differences in mean levels of the parental and home environmental variables are largely related to maternal age. Moreover, some studies have reported positive effects of older maternal age on children's cognitive abilities (see review (23)), even after adjustment for social and economic variables was done. It has been hypothesized that this positive effect of maternal age reflects age-related psychological and emotional attributes (23).

Cognitive and motor abilities that were assessed at the different ages were divided for the modeling procedure in two cognitive and two motor variables, 'early' and 'late' cognitive and motor development. This was done to simplify modeling as well as because of the changing construct of cognitive and motor abilities that are assessed by the developmental

tests during development. Moreover, up until 18 months of age the same test was used to assess cognitive and motor development. In contrast to 'later' cognitive abilities, 'early' cognitive and motor abilities were more affected by prenatal PCB exposure in children born to *older* mothers and raised in less advantageous conditions compared to their counterparts who were born to *younger* mothers. One of the possible explanations for this potential difference in vulnerability to effects of prenatal exposure to PCBs between 'early' and 'late' development may be that compared to younger mothers, older mothers were less inclined to stimulate the early development of their child. Additionally, later development is influenced more strongly by genetic and socio-parental conditions, and potential positive effects, as well as counteracting effects, of maternal age on child development may therefore be more pronounced at more mature age.

Generally, these results may suggest that neurotoxic effects of prenatal exposure to PCBs can be compensated by favorable conditions for child development such as a high level of parental and home environmental conditions. Some epidemiological studies that addressed effects of prenatal exposure to lead and methyl-mercury on child development also reported modification of the effects of prenatal exposure to these neurotoxicants by socio-environmental risk factors (24-27). Moreover, comparable effects have been reported in studies in low birth weight children where in children at high biological risk favorable early parental and home characteristics could compensate for or mask developmental delays (28-32). These findings may be in line with experiments showing significant differences in brain chemistry and anatomy, including increased cortical thickness, in animals raised in enriched environments (33, 34). Moreover, a positive impact of an enriched environment on the effects of brain lesions has been reported in animal studies (34-36).

In the environment PCBs, their metabolites and related compounds, such as dioxins, are present as complex mixtures of various congeners that may vary in metabolism and toxicity. The sum of PCBs 118, 138, 153, and 180 consists of the four most abundant congeners, constituting 46% of the total PCBs (37). In our study, irrespective of the sum of four PCBs in maternal plasma during pregnancy, the sum of the four PCBs was measured in cord blood and breast milk and dioxins, dioxin-like PCBs, and additional nondioxin-like PCBs were assessed in breast milk. PCB levels in maternal and cord blood, and total TEQ (Toxic Equivalent) levels of dioxins and dioxin-like PCBs were highly interrelated (10). Therefore, effect of one of the exposure variables cannot exclude effects of exposure to other compounds. In this model, we included maternal levels of PCBs since they showed the strongest relation with cognitive and motor development at different time points.

In this paper, we have not evaluated effects of postnatal exposure to PCBs and dioxins through lactation. Previous studies in the Rotterdam cohort, in line with most epidemiological PCB studies, hardly gave evidence of negative effects of postnatal exposure to PCBs and dioxins on general cognitive and motor abilities assessed at 3 to 84 months of age, and neither on their development (2-6, 8, 9). In the Dutch PCB/dioxin cohort, psychomotor

abilities measured at 7 months of age appeared to be lower in the highest postnatally exposed children (1). After that age, using multiple regression analysis, no negative effects of lactational exposure to PCBs and dioxins on general cognitive or motor abilities were detected. However, recently, negative effects of lactational exposure to PCBs on general cognitive abilities at 42 months of age have been described in a German cohort of children exposed to environmental levels of PCBs (7). Behavioral animal studies also give evidence of profound effects of lactational exposure to low levels of PCBs (38, 39). Exploration of effects of lactational exposure to PCBs on child development from 3 to 84 months of age will therefore be subject to further detailed study.

In conclusion, SEM is a promising technique for epidemiological neurodevelopmental PCB studies since it enables more proper modeling of relevant variables. This empirical study provides evidence of complex effects of maternal age and other parental and home environmental conditions on the neurotoxic mechanism of PCBs and related neurotoxic compounds and serves as an initial effort to disentangle these mechanisms to increase the knowledge in risk assessment of prenatal exposure to PCBs.

References

1. Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MA, Van der Paauw CG, Tuinstra LG, Sauer PJ. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics 1996;97(5):700-6.
2. Patandin S, Lanting CI, Mulder PG, Boersma ER, Sauer PJ, Weisglas-Kuperus N. Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J Pediatr 1999;134(1):33-41.
3. Rogan WJ, Gladen BC. PCBs, DDE, and child development at 18 and 24 months. Ann Epidemiol 1991;1(5):407-13.
4. Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychorinated biphenyls in utero. N Engl J Med 1996;335:783-9.
5. Gladen BC, Rogan WJ, Hardy P, Thullen J, Tingelstad J, Tully M. Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. J Pediatr 1988;113(6):991-5.
6. Darvill T, Lonky E, Reihman J, Stewart P, Pagano J. Prenatal exposure to PCBs and infant performance on the fagan test of infant intelligence. Neurotoxicology 2000;21(6):1029-38.
7. Walkowiak J, Wiener JA, Fastabend A, Heinzow B, Kramer U, Schmidt E, et al. Environmental exposure to polychlorinated biphenyls and quality of the home environment: effects on psychodevelopment in early childhood. Lancet 2001;358(9293):1602-7.
8. Vreugdenhil HJI, Duivenvoorden HJ, Weisglas-Kuperus N. Prenatal exposure to PCBs and cognitive and motor development from 3 to 84 months of age; a longitudinal study. Submitted.
9. Vreugdenhil HJ, Lanting CI, Mulder PG, Boersma ER, Weisglas-Kuperus N. Effects of prenatal PCB and dioxin background exposure on cognitive and motor abilities in Dutch children at school age. J Pediatr 2002;140(1):48-56.
10. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N, Van der Pauw CG, Tuinstra LGMT, Boersma ER, et al. PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 1994;28(9):1721-1732.
11. Burse VW, Korver MP, Needham LL, Lapeza CR, Jr., Boozer EL, Head SL, et al. Gas chromatographic determination of polychlorinated biphenyls (as Aroclor 1254) in serum: collaborative study. J Assoc Off Anal Chem 1989;72(4):649-59.
12. Bayley N. Manual for the Bayley scales of infant development. New York: The psychological corporation; 1969.
13. Van der Meulen BF, Smorkovsky M. BOS 2-30: Bayley Ontwikkelings-schalen. Lisse, The Netherlands: Swets and Zeitlinger; 1983.
14. Neutel RJ, van der Meulen BF, Lutje Spielber HJ. Groningse Ontwikkelingsschalen, Dutch version of the Kaufman Assessment Battery for Children (K-ABC). Lisse: Swets & Zeitlinger BV, The Netherlands; 1996.
15. Van der Meulen BF, Smorkovsky M. MOS 2 1/5- 8 1/2 McCarthy Ontwikkelingsschalen. Lisse, The Netherlands: Swets & Zeitlinger B.V.; 1985.
16. Stinissen J, Willems PJ, Coetsier P, Hulsman WLL. Manual, Dutch version of the Wechsler Adult Intelligence Scale (WAIS). Amsterdam: Swets & Zeitlinger NV; 1970.
17. Caldwell B, Bradley R. Home observation of the environment. Administration manual. Little Rock, Arkansas; 1984.
18. Bentler PM. Comparative fit indexes in structural models. Psychol Bull 1990;107(2):238-46.
19. Tucker LR, Lewis CA. A reliability coefficient for maximum likelyhood factor analysis. Psychometrika 1973;38:1-10.
20. Browne MW, Cudeck R. Testing structural equation models. In: Long KABJS, editor. Alternative ways of assessing model fit. Thousand Oakes, CA: Sage; 1993.
21. Steiger JH, Shapiro A, Browne MW. On the multivariate asymptomatic distribution of sequential chi-square statistics. Psychometrica 1985;50:253-263.
22. Little RJA, Rubin DB. Statistical analysis with missing data. New York: Wiley; 1987.

23. Stein ZA. A woman's age: childbearing and child rearing. Am J Epidemiol 1985;121(3):327-42.
24. Winneke G, Kraemer U. Neuropsychological effects of lead in children: interactions with social background variables. Neuropsychobiology 1984;11(3):195-202.
25. Dietrich KN, Succop PA, Berger OG, Hammond PB, Bornschein RL. Lead exposure and the cognitive development of urban preschool children: the Cincinnati Lead Study cohort at age 4 years. Neurotoxicol Teratol 1991;13(2):203-11.
26. Davidson PW, Myer GJ, Shamlaye C, Cox C, Gao P, Axtell C, et al. Association between prenatal exposure to methylmercury and developmental outcomes in Seychellois children: effect modification by social and environmental factors. Neurotoxicology 1999;20(5):833-41.
27. Bellinger D, Leviton A, Waternaux C, Needleman H, Rabinowitz M. Low-level lead exposure, social class, and infant development. Neurotoxicol Teratol 1988;10(6):497-503.
28. Weisglas-Kuperus N, Baerts W, Smrkovsky M, Sauer PJ. Effects of biological and social factors on the cognitive development of very low birth weight children. Pediatrics 1993;92(5):658-65.
29. Siegel LS. Reproductive, perinatal, and environmental factors as predictors of the cognitive and language development of preterm and full-term infants. Child Dev 1982;53(4):963-73.
30. Pfeiffer SI, Aylward GP. Outcome for preschoolers of very low birthweight: sociocultural and environmental influences. Percept Mot Skills 1990;70:1367-78.
31. Landry SH, Denson SE, Swank PR. Effects of medical risk and socioeconomic status on the rate of change in cognitive and social development for low birth weight children. J Clin Exp Neuropsychol 1997;19(2):261-74.
32. Aylward GP. The relationship between environmental risk and developmental outcome. J Dev Behav Pediatr 1992;13(3):222-9.
33. Diamond MC, Lindner B, Johnson R, Bennett EL, Rosenzweig MR. Differences in occipital cortical synapses from environmentally enriched, impoverished, and standard colony rats. J Neurosci Res 1975;1(2):109-19.
34. Rosenzweig MR, Bennett EL. Psychobiology of plasticity: effects of training and experience on brain and behavior. Behav Brain Res 1996;78(1):57-65.
35. Brenner E, Mirmiran M, Uylings HB, Van der Gugten J. Growth and plasticity of rat cerebral cortex after central noradrenaline depletion. Exp Neurol 1985;89(1):264-8.
36. Kolb B, Gibb R. Environmental enrichment and cortical injury: behavioral and anatomical consequences of frontal cortex lesions. Cereb Cortex 1991;1(2):189-98.
37. Jacobson JL, Humphrey HE, Jacobson SW, Schantz SL, Mullin MD, Welch R. Determinants of polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), and dichlorodiphenyl trichloroethane (DDT) levels in the sera of young children. Am J Public Health 1989;79(10): 1401-4.
38. Rice DC. Behavioral impairment produced by low-level postnatal PCB exposure in monkeys. Environ Res 1999;80:S113-S121.
39. Rice DC. Effects of postnatal exposure of monkeys to a PCB mixture on spatial discrimination reversal and DRL performance. Neurotoxicol Teratol 1998;20(4):391-400.

PART II

Neurotoxic mechanisms of effects;
effects of perinatal exposure to PCBs and dioxins on play behavior,
neuropsychological and neurophysiological outcome.

5 Effects of perinatal exposure to PCBs and dioxins on play behavior in Dutch children at school age

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Abstract

Polychlorinated biphenyls (PCBs) and dioxins are known as neurotoxic compounds that may modulate sex steroid hormones. Steroid hormones play a mediating role in brain development and may influence behaviors that show sex differences, such as childhood play behavior. In this study, we evaluated the effects of perinatal exposure to environmental levels of PCBs and dioxins on childhood play behavior and whether the effects showed sex differences. As part of the follow-up to the Dutch PCB/dioxin study at school age we used the Pre-School Activity Inventory (PSAI) to assess play behavior in the Rotterdam cohort (n=207). The PSAI assesses masculine or feminine play behavior scored on three subscales: Masculine, Feminine, and Composite. Prenatal exposure to PCBs was defined as the sum of PCB118, 138, 153, 180 in maternal and cord plasma, and in breast milk. For breast milk we measured additional PCBs as well as 17 dioxins. Respondents returned 160 questionnaires (age 7.5 years (± 0.4)). Effects of prenatal exposure to PCBs, measured in maternal and cord plasma, on scores on the Masculine and Composite scales were different for boys and girls. In boys, higher prenatal PCBs levels were related with less masculinized play, whereas in girls higher PCB levels were associated with more masculinized play. Higher prenatal dioxin levels were associated with more feminized play in boys as well as girls, assessed by the Feminine scale.

Conclusion: These effects suggest prenatal steroid hormone imbalances caused by prenatal exposure to environmental levels of PCBs, dioxins and other related organochlorine compounds.

Introduction

Polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (the latter two termed dioxins) are lipophilic and bioaccumulating environmental pollutants that are known for their neurotoxic effects in animals and humans. These fat-soluble toxicants are present in human beings and cross the placenta during pregnancy, thereby exposing children during the rapid development of the central nervous system (CNS). In the last two decades, several prospective epidemiologic studies in industrialized countries have shown subtle effects of exposure to background levels of PCBs and dioxins on health, growth and development in children (1, 2). Many systems of the developing CNS may be affected by the neurotoxic effects of prenatal exposure to PCBs and dioxins (2). One property of PCBs and dioxins is the modulation of the endocrine system, including sex steroid hormones such as estrogens and androgens (3, 4). Steroid hormones play an important mediating role in the development of the CNS and influence not only reproductive but also nonreproductive behaviors that show sex differences (5, 6). In evaluation of steroid hormone disrupting effects of PCBs and dioxins, effects on sexual dimorphic neurobehavior may therefore be important endpoints. Moreover, prenatal sex differences in sex steroid hormone metabolism could cause sex differences in endocrine-disrupting effects of PCBs and dioxins.

Sex-specific effects of perinatal PCB and dioxin exposure have been reported in animal studies on sexual dimorphic neurobehaviors such as sweet preference (7, 8), and spatial learning (9, 10). In humans, sex-specific neurobehavioral effects of prenatal exposure to PCBs and dioxins have been described only for the Yu-Cheng accident (11). In this cohort of children born to mothers who were accidentally exposed to high levels of PCBs and PCDFs in rice oil, cognitive abilities, as assessed by a test that measured predominantly spatial cognitive abilities, were more affected by prenatal exposure to PCBs/PCDFs in boys than in girls.

In animal studies, nonreproductive behaviors that were altered by gonadal steroids include spatial and visual discrimination learning (12, 13), open field exploration (14), and rough and tumble play (15). Especially behaviors that show sex differences were altered by gonadal steroids, whereas no such effect has been reported on behaviors that do not show sex differences. In humans, childhood play behavior shows marked sexual dimorphic differences and gives the clearest evidence for prenatal hormonal influence on human behavioral development (16).

In The Netherlands, a cohort of children born healthy has been prospectively followed from birth to school age to address neurotoxic effects of perinatal exposure to PCBs and dioxins. In this cohort, prenatal PCB exposure was related to lower psychomotor scores at 3 months of age (17) and lower cognitive abilities at 42 months (18). At school age, negative effects of prenatal PCB exposure on cognitive and motor development were seen

in children with relatively low parental and home characteristics, whereas in children raised in relatively more favorable environments these subtle effects of prenatal PCB exposure were not detectable (19).

As part of the follow-up assessment at school age, we measured gender-role play in the Rotterdam cohort, half of the Dutch PCB and dioxin population. Our aim in this study was to evaluate effects of perinatal exposure to PCBs and dioxins on play behavior and whether these effects show sex differences.

Method

Subjects and study design

The study population consisted of 207 healthy Caucasian mother-infant pairs who were recruited from June 1990 to February 1992 in the area of Rotterdam, in The Netherlands. The study design and recruitment process, chemical analysis and PCB and dioxin concentrations have been described in detail elsewhere (20). Pregnancy and delivery were uncomplicated. Only first- or second-born children, born healthy at term, were included. Half of the group of children was breast-fed (BF) (n=105) for at least six weeks, the others were formula-fed (FF) (n=102) during infancy. All FF infants received formula from a single batch (Almiron M2, Nutricia NV, Zoetermeer, The Netherlands) from birth until 7 months of age. In this formula, PCBs and dioxins were not detectable. The medical ethics committee of the University Hospital Rotterdam/ Sophia Children's Hospital approved the study design and the parents gave informed consent.

Assessment of exposure variables

Plasma samples were collected from the mothers during the last month of pregnancy and cord plasma samples were collected directly after birth. These samples were analyzed for four PCB congeners, International Union for Pure and Applied Chemistry (IUPAC) numbers 118, 138, 153 and 180. Two weeks after delivery a 24-hour representative breast milk sample was collected from the mothers who were breast-feeding their children. Breast milk samples were analyzed for 17 dioxins (PCDDs and PCDFs), 6 dioxin-like PCBs (3 planar PCBs and 3 mono-ortho PCBs), and 20 nondioxin-like PCBs. Toxic potency of the mixture of dioxins and dioxin-like PCBs was expressed by using the toxic equivalent (TEQ) approach (21).

We estimated prenatal exposure to PCBs in the total study population by using the sum of the four PCB congeners in maternal ($\Sigma PCB_{maternal}$) and in cord plasma (ΣPCB_{cord}). In the BF group additional prenatal exposure measurements were used: the ΣPCB_{milk} (the sum of PCB 118, 138, 153, and 180), the dioxin, planar, mono-ortho and total TEQ value (the sum

of the TEQ values of the 17 dioxins and the 6 dioxin-like PCBs) and the $\Sigma PCB_{20\ nondioxin\text{-}like}$ (the sum of 20 nondioxin-like PCBs).

Postnatal exposure to PCBs and dioxins through lactation was estimated by multiplying the number of weeks of breast-feeding with respectively ΣPCB_{milk}, the dioxin, planar, mono-ortho and total TEQ, and $\Sigma PCB_{20\ nondioxin\text{-}like}$ concentrations in breast milk.

Assessment of play behavior

Parents were asked to complete the Dutch version of the Pre-School Activities Inventory (PSAI) (22) (Appendix) when the children reached school age. This questionnaire, along with a questionnaire on problem behavior and a health questionnaire, was sent to the parents in two mailings, depending on the age of the child (in 1998 and 1999), near the end of a school year.

The PSAI is designed to discriminate play behavior both within and between the sexes. It consists of 24 questions addressing three aspects of play behavior: type of toys, activities, and child characteristics. Answers are given on a 5-point scale ranging from never to very often. The questions assess either feminine or masculine play behavior from which three scales are derived: a Composite scale, integrating both masculine and feminine play behavior, and a Masculine and Feminine scale. The Composite scale is essentially defined as the difference: Feminine scale minus Masculine scale. A negative score on the Composite scale implies masculine play behavior and a positive score feminine play behavior. A higher score on the Feminine scale indicates more feminine play behavior whereas a higher score on the Masculine scale indicates more masculine play behavior.

The questionnaire has been validated in a group of preschool English children (n= 102), additionally, a test-retest reliability for the scores on the PSAI of .62 for boys, and .66 for girls has been found (22). The PSAI has been assessed in various cohorts for standardization and norming purposes. These cohorts include normal preschool children across several samples in the UK (Pilot study (n=75); Validation study (n=102); and a cohort obtained through the magazine *Practical Parenting* (n=1643)), in the United States (n=203), and also in the Netherlands, using a Dutch translation of the questionnaire (n=341) (23).

Assessment of other variables

Variables that may influence child neurodevelopment have been assessed and included birth weight, duration of gestation, fetal exposure to alcohol and cigarette smoking, maternal age at birth of the child, parity, type of feeding during infancy, duration of breast-feeding, sex, and parental education level. The verbal IQ of the parent who spends the most time with the child (usually the mother) was assessed, during the follow-up session at 42 months by two subtests, Information and Vocabulary from the Dutch version of the

Wechsler Adult Intelligence Scale (WAIS) (24). At 7 years of age, follow-up assessment in this cohort was done at home by a psychologist (HV). During this visit, the child's home environment was assessed by the Home Observation for Measurement of the Environment (HOME) (25).

Data analysis

To compare groups for a single variable we used either the Student's t-test (for continuous variables), the chi-square test (for categorical variables), or the Mann-Whitney *U* test. Plasma and milk PCB and dioxin values were positively skewed and were therefore normalized by natural logarithmic transformation. We studied the effects of PCB and dioxin exposure on the scores for the play behavior scales using multiple linear regression analyses (SPSS, version 9). Variables that were likely to affect play behavior were included in the regression model as a fixed set of variables. These variables were: sex (0/1 = boy/girl), highest education level of either parent (0/1/2 = low (primary school, secondary school not finished)/middle (secondary school finished)/ high (high school finished, professional and university training)), parental verbal IQ, type of feeding during infancy (0/1 = BF/FF), duration of breast-feeding (0 for FF children), HOME score, and assessment age. Additionally, confounding variables, i.e. variables that correlated ($p<0.2$), adjusted for the fixed set of variables, with one of the exposure variables and with scores on one of the three play behavior scales were included in the final regression model. Candidate confounders were alcohol use (0/1 = no/yes) and smoking (0/1 = no/yes) during pregnancy, duration of gestation, birth weight, maternal age at birth, and parity (0/1 = 1st/2nd born). This procedure resulted in the following regression model: sex, parental education level, parental verbal IQ, feeding type, duration of breast-feeding, HOME score, age at assessment, and parity. We studied sex differences in the effects of exposure to PCBs and dioxins by including an interaction term, the product of sex and exposure ('sex*exposure'), in the regression model. The effect of exposure on the outcome variables in boys and in girls, and the difference between these effects (girls minus boys) are estimated through the interaction term sex*exposure in essentially the same regression model by reparameterizing the sex effect. Results were considered significant if $p \leq 0.05$.

Results

In the follow-up assessment at school age, 189 of the 207 children in the original cohort were re-examined and 160 of these parents returned the PSAI questionnaire (84 % were filled out by mothers, 6 % by fathers, and 10 % by both parents). Two children were excluded from data analyses due to circumstances other than PCB and dioxin exposure

that are likely to influence play behavior: a girl with Turner syndrome and a boy with a pervasive developmental disorder. Four questionnaires had missing data and were therefore excluded from data analyses.

Table 5.1 Characteristics of the total study population and BF and FF boys and girls separately.

Characteristics	Total	Breast-fed		Formula-fed	
		Boys	Girls	Boys	Girls
	(n=158)	(n=53)	(n=32)	(n=35)	(n=38)
Duration of breast-feeding (wk)	17 (6-72)	16 (6-72)	19 (6-54)		
Number of 1st born	80 (51 %)	28 (53 %)	17 (53 %)	15 (43 %)	20 (53 %)
Parental education					
Low	15 (10 %)	2 (4 %)	4 (13 %)	6 (17 %)	3 (8 %)
Medium	52 (33 %)	17 (32 %)	6 (19 %)	12 (34 %)	17 (45 %)
High	91 (58 %)	34 (64 %)	22 (69 %)	17 (49 %)	18 (47 %)
Parental verbal IQ	123.6	125.6	129.1	122.4	117.2
	(± 14.9)	(± 13.2)	(± 11.2)#	(± 14.5)	(± 18.1)#
HOME	48.3	48.1	49.6	47.8	48.1
	(± 3.0)	(± 3.1)*	(± 2.7)*#	(± 2.8)	(± 3.1)#
Age at assessment	7.5 (± 0.4)	7.6 (± 0.4)	7.5 (± 0.3)	7.5 (± 0.4)	7.5 (± 0.4)
Exposure variables					
$\Sigma PCB_{maternal}$	2.06	2 .16	2.09	2.04	1.86
(μg/L)	(0.73-5.08)	(0.73-4.21)	(0.87-4.87)	(0.88-5.08)	(0.80-4.71)
ΣPCB_{cord}	0.42	0.44	0.40	0.38	0.40
(μg/L)	(0.08-1.99)	(0.11-1.72)	(0.08-1.99)	(0.09-1.21)	(0.08-1.98)
ΣPCB_{milk}	390	422	350		
(μg/kg fat)	(174-805)	(200-805)	(174-796)		
TEQ_{dioxin}	36.3	36.6	36.0		
(ng/kg fat)	(10.2-66.6)	(16.6-66.6)	(10.2-58.8)		
$TEQ_{planarPCB}$	15.3	14.4	16.4		
(ng/kg fat)	(4.4-45.7)	(4.4-45.7)	(5.3-30.0)		
$TEQ_{monoPCB}$	13.9	14.4	12.4		
(ng/kg fat)	(3.2-25.8)	(6.4-25.8)	(3.2-24.8)		
Total $TEQ_{PCB+dioxin}$	68.1	68.1	67.1		
(ng/kg fat)	(27.7-135.2)	(27.7-135.2)	(28.1-108.9)		
$\Sigma PCB_{20\ nondioxin\text{-}like}$	438	456	370		
(μg/kg fat)	(203-890)	(203-890)	(206-846)		

Values are numbers (percentages), means (± standard deviations) or medians (range).
Parental education: low=primary school, secondary school not finished, middle=secondary school finished, high=high school finished, professional and university training; Parental verbal IQ: score on two subtests of the Wechsler Adult Intelligence Scale, Information and Vocabulary, assessed from one of the parents; HOME: Home Observation for the Measurement of the Environment at school age; $\Sigma PCB_{maternal,cord,milk}$: sum of PCB congeners IUPAC nos. 118, 138, 153, 180 in respectively maternal plasma, cord plasma, breast milk; TEQ: toxic equivalents according to the 1997 WHO TEF values for mono-ortho PCBs: UIPAC nos. 105, 118, 156, planar PCBs: IUPAC nos. 77, 126, 169, and 17 dioxins (PCDDs and PCDFs); $\Sigma PCB_{20\ nondioxin\text{-}like}$: sum of 20 nondioxin-like PCBs in breast milk.
* = P<0.05 comparing sexes within feeding groups. # = p< 0.05 comparing feeding groups within sexes.

Compared to the nonparticipating children (including both children who did not participate in the follow-up at school-age and children whose questionnaires were not returned), prenatal PCB and dioxin exposure levels were comparable with the levels in children whose parents returned the questionnaire. Moreover, the distribution of children over the feeding groups in the participating group was not statistically different from that of the nonparticipating group (n_{BF}=20; n_{FF}=22). In regard to the other variables used in the regression model, these groups were also generally comparable except for the parental education levels (p=0.011), parental verbal IQs (p=0.009), and HOME scores (p=0.021), which were higher in the participating group.

Table 5.2a Results of multiple regression analyses in the total population. Effects of prenatal exposure to PCBs on scores on the PSAI scales: the Composite, Masculine, and Feminine scale.

	Sex*Exposure[a]			Boys[b]			Girls[c]		
	Regr. coef.	SE	p	Regr. coef.	SE	p	Regr. coef.	SE	p
LnΣPCB$_{maternal}$									
Composite	-5.93	2.69	0.029	2.73	1.78	0.127	-3.20	2.13	0.137
Masculine	4.50	2.04	0.029	-2.77	1.35	0.042	1.73	1.62	0.286
Feminine	-1.20	1.95	0.590	-0.04	1.30	0.975	-1.24	1.55	0.423
LnΣPCB$_{cord}$									
Composite	-5.51	2.08	0.009	4.06	1.56	0.011	-1.45	1.46	0.323
Masculine	5.94	1.56	0.001	-3.85	1.17	0.001	2.09	1.10	0.059
Feminine	0.56	1.52	0.712	0.15	1.15	0.898	0.71	1.07	0.508

Table 5.2b Results of multiple regression analyses in the BF group. Effects of prenatal exposure to PCBs and dioxin on scores on the PSAI scales: the Composite, Masculine, and Feminine scale.

	Sex*Exposure[a]			Boys[b]			Girls[c]			Total BF group[d]		
	Regr. Coef.	SE	p	Regr. coef.	SE	p	Regr. coef.	SE	p	Regr. coef.	SE	p
LnΣPCB$_{milk}$												
Composite	-9.53	3.98	0.020	2.15	2.37	0.369	-7.39	3.29	0.028			
Masculine	4.94	3.14	0.121	-0.07	1.87	0.970	4.86	2.59	0.065			
Feminine	-4.60	2.71	0.094	2.08	1.62	0.203	-2.30	0.24	0.263			
LnDioxin TEQ												
Composite	-2.85	4.69	0.546	6.19	3.56	0.088	3.34	3.27	0.312	4.63	2.46	0.066
Masculine	1.70	3.77	0.653	-2.18	2.87	0.449	-0.48	2.63	0.856	-1.25	1.98	0.529
Feminine	-1.15	3.18	0.720	4.00	2.42	0.103	2.86	2.22	0.203	3.38	1.67	0.048

Adjusted for duration of breast-feeding, parity, parental education level, parental verbal IQ, HOME score, age at examination and in total subgroup (Table 5.2a) also for type of feeding. The effect of exposure on the PSAI scores in boys and in girls, and the difference between these effects (girls minus boys) are estimated through the interaction term 'sex*exposure' in essentially the same regression model by reparameterizing the sex effect.
[a] Regression coefficient, standard error, and p-value of the interaction variable 'sex*Exposure' (LnΣPCB$_{maternal}$ or LnΣPCB$_{cord}$ or LnΣPCB$_{milk}$ or LnDioxinTEQ) on the outcome variable when in the regression model boy is coded 0, and girl=1. p<0.05 indicates a significantly different effect of prenatal exposure on PSAI scores between boys and girls. [b] Regression coefficient of exposure, standard error, and p-value, on PSAI scores in boys and girls, respectively. [c] Regression coefficient of exposure, standard error, and p-value, on PSAI scores in the total BF group not including the 'sex*exposure' interaction term in the regression model.

The mean age of the children at assessment was 7.5 (± 0.4) years old. The descriptives for the total study group, and for BF and FF boys and girls separately, are presented in Table 5.1. The characteristics of all boys and girls were not significantly different. Comparing characteristics of boys and girls within feeding groups, the HOME score was significantly higher in BF girls than in BF boys. The HOME score and the parental education level were significantly higher in BF girls than in FF girls.

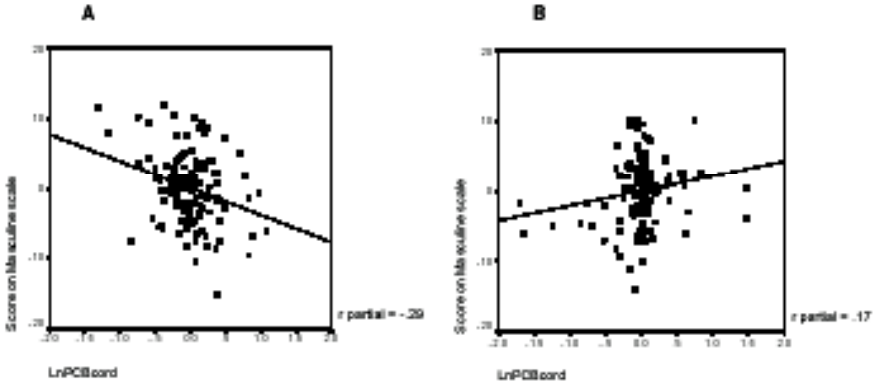

Figure 5.1 Relation in boys (A) and in girls (B) between scores on the Masculine scale and levels of $Ln\Sigma PCB_{cord}$ adjusted for confounding variables; partial regression plot.

Boys and girls scored significantly different on the three PSAI scales (Mean (SD) Composite scale: boys -14.6 (5.8), girls 14.0 (5.3); Masculine scale: boys 24.2 (5.3), girls 12.6 (4.5); Feminine scale: boys 9.6 (3.3), girls 26.4 (6.2); all p-values < 0.001).

Table 5.2a presents effects of PCBs and dioxins on the PSAI scales for boys and girls including the sex difference in effect, adjusted for all other variables. Effects of prenatal exposure to PCBs on the scores on the Composite scale and Masculine scale were significantly different for boys and girls. In boys, higher prenatal PCB exposure was related with higher scores on the Composite scale and lower scores on the Masculine scale, both indicating less masculine play behavior. In girls, effects of prenatal PCB exposure moved in opposite directions on the Composite and Masculine scales; however, relations were not significant. We saw no sex-specific effects of prenatal PCB exposure on scores on the Feminine scale. As an example of the relation between prenatal PCB exposure and play behavior in both sexes, adjusted for confounding variables, the relation between $ln\Sigma PCB_{cord}$ and scores on the Masculine scale are visualized in a partial regression plot (Figure 5.1).

In the BF group (see Table 5.2b), effects of ΣPCB_{milk} on the scores on the Composite scale were also significantly different for boys and girls (p=0.020). In girls, higher exposure to these compounds was related to lower scores (p=0.028), indicating more masculine play behavior, whereas in boys the relation was in the opposite direction, although

not significant (p=0.369). We saw no sex-specific effects of ΣPCB_{milk} on scores on the Masculine and Feminine scale. Effects of prenatal exposure to dioxins, planar and mono-ortho TEQs, total TEQ, and the sum of the 20 nondioxin-like PCBs on play behavior were not significantly different for boys and girls. Prenatal dioxin TEQ levels were significantly related with higher scores on the Feminine scale in the total group of boys and girls (p=0.048), indicating more feminized play behavior in both sexes.

Postnatal exposure, through lactation, to ΣPCB_{milk}, dioxin, planar and mono-ortho TEQs, total TEQ, and $\Sigma PCB_{20\ nondioxin\text{-}like}$ was not related to play behavior in the total BF group nor in boys and girls separately.

Discussion

In this study we described sex-specific effects of prenatal exposure to PCBs on play behavior in healthy Dutch children at school age. Higher prenatal exposure to PCBs was associated with less masculinized play behavior in boys and with more masculinized play behavior in girls. Effects of prenatal exposure to dioxins were seen on feminine play behavior. In boys as well as in girls, higher prenatal dioxin levels were associated with more feminized play behavior. Childhood play behavior shows marked sex differences and is likely to be influenced by the prenatal steroid hormone environment. We therefore suggest that these results may indicate behavioral effects of steroid hormone imbalances early in development related to prenatal exposure to PCBs and dioxins, their metabolites and/or related compounds.

In the Yu Cheng cohort, researchers observed sex-specific effects of prenatal exposure to high levels of PCBs and PCDFs on the scores on the Raven's Colored Progressive Matrices (CPM) and Standardized Progressive Matrices (SPM) (11). These tests are considered to be tests for general cognitive development that appeal more on spatial rather than verbal capabilities. Spatial abilities form another domain of nonreproductive sex-specific behaviors that provide evidence for prenatal steroid hormone involvement. In the Yu Cheng cohort, prenatally exposed boys were affected in their scores on the CPM and SPM tests, whereas in exposed girls no effect was seen. Because boys typically develop better spatial abilities than girls (26, 27), these results were interpreted as demasculinizing or feminizing effects caused by disturbances in steroid hormones by prenatal exposure to PCBs/PCDFs (11). On the basis of results of play behavior studies in several groups of children that were prenatally exposed to abnormal levels of endogenous or exogenous steroid hormones, it has been hypothesized that there is evidence for prenatal androgen influences on sexual differentiation of childhood play (16). Masculinized or defeminized childhood play behavior was reported in genetic females who were exposed to elevated androgens (28, 29), whereas demasculinized or feminized play behavior was associated with prenatal

exposure to progestrogenic compounds that are assumed to interfere with androgen action in genetic females and, more subtly, in genetic males (30).

In adults prenatally exposed to diethylstilbestrol (DES), a group that might be seen as a model group in studying potential estrogenic effects of prenatal PCB and dioxin exposure, childhood play behavior has been studied retrospectively. Males prenatally exposed to DES recalled slightly more masculinized play behavior than nonexposed controls, assessed by an interview covering childhood play behavior (31). In DES females no difference in childhood play, retrospectively assessed by questionnaires filled out by the DES subjects and their mothers, has been reported (32, 33). The effects of prenatal exposure to PCBs and dioxins on childhood play behavior we reported in this study are opposite of the results of these DES studies. This difference in effect can be related to the retrospective nature of these DES studies, and to differences in timing and duration of exposure to these chemicals in these groups. Moreover, differences in behavioral effects can be related to the level of exposure, which is likely to be higher in DES-exposed children. Many studies have reported that effects of exposure to hormones and hormone-mimicking chemicals show nonmonotonic dose-response curves, such as U-shaped or inverted U-shaped (34-38).

The current knowledge on the mechanisms of action of PCBs and dioxins and metabolites, such as hydroxylated PCBs, on prenatal steroid hormone metabolism is still limited. Complex interactions with various steroid hormone systems are suggested, including estrogen and androgen hormone systems (3). These systems can be affected on various levels and estrogenic (39, 40), anti-estrogenic (8, 41-43), and anti-androgen (7) effects have been described in *in vivo* and *in vitro* studies, possibly depending on congener type or metabolites. In this study we lack information on prenatal steroid hormone levels and although play behavior studies suggest that childhood play behavior is mediated predominantly by prenatal androgen action, our data are insufficient to exclude multiple endocrine effects to be involved in the mechanism of action of prenatal exposure to PCBs and dioxins.

In the environment, PCBs and dioxins are present as complex mixtures of various congeners that may vary in metabolism, toxicity, and endocrine-disrupting properties. In this study we measured PCBs 118, 138, 153, and 180 in maternal and cord plasma samples. The sum of these four most abundant congeners constitutes 46% of the total PCBs (44). In the BF group various PCB and dioxin congeners were measured in breast milk. Prenatal levels of ΣPCB_{milk} were associated with masculine play behavior, similar to what was seen using maternal and cord ΣPCB levels as prenatal exposure levels. Dioxin exposure was related with more feminine play behavior. Nondioxin-like PCB levels and dioxin-like PCB and total TEQ levels were not significantly associated with play behavior. Whether these results reflect effects that are specific to PCB or dioxin congeners or the limited power of analyses in this subgroup of BF children cannot be concluded from these results. Moreover,

total TEQ levels, the sum of the nondioxin-like PCBs, and the four PCBs in breast milk and maternal and cord plasma correlated highly with each other (20).

Play behavior in our study was not associated with postnatal exposure to PCBs and dioxins through breast-feeding. We therefore suggest that childhood play behavior is sensitive to endocrine-disrupting behavioral effects of exposure to PCBs and dioxins early in development, as is supported in females with congenital adrenal hyperplasia (29) and by studies in other mammalians (45, 46).

In conclusion, this is the first behavioral study in humans to show effects of prenatal exposure to environmental levels of PCBs and dioxins on behavior that shows marked sex differences. Moreover, sex-specific effects of background prenatal exposure to PCBs have not been previously reported in human PCB studies. The results of this exploratory study give evidence for steroid hormone involvement in the neurotoxic mechanism of action of prenatal exposure to environmental levels of PCBs, dioxins and other related organochlorine compounds. Evaluation of the relation between prenatal steroid hormone status and PCB and dioxin exposure is needed to further confirm these findings; in addition, follow-up of this cohort will be necessary to assess potential implications of these results on later development.

References

1. Weisglas-Kuperus N. Neurodevelopmental, immunological and endocrinological indices of perinatal human exposure to PCBs and dioxins. Chemosphere 1998;37(9-12):1845-53.
2. Brouwer A, Ahlborg UG, Van den Berg M, Birnbaum LS, Boersma ER, Bosveld B, et al. Functional aspects of developmental toxicity of polyhalogenated aromatic hydrocarbons in experimental animals and human infants. Eur J Pharmacol 1995;293(1):1-40.
3. Golden RJ, Noller KL, Titus-Ernstoff L, Kaufman RH, Mittendorf R, Stillman R, et al. Environmental endocrine modulators and human health: an assessment of the biological evidence. Crit Rev Toxicol 1998;28(2):109-227.
4. Vincent DR, Bradshaw WS, Booth GM, Seegmiller RE, Allen SD. Effect of PCB and DES on rat monoamine oxidase, acetylcholinesterase, testosterone, and estradiol ontogeny. Bull Environ Contam Toxicol 1992;48(6):884-93.
5. Matsumoto A. Synaptogenic action of sex steroids in developing and adult neuroendocrine brain. Psychoneuroendocrinology 1991;16(1-3):25-40.
6. Fitch RH, Denenberg VH. A role for ovarian hormones in sexual differentiation of the brain. Behav Brain Sci 1998;21(3):311-27.
7. Hany J, Lilienthal H, Sarasin A, Roth-Harer A, Fastabend A, Dunemann L, et al. Developmental exposure of rats to a reconstituted PCB mixture or aroclor 1254: effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. Toxicol Appl Pharmacol 1999;158(3):231-43.
8. Amin S, Moore RW, Peterson RE, Schantz SL. Gestational and lactational exposure to TCDD or coplanar PCBs alters adult expression of saccharin preference behavior in female rats. Neurotoxicol Teratol 2000;22(5):675-82.
9. Schantz SL, Moshtaghian J, Ness DK. Spatial learning deficits in adult rats exposed to ortho-substituted PCB congeners during gestation and lactation. Fundam Appl Toxicol 1995;26(1): 117-26.
10. Roegge CS, Seo BW, Crofton KM, Schantz SL. Gestational-lactational exposure to Aroclor 1254 impairs radial-arm maze performance in male rats. Toxicol Sci 2000;57(1):121-30.
11. Guo YL, Lai TJ, Chen SJ, Hsu CC. Gender-related decrease in Raven's progressive matrices scores in children prenatally exposed to polychlorinated biphenyls and related contaminants. Bull Environ Contam Toxicol 1995;55(1):8-13.
12. Williams CL, Meck WH. The organizational effects of gonadal steroids on sexually dimorphic spatial ability. Psychoneuroendocrinology 1991;16(1-3):155-76.
13. Bachevalier J, Hagger C. Sex differences in the development of learning abilities in primates. Psychoneuroendocrinology 1991;16(1-3):177-88.
14. Steward J, Cygan D. Ovarian hormones act early in development to feminize adult open-field behavvior in the rat. Horm Behav 1980;14(1):20-32.
15. Ward IL, Stehm KE. Prenatal stress feminizes juvenile play patterns in male rats. Physiol Behav 1991;50(3):601-5.
16. Collaer ML, Hines M. Human behavioral sex differences: A role for gonadal hormones during early development? Psychological Bulletin 1995;118(1):55-107.
17. Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MA, Van der Paauw CG, Tuinstra LG, Sauer PJ. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics 1996;97(5):700-6.
18. Patandin S, Lanting CI, Mulder PG, Boersma ER, Sauer PJ, Weisglas-Kuperus N. Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J Pediatr 1999;134(1):33-41.
19. Vreugdenhil HJ, Lanting CI, Mulder PG, Boersma ER, Weisglas-Kuperus N. Effects of prenatal PCB and dioxin background exposure on cognitive and motor abilities in Dutch children at school age. J Pediatr 2002;140(1):48-56.
20. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N, Van der Pauw CG, Tuinstra LGMT, Boersma ER, et al. PCB and dioxin levels in plasma and human milk of 418 Dutch women and

their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 1994;28(9):1721-1732.
21. Van den Berg M, Birnbaum L, Bosveld ATC, Brunstrom B, Cook P, Feeley M, et al. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 1998;106(12):775-92.
22. Golombok S, Rust J. The Pre-School Activities Inventory: A standardized assessment of gender role in children. Psychological Assessment 1993;5(2):131-136.
23. Golombok S, Rust J. The measurement of gender role behaviour in pre-school children: A research note. Journal of Child Psychology & Psychiatry & Allied Disciplines 1993;34(5):805-811.
24. Stinissen J, Willems PJ, Coetsier P, Hulsman WLL. Manual, Dutch version of the Wechsler Adult Intelligence Scale (WAIS). Amsterdam: Swets & Zeitlinger NV; 1970.
25. Caldwell B, Bradley R. Home observation of the environment. Administration manual. Little Rock, Arkansas; 1984.
26. Voyer D, Voyer S, Bryden MP. Magnitude of sex differences in spatial abilities: a meta-analysis and consideration of critical variables. Psychol Bull 1995;117(2):250-70.
27. Linn MC, Petersen AC. Emergence and characterization of sex differences in spatial ability: a meta-analysis. Child Dev 1985;56(6):1479-98.
28. Dittmann RW, Kappes MH, Kappes ME, Borger D, Stegner H, Willig RH, et al. Congenital adrenal hyperplasia. I: Gender-related behavior and attitudes in female patients and sisters. Psychoneuroendocrinology 1990;15(5-6):401-20.
29. Berenbaum SA, Duck SC, Bryk K. Behavioral effects of prenatal versus postnatal androgen excess in children with 21-hydroxylase-deficient congenital adrenal hyperplasia. J Clin Endocrinol Metab 2000;85(2):727-33.
30. Meyer-Bahlburg HF, Feldman JF, Cohen P, Ehrhardt AA. Perinatal factors in the development of gender-related play behavior: sex hormones versus pregnancy complications. Psychiatry 1988;51(3):260-71.
31. Kester P, Green R, Finch SJ, Williams K. Prenatal 'female hormone' administration and psychosexual development in human males. Psychoneuroendocrinology 1980;5(4):269-85.
32. Lish JD, Ehrhardt AA, Meyer-Bahlburg HF, Rosen LR, Gruen RS, Veridiano NP. Gender-related behavior development in females exposed to diethylstilbestrol (DES) in utero: an attempted replication. J Am Acad Child Adolesc Psychiatry 1991;30(1):29-37.
33. Lish JD, Meyer-Bahlburg HF, Ehrhardt AA, Travis BG, Veridiano NP. Prenatal exposure to diethylstilbestrol (DES): childhood play behavior and adult gender-role behavior in women. Arch Sex Behav 1992;21(5):423-41.
34. Calabrese EJ, Baldwin LA. The frequency of U-shaped dose responses in the toxicological literature. Toxicol Sci 2001;62(2):330-8.
35. Calabrese EJ, Baldwin LA. U-shaped dose-responses in biology, toxicology, and public health. Annu Rev Public Health 2001;22:15-33.
36. Palanza P, Parmigiani S, Liu H, vom Saal FS. Prenatal exposure to low doses of the estrogenic chemicals diethylstilbestrol and o,p'-DDT alters aggressive behavior of male and female house mice. Pharmacol Biochem Behav 1999;64(4):665-72.
37. Palanza P, Parmigiani S, vom Saal FS. Effects of prenatal exposure to low doses of diethylstilbestrol, o,p'DDT, and methoxychlor on postnatal growth and neurobehavioral development in male and female mice. Horm Behav 2001;40(2):252-65.
38. vom Saal FS, Timms BG, Montano MM, Palanza P, Thayer KA, Nagel SC, et al. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. Proc Natl Acad Sci U S A 1997;94(5):2056-61.
39. Bitman J, Cecil HC. Estrogenic activity of DDT analogs and polychlorinated biphenyls. J Agric Food Chem 1970;18(6):1108-12.
40. Korach KS, Sarver P, Chae K, McLachlan JA, McKinney JD. Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: conformationally restricted structural probes. Mol Pharmacol 1988;33(1):120-6.

41. Moore M, Mustain M, Daniel K, Chen I, Safe S, Zacharewski T, et al. Antiestrogenic activity of hydroxylated polychlorinated biphenyl congeners identified in human serum. Toxicol Appl Pharmacol 1997;142(1):160-8.
42. Kramer VJ, Helferich WG, Bergman A, Klasson-Wehler E, Giesy JP. Hydroxylated polychlorinated biphenyl metabolites are anti-estrogenic in a stably transfected human breast adenocarcinoma (MCF7) cell line. Toxicol Appl Pharmacol 1997;144(2):363-76.
43. Jansen HT, Cooke PS, Porcelli J, Liu TC, Hansen LG. Estrogenic and antiestrogenic actions of PCBs in the female rat: in vitro and in vivo studies. Reprod Toxicol 1993;7(3):237-48.
44. Jacobson JL, Humphrey HE, Jacobson SW, Schantz SL, Mullin MD, Welch R. Determinants of polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), and dichlorodiphenyl trichloroethane (DDT) levels in the sera of young children. Am J Public Health 1989;79(10): 1401-4.
45. Goy RW, McEwen BS. Sexual differentiation of the brain. Cambridge MA: MIT Press; 1980.
46. Wallen K. Nature needs nurture: the interaction of hormonal and social influences on the development of behavioral sex differences in rhesus monkeys. Horm Behav 1996;30(4):364-78.

Appendix

Pre-School Activity Inventory
© Susan Golombok and John Rust (22)

Name: Age: Sex: M/F (delete as appropriate)

Instructions

This inventory is about everyday activities of preschool children. It is in three sections: toy preferences, activities, and characteristics. Each question asks how frequently the child plays with particular toys, engages in particular activities or shows particular characteristics. There are five possible answers: (N) Never, (HE) Hardly ever, (S) Sometimes, (O) Often, or (VO) Very Often. Answer each question by circling the response which best describes the child, e.g. N HE S O VO

Please answer all of the questions. If you are unsure about which response best describes the child for any of the questions then please answer according to the response which seems most appropriate.

PART 1: TOYS: Please answer the questions according to how often the child played with the following toys during the past months.

1. Guns (or used objects as guns)	N HE S O VO
2. Jewelry	N HE S O VO
3. Tool set	N HE S O VO
4. Dolls, doll's clothes or doll's carriages	N HE S O VO
5. Trains, cars or airplanes	N HE S O VO
6. Swords (or used objects as swords)	N HE S O VO
7. Tea set	N HE S O VO

PART 2: ACTIVITIES: Please answer these questions according to how often the child engaged in the following activities during the past month.

1. Playing house (e.g. cleaning, cooking)	N HE S O VO
2. Playing with girls	N HE S O VO
3. Pretending to be a female character (e.g. princess)	N HE S O VO
4. Playing at having a male occupation (e.g. soldier)	N HE S O VO
5. Fighting	N HE S O VO
6. Pretending to be a family character	N HE S O VO
7. Sports and ball games	N HE S O VO

8. Climbing (e.g. fences, trees, gym equipment) N HE S O VO
9. Playing at taking care of babies N HE S O VO
10. Showing all interest in real cars, trains and equipment N HE S O VO
11. Dressing up in girlish clothes N HE S O VO

PART3:CHARACTERISTICS:Pleaseanswerquestionsaccordingtohowoftenthechildshowsthefollowing characteristics.

1. Likes to explore new surroundings N HE S O VO
2. Enjoys rough and tumble play N HE S O VO
3. Shows interest in snakes, spiders or insects N HE S O VO
4. Avoids getting dirty N HE S O VO
5. Likes pretty things N HE S O VO
6. Avoids taking risks N HE S O VO

NOW PLEASE CHECK THAT YOU HAVE ANSWERED ALL THE QUESTIONS

6 Effects of perinatal exposure to PCBs on neuropsychological functions in the Rotterdam cohort at 9 years of age

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Abstract

In this study, the effects of perinatal exposure to environmental levels of PCBs and dioxins on different neuropsychological functions were studied. The 26 lowest and 26 highest prenatally PCB exposed children in the breast-fed (BF) and the formula-fed (FF) group (n=104) of the Rotterdam PCB/dioxin cohort, were invited at 9 years of age to participate in a neuropsychological assessment. The assessment included the Rey Complex Figure, Auditory-Verbal Learning Test, Simple Reaction Time Task, Tower of London (TOL). Higher prenatal PCB levels were associated with longer reaction times (RT), more variation in RTs, and lower TOL scores. A longer breast-feeding duration was associated with lower TOL scores and with better spatial organizational strategy skills.

Conclusion: These results are suggestive of multi-focal neurotoxic effects of prenatal exposure to PCBs. The evaluation of effects of breast-feeding is complex since it contains contaminants and brain stimulating substances. In the suggested effects of lactational exposure to PCBs on the TOL scores, processes related to the prefrontal cortex may be involved in the neurotoxic mechanism. A complex task as the TOL may also serve as a sensitive outcome to assess neurodevelopmental risks of early exposure to PCBs and related compounds.

Introduction

In the past decades, evidence for neurotoxic effects of perinatal exposure to environmental levels of PCBs and dioxins in humans has accumulated. Effects of predominantly prenatal exposure to environmental levels of PCBs on neurodevelopmental outcome have been described in healthy infants (1-9) as well as delayed effects have been described on general cognitive development in older children (10-13).

Functional behavioral tests in perinatally PCB exposed monkeys have shown spatial learning delays (21-24) and deficits on discrimination reversal tasks (22, 23, 25, 26). Because of the resemblance of these PCB induced behavioral deficits and deficits in monkeys with lesions in the dorsolateral area of the prefrontal cortex, it has been suggested that perinatal exposure to PCBs may alter performance on tasks that require normal functioning of the dorsolateral area of the prefrontal cortex, or of one of the input or output pathways. Moreover, these behavioral deficits have been suggested to involve chemical alterations of the dopamine input to the dorsolateral prefrontal cortex (27).

In epidemiological studies, specific neuropsychological functions have been marginally addressed in the evaluation of neurotoxic effects of perinatal exposure to environmental levels of PCBs. From the functional domains that were studied, reaction time and attention (10, 28, 29), memory (30), and verbal comprehension (10, 11) were related to prenatal exposure to environmental levels of PCBs. In the Yu-Cheng cohort, spatial reasoning skills were lower in boys exposed to high levels of PCBs and PCDFs, due to accidental contamination of rice oil with these compounds, compared to control children (31).

In The Netherlands, effects of perinatal exposure to PCBs and dioxins were prospectively examined in a cohort of healthy born children from birth to school age. Since one of the aims of this study was to evaluate the merits of breast-feeding considering the relatively high lactational exposure to PCBs and dioxins, half of the group was breast-fed and the other half formula-fed during infancy. At 9 years of age, half of the Rotterdam population (the lowest and highest exposed quartiles per feeding group) was invited to participate in a neuropsychological assessment. Functions that were addressed were spatial perceptional organization, processing speed and sustained attention, memory functions, and executive functions, selected based on behavioral deficits described in animal and human PCB studies. The aim of this study was to gain more insight in neurotoxic effects of perinatal exposure to PCBs and dioxins by exploring effects of perinatal exposure to PCBs on these neuropsychological functions.

Methods

Subjects and study design

The original study population consisted of 207 healthy Caucasian mother-infant pairs who were recruited from June 1990 to February 1992 in the area of Rotterdam, a highly industrialized and densely populated area in The Netherlands. The study design and recruitment process, chemical analysis and PCBs and dioxin concentrations have been described in detail elsewhere (32). Pregnancy and delivery were uncomplicated. Only first or second, at term, born healthy children were included. One hundred and five children were breast-fed (BF) for at least six weeks and 102 children were formula-fed (FF) during infancy. All FF infants received formula from a single batch (Almiron M2, Nutricia NV, Zoetermeer, The Netherlands) from birth until 7 months of age. In this formula, concentrations of PCBs and dioxins were not detectable. During the last months of pregnancy, plasma samples were collected from the mothers. These samples were analyzed for four PCB congeners (International Union for Pure and Applied Chemistry (IUPAC) numbers 118, 138, 153 and 180). Prenatal exposure to PCBs was defined as the sum of these PCB congeners in maternal plasma samples.

At 9 years of age, we invited the 26 lowest and 26 highest prenatally exposed children from the BF group as well as from the FF group of children to participate in a follow-up assessment in the Sophia Children's Hospital in Rotterdam. Children were not eligible for selection when they had not participated in the follow-up at 42 or 84 months of age or when they had moved from the Rotterdam area, since families had to visit the hospital for the assessment. The medical ethics committee of the University Hospital Rotterdam/ Sophia Children's Hospital approved the study design and the parents gave informed consent.

Assessment of neuropsychological outcome variables

The neuropsychological assessment was done as part of an extended assessment of two hours in the Sophia Children's Hospital in Rotterdam by one psychologist (HV), who was unaware of the PCB and dioxin levels and type of feeding during infancy. The neuropsychological assessment consisted of the following tests, in following order:

Rey-Osterrieth Complex Figure Test (33, 34): This test consists of two tasks. In the 1st task, the copy task, a complex figure is presented to the child and the child is instructed to copy it. In the 2nd task, the recall task (presented after the Simple Reaction Time Test), the child is requested to draw the figure again from memory; the child was not notified of this when copying the figure. The drawings were scored according to Osterrieth's scoring criteria (34, 35) (range of raw score for both tasks: 0-36). These scores are used to assess visual perceptional organization (1st task) and visual-spatial memory (2nd task). Additionally the strategy of the configuration of the design was scored for the copy task, according to

Osterrieth's procedural types (described in (35)), running from 1 (= subject begins drawing the large rectangle and adds details later) to 7 (= the drawing is an unrecognizable scrawl).

Simple Reaction Time Test (SRTT) (36): In the six minutes, age-appropriate, version of the SRRT, the child is asked to press a button as quickly as possible after a red square (2.5*2.5 cm) appears on the computer screen. The inter-trial interval (2.5-5.0 sec.) is varied randomly to reduce effects of stimulus anticipation. Maximum response interval is 1 sec. Response latencies of all trials are recorded. The first 16 trials serve as practice; performance is measured over the next 80 trials. A minimum of 40 correct trials (i.e. 100<RT<1000 ms) is required. The two outcome variables of the SRTT that were used in this study were the mean reaction time (RT) and the variation in RT (SD). RT is a measurement of processing speed and sustained attention. SD assesses mainly sustained attention.

Auditory-Verbal Learning Test (Dutch version) (AVLT) (37): In this test, a list of 15 words is orally presented to the child five times. Immediately after each presentation, the child is asked to repeat these words. The sum of the words that were recalled over the five sessions was used as the short-term verbal memory score. A long-term memory score was derived by asking to recall the words after the assessment of the Tower of London task.

Tower of London (TOL) (38): In this test, subjects must look ahead to determine the order of moves necessary to rearrange three colored balls from their initial position in two upright sticks to match a target configuration on one or more sticks. The child is instructed to solve the problem in a certain number of moves, and has to succeed in maximal 3 attempts. The test consist of 12 items with increasing difficulty scored 3, 2, 1, or 0, depending on the number of attempts (respectively 1, 2, 3 or more) needed to solve the problem (range of score 0-36). This test requires planning, an executive function.

Assessment of confounding variables

Variables that may influence child neurodevelopment have been assessed and included birth weight, duration of gestation, fetal exposure to alcohol and cigarette smoking, parity, type of feeding during infancy, duration of breast-feeding, sex, maternal age and parental education level. The child's home environment was assessed by the Home Observation for Measurement of the Environment (HOME) (39) at 7 years of age. The verbal IQ of the parent that spends the most time with the child (usually the mother) was assessed by two subtests, Information and Vocabulary from the Dutch version of the Wechsler Adult Intelligence Scale (40).

Data analysis

In the present study, the outcome of children that were exposed prenatally to low levels of PCBs, as assessed from maternal plasma, was compared with the outcome of a high prenatally exposed group.

To compare groups for a single variable we used the Student's t-test, and the χ^2 test. The difference in neuropsychological outcome between the low and high prenatally exposed groups was studied by means of multiple regression analyses. To prevent that too many variables would be included in the regression model simultaneously, the model building procedure was hierarchical in a sense that variables that were likely to affect the neuropsychological outcome were included in the regression model as a fixed set of variables first. These variables were: sex (0/1=boy/girl), highest education level of either parent (0/1/2 = primary school, secondary school not finished/ secondary school finished/ high school finished, professional and university training), type of feeding and duration of breast-feeding (captured in two dummy variables for FF, BF_{short}=6-17 weeks of breast-feeding, and BF_{long}=≥ 17 weeks of breast-feeding) and age at examination. These variables were a priori included in the model along with the exposure variable ($\Sigma PCB_{low/high}$). Thereafter, remaining candidate confounding variables were tested one by one for adding to the model, by means of evaluating their correlation with both one of the outcome variables as well as the exposure variable, adjusted for the fixed set of variables already in the model. Candidate confounders were alcohol use (0/1=no/yes) and smoking during pregnancy (0/1=no/yes), duration of gestation, birth weight, and parity (0/1=1st/2nd born), parental verbal IQ, and HOME score. The selection procedure was based on a correlation ($p<0.2$) with the exposure variable ($\Sigma PCB_{low/high}$) and with the scores on at least one of the neuropsychological outcome variable, adjusted for the above mentioned fixed set of variables. This procedure resulted in the following additional set of confounders: alcohol use during pregnancy, gestational age, parity, and parental verbal IQ. Results were considered significant if $p \leq 0.05$.

Results

From the invited children (n=104), 83 (80%) were willing to participate (mean±SD age 9.2±0.2). The parents of 21 children were not motivated to participate in this follow-up. Exposure levels of participating and nonparticipating children were comparable. The characteristics of the low and high exposed children are presented in Table 6.1. In the high exposed group, more children were exposed to alcohol during pregnancy and the mean gestational age was shorter. Parental education level and verbal IQ was higher in the high exposed group compared to the low exposed group of children. All prenatal exposure measurements of PCBs and dioxins were significantly higher in the high exposure group, as can be expected from the study design.

In Table 6.2, the mean scores on the neuropsychological tests are presented. All children were able to complete the tests. However, one SSRT assessment was missing due to technical problems and in three cases the strategy of the Rey Complex Figure Test could not be recorded. Univariate analysis showed no statistical differences between the exposure groups on the neuropsychological outcome variables.

Table 6.1 Characteristics of the low and high prenatal PCB exposure groups.

Characteristics	ΣPCB_{low} (n=42)	ΣPCB_{high} (n=41)
Smoking during pregnancy, yes	10 (24 %)	11 (27 %)
Alcohol use during pregnancy, yes*	2 (5 %)	10 (24 %)
Birth weight (gr)	3469 (± 459)	3377 (± 504)
Gestational age (wk) *	40.3 (± 1.1)	39.7 (± 1.4)
Number of BF	21 (50 %)	23 (56 %)
Duration of breast-feeding (wk)	16 (6-62)	17 (6-54)
Number of boys	20 (48 %)	24 (59 %)
Number of 1st born	23 (55 %)	18 (44 %)
Parental education level **		
Low	10 (24 %)	1 (2 %)
Medium	19 (45 %)	16 (39 %)
High	13 (31 %)	24 (59 %)
Parental verbal IQ **	116.3 (± 16.0)	130.0 (± 13.8)
HOME score at 7 years	48.1 (± 2.6)	48.5 (± 2.7)
Age at assessment (yr)	9.1 (± 0.2)	9.2 (± 0.2)
Exposure variables		
$\Sigma PCB_{maternal}$ (µg/L) **	1.40 (0.59-1.93)	3.22 (2.51-5.08)
ΣPCB_{cord} (µg/L) **	0.29 (0.08-0.63)	0.56 (0.25-1.98)
ΣPCB_{milk} (µg/kg fat) **	275.7 (173.7-566.1)	572.4 (333.6-804.5)
$\Sigma PCB_{20\ nondioxin\text{-}like}$ (µg/kg fat) **	297.4 (204.6-578.6)	608.5 (347.2-890.5)
Total $TEQ_{PCB+dioxin}$ (ng/kg fat)**	46.24 (28.06-88.20)	84.05 (58.00-111.41)

Values are means (± standard deviations), medians (range), and numbers (percentages).
* $p<0.05$; ** $p<0.01$ (Students t-test or χ^2 test).
Parental education level: low=primary school, secondary school not finished, middle=secondary school finished, high=high school finished, professional and university training; Parental verbal IQ score on two subtests of the Wechsler Adult Intelligence Scale, Information and Vocabulary, assessed from one of the parents; HOME: Home Observation for the Measurement of the Environment at school age; $\Sigma PCB_{maternal,cord,milk}$: sum of PCB congeners IUPAC nos. 118, 138, 153, 180 in maternal and cord plasma, and breast milk; $\Sigma PCB_{20nondioxin\text{-}like}$: sum of 20 non dioxin-like PCBs in breast milk; Total TEQ: sum of the toxic equivalents according to the 1997 WHO TEF values for mono-ortho PCBs, UIPAC nos. 105, 118, 156, planar PCBs, IUPAC nos. 77, 126, 169, and 17 dioxins (PCDDs and PCDFs).

Table 6.2 Mean scores of the low and high prenatal PCB exposure groups on the neuropsychological tests.

Test	ΣPCB_{low}	ΣPCB_{high}
Rey copy (n=83)[a]	28.3 (± 5.9)	29.0 (± 4.6)
Rey recall (n=83)[a]	15.7 (± 5.8)	15.7 (± 5.9)
Rey copy strategy (n=80) [a]	3.8 (± 0.5)	3.8 (± 0.4)
SRTT RT (n=82) [b]	343.3 (± 47.5)	354.0 (± 52.7)
SRTT SD (n=82) [b]	79.3 (± 25.3)	90.2 (± 32.4)
AVLT short (n=83) [c]	46.1 (± 8.7)	45.8 (± 8.1)
AVLT long (n=83) [c]	10.0 (± 2.5)	10.0 (± 2.4)
TOL (n=83) [d]	29.9 (± 2.7)	29.8 (± 3.2)

Values are means (± standard deviations).
[a] Rey Complex Figure Test; [b] Simple Reaction Time Task, RT=reaction time, SD=variation in reaction time; [c] Auditory-Verbal Learning Test; [d] Tower of London.

Table 6.3 Results of multiple regression analysis on neuropsychological outcome variables.

	ΣPCB_{high} versus ΣPCB_{low} (=0)			**BF_{short} versus FF (=0)**			**BF_{long} versus FF (=0)**			**BF_{long} versus BF_{short} (=0)**			
	Regr. coef.	SE	p	Regr. coef.	SE	p	Regr. coef.	SE	p	Regr. coef.	SE	p	Adj. R^2
Rey copy	-0.95	1.34	0.479	-0.26	1.45	0.858	0.20	1.45	0.894	0.46	1.65	0.784	.03
Rey recall	-0.97	1.51	0.524	1.53	1.64	0.355	1.77	1.64	0.285	0.25	1.87	0.896	.01
Rey copy strategy	0.09	0.11	0.432	-0.27	0.12	0.028	-0.25	0.12	0.038	0.01	0.14	0.927	.08
SRTT RT	26.58	12.76	0.041	18.88	13.79	0.175	20.42	14.03	0.150	1.53	15.70	0.922	.04
SRTT SD	22.04	6.77	0.002	2.48	7.31	0.735	-6.95	7.44	0.354	-9.44	8.33	0.261	.23
AVLT short	-1.35	2.17	0.536	-2.02	2.35	0.395	-1.05	2.36	0.657	0.96	2.68	0.721	.02
AVLT long	0.06	0.61	0.925	-0.89	0.66	0.184	0.17	0.66	0.797	1.06	0.76	0.164	.17
TOL	-1.85	0.67	0.007	-0.39	0.72	0.593	-1.81	0.73	0.015	-1.42	0.82	0.089	.23

Additional variables in the regression models alcohol used during pregnancy, gestational age, sex, parity, parental education level, parental verbal IQ, age at assessment. Regression coefficients were estimated in essentially the same regression model by reparameterizing the effects of the three categories for duration of breast-feeding (FF: 6-17 weeks, ≥17 weeks). FF: formula-fed; BF_{short}: 6-17 weeks of breast-feeding; BF_{long}: ≥17 weeks of breast-feeding; Rey: Rey Complex Figure Test; SRTT: Simple Reaction Time Task, RT = reaction time, SD = variation in reaction time; AVLT: Auditory-Verbal Learning Test; TOL: Tower of London.

The results of multiple linear regression analyses on the neuropsychological outcome variables are presented in Table 6.3 for level of exposure and type of feeding and duration of breast-feeding, adjusted for confounding variables. Compared to the low exposed children, children in the high exposure group had significantly longer RTs and more variation in RT (larger SDs) on the SRTT. High exposed children scored also significantly lower on the TOL. BF children (BF_{short} and BF_{long}) scored significantly lower, indicating better performance, on the strategy score of the Rey Complex Figure test compared to FF children.

Children that were BF for a long period scored significantly lower on the TOL than their FF counterparts. To explore whether this effect was related to lactational exposure to PCBs, the total population was divided in six groups based on prenatal exposure level (ΣPCB_{low} and ΣPCB_{high}), feeding type and duration of breast-feeding (FF, BF_{short} (6-17 weeks) and BF_{long} ($\geq$ 17 weeks)).

In Figure 6.1, the adjusted mean scores on the TOL are presented for these groups. Compared to low exposed FF children (a), high exposed FF children (a_1, p=0.031), and high exposed BF, for short (a_2, p=0.018) or long periods (a_4, p=0.003), scored lower on the TOL. Low exposed children BF for long periods scored also lower on the TOL (b_1, p=0.021) than high exposed children that were BF for short periods. Moreover, low exposed children that were BF for a long period scored lower on the TOL (a_3, p=0.026) than their low exposed FF counterparts.

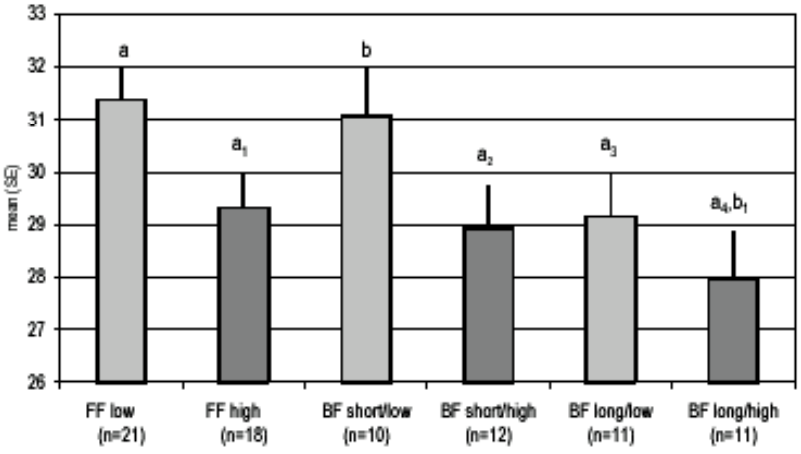

Figure 6.1 Adjusted mean (SD) scores on the Tower of London.

Adjustedforfetalalcoholexposure,gestationalage,sex,parentaleducationlevel,parentalverbalIQ,ageatassessment.
a1, a2, a3, and a4 = adjusted mean scores are significantly different from a
b1 = adjusted mean score is significantly different from b

Discussion

The results of this study suggest that prenatal exposure to environmental levels of PCBs may affect reaction times and the variation in reaction times as well as the scores on the TOL. Moreover, some evidence of negative effects of lactational exposure to PCBs on the scores on the TOL is found.

Reaction time and its variation mainly reflect processing speed as well as the ability to sustain attention. Impaired attention and concentration are amongst the most common mental problems associated with brain damage (41) and indeed attentional deficits have been linked to prenatal exposure to several other neurotoxic agents (such as alcohol (42), and cocaine (43)). Higher prenatal PCB levels were also related to slower processing speed and lower attention skills in the Lake Michigan cohort in 4-year-old children (28) and with less concentration skills in 11-year-old children (10). In a Faeroes cohort, at 7 years of age (29), longer reaction time was also associated with higher prenatal exposure to PCBs, however this relation appeared to be mainly attributable to prenatal exposure to another neurotoxic agent, methyl-mercury.

The TOL is an executive function task. Generally these tasks are complex multi-factorial tasks and performance may reflect frontal lobe functions as well as more posterior related functions. For example, performance on the TOL requires functions such as planning, spatial working memory, attention and response inhibition, the ability to relate and integrate isolated details into a coherent whole, as well as spatial and motor abilities. Affected TOL performance may therefore suggest impairment in either one of these functions. Functional brain imaging studies in adults, and neuropsychological studies in patients with frontal lobe damage (44) have provided evidence that performance on the TOL requires processes that are linked to the prefrontal area of the brain (45-47). Moreover, functional brain imaging studies in adults showed activation during performance on the TOL in other brain areas including the premotor cortex, parietal cortex, anterior cingulate cortex, prestriate cortex and midline cerebellum (45, 48-50).

The authors of behavioral PCB studies in animals have hypothesized that the behavioral deficits suggested that processes related to the prefrontal cortex were involved in the neurotoxic effects of prenatal exposure to PCBs as well as lactational exposure to low levels of PCBs. These deficits included impairment of learning a delayed spatial alteration task (21, 22), impaired performance on discrimination-reversal tasks, more perseverative responding (22, 51), and an inability to inhibit inappropriate responding (21). The PCB-induced dopaminergic alterations that were described in animals studies (16, 52) were suggested to support this hypothesis since the mesocortical dopaminergic system is considered to be one of the most prominent innervating systems of the prefrontal cortex (53). The results of the present study may therefore suggest that the effects of prenatal

exposure to PCBs are diffuse or multi-focal; a spectrum of effects in which processes of the prefrontal cortex may be involved.

The TOL scores were negatively associated with the duration of breast-feeding. Breast milk contains several substances (such as long chain polyunsaturated fatty acids) that may positively affect brain development. In fact, a positive effect of breast-feeding is seen in this study on the strategies used to copy a figure, a complex executive function. The finding of a negative effect of a longer duration of breast-feeding on the TOL scores, therefore, may suggest adverse effects of neurotoxic compounds that breast milk is contaminated by. The duration of breast-feeding in combination with the exposure level is generally used to estimate lactational exposure to PCBs. Since the levels of PCBs measured in breast milk samples that were obtained shortly after birth correlated strongly with PCB levels in maternal plasma (54), we used maternal plasma PCB levels in combination with the duration of breast-feeding to estimate lactational exposure. The results of this study provided some evidence of adverse effects of lactational exposure to PCBs since low exposed children that were BF for a long period scored lower on the TOL than their FF low exposed counterparts.

Apart from the scores on the TOL there was no evidence that the other outcomes that were addressed in this study were negatively affected by a longer duration of breast-feeding. Postnatally, brain development consists mainly of synaptic pruning, elaboration of dendritic arborization and myelination (55-58); the formation and refinement of neuronal networks. The frontal cortex shows delayed myelination and synaptogenesis compared to other brain regions and structural maturation continuous until adolescence or early adulthood (59-61). Since developing brain processes are considered to be especially vulnerable to exposure to neurotoxicants, structure related effects of lactational exposure can be hypothesized. Therefore, in the suggested neurotoxic effects of lactational exposure on the TOL scores, processes that are related to the prefrontal cortex may be involved. It could also be argued that the effects of lactational exposure seen on performance on the TOL are related to a simultaneous appeal on several frontal functions. It may reflect subtle effects of lactational exposure on the neuronal networks supporting the integration of these complex frontal functions, whereas an appeal on one of these functions, such as attention, may not be affected by lactational exposure to PCBs or related compounds. However, these results are not conclusive to exclude neurotoxic effects on other functions that are required in performance on the TOL. Due to the multi-factorial nature of this task, the TOL may serve as a sensitive outcome to measure diffuse effects of exposure to PCBs.

Although BF infants are exposed to relatively large amounts of PCBs and related compounds, neurodevelopmental effects of lactational exposure to PCBs were scarcely detected (8, 12) and currently, prenatal CNS development is considered to be more vulnerable to these neurotoxic agents. However, as described earlier, recent animal behavioral studies do give evidence of negative effects of lactational exposure to low levels

of PCBs (21, 22, 51). The evaluation of the effects of breast-feeding, consisting of both neurotoxic and brain stimulating substances, on the developing brain is very complex. For example, apart from the positive effect of a longer duration of breast-feeding described in this study on spatial organizational skills, neurophysiological assessment in this cohort during the same follow-up session showed a positive effect of a longer duration of breast-feeding on the latency of the cognitive event related potential peak, the P300, whereas prenatal exposure to PCBs affected the latency of this peak negatively (62). Further detailed neuropsychological studies are needed to differentiate these effects more thoroughly.

In this study, no significant effects of perinatal exposure of PCBs on memory and visual perceptional skills were seen. Nonsignificance does not imply that there is no effect, considering the large SE's of the estimated effects and corresponding wide confidence intervals for the true effects in our study. So there is some risk of an effect remaining undetected (type II error). However, these results are in agreement with a study in a Faeroes cohort at 7 years of age in which the scores on the Bender Visual Motor Gestalt Test and the California Verbal Learning Test were not related with prenatal exposure to PCBs (29). In the Lake Michigan cohort, however, at 4 years of age negative effects of prenatal PCB exposure on memory skills have been reported (30). Memory is a complex function, consisting of various different aspects, such as storage, encoding and retrieval of information (63), memory strategies, and attention, in which different brain systems or circuits are suggested to be involved (64). This may explain some of the differences in measured effects of prenatal PCB exposure on memory. For example, the use of strategies in memorizing can be trained to some extent, and may be more susceptible to environmental influences. In our cohort at 7 years of age, effects of prenatal PCB exposure on memory skills were not detectable in children raised in more optimal parental and home environmental conditions, whereas a negative effect of prenatal PCB exposure on memory skills was seen in children raised in less advantaged families (13).

In the present study, we compared a group of low and high prenatally exposed children, based on maternal ΣPCB levels. For this cohort additional exposure measurements were available, including the sum of the four PCBs, IUPAC numbers 118, 138, 153 and 180, measured in cord blood and in breast milk as well as additional measurements of PCB congeners and also dioxins in breast milk. Since relations between prenatal exposure to PCBs and neurodevelopmental aspects were most pronounced in the Dutch PCB/dioxin cohort using maternal PCB levels, we have used this exposure measurement to distinguish the low and high prenatally exposed groups. The levels of dioxin toxic equivalent factors and nondioxin-like PCBs were also significantly different in these exposure groups. In the environment, PCBs, their metabolites and related compounds, such as dioxins, are present as complex mixtures of various congeners that may vary in metabolism and toxicity. Moreover, levels of PCBs and dioxins are interrelated (54) and consequently specific effects of either group of compounds are methodological difficult to detect. We therefore believe

that the difference in outcome between the low and high exposure group could also be related to differences in exposure levels of other PCB congeners, dioxins, and related compounds.

Human PCB studies are of correlational nature which underscores aspects of confounding by, for example, other related neurotoxic compounds. In the Lake Michigan cohort, mothers were selected based on their diet history on Lake Michigan PCB contaminated fish. Fish and other aquatic species form often the source of exposure to PCBs as well as other neurotoxic compounds, such as methyl-mercury. The relations between neurodevelopment and prenatal PCB exposure as described in the Lake Michigan studies, therefore, may have been confounded by exposure to this compound. However, based on the congruence between the results of animal studies and several human cohort studies, it has been suggested that the deficits observed in the Lake Michigan studies result at least in part from PCB exposure (65). In contrast to the Lake Michigan study, the Dutch PCB/dioxin cohort is drawn from the general population. In the Netherlands, PCB and dioxin exposure occurs mainly through dietary intake of predominantly dairy products, as well as processed foods and meat and fish products (66). In this population, lead and cadmium levels in blood samples drawn from 18-months-old children (n=151) were relatively low (67) and not related to cognitive outcome (11).

A point of importance in the interpretation of the results of this study is the question of the magnitude of the observed effects. For example, the difference between the low and high exposure groups observed in the RT is approximately 25 ms, and for the TOL scores less than 2 points. These differences in performance, although identifiable with sensitive neuropsychological tests, may not be of clinical significance but do indicate that exposure at this level may not be sufficiently low to avoid all effects on psychometric performance. Further, it is recognized that multiple testing does run the risk of statistical significance by chance. Nevertheless, the attentional effects described in this study and by others (10, 28, 29) show consistency in that reduced performance was observed in tests concerned with attention/vigilance as was the effect on the TOL in support of behavioral animal studies (22-24, 26). Moreover, the probability of finding at least six significant effects just by chance amongst 24 independent tests is very small, given a test-wise error of 0.05.

In conclusion, the results of this study are suggestive of negative effects of prenatal exposure to PCBs and related compounds on reaction times, the variation in reaction time, and on scores on the TOL. Moreover, these results provide some evidence of negative effects of lactational exposure on performance on the TOL. The evaluation of the effects of breast-feeding, consisting of both neurotoxic and brain stimulating substances, on the developing brain is very complex and a positive effect of a longer duration of breast-feeding was found in this study on spatial organizational strategy skills. Neurotoxic effects of prenatal exposure to PCBs are likely to be multi-focal or diffuse. Postnatally, maturation rates are different for different brain structures and developing brain structures are likely to

be more vulnerable to exposure to PCBs and dioxins. The frontal cortex shows a delayed maturation rate compared to other brain regions. These results may therefore suggest that processes related to the prefrontal cortex are involved in the neurotoxic mechanisms of action of lactational exposure to PCBs and related compounds. It can also be hypothesized that an executive function and therefore complex task as the TOL is a more sensitive outcome to assess neurodevelopmental risks of perinatal exposure to PCBs and related compounds. Although these results are important since they are in support of human and behavioral animal studies addressing perinatal effects of PCB exposure, they require replication in a larger study population. Moreover, this study proved neuropsychological assessment to be an important and sensitive tool. This approach, in combination with behavioral animal studies and *in vivo* and *in vitro* neurochemical studies, will increase our knowledge in neurotoxic mechanisms of effects of perinatal exposure to PCBs and related compounds.

References

1. Rogan WJ, Gladen BC. PCBs, DDE, and child development at 18 and 24 months. Ann Epidemiol 1991;1(5):407-13.
2. Stewart P, Reihman J, Lonky E, Darvill T, Pagano J. Prenatal PCB exposure and neonatal behavioral assessment scale (NBAS) performance. Neurotoxicol Teratol 2000;22(1):21-9.
3. Winneke G, Bucholski A, Heinzow B, Kramer U, Schmidt E, Walkowiak J, et al. Developmental neurotoxicity of polychlorinated biphenyls (PCBS): cognitive and psychomotor functions in 7-month-old children. Toxicol Lett 1998;102-103:423-8.
4. Jacobson JL, Jacobson SW, Fein G, Scwartz P, Dowler J. Prenatal exposure to environmental toxin: A test of the multiple effects model. Developmental Psychology 1984;20(4):523-532.
5. Jacobson SW, Fein GG, Jacobson JL, Schwartz PM, Dowler JK. The effect of intrauterine PCB exposure on visual recognition memory. Child Dev 1985;56(4):853-60.
6. Huisman M, Koopman-Esseboom C, Fidler V, Hadders-Algra M, van der Paauw CG, Tuinstra LG, et al. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. Early Hum Dev 1995;41(2):111-27.
7. Huisman M, Koopman-Esseboom C, Lanting CI, van der Paauw CG, Tuinstra LG, Fidler V, et al. Neurological condition in 18-month-old children perinatally exposed to polychlorinated biphenyls and dioxins. Early Hum Dev 1995;43(2):165-76.
8. Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MA, Van der Paauw CG, Tuinstra LG, Sauer PJ. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics 1996;97(5):700-6.
9. Darvill T, Lonky E, Reihman J, Stewart P, Pagano J. Prenatal exposure to PCBs and infant performance on the fagan test of infant intelligence. Neurotoxicology 2000;21(6):1029-38.
10. Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychorinated biphenyls in utero. N Engl J Med 1996;335:783-9.
11. Patandin S, Lanting CI, Mulder PG, Boersma ER, Sauer PJ, Weisglas-Kuperus N. Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J Pediatr 1999;134(1):33-41.
12. Walkowiak J, Wiener JA, Fastabend A, Heinzow B, Kramer U, Schmidt E, et al. Environmental exposure to polychlorinated biphenyls and quality of the home environment: effects on psychodevelopment in early childhood. Lancet 2001;358(9293):1602-7.
13. Vreugdenhil HJ, Lanting CI, Mulder PG, Boersma ER, Weisglas-Kuperus N. Effects of prenatal PCB and dioxin background exposure on cognitive and motor abilities in Dutch children at school age. J Pediatr 2002;140(1):48-56.
14. Seegal RF, Shain W. Neurotoxicicity of polychlorinated biphenyls. In: Isaacson RL, Jensen KF, editors. The Vulnerable Brain and Environmental Risks. New York: Plenum Press; 1992; 169-195.
15. Morse DC, Plug A, Wesseling W, Vandenberg KJ, Brouwer A. Persistent alterations in regional brain glial fibrillary acidic protein and synaptophysin levels following pre- and postnatal polychlorinated biphenyl exposure. Toxicol Appl Pharmacol 1996;139(2):252-261.
16. Mariussen E, Fonnum F. The effect of polychlorinated biphenyls on the high affinity uptake of the neurotransmitters, dopamine, serotonin, glutamate and GABA, into rat brain synaptosomes. Toxicology 2001;159(1-2):11-21.
17. Seegal RF, Brosch K, Bush B, Ritz M, Shain W. Effects of Aroclor 1254 on dopamine and norepinephrine concentrations in pheochromocytoma (PC-12) cells. Neurotoxicology 1989;10(4):757-64.
18. Seegal RF, Bush B, Brosch KO. Comparison of effects of Aroclors 1016 and 1260 on non-human primate catecholamine function. Toxicology 1991;66(2):145-63.
19. Brouwer A, Longnecker MP, Birnbaum LS, Cogliano J, Kostyniak P, Moore J, et al. Characterization of potential endocrine-related health effects at low- dose levels of exposure to PCBs. Environ Health Perspect 1999;107 Suppl 4:639-49.
20. Weisglas-Kuperus N. Neurodevelopmental, immunological and endocrinological indices of perinatal human exposure to PCBs and dioxins. Chemosphere 1998;37(9-12):1845-53.

21. Rice DC. Behavioral impairment produced by low-level postnatal PCB exposure in monkeys. Environ Res 1999;80:S113-S121.
22. Rice DC, Hayward S. Effects of postnatal exposure to a PCB mixture in monkeys on nonspatial discrimination reversal and delayed alternation performance. Neurotoxicology 1997;18(2):479-94.
23. Schantz SL, Levin ED, Bowman RE. Long-term neurobehavioral effects of perinatal polychlorinated biphenyl (PCB) exposure in monkeys. Environmental Toxicology and Chemistry 1991;10:747-756.
24. Rice DC, Hayward S. Effects of exposure to 3,3',4,4',5-pentachlorobiphenyl (PCB 126) throughout gestation and lactation on behavior (concurrent random interval-random interval and progressive ratio performance) in rats. Neurotoxicol Teratol 1999;21(6):679-87.
25. Schantz SL, Levin ED, Bowman RE, Heironimus MP, Laughlin NK. Effects of perinatal PCB exposure on discrimination-reversal learning in monkeys. Neurotoxicol Teratol 1989;11(3):243-50.
26. Brouwer A, Ahlborg UG, Van den Berg M, Birnbaum LS, Boersma ER, Bosveld B, et al. Functional aspects of developmental toxicity of polyhalogenated aromatic hydrocarbons in experimental animals and human infants. Eur J Pharmacol 1995;293(1):1-40.
27. Levin ED, Schantz SL, Bowman RE. Use of the lesion model for examining toxicant effects on cognitive behavior. Neurotoxicol Teratol 1992;14(2):131-41.
28. Jacobson JL, Jacobson SW, Padgett RT, Brumitt GA, Billings RL. Effects of Prenatal PCB Exposure on Cognitive Processing Efficiency and Sustained Attention. Developmental Psychology 1992;28(2):297-306.
29. Grandjean P, Weihe P, Burse VW, Needham LL, Storr-Hansen E, Heinzow B, et al. Neurobehavioral deficits associated with PCB in 7-year-old children prenatally exposed to seafood neurotoxicants. Neurotoxicol Teratol 2001;23(4):305-17.
30. Jacobson JL, Jacobson SW, Humphrey HE. Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J Pediatr 1990;116(1):38-45.
31. Guo YL, Lai TJ, Chen SJ, Hsu CC. Gender-related decrease in Raven's progressive matrices scores in children prenatally exposed to polychlorinated biphenyls and related contaminants. Bull Environ Contam Toxicol 1995;55(1):8-13.
32. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N, Boersma ER, de Ridder MA, Van der Paauw CG, et al. Dioxin and PCB levels in blood and human milk in relation to living areas in The Netherlands. Chemosphere 1994;29(9-11):2327-38.
33. Rey A. L'Examen psychologique dans les cas d'encephalopathie traumatique. Archives de Psychologie 1941;28:286-340.
34. Osterrieth PA. Le Test de Copie d'une Figure Complex. Archives de Psychologie 1944;30:206-356.
35. Lezak MD. Neuropsychological Assessment. New York: Oxford University Press; 1995.
36. Letz R. NES2 Test users manual (version 6.6). Winchester, MA: Neurobehavioral Systems Inc.; 1994.
37. Kalverboer AF, Deelman BG. Voorlopige selectie van enkele tests voor het onderzoeken van geheugenstoornissen van verschillende aard. Groningen, The Netherlands: Groningen University, Department of Neuropsychology; 1964.
38. Shallice T. Specific impairments of planning. Philos Trans R Soc Lond B Biol Sci 1982;298(1089):199-209.
39. Caldwell B, Bradley R. Home observation of the environment. Administration manual. Little Rock, Arkansas; 1984.
40. Stinissen J, Willems PJ, Coetsier P, Hulsman WLL. Manual, Dutch version of the Wechsler Adult Intelligence Scale (WAIS). Amsterdam: Swets & Zeitlinger NV; 1970.
41. Lezak MD. Subtle sequelae of brain damage. Perplexity, distractibility, and fatigue. Am J Phys Med 1978;57(1):9-15.
42. Streissguth AP, Barr HM, Sampson PD, Parrish-Johnson JC, Kirchner GL, Martin DC. Attention, distraction and reaction time at age 7 years and prenatal alcohol exposure. Neurobehav Toxicol Teratol 1986;8(6):717-25.

43. Richardson GA, Conroy ML, Day NL. Prenatal cocaine exposure: effects on the development of school-age children. Neurotoxicol Teratol 1996;18(6):627-34.
44. Owen AM, Downes JJ, Sahakian BJ, Polkey CE, Robbins TW. Planning and spatial working memory following frontal lobe lesions in man. Neuropsychologia 1990;28(10):1021-34.
45. Dagher A, Owen AM, Boecker H, Brooks DJ. Mapping the network for planning: a correlational PET activation study with the Tower of London task. Brain 1999;122(Pt 10):1973-87.
46. Owen AM, Doyon J, Petrides M, Evans AC. Planning and spatial working memory: a positron emission tomography study in humans. Eur J Neurosci 1996;8(2):353-64.
47. Rezai K, Andreasen NC, Alliger R, Cohen G, Swayze V, 2nd, O'Leary DS. The neuropsychology of the prefrontal cortex. Arch Neurol 1993;50(6):636-42.
48. Lazeron RH, Rombouts SA, Machielsen WC, Scheltens P, Witter MP, Uylings HB, et al. Visualizing brain activation during planning: the tower of London test adapted for functional MR imaging. Am J Neuroradiol 2000;21(8):1407-14.
49. Baker SC, Rogers RD, Owen AM, Frith CD, Dolan RJ, Frackowiak RS, et al. Neural systems engaged by planning: a PET study of the Tower of London task. Neuropsychologia 1996;34(6): 515-26.
50. Rowe JB, Owen AM, Johnsrude IS, Passingham RE. Imaging the mental components of a planning task. Neuropsychologia 2001;39(3):315-27.
51. Rice DC. Effects of postnatal exposure of monkeys to a PCB mixture on spatial discrimination reversal and DRL performance. Neurotoxicol Teratol 1998;20(4):391-400.
52. Seegal RF, Brosch KO, Bush B. Polychlorinated biphenyls produce regional alterations of dopamine metabolism in rat brain. Toxicol Lett 1986;30(2):197-202.
53. Saper CB, Iversen S, Frackowiak RS. The association areas of the cerebral cortex and the cognitive capacities of the brain. In: Kandell ER, Schwartz JH, Jessel TM, editors. Principles of Neural Science. United States of America: McGraw-Hill; 2000; 361.
54. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N, Van der Pauw CG, Tuinstra LGMT, Boersma ER, et al. PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 1994;28(9):1721-1732.
55. Huttenlocher PR. Morphometric study of human cerebral cortex development. Neuropsychologia 1990;28(6):517-27.
56. Changeux JP, Danchin A. Selective stabilisation of developing synapses as a mechanism for the specification of neuronal networks. Nature 1976;264(5588):705-12.
57. Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, et al. Brain development during childhood and adolescence: a longitudinal MRI study. Nat Neurosci 1999;2(10):861-3.
58. Paus T, Zijdenbos A, Worsley K, Collins DL, Blumenthal J, Giedd JN, et al. Structural maturation of neural pathways in children and adolescents: in vivo study. Science 1999;283(5409):1908-11.
59. Huttenlocher PR. Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res 1979;163(2):195-205.
60. Huttenlocher PR, Dabholkar AS. Regional differences in synaptogenesis in human cerebral cortex. J Comp Neurol 1997;387(2):167-78.
61. Sowell ER, Thompson PM, Holmes CJ, Batth R, Jernigan TL, Toga AW. Localizing age-related changes in brain structure between childhood and adolescence using statistical parametric mapping. Neuroimage 1999;9(6 Pt 1):587-97.
62. Vreugdenhil HJI, van Zanten GA, Brocaar M, Weisglas-Kuperus N. Prenatal PCB exposure and breast-feeding and auditory P300 in 9-year-old Dutch children. Developmental Medicine and Child Neurology, in press.
63. Tulving E, Markowitsch HJ. Memory beyond the hippocampus. Curr Opin Neurobiol 1997;7(2): 209-16.
64. Milner B, Squire LR, Kandel ER. Cognitive neuroscience and the study of memory. Neuron 1998;20(3):445-68.
65. Rice DC. Neurotoxicity of lead, methylmercury, and PCBs in relation to the Great Lakes. Environ Health Perspect 1995;103 Suppl 9:71-87.

66. Patandin S, Dagnelie PC, Mulder PG, Op de Coul E, van der Veen JE, Weisglas-Kuperus N, et al. Dietary exposure to polychlorinated biphenyls and dioxins from infancy until adulthood: A comparison between breast-feeding, toddler, and long- term exposure. Environ Health Perspect 1999;107(1):45-51.
67. Weisglas-Kuperus N, Patandin S, Berbers GA, Sas TC, Mulder PG, Sauer PJ, et al. Immunologic Effects of Background Exposure to Polychlorinated Biphenyls and Dioxins in Dutch Preschool Children. Environ Health Perspect 2000;108(12):1203-1207.

7

Prenatal PCB exposure and breast-feeding and auditory P300 latencies in 9-year-old Dutch children

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Abstract

Effects of perinatal PCB exposure on the auditory P300 were evaluated in the prenatally lowest and highest exposed children from the Rotterdam cohort. The 26 lowest and 26 highest prenatally PCB exposed children in the breast-fed (BF) and the formula-fed (FF) group (n=104), were invited at 9 years of age for a P300 assessment, using an auditory simple odd-ball paradigm. In the 83 participating children, 60 assessments satisfied the measurement criteria and were included in the data analyses. Prenatally high exposed children had longer P300 latencies than prenatally low exposed children. Lactational PCB exposure was not related to P300 latencies. Moreover, P300 latencies were shorter in children that were BF for ≥16 weeks compared to children that were BF for 6-16 weeks and to FF children.

Conclusion: These results suggest that prenatal exposure to environmental levels of PCBs and related compounds delays central nervous system mechanisms that evaluate and process relevant stimuli at school age, whereas breast-feeding accelerates these mechanisms.

Introduction

Polychlorinated biphenyls (PCBs) and dioxins are toxic compounds that are detectable in human milk and tissues due to background exposure to these environmental pollutants. The fetus is exposed to maternal levels of these compounds through placental transport. Additionally, a breast-fed infant is exposed to relatively large amounts of PCBs and dioxins in breast milk (1). These compounds are well known for their neurotoxic properties, although the neurotoxic mechanisms of PCBs and dioxins remain largely unknown. Many systems and levels of the developing central nervous system (CNS) were reported to be involved in the complex mechanism of neurotoxic action of PCBs and dioxins (2, 3). These include neuronal and glial cells (4, 5), brain neurotransmitters (6-8), and several hormone systems (9, 10), depending on the type of congener and its metabolites.

Human epidemiological studies have provided accumulating evidence for neurotoxic effects of predominantly prenatal exposure to PCBs by showing relations between exposure levels and neurodevelopmental outcome. In these cohort studies, delayed effects of prenatal exposure to PCBs were suggested on general cognitive and motor development (11-16), processing speed and attention (11, 17, 18), memory (12) verbal comprehension (11, 14) and on planning skills (17).

Neurophysiological techniques may provide a more direct evaluation of CNS function than neurodevelopmental tests. Moreover, the measurement of event-related brain potentials (ERPs) and especially its cognitive P300 component is a useful tool for investigating cognitive function (19-21). ERPs result from intracortical currents induced by excitatory and inhibitory postsynaptic potentials that are triggered by the release of neurotransmitters. The P300 component is a positive ERP that occurs with a latency of about 300 milliseconds when a person is actively processing ('attending to') incoming stimuli (22). The latency of the P300 is considered to be an indicator of the neural activity underlying the processes of attention allocation and immediate memory (19) and a measure of stimulus classification speed (23, 24). The amplitude of the P300 is assumed to reflect the quality with which incoming information is processed when it is incorporated into its memory representations and the context in which the stimulus occurs (19).

In adults, the amplitude and latency of the P300 can discriminate brain pathology from control conditions, including occupationally exposure to neurotoxic chemicals such as organic solvents (25-27), specific neuropathologic states such as Alzheimer's disease (28), or closed head injury (29, 30), and psychiatric disorders, such as schizophrenia (31, 32) and depression (33, 34).

In children P300 abnormalities have been associated with several pathologies including cognitive dysfunction (35, 36), attention deficit disorders (37, 38) and dyslexia (39). Moreover, the latency and amplitude of the P300 are respectively decreasing and increasing with age until adolescence, reflecting CNS maturation processes (40-42).

Effects of prenatal exposure to PCBs and dioxins on the P300 have been addressed in the Yu Cheng cohort, consisting of children born to mothers that were accidentally exposed to high levels of PCBs and polychlorinated dibenzofurans (PCDFs). In the prenatally exposed children, auditory P300 latencies were prolonged, and amplitudes were lower compared to nonexposed matched controls (43). In that study, visual and short-latency somatosensory evoked potentials were not different for the groups.

In The Netherlands, a prospective study into effects of perinatal exposure to PCBs and dioxins on neurodevelopment was launched in 1989. Half of this population of children was breast-fed during infancy and the other half formula-fed. In this cohort, neurotoxic effects of perinatal exposure to environmental levels of PCBs and dioxins have been addressed from birth to school age.

The aim of the present study in the Rotterdam cohort of the Dutch PCB/dioxin study was to gain more insight in the neurotoxic mechanism of perinatal exposure to PCBs by means of exploring effects on a more direct measurement of CNS functioning, the P300 ERP.

Method

Subjects and Study design

The original study population consisted of 207 healthy Caucasian mother-infant pairs who were recruited from June 1990 to February 1992 in the area of Rotterdam, a highly industrialized and densely populated area in The Netherlands. The study design and recruitment process, chemical analysis and PCB and dioxin concentrations have been described in detail elsewhere (44). Pregnancy and delivery were uncomplicated. Only first or second, at term, born healthy children were included. One hundred and five children were breast-fed (BF) for at least six weeks and 102 children were formula-fed (FF) during infancy. All formula-fed infants received formula from a single batch (Almiron M2, Nutricia NV, Zoetermeer, The Netherlands) from birth until 7 months of age. In this formula, concentrations of PCBs and dioxins were not detectable.

At 9 years of age, we invited 104 children of the Rotterdam cohort, the 26 lowest and 26 highest prenatally exposed children (based on $\Sigma PCB_{maternal}$) from both feeding groups, to participate in a follow-up assessment in the Sophia Children's Hospital in Rotterdam. Children were not eligible for selection when they had not participated in the follow-up at 42 or 84 months of age or when they were moved from the Rotterdam area, since families had to visit the hospital for the assessment.

The medical ethics committee of the University Hospital Rotterdam/ Sophia Children's Hospital approved the study design and the parents gave informed consent.

Auditory Event-Related Potentials

An auditory simple odd-ball paradigm was used to elicit the P300 component. Two different sinusoidal tone bursts of two frequencies (1.0 kHz tone; 70 dB nHL, 50 ms duration, 5 ms rise/fall time or 1.5 kHz tone, 66.7 ms duration, 6.7 rise/fall), using a fixed 1.25 s interstimulus interval, were presented binaurally via earphones in pseudo-randomized order. 20% Of these tones were targets (1.5 kHz) and 80% were non-targets (1.0 kHz) (Software package Nicolet Viking, version 4.7.1b). Children were required to lay down on a bed and press a hand held button as quickly as possible in response to target stimuli. ERPs were recorded using Ag/AgCl electrodes placed over the midline frontal, central, parietal (Fz, Cz, and Pz) position referred to linked ears, with forehead ground. Eye movements and blink artefacts were differentially recorded by two electrodes, one lateral inferior to the right eye and another superior to the left eye. Raw potentials were filtered, band pass set at 0.5-30 Hz. Artefact rejection at 9μV was used.

Averaging proceeded until 48 (target) and 192 (non-target) stimuli were accepted. Children were presented two series of 48 successfully averaged target stimuli, and 192 non-target stimuli. Due to artefact rejection (caused by restlessness or tension) in 23 children the assessment took too long to complete averaging, these measurements were not included in the data analysis.

ERP-waveform analysis

The ERP-waveforms were labeled conventionally. For the purpose of this study, the P300 peak was identified in the individual recordings, generally in the first ERP assessment, by two raters who were unaware of the child's exposure levels and type of feeding during infancy. The P300 was identified as the largest positive peak in the area of 250-450 ms (e.g. Figure 7.1). The latency and amplitude of the P300 peak at Fz, Cz and Pz, position were used as outcome variables. For each exposure group, separate grand average ERP waveforms were calculated for the three electrode recordings (Fz, Cz and Pz).

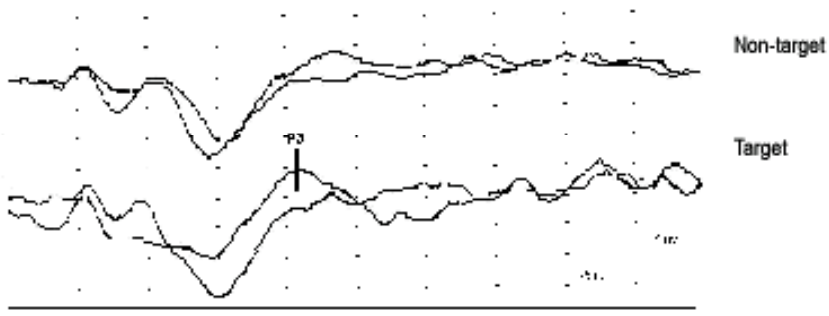

Figure 7.1 ERP waveforms (two assessments) recorded at Pz and P300 peak identification.

Assessment of exposure variables

Plasma samples were collected from the mothers during the last month of pregnancy and cord plasma samples were collected directly after birth. These samples were analyzed for four PCB congeners, International Union for Pure and Applied Chemistry (IUPAC) numbers 118, 138, 153 and 180. Two weeks after delivery a 24-hour representative breast milk sample was collected from the mothers who were breast-feeding their children. Breast milk samples were analyzed for 17 dioxins (PCDDs and PCDFs), 6 dioxin-like PCBs (IUPAC numbers 77, 105, 118, 126, 156, 169) and 20 nondioxin-like PCBs (IUPAC numbers 28, 52, 66, 70, 99, 101, 128, 137, 138, 141, 151, 153, 170, 177, 180, 183, 187, 194, 195, and 202). Toxic potency of the mixture of dioxins and dioxin-like PCBs was expressed by using the toxic equivalent factor (TEQ) approach (45).

In the present study, we compared the outcome of a low exposed group with a high exposed group, based on the sum of the four PCB congeners measured in maternal plasma.

Assessment of confounding variables

Variables that may influence child neurodevelopment have been assessed and included birth weight, duration of gestation, fetal exposure to alcohol and cigarette smoking, parity, type of feeding during infancy, duration of breast-feeding, sex, maternal age and parental education level. The child's home environment was assessed by the Home Observation for Measurement of the Environment (HOME) (46) during the home visit for the follow-up at 7 years of age. The verbal IQ of the parent that spends the most time with the child (usually the mother) was assessed by two subtests, Information and Vocabulary from the Dutch version of the Wechsler Adult Intelligence Scale (47).

Data analysis

To compare groups for a single variable we used either the Student's t-test or the χ^2 test. The difference in outcome between the prenatally low and high exposed groups was studied by means of multiple linear regression analyses (SPSS, version 10). Variables that were likely to affect P300 outcome (latency or amplitude) were included in the regression model as a fixed set of variables. These variables were: sex (0/1=boy/girl), highest education level of either parent (0/1/2=primary school, secondary school not finished/ secondary school finished/ high school finished, professional and university training), type of feeding and duration of breast-feeding (captured in two dummy variables for FF, BF_{short}=6-16 weeks of breast-feeding, and BF_{long} =≥16 weeks of breast-feeding) and age at examination. Additionally, confounding variables, i.e. variables that correlated ($p<0.2$), adjusted for the fixed set of variables, with the exposure variable ($\Sigma PCB_{low/high}$) and with one of the outcome

variables, were added to the regression model. Candidate confounders were alcohol use (0/1=no/yes) and smoking during pregnancy (0/1=no/yes), duration of gestation, birth weight, and parity (0/1=1st/2nd born), parental verbal IQ, and HOME score. This procedure resulted in the following set of explanatory variables included in the regression model for P300 outcome variables: $\Sigma PCB_{low/high}$, alcohol use during pregnancy, sex, type of feeding and duration of breast-feeding, parental education level, and age at assessment. Results were considered significant if $p \leq 0.05$.

Results

From the invited children (n=104), 83 (80%) were willing to participate (age: 8.7-9.6 years; mean±SD 9.2±0.2). The parents of 21 children were not motivated to participate in this follow-up for which they had to visit the hospital. Exposure levels in participating and nonparticipating children were comparable. From the 83 children in whom ERP assessments were done, 60 measurements were complete (i.e. 48 accepted target stimuli) and were included in the data analyses. In Table 7.1 prenatal exposure levels, the number of low and high exposed children, and the type of feeding are presented for the included and excluded children as well as the children that were not willing to participate in this study. The three groups did not show statistical differences in these variables.

Table 7.1 Characteristics of children with complete ERP assessments, incomplete assessments, and of the nonparticipants.

	ERP complete (n = 60)	ERP incomplete (n=23)	Nonparticipants (n=21)
$\Sigma PCB_{maternal}$ µg/L	2.54 (0.59-4.71)	1.71 (0.80-5.08)	2.63 (0.73-7.35)
Number of ΣPCB_{low} (%)	28 (46.7 %)	14 (60.9 %)	10 (57.1 %)
Number of BF (%)	32 (53.3 %)	12 (52.2 %)	9 (42.9 %)

Values are numbers (percentages) or medians (range).
$\Sigma PCB_{maternal}$ = sum of PCB congeners IUPAC nos. 118, 138, 153, 180 in maternal plasma.

The characteristics of the low and high exposed children whose ERP measurements were included in the data analyses are presented in Table 7.2. As described in more detail previously (15), parental education level and verbal IQ were significantly higher in the high exposed group compared to the low exposed group of children. All prenatal exposure measurements of PCBs and dioxins were significantly higher in the high exposure group, which is inherent to the study design. In Table 7.3, the mean latency and amplitude of the P300 are presented.

Table 7.2 Characteristics of low and high prenatal PCB groups with complete ERP assessment.

Characteristics	ΣPCB_{low} (n=28)	ΣPCB_{high} (n=32)
Smoking during pregnancy, yes	7 (25 %)	8 (25 %)
Alcohol use during pregnancy, yes*	2 (7 %)	9 (28 %)
Birth weight (kg)	3406 (± 404)	3344 (± 535)
Gestational age (wk)	40.2 (± 1.1)	39.7 (± 1.3)
Number of BF	13 (46 %)	19 (59 %)
Duration of breast-feeding (wk)	16 (6-40)	16 (6-62)
Number of boys	13 (46 %)	19 (59 %)
Number of 1st born	15 (54 %)	16 (50 %)
Parental education level **		
Low	8 (29 %)	1 (3 %)
Medium	12 (43 %)	13 (41 %)
High	8 (29 %)	18 (56 %)
Parental verbal IQ **	117.0 (± 16.6)	127.2 (± 14.8)
HOME score at 7 years	47.8 (± 2.6)	48.5 (± 2.8)
Age at assessment (yr)	9.2 (± 0.2)	9.2 (± 0.2)
Exposure variables		
$\Sigma PCB_{maternal}$ µg/L**	1.40 (0.59-1.93)	3.24 (2.51-4.71)
ΣPCB_{cord} µg/L**	0.31 (0.08-0.63)	0.58 (0.29-1.98)
ΣPCB_{milk} µg/kg fat**	242.5 (173.7-371.1)	572.4 (333.6-804.5)
$\Sigma PCB_{20\ nondioxin\text{-}like}$ µg/kg fat **	255.2 (204.6-466.1)	608.5 (347.2-858.1)
Total TEQ ng/kg fat **	43.82 (28.06-88.20)	84.05 (58.00-111.41)

Values are numbers (percentages), means (± standard deviations) or medians (range).
* $p<0.05$, ** $p<0.01$ (Students t-test or χ^2 test)
Parentaleducationlevel:low=primaryschool,secondaryschoolnotfinished,middle=secondaryschoolfinished,high=highschool finished,professionalanduniversitytraining;Parentalverbal IQscoreontwosubtestsoftheWechslerAdultIntelligenceScale,InformationandVocabulary,assessedfromoneoftheparents;HOME:HomeObservationfortheMeasurementoftheEnvironmentatschoolage; $\Sigma PCB_{maternal,cord,milk}$:sumofPCBcongenersIUPACnos.118,138,153,180inmaternalandcordplasma,andbreastmilk;$\Sigma PCB_{20nondioxin\text{-}like}$:sumof20 nondioxin-likePCBsinbreastmilk.TotalTEQ:sumoftoxicequivalentsaccordingtothe1997WHOTEFvaluesformono-orthoPCBs(UIPACnos. 105, 118, 156), planar PCBs (IUPAC nos. 77, 126, 169) and 17 dioxins (PCDDs and PCDFs).

Table 7.3 Descriptives of P300 latencies and amplitudes.

P300	Latency (ms)	Amplitude (V)
Frontal (n=60)	336 (± 35) (273; 437)	6 (± 4) (-3; 15)
Central (n=60)	334 (± 36) (255; 429)	7 (± 4) (-3; 19)
Parietal (n=60)	333 (± 35) (254; 424)	8 (± 4) (0.2; 20)

Values are means (±SD) and (minimum; maximum).

The grand averages of the ERP waveforms are presented for the two exposure groups, not adjusted for confounding differences between the exposure groups in Figure 7.2. The grand average waveform for the low exposed group showed a better peak pronunciation compared to the grand average waveform for the high exposed group, especially for the

parietal as well as for the central (data not shown) recordings. The P300 latency of high exposed children was prolonged compared to the P300 latency of low exposed children.

The results of multiple regression analyses on the P300 peak latencies are presented in Table 7.4. Especially for the central as well as the parietal recordings, the P300 latencies were significantly longer in prenatally high exposed compared to low exposed children, adjusted for confounding variables. Moreover, for frontal, central as well as the parietal recordings, children that were BF for a long period (BF_{long}) had significantly shorter P300 latencies than children that were BF for a short period (BF_{short}). For the parietal recording, BF_{long} children had also a shorter P300 latency compared to FF children. P300 amplitudes were not statistically different for low and high exposed children, nor for the three feeding groups, when adjusted for confounding variables.

Table 7.4 Results of multiple regression analysis on the P300 latencies (ms) measured at Fz, Cz, Pz.

	ΣPCB_{high} vs ΣPCB_{low}			BF_{short} vs FF			BF_{long} vs FF			BF_{long} vs BF_{short}		
	Regr. coef.	SE	p	Regr. coef.	SE	p	Regr. coef.	SE	p	Regr. coef.	SE	p
$P300_{fz}$	14.3	9.5	0.140	15.0	10.5	0.160	-19.8	10.8	0.073	-34.7	12.1	0.006
$P300_{cz}$	25.6	9.6	0.011	13.0	10.6	0.229	-20.2	10.9	0.070	-33.2	12.2	0.009
$P300_{pz}$	22.0	9.4	0.023	12.0	10.3	0.251	-22.5	10.6	0.039	-34.5	11.9	0.005

Additional variables in the regression models fetal exposure to alcohol, sex, parental education level, age at assessment. Regression coefficients were estimated in essentially the same regression model by reparameterizing the effects of the three categories for duration of breast-feeding (FF; 6-16 weeks; ≥ 16 weeks).

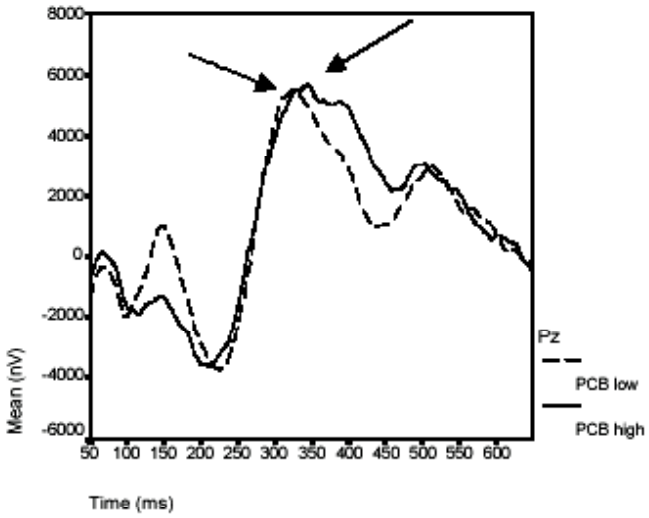

Figure 7.2 Grand average ERP at Pz for prenatally low and high PCB exposed children.

Arrows point to P300 for the prenatally low exposed group (⟶) and for the prenatally high exposed group (⟶).

To estimate effects of postnatal exposure through lactation, the group of BF children was divided in four groups based on prenatal exposure levels and duration of breast-feeding (BF_{short}: <16 weeks; BF_{long}: ≥16 weeks). In Figure 7.3 the mean adjusted latencies measured on Pz are presented for these four groups and the low and high exposed FF groups. In the figure, the significant differences in mean adjusted latencies between the six feeding groups are indicated. Low exposed BF_{long} children (E) had significantly shorter P300 latencies than their high (p_{fz}= 0.013; p_{cz}= 0.002; p_{pz}=0.005) (E') and low (p_{fz}= 0.040; p_{cz}= 0.046; p_{pz}=0.114) exposed BF_{short} counterparts. In the high exposed BF_{long} group (F), latencies were also generally shorter than in high exposed BF_{short} children (p_{fz}= 0.061; p_{cz}= 0.086; p_{pz}= 0.021) (F').

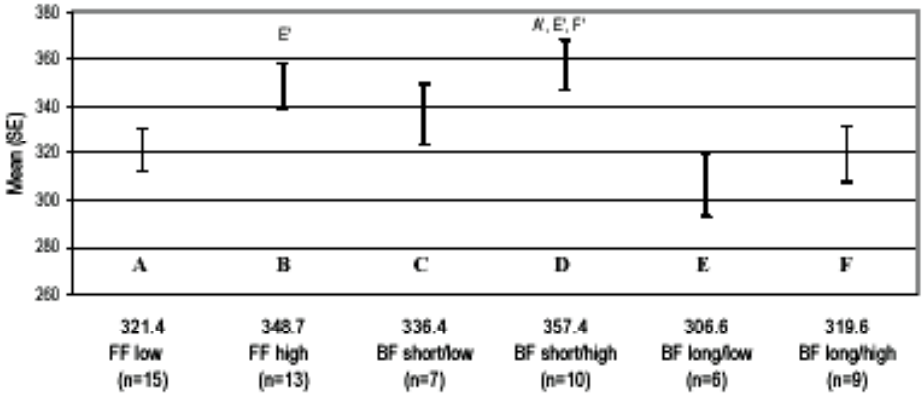

Figure 7.3 Adjusted mean and SE of the P300 latencies (ms) at Pz.

A' = significantly different (p<0.05) from A
E' = significantly different (p<0.05) from E
F' = significantly different (p<0.05) from F

Discussion

In this study, prenatally high PCB exposed children showed prolonged P300 latencies compared to low PCB exposed children. Moreover, a longer breast-feeding duration was related to shorter P300 latencies compared to a shorter duration of breast-feeding, and the FF condition. The P300 amplitudes were not statistically different for the high and low exposure groups nor for the three feeding groups. These results suggest that prenatal PCB exposure is related with slower CNS mechanisms that evaluate and process relevant stimuli, whereas a long duration of breast-feeding accelerates these mechanisms.

In the Yu Cheng cohort, delayed P300 latencies have been reported in 7 to 12-year-old children that were accidentally exposed to relatively high prenatal levels of PCBs and PCDFs. Although the exposure levels we describe are expected to be much lower

than in the Yu Cheng study, the difference in P300 latency between the exposed group and the control group in the Yu Cheng study (Cz = 26.7ms; Pz = 25.2ms) (43) and in the present study between PCB_{high} and PCB_{low} (Cz = 25.6ms; Pz = 22.0ms) are equal within the measurement error. In the Lake Michigan cohort at 11 years of age (11), an American cohort in which neurodevelopmental effects of perinatal exposure to environmental levels of PCBs are addressed, the magnitude of effects of prenatal exposure to PCBs on IQ was also comparable to the difference seen in exposed and nonexposed children in the Yu Cheng study.

In contrast to the Yu Cheng study, in the present study, the P300 amplitude was not statistically different for the two exposure groups. The latency of the P300 is considered to be an indicator of the neural activity underlying the processes of attention allocation and immediate memory (19) and a measure of stimulus classification speed (23, 24). The amplitude of the P300 is assumed to reflect the quality with which incoming information is processed when it is incorporated into its memory representations and the context in which the stimulus occurs (19). The amplitude is, amongst others, considered to be related to the discrepancy between the expected and actual stimulus properties, whereas the latency reflects the duration of the stimulus-evaluation process. Specific neuropathological states and their cognitive deficits seem to be more often related to prolonged latency of P300 (26, 28, 48-50), whereas decrements in P300 amplitude are more often associated with the presence of psychiatric disorders such as, schizophrenia (51, 52), and depression (34, 53, 54). We hypothesize that the difference in the observed effects of prenatal exposure to PCBs on the P300 amplitude in the Yu Cheng cohort and in the Dutch PCB/dioxin may reflect differences in exposure levels and mixture content or subtle differences in the assessment of the P300.

Correlation analysis of the P300 outcome variables and neuropsychological outcome variables that were assessed during the same follow-up session (i.e. the Rey Complex Figure Task, the Auditory-Verbal Learning Test, Simple Reaction Time Task, and the Tower of London (personal communication)), showed no statistical significant interrelationships. ERPs however, are believed to measure only a fraction of the neural activity associated with stimulus processing and do not measure the more elaborated neuronal processes of cognitive processes (55).

The effect of a longer duration of BF on the P300 latency may suggest positive effects of brain development stimulating substances in breast milk, such as long-chain polyunsaturated fatty acids. The brain is 60 % structural lipid and uses arachidonic acid and docosahexaedonic acid, which are deposited in the nonmyelin membranes of the developing nervous system and are believed to be essential for CNS growth, function and integrity (56, 57). These acids were not available for FF children, and children who were BF for a shorter period may have received smaller amounts of these compounds than children that were BF for a longer period. These results do illustrate the complexity of

risk assessment of exposure to environmental persistent compounds, especially in regard to breast-feeding. Assessment of more specific cognitive functions may help to refine our knowledge into neurotoxic effects of exposure to PCBs and dioxins on different stages in development.

In the present study, we compared groups of low and high prenatally exposed children, based on maternal ΣPCB levels. The levels of both dioxin TEQs and nondioxin-like PCBs are highly correlated with maternal ΣPCB levels (58) and were consequently also significantly different in these exposure groups. In the environment, PCBs, their metabolites and related compounds such as dioxins are present as complex mixtures of various congeners that may vary in metabolism and toxicity. Hence, specific effects of either group of compounds are methodological difficult to detect. We, therefore, believe that the difference in outcome between the low and high exposure group could be related to differences in exposure levels of other PCB congeners, dioxins, and related compounds and their metabolites.

The results of this study suggest a negative effect of prenatal exposure to environmental levels of PCBs and dioxins on the P300 latency in a cohort of normal 9-year-old children. Prenatal exposure to PCBs and dioxins are suggested to slow down CNS mechanisms that evaluate and process relevant stimuli. No evidence for effects of postnatal exposure to PCBs and dioxins through lactation on the P300 was seen. Moreover, an accelerating effect of a longer duration of breast-feeding on P300 latencies was found. These results may indicate that at the time of this study, the Dutch PCB and dioxin levels in pregnant women were high enough to make neurophysiological effects noticeable in their children at school age. Breast-feeding for a long duration has a positive effect on the P300 and should therefore not be discouraged considering the Dutch PCB and dioxin levels in breast milk.

References

1. Patandin S, Weisglas-Kuperus N, de Ridder MA, Koopman-Esseboom C, van Staveren WA, van der Paauw CG, et al. Plasma polychlorinated biphenyl levels in Dutch preschool children either breast-fed or formula-fed during infancy. Am J Public Health 1997;87(10):1711-4.
2. Brouwer A, Ahlborg UG, Van den Berg M, Birnbaum LS, Boersma ER, Bosveld B, et al. Functional aspects of developmental toxicity of polyhalogenated aromatic hydrocarbons in experimental animals and human infants. Eur J Pharmacol 1995;293(1):1-40.
3. Tilson HA, Kodavanti PR. The neurotoxicity of polychlorinated biphenyls. Neurotoxicology 1998;19(4-5):517-25.
4. Morse DC, Plug A, Wesseling W, Vandenberg KJ, Brouwer A. Persistent alterations in regional brain glial fibrillary acidic protein and synaptophysin levels following pre- and postnatal polychlorinated biphenyl exposure. Toxicol Appl Pharmacol 1996;139(2):252-261.
5. Seegal RF, Shain W. Neurotoxicicity of polychlorinated biphenyls. In: Isaacson RL, Jensen KF, editors. The Vulnerable Brain and Environmental Risks. New York: Plenum Press; 1992. p. 169-195.
6. Mariussen E, Fonnum F. The effect of polychlorinated biphenyls on the high affinity uptake of the neurotransmitters, dopamine, serotonin, glutamate and GABA, into rat brain synaptosomes. Toxicology 2001;159(1-2):11-21.
7. Seegal RF, Brosch K, Bush B, Ritz M, Shain W. Effects of Aroclor 1254 on dopamine and norepinephrine concentrations in pheochromocytoma (PC-12) cells. Neurotoxicology 1989;10(4):757-64.
8. Seegal RF, Bush B, Brosch KO. Comparison of effects of Aroclors 1016 and 1260 on non-human primate catecholamine function. Toxicology 1991;66(2):145-63.
9. Brouwer A, Longnecker MP, Birnbaum LS, Cogliano J, Kostyniak P, Moore J, et al. Characterization of potential endocrine-related health effects at low- dose levels of exposure to PCBs. Environ Health Perspect 1999;107 Suppl 4:639-49.
10. Weisglas-Kuperus N. Neurodevelopmental, immunological and endocrinological indices of perinatal human exposure to PCBs and dioxins. Chemosphere 1998;37(9-12):1845-53.
11. Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychorinated biphenyls in utero. N Engl J Med 1996;335:783-9.
12. Jacobson JL, Jacobson SW, Humphrey HE. Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J Pediatr 1990;116(1):38-45.
13. Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MA, Van der Paauw CG, Tuinstra LG, Sauer PJ. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics 1996;97(5):700-6.
14. Patandin S, Lanting CI, Mulder PG, Boersma ER, Sauer PJ, Weisglas-Kuperus N. Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J Pediatr 1999;134(1):33-41.
15. Vreugdenhil HJ, Lanting CI, Mulder PG, Boersma ER, Weisglas-Kuperus N. Effects of prenatal PCB and dioxin background exposure on cognitive and motor abilities in Dutch children at school age. J Pediatr 2002;140(1):48-56.
16. Walkowiak J, Wiener JA, Fastabend A, Heinzow B, Kramer U, Schmidt E, et al. Environmental exposure to polychlorinated biphenyls and quality of the home environment: effects on psychodevelopment in early childhood. Lancet 2001;358(9293):1602-7.
17. Vreugdenhil HJI, Mulder PG, Emmen HH, Weisglas-Kuperus N. Effects of perinatal exposure to PCBs on neuropsychological functions in the Rotterdam cohort at 9 years of age. Neuropsychology, in press.
18. Jacobson JL, Jacobson SW, Padgett RT, Brumitt GA, Billings RL. Effects of Prenatal PCB Exposure on Cognitive Processing Efficiency and Sustained Attention. Developmental Psychology 1992;28(2):297-306.
19. Polich J, Herbst KL. P300 as a clinical assay: rationale, evaluation, and findings. Int J Psychophysiol 2000;38(1):3-19.

20. Magliero A, Bashore TR, Coles MG, Donchin E. On the dependence of P300 latency on stimulus evaluation processes. Psychophysiology 1984;21(2):171-86.
21. Pritchard WS. Psychophysiology of P300. Psychol Bull 1981;89(3):506-40.
22. Sutton S, Braren M, Zubin J, John ER. Evoked-potential correlates of stimulus uncertainty. Science 1965;150(700):1187-8.
23. Kutas M, McCarthy G, Donchin E. Augmenting mental chronometry: the P300 as a measure of stimulus evaluation time. Science 1977;197(4305):792-5.
24. Polich J. Attention, probability, and task demands as determinants of P300 latency from auditory stimuli. Electroencephalogr Clin Neurophysiol 1986;63(3):251-9.
25. Steinhauer SR, Morrow LA, Condray R, Dougherty GG. Event-related potentials in workers with ongoing occupational exposure. Biol Psychiatry 1997;42(9):854-8.
26. Morrow LA, Steinhauer SR, Hodgson MJ. Delay in P300 latency in patients with organic solvent exposure. Arch Neurol 1992;49(3):315-20.
27. Morrow LA, Steinhauer SR, Condray R. Predictors of involvement in P300 latency in solvent-exposed adults. Neuropsychiatry Neuropsychol Behav Neurol 1998;11(3):146-50.
28. Neshige R, Barrett G, Shibasaki H. Auditory long latency event-related potentials in Alzheimer's disease and multi-infarct dementia. J Neurol Neurosurg Psychiatry 1988;51(9):1120-5.
29. Papanicolaou AC, Levin HS, Eisenberg HM, Moore BD, Goethe KE, High WM, Jr. Evoked potential correlates of posttraumatic amnesia after closed head injury. Neurosurgery 1984;14(6): 676-8.
30. Keren O, Ben-Dror S, Stern MJ, Goldberg G, Groswasser Z. Event-related potentials as an index of cognitive function during recovery from severe closed head injury. J Head Trauma Rehabil 1998;13(3):15-30.
31. Blackwood D. P300, a state and a trait marker in schizophrenia. Lancet 2000;355(9206):771-2.
32. Ford JM, Mathalon DH, Marsh L, Faustman WO, Harris D, Hoff AL, et al. P300 amplitude is related to clinical state in severely and moderately ill patients with schizophrenia. Biol Psychiatry 1999;46(1):94-101.
33. Bruder GE, Tenke CE, Stewart JW, Towey JP, Leite P, Voglmaier M, et al. Brain event-related potentials to complex tones in depressed patients: relations to perceptual asymmetry and clinical features. Psychophysiology 1995;32(4):373-81.
34. Yanai I, Fujikawa T, Osada M, Yamawaki S, Touhouda Y. Changes in auditory P300 in patients with major depression and silent cerebral infarction. J Affect Disord 1997;46(3):263-71.
35. Finley WW, Faux SF, Hutcheson J, Amstutz L. Long-latency event-related potentials in the evaluation of cognitive function in children. Neurology 1985;35(3):323-7.
36. Kaneko WM, Ehlers CL, Philips EL, Riley EP. Auditory event-related potentials in fetal alcohol syndrome and Down's syndrome children. Alcohol Clin Exp Res 1996;20(1):35-42.
37. Holcomb PJ, Ackerman PT, Dykman RA. Cognitive event-related brain potentials in children with attention and reading deficits. Psychophysiology 1985;22(6):656-67.
38. Satterfield JH, Schell AM, Nicholas TW, Satterfield BT, Freese TE. Ontogeny of selective attention effects on event-related potentials in attention-deficit hyperactivity disorder and normal boys. Biol Psychiatry 1990;28(10):879-903.
39. Taylor MJ, Keenan NK. Event-related potentials to visual and language stimuli in normal and dyslexic children. Psychophysiology 1990;27(3):318-27.
40. Sangal RB, Sangal JM, Belisle C. P300 latency and age: a quadratic regression explains their relationship from age 5 to 85. Clin Electroencephalogr 1998;29(1):1-6.
41. Polich J, Ladish C, Burns T. Normal variation of P300 in children: age, memory span, and head size. Int J Psychophysiol 1990;9(3):237-48.
42. Martin LJ, Barajas JJ, Fernandez R. Auditory P3 development in childhood. Scand Audiol Suppl 1988;30:105-9.
43. Chen YJ, Hsu CC. Effects of prenatal exposure to PCBs on the neurological function of children: a neuropsychological and neurophysiological study. Dev Med Child Neurol 1994;36(4):312-20.
44. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N, Boersma ER, de Ridder MA, Van der Paauw CG, et al. Dioxin and PCB levels in blood and human milk in relation to living areas in The Netherlands. Chemosphere 1994;29(9-11):2327-38.

45. Van den Berg M, Birnbaum L, Bosveld ATC, Brunstrom B, Cook P, Feeley M, et al. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 1998;106(12):775-92.
46. Caldwell B, Bradley R. Home observation of the environment. Administration manual. Little Rock, Arkansas; 1984.
47. Stinissen J, Willems PJ, Coetsier P, Hulsman WLL. Manual, Dutch version of the Wechsler Adult Intelligence Scale (WAIS). Amsterdam: Swets & Zeitlinger NV; 1970.
48. Morrow LA, Steinhauer SR, Condray R. Differential associations of P300 amplitude and latency with cognitive and psychiatric function in solvent-exposed adults. J Neuropsychiatry Clin Neurosci 1996;8(4):446-9.
49. Polich J. P300 and Alzheimer's disease. Biomed Pharmacother 1989;43(7):493-9.
50. Polich J. P300 in the evaluation of aging and dementia. Electroencephalogr Clin Neurophysiol Suppl 1991;42:304-23.
51. Ford JM. Schizophrenia: the broken P300 and beyond. Psychophysiology 1999;36(6):667-82.
52. Jeon YW, Polich J. P300 asymmetry in schizophrenia: a meta-analysis. Psychiatry Res 2001;104(1):61-74.
53. Bruder GE. P300 findings for depressive and anxiety disorders. Ann N Y Acad Sci 1992;658: 205-22.
54. Blackwood DH, St Clair DM, Blackburn IM, Tyrer GM. Cognitive brain potentials and psychological deficits in Alzheimer's dementia and Korsakoff's amnesic syndrome. Psychol Med 1987;17(2):349-58.
55. Rugg MD, Coles MGH. The ERP and cognitive psychology: conceptual issues. In: Rugg MD, Coles MGH, editors. Electrophysiology of Mind-Event-related brain potentials and Cognition. Oxford UK: Oxford Univestity Press; 1995; 27-39.
56. Uauy R, Hoffman DR, Peirano P, Birch DG, Birch EE. Essential fatty acids in visual and brain development. Lipids 2001;36(9):885-95.
57. Uauy R, Mena P. Lipids and neurodevelopement. Nutr Rev 2001;59:S34-46.
58. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N, Van der Pauw CG, Tuinstra LGMT, Boersma ER, et al. PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 1994;28(9):1721-1732.

8 Summary & General discussion & Future perspectives & Conclusions

8.1 Summary

PCBs and dioxins are lipophilic and bioaccumulating environmental pollutants. These compounds are able to cross the placenta and are excreted into mother's milk. Animal studies have shown complex neurotoxic properties of PCBs and dioxins, particularly in regard to the developing brain. In humans, prenatal exposure to *high* levels of PCBs and PCDFs has been associated with neurodevelopmental delays in children born after the Yu Cheng accident (1-3). In the past two decades several cohort studies have studied whether perinatal exposure to *environmental* levels to PCBs and dioxins may cause neurodevelopmental decrements (4-11). The results of these epidemiological studies are not conclusive and only a few cohort studies have addressed effects of perinatal exposure to PCBs on later and more specific neurodevelopmental aspects.

In this thesis, relations are described between perinatal exposure to environmental levels of PCBs and dioxins and several neurodevelopmental outcomes in school age children that are enrolled in the Dutch PCB/dioxin study. This study was performed in the scope of the Dutch PCB/dioxin study that was initiated in 1989. Healthy mother-infant pairs were recruited by two study centers, Rotterdam (n=207) and Groningen (n=211). At school age (6/7 years of age), all children enrolled in the Dutch PCB/dioxin study were invited to participate in a follow-up study, in which (general) cognitive and motor abilities were assessed. Moreover, in the Rotterdam cohort, play behavior was assessed as part of the school age follow-up and at 9 years of age, neuropsychological and neurophysiological outcomes were assessed in half of the Rotterdam cohort.

In **Chapter 1** a general introduction on PCBs and dioxins and their neurotoxic properties is presented as well as an overview of the results of the main epidemiological studies that address neurodevelopmental effects of perinatal exposure to environmental levels of PCBs and dioxins, including the Dutch PCB/dioxin study. In the Dutch PCB/dioxin cohort, studied from birth up to 42 months of age, prenatal PCB exposure was related to poorer neurological condition at birth and 18 months (12, 13), lower psychomotor abilities at 3 months (8), lower general cognitive abilities and verbal comprehension skills and lower attention abilities at 42 months of age (14). At 42 months of age, negative effects of prenatal PCB exposure on cognitive abilities were more pronounced in the formula-fed (FF) group of children compared to the breast-fed (BF) group. Compared to FF children, BF children in the Dutch PCB/dioxin cohort had older mothers, higher educated parents with higher verbal IQs and higher scores on the HOME environment questionnaire. Postnatal exposure to PCBs and dioxins, through lactation, was related to lower psychomotor abilities at 7 months of age (8).

Additionally in Chapter 1 the aims of the studies in this thesis are presented. The general aim was to evaluate neurodevelopmental effects of perinatal exposure to environmental levels of PCBs and dioxins in normal Dutch children at school age. In addition, the

goal was to gain more insight into potential compensating effects of parental and home environmental conditions and breast-feeding as well as into neurotoxic mechanisms of action of perinatal exposure to these compounds on the developing central nervous system (CNS). Finally, the design of the Dutch PCB/dioxin study is described in Chapter 1.

In the studies in the first part of the thesis (**Chapter 2, 3, and 4**), relations between perinatal exposure to environmental levels of PCBs and dioxins and cognitive and motor abilities at school age, and the development of these abilities from 3 to 84 months of age, are described. Moreover, modification of neurodevelopmental effects of perinatal exposure to PCBs and dioxins by parental and home environmental conditions are explored.

Whether the negative effects of exposure to environmental levels of PCBs and dioxins on cognitive abilities persisted until school age was addressed in **Chapter 2**. Moreover, in this study, potential differences of these effects in FF and BF children were explored as well as whether these effects were related to differences between these feeding groups in parental and home environmental characteristics (i.e. maternal age at birth, parental education level and verbal IQ and HOME score). At school age, the Dutch version of the McCarthy Scales of Children's Abilities was used to assess the general cognitive abilities (General Cognitive Index), and memory and motor skills in children of the Rotterdam and Groningen cohort (n=418). From the original cohort, 90 % (n=376) was willing to participate in this follow-up (mean age 6.7 years ± 0.3). The data of four children were excluded from the data analysis because of potential confounding pathology. Prenatal PCB and dioxin levels were comparable for the nonparticipating and participating children. Multiple linear regression analysis showed that, adjusted for confounding variables, prenatal exposure to PCBs and dioxins was not significantly related to cognitive and motor development at school age. Moreover, effects of prenatal exposure on cognitive and motor abilities were not statistically different for the two feeding groups. However, it appeared that effects of prenatal exposure to PCBs and dioxins on cognitive and motor abilities were modified by parental and home environmental conditions (i.e. maternal age, parental education level and verbal IQ, and HOME score). In the Dutch cohort, these parental and home environmental conditions are strongly related to each other. Older maternal age is related to a higher parental education level and verbal IQ and higher scores on the HOME questionnaire, conditions that are considered to be relatively more favorable to child development. The impact of negative effects of prenatal exposure to PCBs and dioxins on cognitive and motor abilities was suggested to increase as parental and home environmental conditions were lower. In children raised in relatively more favorable parental and home environmental conditions, subtle effects of prenatal exposure to PCBs and dioxins were not detectable. Effect modifications of PCB and dioxin exposure by maternal age, parental education level and verbal IQ, and HOME scores could not be explored simultaneously in one regression analysis due to the problem of multicollinearity. The results did not show evidence of negative effects of lactational exposure to PCBs and dioxins on cognitive and motor

outcome, neither of effect modification of lactational exposure by parental and home environmental characteristics. We concluded that neurotoxic effects of prenatal exposure to environmental levels of PCBs and dioxins may persist into school age and may result in subtle cognitive and motor delays. The results of this study suggest that parental and home environmental conditions influence the consequences of the neurotoxic effects on cognitive and motor development.

A disadvantage of studying relations between perinatal exposure to PCBs and dioxins and cognitive and motor abilities at a certain age is that the developmental course of these abilities is not captured. Therefore, in **Chapter 3** effects of perinatal exposure to PCBs, measured in maternal plasma, on the development of cognitive and motor abilities, as assessed in the Rotterdam cohort at 3, 7, 18, 42 and 84 months of age, are described using the method of random regression modeling (RRM). Moreover, important predictors of general cognitive and motor development from 3 to 84 months of age were identified in this study. Data on cognitive and motor abilities were available for all analyzable children (excluding children with potential confounding pathology, n=3). In the initial RRM models, all selected variables of potential relevance to cognitive and motor development were included. In these models, higher levels of prenatal PCB exposure were significantly related to a lower level of lower cognitive and motor development from 3 to 84 months of age. In this study, the problem of multicollinearity, when including the four previously described interaction variables of prenatal PCB exposure and parental and home environmental variables simultaneously in the regression model, was solved by centering these variables as well as their main terms. Simultaneous inclusion of these variables showed that effects of prenatal exposure to PCBs on the level of cognitive development were significantly modified by maternal age, overruling effect modification by the other parental and home environmental conditions. In children born to younger mothers, effects of prenatal exposure to PCBs on cognitive development were suggested to be more pronounced than in children born to older mothers, a condition that is likely to reflect more favorable parental and home environmental conditions for child development. Prenatal PCB levels, and its modification by maternal age, along with parental education level and verbal IQ and HOME scores were important determinants of the level of cognitive development. Motor development was efficiently estimated by prenatal PCB levels including its modification by HOME scores along with parental education levels. Effects of prenatal PCB exposure on motor development were more pronounced when the HOME scores were lower. The results provided no evidence of negative effects of lactational exposure to PCBs on cognitive or motor development and neither were maternal (i.e. prenatal) thyroid hormone levels related to these outcomes.

These results provided evidence of negative effects of prenatal exposure to PCBs on the level of cognitive and motor development, effects that may be modified by conditions that are important to child development. Compared to the large positive effects of more optimal

parental and home environmental conditions, the negative effects of prenatal PCB exposure on cognitive development from 3 to 84 months of age were relatively small. Effects of prenatal exposure were more pronounced for motor than for cognitive development. Motor development may therefore be a more sensitive outcome to detect effects of prenatal exposure to PCBs and related compounds than cognitive development.

Due to the low secretion rate of PCBs and dioxins and their accumulation in human tissues, prenatal PCB and dioxin levels are strongly related to maternal age at birth. This feature makes maternal age a very complex variable when studying neurodevelopmental effects of prenatal PCB and dioxin exposure since, as is described previously, it is also positively related with parental education levels and verbal IQs as well as with HOME scores. In an effort to disentangle these interrelationships of prenatal exposure levels, parental and home environmental conditions and cognitive and motor development, we applied the method of structural equation modeling (SEM). This method enables more proper modeling of these variables and the use of multiple outcome variables. In **Chapter 4**, the results of SEM on the Rotterdam data are described. First, the interrelationships of parental education level and verbal IQ and HOME scores and cognitive and motor development were identified. Subsequently, four groups were composed by dichotomizing the population at the median of maternal ΣPCB levels (2.04 µg/L) and at the median of maternal age (29 years of age) (PCB_{low}/M_{young}; PCB_{low}/M_{old}; PCB_{high}/M_{young}; PCB_{low}/M_{old}). These groups were compared on both the level and interrelationships of the outcome and the determinants. The four groups were significantly different in the level of both cognitive/motor outcome and determinants, as well as in the relations between determinants and cognitive outcome (and indirectly motor outcome). Prenatal PCB exposure was suggested to be related with larger decrements in cognitive and motor abilities in children raised in relatively low parental and home environmental conditions compared to children in which these conditions were more favorable. For early cognitive and motor development (from 3 to 18 months of age), this difference in effect of prenatal PCB exposure was suggested to be more pronounced in children born to older mothers, whereas for later cognitive and motor development (42 to 84 months of age) the difference was more pronounced in children born to younger mothers. This study provides evidence of complex effects of maternal age and other parental and home environmental conditions on the neurotoxic mechanism of PCBs and related neurotoxic compounds and serves as an initial effort to disentangle these mechanisms to increase the knowledge in risk assessment of prenatal exposure to PCBs and dioxins.

In the second part of the thesis, effects of perinatal exposure on more specific neurodevelopmental aspects are described. PCBs and dioxins are known to have sex steroid hormone modulating properties. Steroid hormones play a mediating role in brain development and may influence not only reproductive but also nonreproductive behaviors that show sex differences, such as childhood play behavior. In **Chapter 5**, (sex-specific)

effects of perinatal exposure to PCBs and dioxins on child play behavior are described. As part of the first follow-up study at school age, play behavior was assessed by means of the Pre-School Activity Inventory (PSAI) in the Rotterdam cohort. The PSAI assesses masculine and feminine play behavior scored on three subscales: Masculine, Feminine, and Composite. One hundred and sixty PSAI questionnaires were returned (mean age ± SD: 7.5 years ±0.4). Higher prenatal PCB levels were related with less masculinized play behavior in boys and with more masculinized play behavior in girls. Higher prenatal dioxin levels, available for BF children, were associated with more feminized play in boys as well as in girls, assessed by the Feminine scale. There was no evidence that lactational exposure to PCBs and dioxins was related to play behavior in the total BF group and neither in boys and girls separately. The results are suggestive of steroid hormone involvement in the neurotoxic mechanism of action of prenatal exposure to environmental levels of PCBs and dioxins.

Half of the Rotterdam cohort, the lowest prenatally exposed (p25; n=26) and the highest prenatally exposed children (p75; n=26) of both feeding groups (total n=104) were invited to participate in neuropsychological (see **Chapter 6**) and neurophysiological (see **Chapter 7**) assessments at 9 years of age. From the invited children 80% (n=83) was willing to participate in this follow-up study (mean age±SD: 9.2±0.2). Exposure levels of the participating and nonparticipating children were comparable.

Chapter 6 describes relations between perinatal exposure of PCBs and dioxins and several neuropsychological functions. The assessment included the Rey Complex Figure Task, the Auditory-Verbal Learning Test, the Simple Reaction Time Task, and the Tower of London. Prenatally high exposed children had, adjusted for confounding variables, longer reaction times and more variation in their reaction times, and lower scores on the Tower of London (TOL) than prenatally low exposed children. On the latter task, assessing predominantly executive or planning functions, in contrast to the other tasks, children that were BF for a long period (≥17 weeks) scored significantly lower than FF children. The results of this study are suggestive of multi-focal or diffuse neurotoxic effects of prenatal exposure to PCBs and related compounds. For lactational exposure, the negative effect on the TOL scores may suggest that processes related to the prefrontal cortex are involved in the neurotoxic mechanism of exposure to PCBs and related compounds. This can be hypothesized since the frontal cortex shows a delayed maturation rate compared to other brain regions and developing brain structures are more vulnerable to exposure to PCBs and dioxins. A complex task as the TOL may also serve as a sensitive outcome parameter to assess neurotoxic effects of early exposure to PCBs and related compounds.

Chapter 7 describes relations between perinatal exposure to PCBs and dioxins and a neurophysiological measurement, the auditory P300 latency and amplitude. The P300 is considered to be a cognitive component of event-related brain potentials and occurs with a latency of about 300 milliseconds when a person is actively processing ('attending to')

incoming stimuli. Prenatally high exposed children had significantly longer P300 latencies than prenatally low exposed children, adjusted for confounding variables. The results gave no evidence of differences in P300 latencies related to lactational exposure to PCBs and dioxins. In stead, a longer duration of breast-feeding (≥16 weeks) was associated with shorter P300 latencies compared to children that were BF for 6-16 weeks as well as compared to FF children. No differences in P300 amplitudes were seen relative to prenatal or postnatal exposure to PCBs and dioxins, or to the duration of breast-feeding. These results suggest that prenatal exposure to PCBs and dioxins delays CNS mechanisms that evaluate and process relevant stimuli at school age, whereas breast-feeding accelerates these mechanisms.

8.2 General discussion

8.2.1 Neurotoxic mechanisms of neurodevelopmental effects of perinatal exposure to PCBs and dioxins

Prenatal exposure to PCBs and dioxins can be regarded as chronic exposure of the developing CNS and many processes of the CNS are likely to be sensitive to exposure to PCBs and dioxins, including neuronal and glial cells, neurotransmitters, and endocrine systems (15-18). Consequently, effects of prenatal exposure to PCBs and dioxins are likely to be of multi-focal or diffuse nature. The results the Dutch PCB/dioxin study and other prospective human PCB studies suggest effects of prenatal exposure to PCBs on several neurodevelopmental outcome variables, including general cognitive and motor development (5-8, 11, 19-21), verbal comprehension skills (5, 6), processing speed (4, 22), attention and concentration (5, 14, 22), memory skills (19, 23), planning or executive functions (22), and on a neurophysiological endpoint that assesses processing and evaluation of auditory stimuli (24). In the neuropsychological study described in this thesis, scores on some tests were not related to perinatal exposure to PCBs and dioxins. This may reflect differences in sensitivity of neuropsychological tests to measure *subtle* neurotoxic effects of perinatal exposure to neurotoxic compounds. The difference in sensitivity in a relatively small cohort may affect the power to detect effects and may, therefore, result in missing effects of perinatal exposure to PCBs and dioxins (increasing Type II errors).

Postnatally, maturation of different areas in the brain occurs at different rates. The frontal cortex shows the slowest maturation rate. Since developing CNS structures are known to be especially vulnerable to adverse effects of exposure to PCBs and dioxins, structure related effects of lactational exposure can be hypothesized. Some evidence in support of this hypothesis can be found in the finding that performance on the TOL was the only outcome that was suggested to be related to lactational exposure to PCBs. In planning or executive functions, processes of the prefrontal cortex are especially involved, in which higher cortical functions from several areas of the brains are integrated. In monkeys that were only

exposed to PCBs through lactation, PCB-induced behavioral deficits were also suggestive of prefrontal cortex involvement (25). Moreover, brain dopaminergic systems have been shown to be affected (26, 27) by exposure to PCBs and some major dopaminergic pathways are known to serve the prefrontal cortex (28).

Another important aspect of neurotoxic effects of prenatal exposure to PCBs and dioxins described in this thesis is that the neurodevelopmental consequences of neurotoxic actions of prenatal exposure to environmental levels of PCBs and dioxins may be influenced by parental and home environmental conditions. In these studies, the neurodevelopmental outcomes were global measurements of cognitive and motor abilities that are relatively strongly related to parental and home environmental conditions. The results are suggestive of compensation of negative effects of perinatal exposure on cognitive and motor development in children raised in more favorable parental and home environmental conditions or of cumulative deficits in children raised in less favorable conditions. These results may be in line with animal studies that show a positive impact of an enriched environment on the effects of brain lesions (29-31) and with some human studies addressing effects of perinatal exposure to lead and methyl mercury (32, 33) and the outcome in very low birth weight children (34-36).

Neurotoxic effects of perinatal exposure to PCBs and dioxins may be mediated by hormone-disrupting properties of PCBs and dioxins, for example in regard to steroid and thyroid hormone systems. The (sex-specific) effects of perinatal exposure to PCBs and dioxins on childhood play behavior suggest mediation of behavioral effects of prenatal PCB and dioxin exposure by the sex-steroid hormone system. However, evaluation of the relation between prenatal steroid hormone status and PCB and dioxin exposure is needed to further confirm these findings. In this cohort, maternal and infant thyroid hormone levels were related to maternal levels of PCBs and dioxins. Prenatal alterations in prenatal thyroid hormone levels may cause long-lasting neurodevelopmental deficits (37, 38). However, in this study, maternal thyroid hormone status was not statistically related to the level of cognitive and motor development from 3 to 84 month of age. The presently used analyses, therefore, do not provide evidence that prenatal thyroid hormone status is the one of the key mechanisms in the neurotoxic effects of prenatal exposure to PCBs and dioxins on general cognitive and motor development.

Animal studies show differences in neurotoxic effects of nonplanar PCBs and dioxins and dioxin-like PCBs (18, 39). Humans are exposed to complex mixtures of PCBs and dioxins and their related compounds such as hydroxilated PCBs. Not finding associations between outcome variables and dioxin toxic equivalents (TEQs) or total TEQs may suggest that neurotoxic effects of PCBs and dioxins were not mediated by the Ah receptor, as is in line with animal studies that report more pronounced neurotoxic actions of nonortho substituted PCB congeners than of dioxins and dioxin-like PCBs. The studies presented in this thesis, show more pronounced effects on general cognitive and motor abilities of the

four nonplanar PCBs (IUPAC nos. 118, 138, 153, and 180) as assessed in plasma compared to the dioxin TEQs and total TEQs assessed in breast milk. However, based on these results we believe that we cannot differentiate effects of different types of congeners, since the levels of different types of congeners were strongly related (40). Moreover, dioxins as well as a more elaborate number of PCB congeners were assessed in breast milk that was available for only half of the cohort. Analyses in this subpopulation may have increased the risk of Type II errors, which may consequently increase the risk of missing associations. However, in regard to play behavior some evidence of different neurotoxic effects of PCBs and dioxins can be hypothesized. Prenatal exposure to the sum of the four nonplanar PCBs was suggested to be related with opposite effects in boys and girls on masculine play behavior, whereas higher levels of prenatal exposure to dioxins, expressed in TEQs, were related to more feminized play behavior in both boys and girls.

In **conclusion**, the mechanisms of neurotoxic effects of prenatal exposure to PCBs and dioxins may include multi-focal, or diffuse, neurodevelopmental impairments. Due to differences in the maturation of the CNS, lactational exposure may be related to more focal effects, in which processes related to the prefrontal cortex are suggested to be involved. Neurotoxic effects on neurodevelopmental outcome that is more strongly related to parental and home environmental conditions may be modified by these conditions. Moreover, steroid hormones are suggested to be involved in the neurotoxic mechanism of effects of prenatal exposure to PCBs on a sex-specific nonreproductive behavior.

8.2.2Is breast-feeding still safe in the Netherlands?

Although BF children are exposed to relatively large amounts of PCBs and dioxins, negative effects of lactational exposure to PCB and dioxins are only suggested on the scores on the TOL. The results of this study, therefore, may indicate that effects of prenatal exposure to PCBs and dioxins are more pronounced than effects of exposure to PCBs and dioxins through lactation. This is in agreement with most of the human studies that address perinatal exposure to environmental levels of PCBs and dioxins (5, 20, 21, 23). Only two studies have described negative effects of lactational exposure on scores on these developmental tests. Lactational exposure to PCBs and dioxins was related to lower psychomotor abilities at 7 months of age (8) in the Dutch study and to lower general cognitive abilities at 42 months of age in the German cohort (7).

However, there are some methodological aspects that should be considered in risk assessment studies that address neurodevelopmental effects of lactational exposure to PCBs and dioxins, especially in Western societies. Based on the studies described in this thesis it can be hypothesized that negative effects of lactational exposure may, similarly to effects of prenatal exposure, be counteracted or masked by optimal parental and home environmental conditions. In The Netherlands, comparable to most Western societies,

the parents' choice for breast-feeding their child generally reflects also differences in for example levels of parental and home environmental conditions. Studies that explore subtle negative effects of lactational exposure to PCBs and dioxins may therefore benefit from more advanced modeling techniques in which the interrelationships of these neurodevelopmental determinants and exposure can be more properly modeled.

Moreover, more insight is needed into potential positive effects of breast-feeding, such as the effects of brain stimulating substances that are provided by breast milk and not by formula milk. The design and aims of the studies described in this thesis are not adequate to address this aspect of breast-feeding. However, the results of the neurophysiological study presented in this thesis may suggest positive effects of a longer duration of breast-feeding in which potentially brain stimulating effects of substances in breast milk are involved.

Animal studies show evidence of profound neurodevelopmental effects in monkeys that were only exposed to low levels of PCBs and dioxins through lactation (25, 41-43). These results indicate the potential for neurodevelopmental effects of lactational exposure to PCBs and dioxins in humans. Moreover, these behavioral deficits in animals were suggestive of prefrontal cortex involvement. Since structure related effects of lactational exposure can be hypothesized considering maturation differences of brain structures, risk assessment studies that address lactational exposure should include a more elaborate neuropsychologal test battery and larger study populations in which children were breast-fed for longer durations than in the Dutch cohort (median of breast-feeding duration 17 weeks) than in the neuropsychological study that is described in this thesis. This may increase the knowledge of neurotoxic effects of lactational exposure as well as help to differentiate effects of prenatal and lactational exposure to PCBs and dioxins.

In **conclusion**, although infants are exposed to relatively large amounts of PCBs and dioxins through lactation, neurodevelopmental effects of prenatal exposure to environmental levels of PCBs and dioxins were generally more pronounced. However, subtle effects of postnatal exposure to PCBs and dioxins were suggested on one of the neurodevelopmental outcome variables that were explored in this study. On the other hand, the results of the neurophysiological study presented in this thesis may suggest positive effects of a longer duration of breast-feeding in which potentially brain stimulating effects of substances in breast milk are involved. These results do not warrant restrictions on breast-feeding or reductions of the period of breast-feeding in the Western societies. Neurodevelopmental effects of lactational exposure to PCBs and dioxins and effects of breast milk brain stimulating substances should be studied more thoroughly, using advanced modeling techniques in addition to addressing specific cognitive domains in larger cohorts as well as animal research.

8.2.3 Magnitude of estimated neurodevelopmental effects

The magnitude of neurodevelopmental effects that were associated with PCBs and dioxin exposure is relatively small in the Dutch cohort, and is not likely to be clinically relevant to the individual child. The level of cognitive development from 3 to 84 months of age, for example in children born to younger mothers, was approximately 3 points lower in high prenatally exposed children (75 % equivalent) compared to their low exposed counterparts (25 % equivalent).

The results presented in this thesis suggest that under less favorable parental and home environmental conditions the magnitude of cognitive and motor decrements may be larger. The Dutch cohort consists of families that were willing to participate for at least 7 years in this study, at a voluntary basis. Parental and home environmental characteristics of this group are therefore likely to be more advantaged than in the average Dutch population or in populations in which educational possibilities or potential for cognitive stimulation are limited.

When considering these subtle effects in a large population, a lower average IQ shifts the distribution and increases the number of individuals who can be classified as retarded (IQ <85). Additionally, it decreases the number of gifted and exceptionally gifted individuals (IQ >130). For example, if the average IQ is shifted by 5 points (in a normal distribution with a mean of 100 and a standard deviation of 15) the number of children that score below 70 increases by a factor 2 (44).

Concluding, neurodevelopmental effects of perinatal exposure to PCBs and dioxins are detectable in a cohort of normal children. The magnitude of the effects is relatively small and not likely to be clinically relevant to the individual child. The magnitude of neurodevelopmental effects may be somewhat larger in populations in which conditions for child development are less favorable. For the whole society, however, these subtle decrements may have long-term consequences.

8.3 Future perspectives

The studies in this thesis as well as the results of other epidemiologic PCB studies draw attention to a number of important aspects that should be considered in this type of prospective follow-up risk assessment studies that address effects of perinatal exposure to PCBs and dioxins on neurodevelopmental outcome.

First, the results of these studies may illustrate the importance of paying attention to nonrandom attrition of the subjects of the original cohort. In the follow-up at school age, 10 % of the original cohort was lost to follow-up. Although exposure levels were not statistically different between participating and nonparticipating children, the latter group was significantly more FF, BF for shorter periods, and maternal age, parental education

levels and verbal IQ's were significantly lower in this lost to follow-up group. Since effects of prenatal exposure to PCBs and dioxins were suggested to be modified by parental and home environmental conditions, this is an important change in the study population. At preschool age, negative effects of prenatal PCB exposure on cognitive development were seen in the total cohort, whereas at school age significant negative effects were only seen when parental and home characteristics were less optimal. The higher mean levels of these background variables in the population at school age might explain that no effect of prenatal PCB exposure is seen in the total cohort, adjusting for the mean population levels of the confounders. Therefore, changes in the distribution of these variables in a cohort are a point of great attention in prospective follow-up studies that address neurodevelopmental risks of perinatal exposure to PCBs and dioxins, since it may cause missing neurodevelopmental effects in older children.

Secondly, the choice of neurodevelopmental outcomes to detect harmful effects of prenatal and lactational exposure to PCBs and dioxins should be dealt with great care in risk assessment studies. For example, general cognitive abilities, as measured with developmental tests, may not be the most sensitive outcome to detect neurotoxic effects of lactational exposure to PCBs and dioxins since this outcome is particularly sensitive to parental and home environmental conditions. The process of learning or more specific neuropsychological functions as well as motor development, developmental aspects that are to a smaller extent related to these conditions, may therefore be more sensitive outcomes in risk assessment studies addressing subtle effects of lactational exposure to neurotoxicants than the scores on developmental tests. Moreover, outcomes such as specific neuropsychological domains and neurophysiological assessments may be more sensitive to differentiate effects of prenatal and lactational exposure to PCBs and dioxins than more global measurements of cognitive functioning.

Thirdly, due to the complex interrelationships of various neurodevelopmental determinants and maternal PCB and dioxin levels, risk assessment studies may benefit from using sophisticated statistical modeling techniques. Moreover, these analyses make it possible to address the developmental course of functions.

Forth, due to differences in eating habits or area differences in environmental PCB and dioxin mixtures, populations world wide are not exposed to similar mixtures of PCBs and dioxin. For example, one of the more recently recruited PCB cohorts (the Oswego Study) has reported that the cord blood of women that consumed Lake Ontario fish contained a significantly higher proportion of the most heavily chlorinated PCBs relative to nonfish eaters. Levels of PCBs of lighter chlorination as well as the total PCB levels were similar in these groups (45). Moreover, the cord blood levels of the highly chlorinated PCBs correlated more strongly with breast milk PCB levels than lower chlorinated PCBs. The results of the neurodevelopmental analyses in this cohort, in children from birth to 12 months of age, showed some evidence of more pronounced neurodevelopmental effects

of exposure to the higher chlorinated PCBs (46). The initial findings of this study as well as the results of laboratory studies therefore suggest that risk assessment studies may benefit from addressing more thoroughly the effects of different types of congeners that children are exposed to.

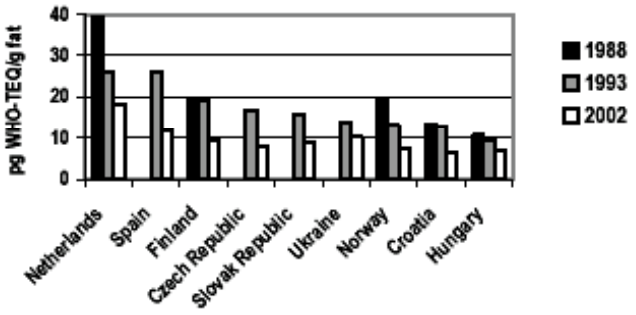

Figure 8.1 Temporal trends of levels of PCDD/PCDF in human milk (48).

Fifth, environmental levels of PCBs and dioxins are generally declining, due to worldwide control of sources, regulations of disposal practices, elimination of production, and natural attenuation. In The Netherlands, efforts to minimize dioxin emissions, as were performed from the late 1980s, clearly show decreasing levels of PCBs and dioxins in food in the past 10 years (47). In breast milk, dioxin levels even decreased up to 50 % during the past decade (47, 48) (see Figure 8.1). Children, however, are perinatally exposed to a large number of other potentially neurotoxic persistent environmental pollutants such as heavy metals, pesticides and insecticides, flame retardants, cleansers. For example, although breast milk levels of PCBs and dioxins have decreased over the past years, an increase is seen in the levels of another group of persistent organic pollutants, polybrominated diphenyl ethers (PBDEs) (49). PBDEs are used as flame-retardants and are presently applied throughout the world. The chemical structure of the PBDEs resembles the structure of PCBs and dioxins and their neurotoxic properties have been recently recognized (50). Furthermore, of the over 80.000 chemicals that are used in commerce and industry, only a small proportion has undergone testing for developmental toxicity. PCBs and dioxins are among the few contaminants that underwent extensive research to explore their neurotoxic properties and neurodevelopmental consequences for humans exposed to environmental levels of these compounds. The subtle neurodevelopmental decrements described in the prospective follow-up studies in healthy born children that were perinatally exposed to relatively low levels of PCBs and dioxins may be illustrative for the potential risks of exposure to other man made neurotoxic compounds.

8.4 Conclusions

The studies in this thesis suggest that prenatal exposure to environmental levels of PCBs and dioxins and related compounds can be related with negative neurodevelopmental effects in children at school age. Subtle effects of prenatal exposure to PCBs and dioxins were seen on general cognitive and motor development, memory skills, reaction time and variation in reaction time, TOL score, P300 latency and play behavior. The magnitude of the effects on general cognitive and motor development may be larger when parental and home environmental conditions are not optimal for child development. Negative neurodevelopmental effects of lactational exposure to PCBs and dioxins were less pronounced than effects of prenatal exposure to PCBs and dioxins. However, subtle negative effects of lactational exposure to PCBs were suggested on planning abilities or executive functioning. Given the results of the neurophysiological study presented in this thesis that suggest positive effects of a longer duration of breast-feeding in addition to the decline in contamination of breast milk with PCBs and dioxins, we conclude that the results of these studies do not warrant restrictions on breast-feeding or reductions in the period of breast-feeding in The Netherlands. Risk assessment studies may benefit from addressing the development of cognitive and motor abilities as well as from statistical techniques that allow more proper modeling of the predictors of neurodevelopmental outcome in studying effects of perinatal exposure to PCBs and dioxins. Additionally, specific neurodevelopmental outcomes may be particularly sensitive tools to detect neurotoxic effects of perinatal exposure to PCBs and dioxins and may increase the knowledge into neurotoxic effects of exposure as well as serve to differentiate effects of prenatal and lactational exposure to PCBs and dioxins. Generally, the results of this study emphasize efforts to reduce environmental levels of PCBs and dioxins and related compounds, to reduce maternal body burdens of PCBs and dioxins. The results of this study may be illustrative of potential neurodevelopmental risks of developmental exposure to various other neurotoxic agents. These environmental contaminants deserve serious consideration since 'The ultimate pollution is the chemical contamination of the brain, mind and intelligence (51)'.

References

1. Chen YC, Guo YL, Hsu CC, Rogan WJ. Cognitive development of Yu-Cheng ("oil disease") children prenatally exposed to heat-degraded PCBs. Jama 1992;268(22):3213-8.
2. Guo YL, Lai TJ, Chen SJ, Hsu CC. Gender-related decrease in Raven's progressive matrices scores in children prenatally exposed to polychlorinated biphenyls and related contaminants. Bull Environ Contam Toxicol 1995;55(1):8-13.
3. Harada M. Intrauterine poisoning: Clinical and epidemiological studies and significance of the problem. Bull Inst Const Med Kumamoto Univ. 1976;25:1-69.
4. Grandjean P, Weihe P, Burse VW, Needham LL, Storr-Hansen E, Heinzow B, et al. Neurobehavioral deficits associated with PCB in 7-year-old children prenatally exposed to seafood neurotoxicants. Neurotoxicol Teratol 2001;23(4):305-17.
5. Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychorinated biphenyls in utero. N Engl J Med 1996;335:783-9.
6. Patandin S, Lanting CI, Mulder PG, Boersma ER, Sauer PJ, Weisglas-Kuperus N. Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J Pediatr 1999;134(1):33-41.
7. Walkowiak J, Wiener JA, Fastabend A, Heinzow B, Kramer U, Schmidt E, et al. Environmental exposure to polychlorinated biphenyls and quality of the home environment: effects on psychodevelopment in early childhood. Lancet 2001;358(9293):1602-7.
8. Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MA, Van der Paauw CG, Tuinstra LG, Sauer PJ. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics 1996;97(5):700-6.
9. Lanting CI, Patandin S, Fidler V, Weisglas-Kuperus N, Sauer PJ, Boersma ER, et al. Neurological condition in 42-month-old children in relation to pre- and postnatal exposure to polychlorinated biphenyls and dioxins. Early Hum Dev 1998;50(3):283-92.
10. Lonky E, Reihman J, Darvill T, Mather JE, Daly H. Neonatal Behavioral Assessment Scale performance in humans influenced by maternal consumption of environmentally contaminated Lake Ontario fish. Journal of Great Lakes 1996;22(2):198-212.
11. Winneke G, Bucholski A, Heinzow B, Kramer U, Schmidt E, Walkowiak J, et al. Developmental neurotoxicity of polychlorinated biphenyls (PCBS): cognitive and psychomotor functions in 7-month old children. Toxicol Lett 1998;102-103:423-8.
12. Huisman M, Koopman-Esseboom C, Lanting CI, van der Paauw CG, Tuinstra LG, Fidler V, et al. Neurological condition in 18-month-old children perinatally exposed to polychlorinated biphenyls and dioxins. Early Hum Dev 1995;43(2):165-76.
13. Huisman M, Koopman-Esseboom C, Fidler V, Hadders-Algra M, van der Paauw CG, Tuinstra LG, et al. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. Early Hum Dev 1995;41(2):111-27.
14. Patandin S, Veenstra J, Mulder PGH, Sewnaik A, Sauer PJJ, Weisglas-Kuperus N. Attention and activity in 42-month-old Dutch children with environmental exposure to polychlorinated biphenyls and dioxins. In: Effects of environmental exposure to polychlorinated biphenyls and dioxins on growth and development in young children; doctoral thesis. Rotterdam; 1999; 123-142.
15. Brouwer A, Morse DC, Lans MC, Schuur AG, Murk AJ, Klasson-Wehler E, et al. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. Toxicol Ind Health 1998;14(1-2):59-84.
16. Brouwer A, Ahlborg UG, Van den Berg M, Birnbaum LS, Boersma ER, Bosveld B, et al. Functional aspects of developmental toxicity of polyhalogenated aromatic hydrocarbons in experimental animals and human infants. Eur J Pharmacol 1995;293(1):1-40.
17. Mariussen E, Fonnum F. The effect of polychlorinated biphenyls on the high affinity uptake of the neurotransmitters, dopamine, serotonin, glutamate and GABA, into rat brain synaptosomes. Toxicology 2001;159(1-2):11-21.
18. Tilson HA, Kodavanti PR. Neurochemical effects of polychlorinated biphenyls: an overview and identification of research needs. Neurotoxicology 1997;18(3):727-43.

19. Vreugdenhil HJ, Lanting CI, Mulder PG, Boersma ER, Weisglas-Kuperus N. Effects of prenatal PCB and dioxin background exposure on cognitive and motor abilities in Dutch children at school age. J Pediatr 2002;140(1):48-56.
20. Rogan WJ, Gladen BC. PCBs, DDE, and child development at 18 and 24 months. Ann Epidemiol 1991;1(5):407-13.
21. Gladen BC, Rogan WJ, Hardy P, Thullen J, Tingelstad J, Tully M. Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. J Pediatr 1988;113(6):991-5.
22. Vreugdenhil HJI, Mulder PG, Emmen HH, Weisglas-Kuperus N. Effects of perinatal exposure to PCBs on neuropsychological functions in the Rotterdam cohort at 9 years of age. Neuropsychology, in press.
23. Jacobson JL, Jacobson SW, Humphrey HE. Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J Pediatr 1990;116(1):38-45.
24. Vreugdenhil HJI, Van Zanten GA, Brocaar MP, Mulder PGH, Weisglas-Kuperus N. Prenatal PCB exposure and breast-feeding affect auditory P300 latencies in 9-year-old Dutch children. Developmental Medicine and Child Neurology, in press.
25. Rice DC. Behavioral impairment produced by low-level postnatal PCB exposure in monkeys. Environ Res 1999;80(2 Pt 2):S113-S121.
26. Seegal RF, Brosch KO, Okoniewski RJ. Effects of in utero and lactational exposure of the laboratory rat to 2,4,2',4'- and 3,4,3',4'-tetrachlorobiphenyl on dopamine function. Toxicol Appl Pharmacol 1997;146(1):95-103.
27. Mariussen E, Andersson PL, Tysklind M, Fonnum F. Effect of polychlorinated biphenyls on the uptake of dopamine into rat brain synaptic vesicles: a structure-activity study. Toxicol Appl Pharmacol 2001;175(2):176-83.
28. Saper CB, Iversen S, Frackowiak RS. The association areas of the cerebral cortex and the cognitive capacities of the brain. In: Kandell ER, Schwartz JH, Jessel TM, editors. Principles of Neural Science. United States of America: McGraw-Hill; 2000; 361.
29. Brenner E, Mirmiran M, Uylings HB, Van der Gugten J. Growth and plasticity of rat cerebral cortex after central noradrenaline depletion. Exp Neurol 1985;89(1):264-8.
30. Diamond MC, Lindner B, Johnson R, Bennett EL, Rosenzweig MR. Differences in occipital cortical synapses from environmentally enriched, impoverished, and standard colony rats. J Neurosci Res 1975;1(2):109-19.
31. Kolb B. Brain development, plasticity, and behavior. Am Psychol 1989;44(9):1203-12.
32. Winneke G, Kraemer U. Neuropsychological effects of lead in children: interactions with social background variables. Neuropsychobiology 1984;11(3):195-202.
33. Bellinger D, Leviton A, Waternaux C, Needleman H, Rabinowitz M. Low-level lead exposure, social class, and infant development. Neurotoxicol Teratol 1988;10(6):497-503.
34. Landry SH, Denson SE, Swank PR. Effects of medical risk and socioeconomic status on the rate of change in cognitive and social development for low birth weight children. J Clin Exp Neuropsychol 1997;19(2):261-74.
35. Pfeiffer SI, Aylward GP. Outcome for preschoolers of very low birthweight: sociocultural and environmental influences. Percept Mot Skills 1990;70(3 Pt 2):1367-78.
36. Weisglas-Kuperus N, Baerts W, Smrkovsky M, Sauer PJ. Effects of biological and social factors on the cognitive development of very low birth weight children. Pediatrics 1993;92(5):658-65.
37. Rovet JF, Ehrlich RM. Long-term effects of L-thyroxine therapy for congenital hypothyroidism. J Pediatr 1995;126(3):380-6.
38. Porterfield SP. Vulnerability of the developing brain to thyroid abnormalities: environmental insults to the thyroid system. Environ Health Perspect 1994;102(Suppl 2):125-30.
39. Fischer LJ, Seegal RF, Ganey PE, Pessah IN, Kodavanti PR. Symposium overview: toxicity of non-coplanar PCBs. Toxicol Sci 1998;41(1):49-61.
40. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N, Van der Pauw CG, Tuinstra LGMT, Boersma ER, et al. PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 1994;28(9):1721-1732.

41. Rice DC, Hayward S. Effects of postnatal exposure of monkeys to a PCB mixture on concurrent random interval-random interval and progressive ratio performance. Neurotoxicol Teratol 1999;21(1):47-58.
42. Rice DC. Effect of postnatal exposure to a PCB mixture in monkeys on multiple fixed interval-fixed ratio performance. Neurotoxicol Teratol 1997;19(6):429-34.
43. Rice DC, Hayward S. Effects of postnatal exposure to a PCB mixture in monkeys on nonspatial discrimination reversal and delayed alternation performance. Neurotoxicology 1997;18(2):479-94.
44. Rice DC. Issues in developmental neurotoxicology: interpretation and implications of the data. Can J Public Health 1998;89 Suppl 1:S31-6, S34-40.
45. Stewart P, Darvill T, Lonky E, Reihman J, Pagano J, Bush B. Assessment of prenatal exposure to PCBs from maternal consumption of Great Lakes fish: an analysis of PCB pattern and concentration. Environ Res 1999;80(2 Pt 2):S87-S96.
46. Darvill T, Lonky E, Reihman J, Stewart P, Pagano J. Prenatal exposure to PCBs and infant performance on the fagan test of infant intelligence. Neurotoxicology 2000;21(6):1029-38.
47. Liem AK, Furst P, Rappe C. Exposure of populations to dioxins and related compounds. Food Addit Contam 2000;17(4):241-59.
48. Van Leeuwen FXR, Malisch R. Results of the third round of the WHO-coordinated exposure study on the levels of PCBs, PCDDs, and PCDFs in human milk. Organohalogen Compounds 2002;56:311-316.
49. Meironyte D, Noren K, Bergman A. Analysis of polybrominated diphenyl ethers in Swedish human milk. A time-related trend study, 1972-1997. J Toxicol Environ Health A 1999;58(6):329-41.
50. Darnerud PO, Eriksen GS, Johannesson T, Larsen PB, Viluksela M. Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology. Environ Health Perspect 2001;109 Suppl 1:49-68.
51. Muir T, Zegarac M. Societal costs of exposure to toxic substances: economic and health costs of four case studies that are candidates for environmental causation. Environ Health Perspect 2001;109 Suppl 6:885-903.

Samenvatting

Dit proefschrift beschrijft resultaten van het Nederlandse PCB/dioxine onderzoek. Het Nederlandse PCB/dioxine onderzoek is een prospectieve follow-up studie bij kinderen die borstvoeding hebben gekregen en bij kinderen die zijn flesgevoed. In dit onderzoek worden de mogelijke schadelijke effecten van prenatale blootstelling aan PCB's en dioxinen en ook blootstelling aan deze stoffen door borstvoeding op de ontwikkeling van het kind geëvalueerd.

PCB's (polychloorbiphenylen) en dioxinen (PCDDs en PCDFs respectievelijk polychloor-dibenzo-p-dioxinen en polychloor-dibenzo-furanen) zijn (neuro)toxische stoffen die wereldwijd in het milieu worden aangetroffen als gevolg van milieuverontreiniging. Doordat deze stoffen zich ophopen in het vetweefsel en slechts langzaam daaruit kunnen worden verwijderd, accumuleren zij in de voedselketen. De voornaamste blootstellingsbron (90 %) voor de mens is dan ook voedsel van dierlijke oorsprong zoals zuivelproducten, vis en vlees. PCB's en dioxinen worden via de vetfractie van het bloed getransporteerd in het lichaam en daardoor kan ook de foetus blootgesteld worden aan deze stoffen. Bovendien worden deze stoffen uitgescheiden in moedermelk waardoor het jonge kind aan relatief hoge niveaus van PCB's en dioxinen kan worden blootgesteld.

Dieronderzoek heeft aangetoond dat blootstelling aan PCB's en dioxinen kan resulteren in een complex scala aan neurotoxische effecten, vooral als het zich nog ontwikkelende centraal zenuwstelsel (CZS) wordt blootgesteld aan deze stoffen. Bij een groep kinderen, waarvan de moeders blootgesteld waren aan *hoge* concentraties van PCB's en dioxinen, bleek dat prenatale blootstelling aan PCB's en dioxinen ontwikkelingsachterstanden kan veroorzaken. De afgelopen 20 jaar is in een aantal cohorten onderzocht of perinatale blootstelling (prenatale blootstelling en blootstelling door borstvoeding) aan lagere niveaus van PCB's en dioxinen zoals deze bij mensen in geïndustrialiseerde landen voorkomen (achtergrondniveaus) ook schadelijk kan zijn voor de ontwikkeling van een kind. Eén van deze cohort studies is het Nederlands PCB/dioxine onderzoek. In dit onderzoek worden de effecten van prenatale blootstelling aan PCB's en dioxinen en blootstelling via moedermelk (postnatale blootstelling) op de groei, gezondheid, de neurologische, cognitieve, en motorische ontwikkeling en het gedrag van Nederlandse kinderen geëvalueerd om daarmee onder andere te onderzoeken of het geven van borstvoeding nog wel veilig is in Nederland. Tussen 1990 en 1992 werd daartoe een cohort van gezond geboren kinderen samengesteld door twee studiecentra, in Rotterdam (Sophia Kinderziekenhuis/Erasmus Universiteit te Rotterdam) en in Groningen (Rijksuniversiteit te Groningen). Deze klinische studie werd gestart in samenwerking met dierexperimentele onderzoekscentra (Landbouw Universiteit Wageningen en TNO te Rijswijk) en laboratoria (TNO in Zeist en RIKILT in Wageningen), waar de PCB's en dioxinen in plasma en moedermelk werden geanalyseerd.

Het Nederlandse PCB/dioxine cohort bestaat uit 418 gezond geboren kinderen (207 in Rotterdam, 211 in Groningen) waarvan in elk studiecentrum de helft van de groep borstvoeding (BV) heeft gekregen voor tenminste 6 weken en de andere helft werd gevoed met flesvoeding (FV) waarin geen meetbare niveaus van PCB's en dioxinen werden aangetroffen. De FV groep vormt hiermee een groep die met name prenataal aan PCB's en dioxinen is blootgesteld. Als maat voor prenatale blootstelling aan PCB's werd de som genomen van de concentraties vier PCB's (IUPAC nummers 118, 138, 153, en 180) die bepaald waren in het bloed van de moeder tijdens de zwangerschap en in het navelstrengbloed. In moedermelk, die twee weken na de geboorte werd verzameld, kon een groter aantal PCB's en ook dioxinen worden bepaald. Doordat deze bepalingen in moedermelk gedaan zijn kort na de geboorte van het kind geven deze concentraties ook een indicatie van de prenatale blootstelling aan PCB's en dioxinen. Postnatale blootstelling aan PCB's en dioxinen, als gevolg van de borstvoeding, wordt geschat door de PCB en dioxine concentraties in moedermelk te vermenigvuldigen met het aantal weken dat een kind borstvoeding heeft gehad.

In het kader van het PCB/dioxine onderzoek zijn verschillende ontwikkelingsparameters onderzocht bij kinderen op de leeftijd van 2 weken, 3, 7, 18 en 42 maanden. Ook zijn bij deze onderzoeken parameters bepaald die van belang zijn voor de ontwikkeling van het kind, zoals ouder- en opvoedingsklimaatfactoren.

Omdat het CZS zich nog lang na de geboorte blijft ontwikkelen en daarmee meer specifieke cognitieve aspecten zich pas op latere leeftijd ontwikkelen, is het van belang om te onderzoeken of perinatale blootstelling aan PCB's en dioxinen ook na de peuterleeftijd invloed heeft op de ontwikkeling van een kind. Daarom werden de kinderen van het hele Nederlandse PCB/dioxine cohort uitgenodigd om deel te nemen aan een onderzoek toen zij 6/7 jaar oud waren. Bovendien werd de helft van het Rotterdamse cohort benaderd om te participeren in een neuropsychologisch onderzoek op 9-jarige leeftijd.

In dit proefschrift worden de resultaten van deze onderzoeken beschreven. De onderzoeken zijn met name gericht op de *neurotoxische* effecten van PCB's en dioxinen die geëvalueerd werden door het bestuderen van potentiële relaties tussen perinatale blootstelling aan PCB's en dioxinen en cognitieve, motorische en neurofysiologische uitkomstmaten en ook het speelgedrag.

In **Hoofdstuk 1** wordt een beschrijving gegeven van het brede scala van neurotoxische eigenschappen van PCB's en dioxinen. Uit dieronderzoek blijkt dat PCB's en dioxinen veranderingen kunnen aanbrengen in neuronen, gliacellen, neurotransmitters en ook hormoonsystemen. Ook worden in Hoofdstuk 1 de resultaten van de verschillende epidemiologische onderzoeken naar neurotoxische effecten van perinatale blootstelling aan PCB's en dioxinen beschreven, inclusief de resultaten van het Nederlandse PCB/dioxine onderzoek bij kinderen van 2 weken tot 42 maanden. In het Nederlandse PCB/

dioxine cohort was een hogere prenatale blootstelling aan PCB's gerelateerd aan een minder optimale neurologische conditie op de leeftijd van 2 weken en 18 maanden, aan lagere psychomotore vaardigheden op de leeftijd van 3 maanden, en aan lagere globale cognitieve vaardigheden en een lager verbaal begrip bij kinderen op de leeftijd van 42 maanden. Bij het onderzoek op 42 maanden bleek dat bij FV kinderen de negatieve effecten van prenatale blootstelling aan PCB's groter waren dan bij de BV kinderen. De BV groep verschilt in veel opzichten van de FV groep kinderen. In vergelijking tot de FV groep, heeft de BV groep van het Nederlandse cohort oudere moeders, hoger opgeleide ouders met een hoger verbaal IQ en ouders die hoger scoren op de HOME-vragenlijst. De HOME-vragenlijst brengt de kwaliteit van het opvoedingsklimaat in kaart wat betreft de stimulering van de cognitieve en sociaal-emotionele ontwikkeling van het kind. Postnatale blootstelling aan PCB's en dioxinen bleek een negatief effect te hebben op één uitkomstmaat, namelijk de psychomotore ontwikkeling op 7 maanden.

Als laatste wordt in Hoofdstuk 1 de opzet van het Nederlands PCB/dioxine onderzoek gepresenteerd en worden de doelstellingen van de onderzoeken in dit proefschrift beschreven. Het algemene doel was om ontwikkelingseffecten van perinatale blootstelling aan achtergrondniveaus van PCB's en dioxinen bij kinderen van de schoolleeftijd te evalueren. Daarnaast was het doel om meer inzicht te krijgen in mogelijke compenserende effecten van ouder- en opvoedingsklimaatfactoren en borstvoeding, en om meer inzicht te krijgen in neurotoxische mechanismen van effecten van perinatale blootstelling aan deze stoffen gedurende de ontwikkeling van het CZS.

Het eerste deel van dit proefschrift (**Hoofdstuk 2, 3 en 4**) beschrijft onderzoeken die de relatie evalueren tussen perinatale blootstelling aan PCB's en dioxinen en cognitieve en motorische vaardigheden bij kinderen op de schoolleeftijd en ook op de *ontwikkeling* van deze vaardigheden van 3 maanden tot de schoolleeftijd. Daarnaast werd onderzocht of de effecten van PCB's en dioxinen op de ontwikkeling van het kind beïnvloed werden door ouder- en opvoedingsklimaatfactoren (leeftijd van de moeder, opleidingsniveau van de ouders, verbaal IQ van de ouders en de score op de HOME-vragenlijst).

Hoofdstuk 2 beschrijft de relaties tussen perinatale blootstelling aan PCB's en dioxinen en cognitieve en motorische vaardigheden van 6/7 jarige kinderen. De Nederlandse versie van de McCarthy Scales of Children's Abilities werd gebruikt om de globale cognitieve ontwikkeling te bepalen (Globale Cognitieve Index, GCI), geheugen en motorische vaardigheden werden bepaald met twee andere subschalen van deze test. Van het originele cohort van 418 kinderen was 90% (n=376) bereid om mee te doen aan dit onderzoek (gemiddelde leeftijd 6.7 jaar ± 0.3 jaar). Multiple lineaire regressie analyses lieten zien dat, gecorrigeerd voor covariabelen, prenatale blootstelling aan PCB's en dioxinen niet significant gerelateerd was aan de GCI scores, of aan de scores op de geheugen en motorische schalen. Het bleek echter dat de effecten van prenatale blootstelling aan PCB's op deze cognitieve en motorische maten werden gemodificeerd door ouder- en

opvoedingsklimaatfactoren (leeftijd van de moeder, opleidingsniveau van de ouders, verbaal IQ van de ouders en de score op de HOME-vragenlijst). In het Nederlandse cohort zijn deze variabelen sterk aan elkaar gerelateerd. Een oudere leeftijd van de moeder bij de geboorte van haar kind is gerelateerd aan een hoger opleidingsniveau en een hoger verbaal IQ van de ouders en ook aan hogere scores op de HOME, condities die gezien worden als relatief gunstig voor de ontwikkeling van een kind. De impact van negatieve effecten van prenatale blootstelling aan PCB's en dioxinen op de cognitieve en motorische variabelen bleek groter te worden naarmate deze ouder- en opvoedingsklimaatfactoren minder optimaal waren. Bij kinderen waarbij deze factoren meer optimaal waren, waren deze subtiele negatieve effecten niet meetbaar. De resultaten van dit onderzoek wezen niet op negatieve effecten van blootstelling door middel van borstvoeding. We concluderen dat de neurotoxische effecten van prenatale blootstelling aan achtergrondniveaus van PCB's en dioxinen ook op schoolleeftijd kunnen resulteren in kleine cognitieve en motorische achterstanden, met name bij kinderen waarbij de ouder- en opvoedingsklimaatfactoren minder optimaal zijn voor hun ontwikkeling.

Een nadeel van het onderzoeken van de relatie tussen perinatale blootstelling aan PCB's en dioxinen en de cognitieve en motorische vaardigheden op een bepaalde leeftijd is dat het ontwikkelingsverloop van deze vaardigheden op verschillende leeftijden niet kan worden belicht. Daarom hebben we ook bestudeerd of perinatale blootstelling aan PCB's gerelateerd was aan de ontwikkeling van de globale cognitieve en motorische vaardigheden, zoals gemeten in het Rotterdamse cohort op de leeftijd van 3, 7, 18, 42 en 84 maanden. In **Hoofdstuk 3** worden de resultaten van deze Random Regression Modeling (RRM) analyses beschreven. Een tweede doel van deze analyse bestond uit het identificeren van de belangrijkste voorspellers van de globale cognitieve en motorische ontwikkeling van 3 tot 84 maanden. Gegevens over de globale cognitieve en motorische scores waren beschikbaar voor alle kinderen van het Rotterdamse cohort. In de eerste fase van de analyse werden alle variabelen die mogelijk relevant zijn voor de cognitieve en motorische ontwikkeling in de RRM modellen opgenomen. In deze modellen was een hogere prenatale PCB-concentratie significant gerelateerd aan een lager niveau van globale cognitieve en motorische ontwikkeling van 3 tot 84 maanden. In de tweede fase werden de eerder beschreven 4 interactievariabelen van prenatale PCB-blootstelling en ouder- en opvoedingsklimaatfactoren tegelijk in het model opgenomen, hetgeen mogelijk was door deze interactievariabelen en hun hoofdtermen te centreren. Bij simultane inclusie van deze interactievariabelen bleek dat de effecten van prenatale blootstelling aan PCB's op de cognitieve ontwikkeling significant werden gemodificeerd door de leeftijd van de moeder. De modificatie van het PCB effect door de leeftijd van de moeder bleek de effect modificatie van de andere ouder- en opvoedingsklimaatfactoren te overschaduwen. Bij kinderen met jongere moeders bleken de negatieve cognitieve effecten van prenatale blootstelling aan PCB's groter te zijn dan bij kinderen met oudere moeders. Prenatale PCB-concentraties, en

de effect modificatie door de leeftijd van de moeder, samen met het opleidingsniveau en verbaal IQ van de ouders en de HOME-scores bleken de belangrijkste voorspellers van de cognitieve ontwikkeling te zijn. Voor de motorische ontwikkeling waren dat de prenatale PCB niveaus en de effect modificatie door de HOME-scores, en het opleidingsniveau van de ouders. Bij lagere HOME-scores werden meer uitgesproken negatieve effecten van prenatale PCB-blootstelling op de motorische ontwikkeling gevonden. In dit onderzoek werden geen negatieve effecten van postnatale blootstelling aan PCB's en dioxinen door middel van borstvoeding op de ontwikkeling aangetoond. Prenatale schildklierhormoon niveaus, gemeten tijdens de zwangerschap in het bloed van de moeder, waren ook niet gerelateerd aan deze ontwikkelingsmaten.

De resultaten van dit onderzoek suggereren negatieve effecten van prenatale blootstelling aan PCB's op het niveau van de globale cognitieve en motorische ontwikkeling. Deze negatieve effecten kunnen mogelijk worden beïnvloed door ouder- en opvoedingsklim aatcondities die van invloed zijn op de ontwikkeling van het kind. Vergeleken met de grote, positieve effecten van meer optimale ouder –en opvoedingsklimaatcondities waren de negatieve effecten van prenatale PCB-blootstelling op de cognitieve ontwikkeling van 3 tot 84 maanden relatief klein. De effecten van prenatale blootstelling aan PCB's op de motorische ontwikkeling waren meer uitgesproken vergeleken met de effecten op de cognitieve ontwikkeling. De motorische ontwikkeling is daarom mogelijk een meer gevoelige uitkomstmaat om effecten van blootstelling aan PCB's en gerelateerde stoffen te bepalen dan de globale cognitieve ontwikkelingsmaat.

Door de trage afbraaksnelheid van PCB's en dioxinen accumuleren zij in menselijk weefsel, en zijn de prenatale PCB en dioxine concentraties sterk gerelateerd aan de leeftijd van de moeder tijdens de zwangerschap. Een oudere leeftijd van de moeder bij geboorte is dan ook geassocieerd met een hogere PCB-spiegel maar ook met een hoger opleidingsniveau en verbaal IQ van de ouders, en hogere HOME-scores. Dit aspect maakt de variabele 'leeftijd van de moeder' erg complex als effecten van PCB's worden geëvalueerd op ontwikkelingsaspecten van het kind. In een poging om meer inzicht te krijgen in dit complexe netwerk van interrelaties van prenatale PCB niveaus, ouder- en opvoedingsklimaatfactoren en de cognitieve en motorische ontwikkeling van het kind hebben we de data van het Rotterdamse cohort geanalyseerd met de Structural Equation Modeling (SEM) methode. In **Hoofdstuk 4** worden de resultaten van deze analyses beschreven. De analyse begon met het identificeren van de relaties tussen het opleidingsniveau van de ouders, het verbaal IQ van de ouders, de HOME-score en de cognitieve en motorische ontwikkeling van de kinderen van 3 tot 84 maanden. Vervolgens is de hele populatie verdeeld in 4 groepen op basis van de mediaan van de prenatale PCB-concentraties (2.04 µg/L) en de mediaan van de leeftijd van de moeder (29 jaar) (PCB_{laag}/M_{jong}; PCB_{laag}/M_{oud}; PCB_{hoog}/M_{jong}; PCB_{hoog}/M_{oud}). Het geïdentificeerde model werd daarop gebruikt om verschillen tussen deze 4 groepen te evalueren wat betreft het gemiddelde

niveau van de variabelen in het model als ook de relaties tussen de ontwikkelingsvariabelen en hun determinanten (opleidingsniveau van de ouders, het verbaal IQ van de ouders, de HOME-score). De 4 groepen bleken significant verschillend te zijn zowel in het *niveau* van de cognitieve/motorische uitkomsten en hun determinanten, als ook in de *relaties* tussen de determinanten en de cognitieve ontwikkeling (en indirect ook de motorische ontwikkeling). De resultaten van dit onderzoek suggereerden dat effecten van prenatale blootstelling aan PCB's meer uitgesproken waren bij kinderen die opgroeien met relatief minder optimale ouder –en opvoedingsklimaatcondities, dan bij kinderen waarbij deze factoren meer optimaal lijken. Voor de vroege cognitieve en motorische ontwikkeling (van 3 tot 18 maanden) werd dit verschil in de impact van prenatale blootstelling aan PCB's met name gesuggereerd bij kinderen met oudere moeders, terwijl dit verschil bij de latere cognitieve en motorische ontwikkeling (van 42 tot 84 maanden) gezien werd bij de kinderen met jongere moeders. De resultaten van dit onderzoek suggereren dat er een complexe interactie bestaat tussen factoren zoals de leeftijd van de moeder en andere ouder- en opvoedingsklimaatfactoren en de neurotoxisch effecten van PCB's en verwante neurotoxische stoffen. Dit onderzoek wordt beschouwd als eerste poging om meer inzicht te verkrijgen in deze interactie.

In het tweede deel van het proefschrift (**Hoofdstuk 5, 6, 7**) worden effecten van perinatale blootstelling aan PCB's en dioxinen op meer specifieke ontwikkelingsmaten beschreven. PCB's en dioxinen staan mede bekend om hun hormoonverstorende capaciteiten. In de ontwikkeling van de hersenen spelen hormonen ook een rol. Geslachtshormonen, bijvoorbeeld, beïnvloeden mogelijk ook gedrag dat sekseverschillen vertoont, zoals speelgedrag. In **Hoofdstuk 5** worden (seksespecifieke) effecten beschreven van prenatale blootstelling aan PCB's en dioxinen op het speelgedrag van kinderen. Speelgedrag werd in het Rotterdamse cohort gemeten door middel van de Pre-School Activity Inventory (PSAI). Deze vragenlijst, die door de ouders ingevuld dient te worden, meet sekseverschillen in speelgedrag. 160 PSAI vragenlijsten werden door de ouders geretourneerd (gemiddelde leeftijd van de kinderen ± SD: 7.5 ± 0.4 jaar). Hogere prenatale concentraties van PCB's waren bij jongens gerelateerd aan minder mannelijk speelgedrag en bij meisjes aan meer mannelijk speelgedrag. Hogere prenatale concentraties van dioxinen, bepaald in moedermelk, waren gerelateerd aan meer vrouwelijk speelgedrag bij jongens en bij meisjes. Postnatale blootstelling door borstvoeding was niet gerelateerd aan speelgedrag en er waren ook geen sekseverschillen in de effecten van postnatale blootstelling aan PCB's en dioxinen op speelgedrag. Deze resultaten suggereren betrokkenheid van geslachtshormonen in het neurotoxische mechanisme van prenatale blootstelling aan achtergrond niveaus van PCB's en dioxinen.

De helft van het Rotterdamse cohort, de laagste groep prenataal belaste (p25; n=26) en hoogste groep prenataal belaste kinderen (p75; n=26) van de beide voedingsgroepen (n_{totaal}=104), werden op 9-jarige leeftijd uitgenodigd om deel te nemen aan

neuropsychologisch (zie **Hoofdstuk 6**) en neurofysiologisch onderzoek (zie **Hoofdstuk 7**) in het Sophia Kinderziekenhuis. Van de benaderde kinderen was 80 % (n=83) bereid deel te nemen aan dit onderzoek (gemiddelde leeftijd ± SD: 9.2 ± 0.2 jaar). De blootstellingniveaus van de participerende en niet participerende groep kinderen waren vergelijkbaar.

Hoofdstuk 6 beschrijft de relaties tussen perinatale concentraties van PCB's en dioxinen en de scores op verschillende neuropsychologische testen. Het neuropsychologisch onderzoek bestond uit de Rey Complex Figure Test, de 15 Woorden Taak, de Simple Reaction Time Task, en de Tower of London test (TOL). De prenataal hoog belaste kinderen hadden, gecorrigeerd voor verstorende variabelen, langere reactietijden met meer variatie in de reactietijd en lagere scores op de TOL in vergelijking met de laag belaste kinderen. Bovendien bleek dat de scores op de laatste test, een test die met name planningsfuncties of executieve functies meet, lager waren bij kinderen die voor een lange periode (≥ 17 weken) borstvoeding hadden gehad in vergelijking tot de TOL-scores van FV kinderen. De resultaten van dit onderzoek suggereren multi-focale of diffuse neurotoxische effecten van prenatale blootstelling aan PCB's en verwante stoffen. Wat betreft de mogelijk negatieve effecten van blootstelling door borstvoeding suggereert het negatieve effect op de TOL-scores dat processen gerelateerd aan de prefrontale cortex betrokken zijn bij het neurotoxische mechanisme van effect van PCB's en verwante stoffen, omdat de frontale cortex in vergelijking tot de overige hersenstructuren zich trager ontwikkelt en daarmee mogelijk kwetsbaarder is voor neurotoxische effecten van PCB's en dioxinen. Een complexe taak als de TOL kan ook beschouwd worden als een gevoelige maat voor meer diffuse neurotoxische effecten van vroege blootstelling aan PCB's en verwante stoffen.

Hoofdstuk 7 beschrijft de relaties tussen perinatale blootstelling aan PCB's en dioxinen en een neurofysiologische maat, de latentietijd en amplitude van de auditieve P300. De P300 wordt beschouwd als een cognitieve component van een event-related potential. Deze positieve piek treedt ongeveer 300 milliseconden nadat een stimulus wordt aangeboden op als een persoon de stimulus actief of bewust verwerkt. De latentietijd en de amplitude van de P300 werden gebruikt als uitkomstmaten in dit onderzoek.

De prenataal hoog belaste kinderen hadden een langere P300 latentietijd dan de prenataal laag belaste kinderen, na correctie voor covariaten. De resultaten van dit onderzoek wezen niet op effecten van postnatale blootstelling, door borstvoeding, op de P300 latentietijd. De P300 latentietijd bleek korter te zijn bij kinderen die voor een langere tijd borstvoeding hebben gehad (≥ 16 weken) dan bij kinderen die voor een kortere duur borstvoeding hebben gehad (6-16 weken). De P300 latentietijd van kinderen die voor een langere duur borstvoeding hadden gekregen was ook korter dan de P300 latentietijd van FV kinderen. Prenatale en postnatale blootstelling aan PCB's en dioxinen, en de borstvoedingsduur waren niet gerelateerd aan de amplitude van de P300. Deze resultaten suggereren dat prenatale blootstelling aan PCB's en dioxinen op de schoolleeftijd mogelijk een vertragend

effect heeft op CZS-mechanismen die relevante stimuli evalueren en verwerken, terwijl een langere periode van borstvoeding dit mechanisme mogelijk versnelt.

In **Hoofdstuk 8** wordt een samenvatting van de resultaten van deze onderzoeken gepresenteerd. Daarnaast worden in dit hoofdstuk de mogelijke neurotoxische mechanismen besproken die op grond van deze studie samen met de resultaten van andere onderzoeken mogelijk een rol spelen. Ook wordt in dit hoofdstuk de vraag belicht of het geven van borstvoeding nog wel veilig is in Nederland en wordt de grootte van de geobserveerde negatieve effecten in dit hoofdstuk geëvalueerd. Een aantal aandachtspunten voor onderzoek op dit gebied wordt ook in Hoofdstuk 8 gepresenteerd.

Conclusie

De resultaten van het Nederlandse PCB/dioxine onderzoek suggereren dat prenatale blootstelling aan achtergrondniveaus van PCB's en dioxinen geassocieerd is met negatieve ontwikkelingseffecten bij kinderen op de schoolleeftijd. Relatief kleine negatieve effecten van prenatale blootstelling aan PCB's en dioxinen werden gezien op de globale cognitieve en motorische ontwikkeling, het geheugen, de reactietijd en de variatie van de reactietijd, de scores op de TOL, de P300 latentietijd, en het speelgedrag. De negatieve effecten van prenatale blootstelling aan PCB's op de globale cognitieve en motorische ontwikkeling waren groter als ouder- en opvoedingsklimaatcondities minder optimaal waren voor de ontwikkeling van kinderen.

De resultaten van dit onderzoek geven geen aanleiding om het geven van borstvoeding te ontraden, of om te adviseren om de duur van borstvoeding te verkorten. Ook al worden kinderen door borstvoeding aan relatief hoge concentraties van PCB's en dioxinen blootgesteld, een negatief effect van postnatale blootstelling door borstvoeding wordt slechts gesuggereerd bij één van de uitkomstmaten, de scores op de TOL. Bovendien suggereren de resultaten van het neurofysiologisch onderzoek een positief effect van een langere borstvoedingsduur en neemt de contaminatie van borstvoeding met PCB's en dioxinen in Nederland het laatste decennium af.

De onderzoeken in dit proefschrift laten zien dat de evaluatie van potentiële neurotoxische effecten van stoffen als PCB's en dioxinen baat kan hebben bij het gebruik van meer geavanceerde statische technieken waarmee de betrokken variabelen beter gemodelleerd kunnen worden en ook rekening gehouden kan worden met de ontwikkeling van een uitkomstvariabele. Uit de onderzoeken blijkt ook dat de keuze voor ontwikkelingsmaten om neurotoxische effecten van perinatale blootstelling aan PCB's en dioxinen te evalueren van belang is voor de detectie van neurotoxische effecten. De globale cognitieve ontwikkeling, een uitkomstmaat die in de meeste epidemiologische PCB onderzoeken wordt gebruikt, is misschien niet de meest sensitieve uitkomstvariabele mede omdat het een globale

indicatie geeft van de cognitieve ontwikkeling en omdat deze variabele sterk gerelateerd is aan ouder- en opvoedingsklimaatfactoren. De motorische ontwikkeling en een aantal meer specifieke neuropsychologische en neurofysiologische maten, zoals de reactietijd en de variatie in reactietijd, de TOL, en de P300, lijken meer sensitief om neurotoxische effecten van vroege blootstelling aan PCB's en dioxinen te evalueren. Deze meer specifieke uitkomstmaten kunnen mogelijk ook bijdragen aan het beter onderscheiden van effecten van prenatale en postnatale blootstelling aan PCB's en dioxinen.

De resultaten van deze onderzoeken wijzen op het belang van maatregelen om de achtergrondniveaus van PCB's en dioxinen te doen afnemen. Bovendien kunnen zij een illustratie vormen voor het evalueren van mogelijke risico's voor kinderen van blootstelling aan andere bioaccumulerende neurotoxische stoffen die in het milieu aanwezig zijn.

Dankwoord

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gewaardeerd. Ik heb de 'neo-neuro-uurtjes' die door Nynke en jou werden georganiseerd als erg leerzaam ervaren; misschien is er in de toekomst toch een taak voor de neuropsycholoog bij de afdeling weggelegd?

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Conclusie: Promoveren is helemaal niet zo'n kluizenaarsbestaan als het soms lijkt!

Curriculum Vitae

Hestien Vreugdenhil was born on December 10, 1969 in Koudekerk aan den Rijn. After finishing high school in 1988 (VWO-B; Christelijk Lyceum, Alphen aan den Rijn), she studied one year at Calvin College (Grand Rapids, USA). Thereafter, she was dedicated to study Psychology at the Free University in Amsterdam. She specialized in Child Neuropsychology and did a clinical internship at the Academical Hospital Amsterdam (AMC), at the Department of Social-Psychology of the Emma Children's Hospital. She performed a research internship at the National Institutes of Health (Bethesda, USA) under supervision of Prof.dr. E.P. Brouwers (subject: spontaneous eye blinking, a measure of dopaminergic function, in children with AIDS). In 1997, she joined the Rotterdam research team of the Dutch PCB/dioxin study (Erasmus MC-Sophia, Department of Pediatrics), headed by dr. N. Weisglas-Kuperus, which has led to the present thesis (promotor: Prof.dr. H.A. Büller). Presently, she is working as a Neuropsychologist in a psychiatric center for children, the RMPI in Barendrecht. She has been admitted to the transitional arrangement for registered healthcare psychologists (Dutch BIG registration).

Abbreviations

Ah receptor	Aryl hydrocardon receptor
β	Regression coefficient
BF	Breast-fed
CFI	Comparative fit index
CNS	Central nervous system
C_z	ERP recording at central position (midline)
ERP	Event-related potential
FF	Formula-fed
FT	Feeding type (BF or FF)
FT_4	Free thyroxine
F_z	ERP recording at frontal position (midline)
GC-ECD	Gas chromatography with electron capture detection
GC-HRMS	Gas chromatography-high-resolution mass spectrometry
GCI	General cognitive index
HOME	Home observation for measurement of the environment
IUPAC	International union of pure and applied chemistry
K-ABC	Kaufman Assessment Battery for Children
MDI	Mental developmental index (Bayley Scales of Infant Development)
NOS	Neurological optimality score
PBDE	Polybrominated diphenyl ether
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-p-dioxin
PCDF	Polychlorinated dibenzofuran
PDI	Psychomotor developmental index (Bayley Scales of Infant Development)
PSAI	Pre-school activity inventory
P_z	ERP recording at parietal position (midline)
RMSEA	Root mean square error of approximation
RRM	Random regression modeling
RT	Reaction time
SEM	Structural equation modeling
SD	Standard deviation
SRMR	Standardized root mean square residual
SRTT	Simple reaction time test
ΣPCB	Sum of PCBs IUPAC numbers 118, 138, 153, 180
TCDD	2,3,7,8-Tetrachlorodibenzo-p-dioxin
TEF	Toxic equivalent factor
TEQ	Toxic equivalent
TLI	Tucker-Lewis index
TOL	Tower of London
TSH	Thyroid stimulating hormone
TT_3	Total triiodothyronine
TT_4	Total thyroxine
TTEQ	Total TEQ: sum of the dioxin and dioxin-like PCB TEQs
WAIS	Wechsler Adult Intelligence Scale (Dutch version)