Polychlorinated and hydroxypolychlorinated biphenyls

Soechitram, Shalini-Devi

Polychlorinated and Hydroxypolychlorinated Biphenyls

Influence on child neurological and endocrine development

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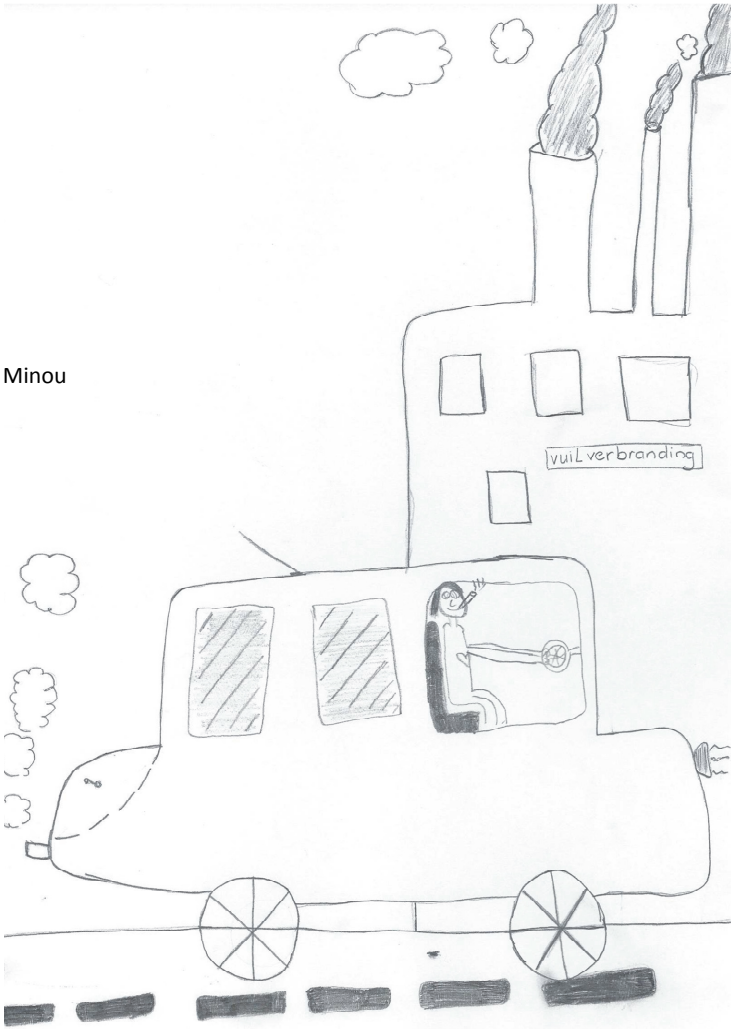
“Together we can help create a peaceful, just, sustainable and healthy world.”
Deepak Chopra

Voor Anousha en Minou



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Abbreviations

Ah receptor	aryl-hydrocarbon receptor
BMI	body mass index
CALUX	chemical activated luciferase gene expression
D1	type I iodothyronine deiodinase
D2	type II iodothyronine deiodinase
D3	type III iodothyronine deiodinase
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltricholoroethane
FMs	fidgety movements
GC/ MS	gas chromatography/ Mass spectrometry
MDI	mental developmental index
mg/l	microgram per liter
MOS	motor optimality score
mU/l	micro units per liter
n	number (frequency)
ng/g	nanogram per gram
OH-PCB	hydroxylated polychlorinated bipheneyls
OHS	organohalogenated substances
OR	odds ratio
PCAH	polychlorinated aromatic hydrocarbons
PCB	polychlorinated biphenyls
PDI	psychomotor developmental index
pg/g	picogram per gram
pmol/l	picomol per litre
POPs	persistent organic pollutants
ΣPCB	sum PCB
rT3	3,3’,5’-triiodothyronine (reverse T3)
sd	standard deviation
T2	3,3’-diiodothyronine
T3	3,3',5-triiodothyronine
T4	thyroxin
T4S	thyroxin sulfontransferase
TBG	thyroxin-binding globulin
TCDD	tetrachlorodibenzo-p-dioxin
TEQ	toxic equivalent quotient
TSH	thyroid-stimulating hormone
TTR	transthyretin receptor

Chapter 1

GENERAL INTRODUCTION

Introduction

The research described in this thesis is part of the European Community funded program on the Risk of Endocrine Contaminants on human health, called the RENCO study. This program started, because it became evident that some of the persistent organic pollutants are active as endocrine disrupters. The well-known polychlorinated biphenyls (PCBs) and dioxins are still the most abundant pollutants in wildlife and humans. Based on studies done with animals, concern has risen about toxic effects of metabolites of PCBs, the hydroxylated polychlorinated biphenyls (OH-PCBs). Studies with pregnant rats, seals and mice showed that these metabolites could accumulate in the fetus. Studies with animals also showed that these compounds might act as endocrine disruptors. Because of the endocrine activity of these OH-PCBs we started our research with pregnant women and their infants.

1. Background

Dioxins and Polychlorinated biphenyls (PCBs) are persistent environmental contaminants. Because of their lipophilic nature and stability they are the most dominating classes of environmental pollutants worldwide. They are resistant to both chemical and biologic degradation and accumulate in the food chain. Human exposure happens largely through the diet, especially through the consumption of animal products and dairy products. In breastfed infants daily exposure to PCBs and dioxins can be 10-20 times higher than in adults[1]. For the nursing infant lactational transfer is the major route. The fetus is exposed by prenatal transfer through the placenta. Developmental effects on neurobehavioral, reproductive and endocrine parameters were observed via experiments with animals and with human infants after in utero and lactational exposure to PCBs and dioxins[2].

Concern about the toxic effects of PCBs raised after two poisoning incidents in Asia. Cooking oil was contaminated with PCBs and related compounds in Japan, the Yusho accident in 1968[3] and in Taiwan, the Yu-Cheng incident, in 1979[4]. Children exposed prenatally to large amounts of these pollutants showed low birth weight, abnormal skin pigmentation, lower IQ and gastrointestinal and immunological symptoms compared to unexposed siblings. Near Seveso (Italy) large amounts of dioxins were spoiled after an explosion in 1976 during the production of chlorophenol. Especially chloracne was reported, but also reversible peripheral neuropathy and an increase of breast cancer[5] and a change in sex ratio at birth (Mocarelli) were reported. These accidents demonstrated that human infants are more sensitive than adults to the toxic effects of these chemicals[6]. Expressed per kg body weight children have a higher exposure than adults do to any toxins present in water, food or air. Especially prenatal exposure, when fetal brain and endocrine/immunological systems are developed, might have life-long effects. Of great concern are the studies suggesting that exposure to background levels of PCBs and dioxins during utero and the lactational period

influence growth and development in children. Different longitudinal prospective studies following infants till childhood were performed and showed observable effects like growth retardation, reduced activity and cognitive impairment in the exposed children. In North Carolina (US) 930 children[7], in Michigan (US) 242[8,9] and in the Netherlands 418 (in 1992) were studied. In the Dutch study in 1992 newborns were followed untill the age of 18 month and this same cohort was examined at a later stage (in 1994) at pre-school age (42 months). The Dutch study showed that preschool children who were breastfed during infancy, have a four times higher PCB body burden than their formula fed counterparts at 34 months of age[1]. The daily PCB/dioxin TEQ intake exceeded the Tolerable Daily Intake of 1-4 pg TEQ/kg body weight at all ages, beginning at the preschool years. In the Dutch cohort PCB and dioxin levels measured in human milk (mean TEQ dioxin 30 pg TEQ/g fat; TEQ-non-ortho-PCBs 16; TEQ-mono-ortho-PCB 14) are higher than in most of the other countries in the world[5]. In this study significant relations were found between perinatal background PCB/Dioxin exposure and adverse effects on growth, immunologic parameters, thyroid hormone metabolism, neurodevelopment and behavior.

In the last few years more research has been done to determine levels of OH-PCBs in humans[10]. OH-PCBs are the major metabolites of PCBs[11]. These metabolites are being formed by oxidative metabolism of PCBs, mediated by the cytochrome P450 enzymatic system, that generally involves an arene oxide intermediate[12]. OH-PCB congeners have been found to accumulate in humans and animals. The most abundant OH-PCB congeners are metabolites of CB105, CB118, CB138, CB153 and CB187[13] all known to be amongst the most persistent and bioaccumulative PCB congeners. Research in newborns and preschool children regarding their exposure to OH-PCBs has rarely been done. This RENCO study was the first to measure OH-PCBs in mothers and corresponding umbilical cord samples.
PCBs and its metabolites, the OH-PCBs are found in animals to function as potential endocrine disrupters and influence neurodevelopmental pathways.

2. Effects on thyroid function

Thyroid hormone is required for normal brain maturation, brain function and development of the central nervous system[14]. PCBs and dioxins alter the thyroid hormone status in animals by decreasing the circulating thyroxin level and thereby increasing thyroid stimulating hormone (TSH)[15,16]. Particularly the OH-metabolites of PCBs have a marked structural resemblance to the T4 binding pocket on TTR (transthyretin). Transthyretin is the major T4 transport protein in the plasma of rodents. Because of the competitive binding of OH-PCBs on TTR, these metabolites can reduce fetal plasma T4 and accordingly fetal brain T4 level[17,18]. In the Netherlands relatively subtle thyroid hormone alterations have been observed in pregnant mothers and their infants, who were exposed to background levels of PCBs and

dioxins[19]. Based on animal studies, the altered thyroid hormone levels found in pregnant women and their infants, might be caused by competitive binding of OH-PCB metabolites to TTR. In humans, however, thyroxin-binding globulin (TBG) transports most of the T4 in blood and TTR approximately 20%. The OH-PCBs can inhibit T4 binding to TTR, but not to TBG and thus may cause different effects in rodents than in humans[20].

Three different mechanisms can be described with regard to how endocrine disrupters like PCBs might decrease thyroid hormone levels, namely a) in animal experiments PCBs have their influence on the thyroid follicular cells and reduce thyroid hormone levels in the neonate[21] b) metabolism of thyroid hormone, where PCBs facilitate biliary excretion of T4 by induction of UDP glucuronyltransferase activity and reduce thyroid hormone and c) interactions of mainly OH-PCBs with the transport binding protein, TTR. Regarding thyroid hormone metabolism, PCBs strongly induce UDP-glucuronyltransferase activities in the rat, thus increasing the hepatic clearance of thyroid hormone[22], while OH-PCBs inhibit D1 activity and iodothyronine sulfotransferase activity[23].

3 . Effects on sexual development

Environmental contaminants, which act as endocrine disruptors, and their effects on sexual development have become a topic of scientific and public discussion. Prenatal exposure of the male fetus to endocrine disruptors may be responsible for a series of outcomes, such as hypospadias, cryptorchidism, falling sperm count and testicular cancer. A meta-analysis of the international literature on semen quality since 1930-1991 showed a significant decrease in mean sperm count from 113 million/ml to 66 million/ml and a decline in seminal volume from 3.40 ml to 2.75 ml[24-26].

The effects of PCBs and dioxin exposure on body weight and penis length, on duration of gestation and lactation and on the male/female ratio in offspring might be due to the hormonal effects of these toxins. However, a direct causal relation between these xenobiotics and human reproductive health and sexual maturation has yet to be established. The effects of endocrine disruptors in longer-lived humans may not be as easily discerned as in shorter-lived laboratory or animal species. PCBs and dioxins have been reported to adversely affect reproduction in animals by reducing the incidence of breeding. Also the survival rate of young animals was reduced[27]. A Flemish study, den Hond et al., compared genital development and pubic hair growth in boys between two area's in Belgium with a different degree of pollution. Indices of sexual development were inversely associated with the serum concentrations of marker PCBs. Testicular volume was significantly lower in polluted areas, but not significantly correlated with exposure to polychlorinated aromatic hydrocarbons (PCAHs)[28]. PCBs may interact with the receptors of sex steroid hormones, by which they may exert estrogenic, androgenic or ant estrogenic effects[2]. Dioxins interact with

the Ah receptor, which may influence the synthesis of hormones or their transport proteins[2].

Sharpe and Skakkebaek hypothesized that PCAHs may interfere with the development of Sertoli and Leydig cells during fetal development[29]. Skakkebaek introduced the term testicular dysgenesis syndrome, consisting of hypospadias, undescended testis, poor semen quality and testicular cancer. He suggested that the testicular dysgenesis syndrome is the result of disruption of gonadal development during fetal life, and may be increasingly common due to adverse environmental effects[30].
The prevalence of cryptorchidism increased in some regions[31,32] over the past decades. A study conducted in Finland and Denmark between 1997 and 2001 showed that congenital cryptorchidism was associated with persistent pesticides in breast milk as a proxy for maternal exposure suggesting that testicular descent in the fetus may be adversely affected[33]. The occurrence of testicular cancer in young men in Europe and United States has increased in the last twenty years[34]. Abnormal intrauterine hormone levels i.e. decreased androgen and/or increased estrogen levels may play an important role in the occurrence of testicular cancer[29].

4. Effects on neurological development

Adverse neurodevelopmental effects of PCBs are well described in the past decade in animal models as well as in human studies[9,35-39].

In the past decades animal studies have shown that PCBs are toxic to the nervous system through influences on brain dopamine concentrations[40], changes in thyroid hormone metabolism[22,37] and estrogen activity[41]. Also studies with children show neurotoxic effects at background levels and accidental exposure to PCBs. There are three major studies in the USA, the Michigan cohort[42], the Oswego cohort[43] and the North Carolina cohort[7]. In Europe follow-up studies with infants and preschool children have been done in the Netherlands (Huisman, Koopman-Esseboom and Patandin), Faroe Island (Steuerwald) and Germany (Wilhelm). More recently Park (Slovakia) evaluated the associations between OH-PCBs and neurodevelopment in children at 16 months of age.
PCBs are related to a higher incidence of hypotonia in neonates[35] and have a negative effect on the neurological optimality score at 18 months[44]. Also adverse effects are reported for psychomotor and mental development (lower scores on the Bayley scales of infant development)[45]. Patandin did not find negative neurological effects at 42 months of age in the same cohort as Koopman-Esseboom and Huisman. The author's explanation for this outcome is the maturation of the brain as an ongoing process from conception to many years after birth. This implicates that the type and severity of effects of PCBs on the brain is related to the developmental stage of the brain[39,45]. Studies in the USA have shown cognitive impairment at 2 years, but not at

3,4,5 years of age, and later on a lower verbal IQ score at 11 years of age[9,46,47]. So the pattern of adverse neurodevelopmental condition can change over the years.
The effects of OH-PCBs on neurological outcome have been studied in the last ten years. OH-PCBs are considered to be endocrine disruptors, affecting thyroid hormone metabolism and sex steroid metabolism. Meerts et al. (2004) showed neurotoxicity of OH-107 in rats, altered latency in movement onset, which is indicative for deficits in learning abilities and memory. Park discovered an association between neurocognitive impairment at the age of 16 months, a decreased Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI) and cord levels of OH-107. Maternal concentrations of the same congener showed only a decrease in MDI and not in PDI[38]. The authors could not explain this difference. They assumed that OH-107 affects neurodevelopment by enhancing estrogen through an indirect pathway, the inhibition of estrogen sulfotransferase[41]. Another OH-study done by Roze et al with schoolchildren showed a decrease in fine manipulative abilities, better attention and visual perception in relation to background PCB and OH-PCB levels[48]. Still there is limited information about the impact of background levels of OH-PCBs on neurodevelopment of neonates and infants.

5. Objectives of this study

PCBs, dioxins and OH-PCBs are present in humans with such concentrations that make them toxicologically relevant. These manmade chemicals look alike, or may interfere with, endogenously produced hormones, neurotransmitters, growth factors and inhibiting substances. They may change the course of prenatal human development. Cognitive and neuromotor changes, reduced semen quality, testicular dysgenesis syndrome, loss of fertility and influences on thyroid hormone axis can all be associated with human prenatal exposure to PCB and its metabolites (OH-PCBs). It is therefore of extreme importance to study the health of the offspring of exposed individuals.
This study is part of a larger program on the assessment of risk factors in humans regarding adverse effects of endocrine active environmental organohalogen contaminants, and is being supported by the Environment and Climate program of the European Union. The general objective of this project is to provide a scientific explanation for potential adverse health effects following human background exposure to endocrine active organohalogenated substances (OHS). The human part of the study is the last study in this program, which is to be finished through this thesis. In the human part we have investigated the neurodevelopmental, endocrinological and reproductive effects of perinatal exposure to PCBs and OH-PCBs at background levels in 2000 in a Dutch cohort of healthy, developing children.

6. Questions raised in this thesis

- What are the levels of PCBs and OH-PCBs in pregnant woman and their infants in a population exposed to background pollution?
- Are the levels different between a densely populated area like The Netherlands and a densely populated city like Hong Kong?
- Are the levels of PCBs decreasing in the Netherlands as result of the ban on the production of these compounds?
- Are the levels of PCBs and OH-PCBs related to testicular volume in newborn infants?
- Are the levels of PCBs and OH-PCBs as found in the Netherlands related to levels of thyroid hormones?
- Are the levels of PCBs and OH-PCBs related to indicators of neuromotor function in newborn infants?

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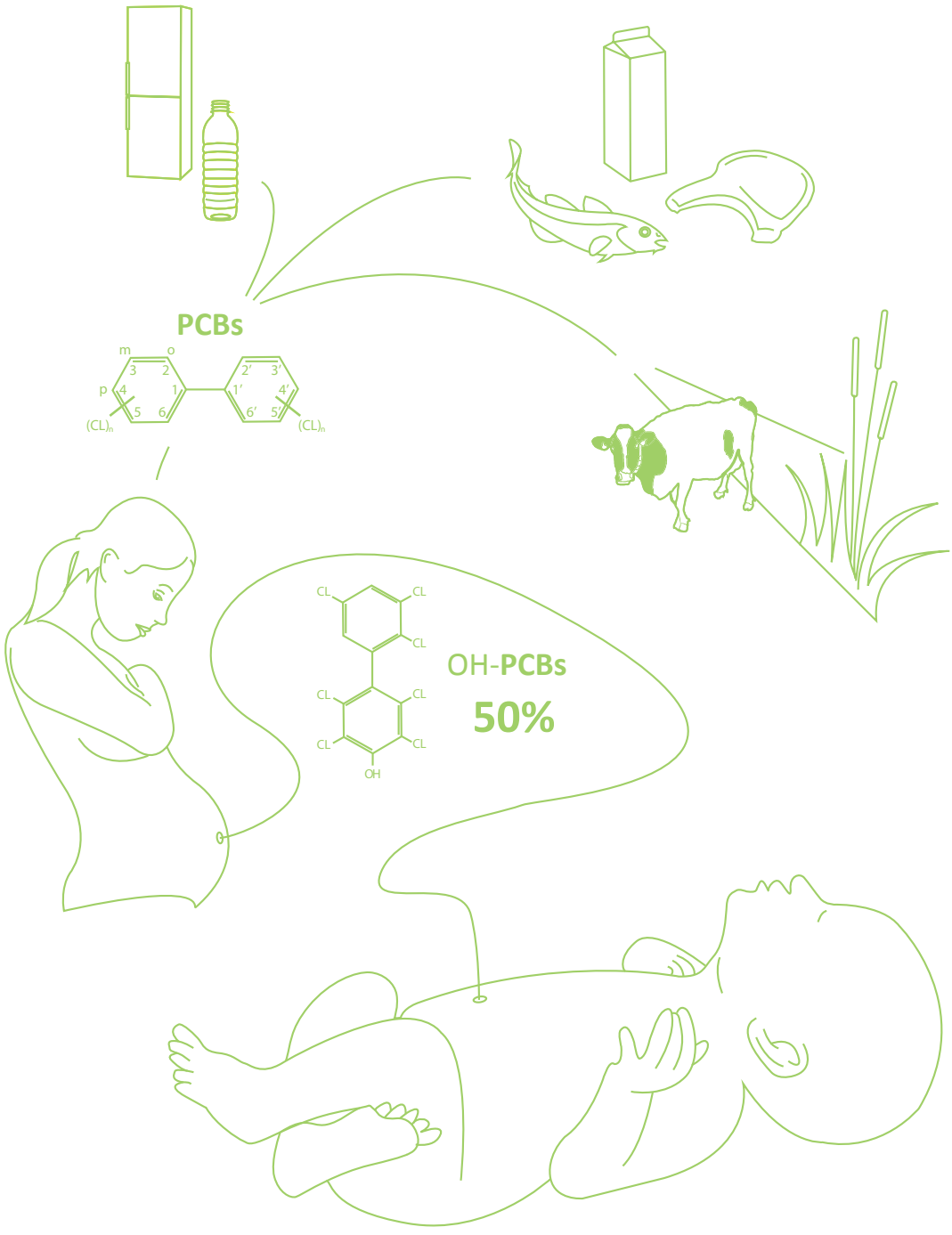
Levels of PCBs and OH-PCBs



Chapter 2

FETAL EXPOSURE TO PCB AND THEIR HYDROXYLATED METABOLITES IN A DUTCH COHORT

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Abstract

Polychlorinated Biphenyls (PCBs) are still the most abundant pollutants in wildlife and humans. Hydroxylated PCB metabolites (OH-PCBs) are known to be formed in humans and wildlife. Studies in animals show that these metabolites cause endocrine-related toxicity. The health effects in humans have not yet been evaluated, especially the effect on the fetus and new-born. The aim of this study is to measure the levels of PCBs and OH-PCBs in maternal and cord blood samples in a population with background levels of PCBs. We analysed 51 maternal and corresponding cord blood samples in the Northern part of the Netherlands. The PCB concentrations in maternal plasma ranged from 2 to 293 ng/g lipid and OH-PCB concentrations from nondetectable (ND) to 0.62 ng/g fresh weight. In cord plasma PCB concentrations are 1-277 ng/g lipid and OH-PCB concentrations, ND to 0.47 ng/g fresh weight. The cord versus maternal blood calculated ratio was 1.28± for PCBs and 2.11±1.33 for OH-PCBs, expressed per gram of lipid. When expressed per gram fresh weight, the ratio is 0.32±0.15 and 0.53±0.23 for PCBs and OH-PCBs, respectively. A significant correlation between the respective maternal and cord levels for both PCBs and OH-PCBs was found. Our results indicate that OH-PCBs and PCBs are transferred across the placenta to the fetus in concentrations resulting in levels of approximately 50 and 30%, respectively, of those in maternal plasma. More research in humans is needed to evaluate potential negative effects of these endocrine disruptors on the fetus.

Introduction

Polychlorinated biphenyls (PCBs) are, together with DDT and DDT related chemicals, the most dominating classes of environmental pollutants worldwide, with concentrations varying in wildlife and in humans from different areas of the globe[1]. Although all PCB congeners, either present in commercial mixtures or as single chemicals, are lipophilic substances with low water solubility, only some of them, even within the same class of chemicals, have a strong tendency to accumulate in higher organisms. This is shown by a few strongly dominating PCB congeners retained in, for example, humans[2,3], grey seals[4] and polar bears[5], all acting at top of the food chain. PCB concentrations are generally in the low micrograms PCB per gram lipid range among humans[3,6] but higher levels are occasionally found in individuals with a heavy consumption of fatty fish from contaminated waters[6] or with a pronounced diet based on subsistence food items[2,7]. The level of PCBs have been shown to slowly decrease[8] as a result of the legislative measures taken to prohibit the production of PCBs in the early 1970's, leading to lower environmental releases.

Studies have shown negative effects of PCBs in animals and humans, especially in newborn infants[9,10]. Reported effects of background exposure in infants include reduced birth weight, less postnatal growth[11,12] neonatal hypotonia[13,14], impaired development and impaired immune response[15,16] and lower thyroid hormone levels[3,17-20]. With few exceptions[21], most negative effects of background levels of PCBs were primarily related to antenatal exposure, whereas postnatal effects from PCBs were related mainly to accidental exposure of infants to rather high levels of PCBs and other organohalogen substances[22]. It is unknown whether these effects are caused by PCBs themselves or by their metabolites.

Hydroxylated PCBs (OH-PCBs), or polychlorobiphenylols, are major metabolites of PCBs[23]. These metabolites are formed by oxidative metabolism of PCBs, mediated by the cytochrome P450 enzymatic system, that generally involves an arene oxide intermediate[24]. OH-PCBs, like most phenolic compounds, are readily conjugated and excreted, but several OH-PCB congeners and some other halogenated phenolic compounds have been found to be retained in human and wildlife blood[2,25-27]. The OH-PCB concentrations so far reported have been 10-20% of the PCB level in humans but were found to be higher in for example, polar bear blood[27]. The three most abundant OH-PCB congeners retained in the blood are metabolites of CB105, CB118, CB138, CB153 and CB187[26], all known to be among the most persistent and bioaccumulative PCB congeners.

The toxicological impact of OH-PCBs is still not known, but several studies indicate that these metabolites may have adverse effects in mammals[28]. In animals, OH-PCBs are transferred over the placenta[29-31]. It is not yet known at what rate OH-PCBs are

transferred to the human fetus[32]. The objective of the present study was to assess PCB and OH-PCB levels in mothers and children at birth and to determine the transplacental transfer of PCBs and OH-PCBs.

Materials and methods

Cohort

From September 1998 through December 2000, pregnant women from the northern part of The Netherlands were invited by their midwife or obstetrician to participate in a study on exposure to PCBs and OH-PCBs and their potential effects on the development of the newborn infant. The mothers had to be of Western European origin, and Dutch had to be their language. To establish an optimal study population, pregnancy and delivery had to involve no serious illness or complications. Only infants born at term (37-42 weeks of gestation) without congenital anomalies or diseases were included. Admission of an infant at a hospital for more than 1 day after birth was an exclusion criterion. The medical ethics committee of the University of Groningen approved the study. A blood sample was taken from the pregnant women in the second and/or third trimester of their pregnancy. Blood samples of the umbilical cord were taken directly after delivery. Blood was collected in a vacuum system EDTA-tube (Ritmeester, Utrecht, The Netherlands) and centrifuged within 24 hours for 5 min at 4,000 rpm. The plasma was transferred to separate glass tubes with a screw caps with Teflon inlayers and stored at –18 to –20°C until analysis. A total of 51 paired maternal and cord blood plasma samples were analysed in the present study. An additional 29 maternal blood samples and 11 cord blood samples were also analysed but these samples were not paired and are not included in this study. The 51 samples were analysed at the analytical laboratory participating in the study.

Chemicals

Hexane and dichlormethane were of pesticide grade (Fisons, Leicestershire, England). Methyl *tert*-butyl ether (MTBE), 2-propanol, and potassium hydroxide (Eka Nobel AB, Bohus, Sweden), as well as potassium chloride (Merck, Darmstadt, Germany) and sulfuric acid (98%; BDH laboratory Supplies, Poole, England) were of analytic quality. Ethanol (99.5%) was purchased from Kemetyl (Haninge, Sweden). Diazomethane was synthesized as described by Furniss et al. (1989)[33]. Silica gel (<0.063 mm; Macherey-Nagel, Düren, Germany) was activated by heating it overnight at 280^{o}C, and allowed to cool to room temperature before use. All glassware was heated at 300^{o}C overnight before use.

Instruments

Gas chromatography (GC) was performed on a Varian 3400 GC equipped with an electron capture detector, a Varian 8200 autosampler, and a split/splitless injector operated in the splitless mode. The fused silica capillary column used was a nonpolar column, CP-SIL 8CB (25 m x 0.15 mm x 0.12 µm), from Chrompack (EA Middelburg, The Netherlands). The column oven temperature was programmed as follows: for analysis of metoxylated derivatives of OH-PCBs: 80°C (2 min), then 50°C/min to 200°C, then 1°C/min to 230C°, then 30°C/min to 330°C (3 min); for analysis of PCBs: 80°C (1 min), then 20°C/min to 300°C (10 min). The injector and detector temperatures were 250°C and 360°C, respectively. Hydrogen was used as carrier gas, and nitrogen was used as make-up gas.
For the evaporation of solvents during cleanup of the samples, we used a centrifugal concentrator (Genevac SF50 Sales development Ltd, Ipswich, England). For the phase separation during the extraction and lipid removal with concentrated sulfuric acid using test tubes, we used a Wifug centrifuge (Wifug Ltd, Parry lane, Bradford, England).

Analysis

The following PCB congeners were used as analytical standards: 2,3,3', 4,4'-pentachlorobiphenyl (CB105), 2,2', 3,4,4', 5'-hexachlorobiphenyl (CB138), 2,2', 3,4', 5,5'-hexachlorobiphenyl (CB146), 2,2', 3,3', 4,4', 5-heptachlorobiphenyl (CB170), 2,2', 3,4,4', 5,5'-heptachlorobiphenyl (CB180) and 2,2', 3,4', 5,5', 6-heptachlorobiphenyl (CB187) and they were purchased from Promochem AB (Ulricehamn, Sweden). 2,2',4,4',5,5'-Hexachlorobiphenyl (CB153), 2,3,3',4,4',5-hexachlorobiphenyl (CB156), 2,2',3,4,4',5',6-heptachlorobiphenyl (CB183) were synthesized in house[34,35]; 2,3',4,4',5-pentachlorobiphenyl (CB118) and 2,3,3',4,4', 5,5'-heptachlorobiphenyl (CB189, internal standard) were synthesized as described elsewhere[35]. A larger number of PCB congeners are given here than are given in "Results". However, for this study, the ones mentioned here were all analysed. The PCB-numbering system as suggested by Ballschmiter et al. (1993)[36] is applied in the present study.

The parent PCB compound and its OH-PCB metabolites are given in Table 2.1. The following methoxylated (MeO-PCB) congeners were used as authentic reference standards to quantify the methyl ether derivatives of the hydroxylated PCBs: 4-methoxy-2,3,3',4',5-pentachlorobiphenyl (4-MeO-CB107), 3-methoxy-2,2',3',4,4', 5-hexachlorobiphenyl (3'-MeO-CB138), 4-methoxy-2,2',3,4',5,5'-hexachlorobiphenyl (4-MeO-CB146), 3-methoxy-2,2',4,4',5,5'-hexachlorobiphenyl (3-MeO-CB153), 4-MeO-2,2',3,3',4',5,5'-heptachlorobiphenyl (4'-OH-CB172), and 4-methoxy-2,2',3,4',5,5', 6-heptachlorobiphenyl (4-MeO-187). 4-Methoxy-2,3,3',4',5,5',6-heptachlorobiphenyl (4-MeO-CB193) and 4-hydroxy-2,3,3',4',5,5',6-heptachlorobiphenyl (4-OH-CB193) were synthesized in house[37] and applied as internal standards. The MeO-PCB and

OH-PCB congeners are numbered according to Letcher et al. (2000). For this study the following OH-PCBs were measured: OH-CB107, OH-CB153, OH-CB146, OH-CB138, OH-CB187, OH-CB172 (all presented " Results").

Table 2.1 PCB congeners and their respective OH-PCB metabolites.

Parent PCB	Metabolite
CB105	4-OH-CB107
CB118	4-OH-CB107
CB138	4-OH-CB146
CB138	3'-OH-CB138
CB153	4'-OH-CB146
CB153	3'-OH-CB153
CB170	4'-OH-CB172
CB180	4'-OH-CB172
CB187	4'-OH-CB187

Extraction and cleanup

The extraction procedure applied in this study is identical to the method described by Hovander et al. (2000)[38]. The cleanup procedure that was required to obtain samples pure enough for analysis was a combination of sulfuric acid treatment and silica gel/sulfuric acid column chromatography separations. Both methods are described by Hovander et al. (2000)[38]. Before extraction, the samples were spiked with CB189 (2 ng/sample) and 4-OH-CB193 (1 ng/sample). Sample volumes smaller than approximately 4 g of plasma was adjusted to 5 g with an aqueous 1% potassium chloride solution before extraction. For each 10 samples, a solvent blank was run.

The procedure applied for cleanup may be summarised as follows: To approximately 5 g plasma spiked with internal standards, 1 ml 6 M hydrochloric acid was added and mixed well. Thereafter 2-propanol (6 ml) was added and mixed well; each sample was extracted and reextracted with 6 and 3 ml of Hexane:MTBE (1:1), respectively. The phases (a water phase and an organic phase) were separated by centrifugation. The organic phase also underwent a washing step with 4 ml aqueous 1% potassium chloride before reduction of the organic solvent. The lipid residue was determined gravimetrically.

The phenolic compounds were isolated after the extraction from the plasma by using potassium hydroxide (0.5 M in 50% ethanol) and were derivatized to their corresponding methyl ethers by addition of ethereal diazomethane (0.5 ml, 3 hr at 4-8°C) before cleanup and analysis[38].

Results

In total, 214 pregnant women expressed interest in participating in this study; 104 of them actually participated in the study. Of these 104, a random sample of 51 mother-infant pairs were included in the study.
Clinical characteristics of the mothers and infant are given in Table 2.2. The mean ± SD maternal age was 31±4 years; mean body mass index (BMI) was 20±3. Infants were born after a gestational age of 40±1 weeks with a birth weight of 3,714±461 g. Fifty five percent of the infants were male.

Table 2.2 Characteristics of the study group (n=51).

Maternal age (years; mean ± SD)	31 ± 4
Weight gain during pregnancy (kg; mean ± SD)	13.3 ± 6
Mother's BMI (mean ± SD)	20 ± 3
Parity, first-born /second- or third-born (%)	33 / 67
Smoking during pregnancy, yes / no (%)	21 / 79
Alcohol during pregnancy, yes / no (%)	23 / 77
Sex of child, male / female (%)	55 / 45
Gestational age (weeks; mean ± SD)	40 ± 1
Apgar score 1 min[median (range)]	9 (4-10)
Birth weight (g; mean ± SD)61	3714 ± 461

The results of all PCB and OH-PCB measurements are presented in Table 2.3 [mean(range)]. The sum PCB (sum of six congeners) in maternal plasma was 268 (113-619) ng/g lipid [mean (range)], compared with 345 (78-809)ng/g lipid in cord blood. The sum OH-PCB (sum of six congeners) in maternal blood was 54 (14-125)ng/g lipid weight compared with 114 (49-244)ng/g lipid in cord blood. Expressed per gram fresh weight- a more suitable way of expressing OH-PCB values because they are more hydrophilic than are PCBs- the levels were 0.340 (non-detectabel to 0.622) ng/g fresh weigth in maternal blood compared with 0.180 (non-detectable to 0.407) ng/g fresh weight in cord blood. The lipid content of maternal plasma was considerably higher than that of the fetus, 0.7 versus 0.2 g/100 mg plasma.
The ratios of cord versus maternal plasma concentrations for PCB and OH-PCB congeners, expressed in nanograms per gram lipid as well as nanograms per gram plasma, are shown in Figure 2.1. The sum PCBs have a ratio ± SD of 1.3 ± 0.56 when expressed per gram of lipid. The sum OH-PCBs have a ratio of 2.2 ± 1.33 when expressed per gram of lipid and a ratio of 0.5 ± 0.23 when expressed per gram of plasma.

Table 2.3 PCB and OH-PCB concentrations in corresponding maternal and cord plasma samples [mean (range); n=51].

PCB	Maternal PCB		Cord PCB		OH-CB	Maternal OH-PCB		Cord OH-PCB	
congener	ng/g l.w.	ng/g f.w.	ng/g l.w.	ng/g f.w.	congener	ng/g l.w.	ng/g f.w.	ng/g l.w.	ng/g f.w.
CB118	28	0.188	56	0.093	4-OH-CB107	10	0,060	14	0.022
	(8-69)	(0.054-0.452)	(9-277)	(0.013-0.470)		0.8-38	n.d.-0.183	4-354	n.d.-0.048
CB146	10	0.069	30	0.050	3-OH-CB153	5	0.035	14	0.021
	(2-29)	(0.015-0.312)	(1-106)	0.002-0.154		1.4-13	n.d.-0.101	3-38	n.d.-0.063
CB153	101	0.700	115	0.193	4-OH-CB146	10	0.063	23	0.036
	(43-293)	(0.248-3.514)	(25-252)	0.037-0.412		3-27	n.d-0.129	8-58	n.d.-0.097
CB138	73	0.496	77	0.130	3′-OH-CB138	7	0.045	18	0.028
	(32-171)	(0.183-2.040)	(20-213)	0.029-0.399		1.3-26	n.d.-0.166	6-50	n.d.-0.079
Cb156	12	0.084	29	0.047	4-OH-CB187	20	0.022	38	0.061
	(4-22)	(0.028-0.215)	(7-96)	0.013-0.135		7-49	n.d.-0.048	17-69	n.d.-0.115
CB180	44	0.300	37	0.063	4′-OH-Cb172	2	0.015	7	0.011
	(15-93)	(0.090-0.961)	(11-88)	0.019-0.144		0.4-6	n.d.-0.034	2-18	n.d.-0.030
ΣPCB	268	1.837	345	0.585	ΣOH-PCB	54	0.340	114	0.180
	(113-619)	(0.645-7.432)	(78-809)	0.134-1.370		14-125	n.d.-0.622	49-244	n.d.-0.407
Lipid (%)	0.7		0.2		lipid %	0,7		0,2	
	(0.4-1.2)		(0.1-0.3)			0.4-1.2		0.1-0.3	

Table 2.4 shows the correlation between the maternal and cord levels for both the PCBs and the OH-PCBs. Except for CB118 and CB156, there was a significant correlation between the maternal and cord plasma levels. This indicates a transfer of these compounds across the placental barrier. Figure 2.2 shows the correlation between PCB and OH-PCB levels between maternal and cord plasma.

Table 2.4 Correlation between maternal and cord plasma levels of PCBs.

PCB congener (g lipid)	Correlation coefficient	OH-PCB congener (g fresh weight)	Correlation coefficient
CB118	0.03	4-OH-CB107	0.15
CB146	0.34	3-OH-CB153	0.54
CB153	0.57	4-OH-CB146	0.54
CB138	0.36	3′-OH-CB138	0.57
CB156	0.15	4-OH-CB187	0.55
CB180	0.79	4′-OH-CB172	0.58
Sum	0.43	Sum	0.52

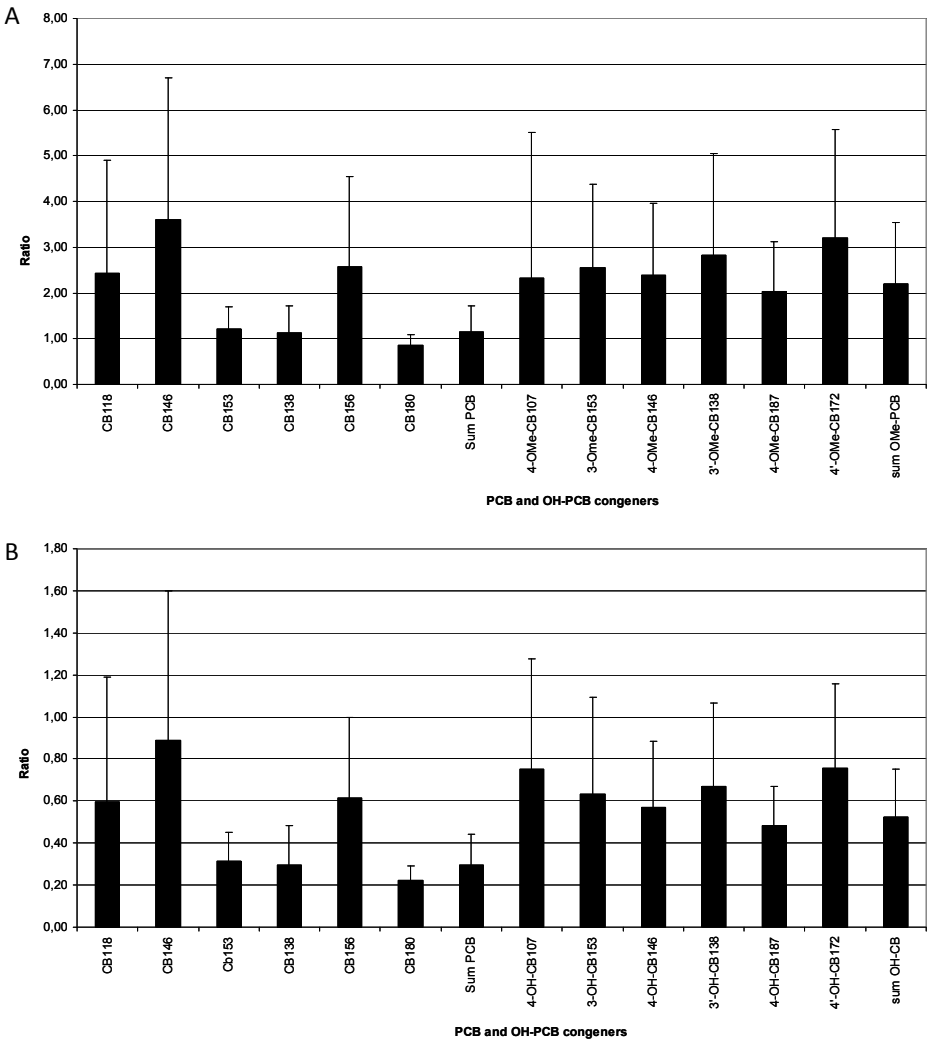

Figure 2.1 Ratio cord versus maternal plasma expressed (A) per lipid weight and (B) per fresh weight (mean ± SD).

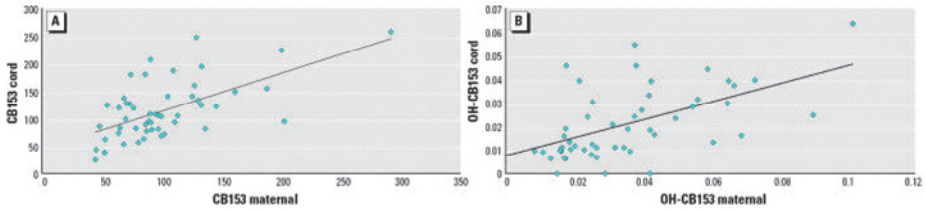

Figure 2.2 Correlation between maternal and cord plasma levels for (***A***) PCB-153 (expressed per lipid weight) and (***B***) OH-PCB-153 (expressed per fresh weight).

The correlations between the parent compound and the resulting OH-PCB for both maternal plasma and cord plasma are given in Table 2.5. There was a significant correlation between the parent compound and resulting OH metabolite for all congeners.

Table 2.5 Correlation between parent compound and hydroxy metabolite in maternal and cord plasma.

Parent PCB congener (g lipid)	OH-PCB metabolite congener (g fresh weight)	Correlation coefficient in maternal sample	Correlation coefficient in cord sample
CB118	4-OH-CB107	0.21	0.46
CB138	4-OH-CB146	0.38	0.58
CB138	3'-OH-CB138	0.26	0.33
CB153	4-OH-CB146	0.39	0.49
CB153	3-OH-CB153	0.27	0.33
CB180	4'-OH-CB172	0.48	0.45

Discussion

In this study, we found that OH-PCBs, metabolites of PCBs, are detectable in plasma of pregnant woman who are exposed to background levels of PCBs in the Netherlands. Both the PCB pollutants and their OH-PCB metabolites are also detectable in cord plasma. Second, plasma levels of OH-PCBs in umbilical cord plasma are 50% of the levels in the mothers, indicating a considerable placental transfer. The placental transfer of OH-PCBs may be explained from their strong binding to transthyretin (TTR) and active transport across the placenta. PCBs, in contrast, are neutral lipophilic compounds strongly distributed to lipids and therefore less readily cross the placenta. The low ratio in PCBs when expressed per gram of plasma may be explained by the fact that a newborn infant consists of 15% fat in contrast to 25% fat in adults. In our study, the lipid content of cord plasma is about 0.2% lipids compared with 0.7% lipids in the mother. The PCB body burden of the infant expressed per gram of body weight therefore is lower than the body burden of the mother, although the PCB levels are equal or slightly higher when expressed per gram of lipid.

Because of the different transfer mechanism of OH-PCBs, the infant will have a body burden, expressed per bodyweight of OH-PCBs, of 50-70% as their mothers, an intriguing observation making OH-PCBs possibly more important from a risk assessment viewpoint than initially thought.

Our finding that there is a correlation between the maternal and cord levels of both PCBs and OH-PCBs further supports the transplacental transfer of both compounds.
PCBs can reach the fetus only by transplacental transfer. OH-PCBs in the fetus can be the result of transplacental transfer as well as hydroxylation by the fetus itself. From our observational study, no firm conclusions can be drawn regarding the source of OH-PCBs in the fetus. Levels of OH-PCBs were, on average, approximately 50% of maternal levels. At the same time, correlation between parent PCB and resulting OH-PCBs was stronger in the fetus than in the pregnant mother. The fetus excretes OH-PCBs to the mother, wehereas the mother excretes OH-PCBs in feces and/or urine. That the correlation between the parent compound and the OH congener is rather weak in the mother can be explained by differences in kinetics between the PCBs and OH-PCBs. PCBs have a half-life longer than that of OH-PCBs.

Levels of PCBs found in this study are almost equal to levels found by us in the Netherlands in a comparable group of healthy pregnant women[3,39]. Plasma levels found in this study versus levels found 10 years ago are, for CB118, 0.19 versus 0.16 ng/g plasma; for CB138, 0.50 versus 0.60 ng/g; for CB153, 0.70 versus 0.91 ng/g; and CB180, 0.30 versus 0.54 ng/g. Although the samples are analysed in different laboratories by different methods, these results might indicate that PCB levels in the Netherlands do seem to have hardly declined in these 10 years. We cannot compare the OH-PCB levels because OH-PCB levels have not been measured before in the Dutch population.

One limitation to this study is the time difference between the blood taken from the pregnant mother and cord blood. We do not believe, however, that this time difference influenced our results. PCB levels in the mother are the result of lifelong exposure and do not change during pregnancy[3]. Most likely, the conversion of PCB and OH-PCBs do not change during pregnancy. Although we did not measure the level of OH-PCBs at more times during pregnancy, we expected the levels of both PCBs and OH-PCBs to be constant during pregnancy and therefore accepted the time difference between maternal samples and cord samples, caused by practical considerations.
The mother-infant pairs included in this study were a random selection from a larger cohort of mother-infant pairs. The total group was included on a voluntary basis in our study. Although we expected to have a random sample of mothers in our region, we cannot exclude some bias in the mothers who volunteered to enter the study. Mothers might have had some concerns regarding these environmental compounds. These could be mothers who select their food carefully, but also mothers without

such opportunities for food selection who are therefore concerned. Altogether we believe, however, that our population is a valid representation of the population in our region.

Sjodin et al. (2000)[6] measured PCB and OH-PCB levels in Swedish and Latvia fisherman consuming either low or high amounts of fish from the Baltic Sea, known to be highly polluted by contaminants. They found CB153 levels in Swedish fisherman ranging from 226 ng/g lipid in low fish consumers to 534 ng/g lipid in high fish consumers. The CB153 levels in the pregnant woman in our study were 101 ng/g lipid (range, 43-293). Sjodin et al. (2000)[6] also measured OH-PCB levels in their subjects. The 4-OH-CB187 levels ranged from 31 ng/g lipid in low fish consumers to 176 ng/g lipid in high fish consumers from Latvia and from 43 to 75 ng/g lipid in their Swedish counterparts. In the pregnant woman in our study we found much lower 4-OH-CB 187 levels of 20 ng/g lipid (range, 6.6-49).

Recently, Sandau et al. (2002)[40] also analysed OH-PCBs in umbilical cord plasma of neonates from coastal populations in Québec. They measured the OH-PCB levels in three different areas. In one area (southern Québec), individuals were exposed to background levels of PCBs and in the other two (Nunavik and Lower North Shore) they were selected because of their high fish consumption. The OH-PCB concentrations range for OH-CB187 was 10-250 pg/g plasma; OH-CB146, 4-507 pg/g plasma; OH-CB153, 3-74 pg/g plasma; OH-CB107, 3-168 pg/g plasma; OH-CB138, 3-92 pg/g plasma; and OH-CB172, 1-75 pg/g plasma[40]. The concentrations measured in Québec are higher than those found in our study. This can be explained by the level of contamination and the mainly fishdiet of Québec. Also remarkable is a different pattern in OH-PCBs between the study in Québec and our study. This might indicate a different source of PCBs.

OH-PCBs are considered endocrine disrupters in animals, with effects on thyroid hormones, estrogens, and testosterone. Animal studies have shown a significant reduction of thyroid hormones in the brain after exposure to OH-PCBs. The reduction in thyroid hormones is related to the binding of OH-PCBs to TTR, which can be explained by the strong structural resemblance between OH-PCB and thyroxine[17,41]. Some OH-PCBs, have >60% higher affinity for TTR than does thyroxine itself[28,41,42]. In humans, thyroid hormones are mainly bound to thyroxine- binding globulin (TBG)[18]. Whether the binding of OH-PCBs to TTR has negative effects in humans is unknown.
Meerts (2001)[43] observerd a significant prolongation of estrous cycles in the offspring of pregnant rats exposed to OH-CB107 but found no effect on their reproductive performance[43]. Also, Meerts (2001)[43] observed an impaired habituation in male rat offspring but not in female offspring.

Whether OH-PCBs do have an effect in the human on either the thyroid or sex hormones is unknown. TBG and not TTR is the main transport protein of thyroxine in humans. TTR, however, might be important for the transfer of thyroid hormones into the brain. Further studies in humans are needed to elucidate if OH-PCBs have a negative effect on the human fetus or older individuals.

Conclusion

Our study indicates that OH-PCBs can be found in the plasma of healthy pregnant women in the Netherlands. The level of OH-PCBS in cord plasma is approximately 50% of levels found in the mother. So both PCBs and OH-PCBs cross the placenta. In both cord and maternal plasma, OH-PCBs are correlated with the respective parent PCBs. The only way to reduce the exposure of the fetus to these potentially toxic compounds is to reduce the body burden of PCBs in pregnant women.

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Chapter 3

COMPARISON OF DIOXIN AND PCB CONCENTRATIONS IN HUMAN BREAST MILK SAMPLES FROM HONG KONG AND THE NETHERLANDS

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Abstract

Objectives

The adverse effects of dioxins and polychlorinated biphenyls(PCBs) on human health are of increasing concern. These lipophilic compounds are concentrated through the food chain and are present in human milk. This study compares PCB levels in human milk samples from Hong Kong and Dutch mothers.

Methods

Ten breast milk samples from Hong Kong and ten from the Netherlands were collected during home visits between 2 and 6 weeks postpartum. Total toxic equivalence (TEQ) of dioxin and PCBs were determined using the DR-CALUX bioassay.

Results

The total dioxin and PCB levels in breast milk samples for Hong Kong ranged from 3.1–29.9 pg TEQ/g lipid and for the Netherlands from 8.9 to 89.5 pg TEQ/g lipid.

Conclusions

Despite Hong Kong's high degree of industrialization, the levels of dioxin and PCBs were fourfold lower in the Hong Kong than in the Dutch samples. This may be due to a lower dietary intake of dioxins and PCBs in Hong Kong because of lower background levels of these contaminants or to different food habits.

Introduction

In recent years, there has been increasing concern about the adverse effect of polychlorinated biphenyls (PCBs) and dioxins on human health. These toxicants are very stable, persistent substances used for many industrial purposes. A disadvantage of this stability, however, is that they are very resistant to degradation and therefore persist for long periods in the environment. As many of these substances are lipophilic, they concentrate in the food chain and are present in high concentrations in certain food products. Food intake probably accounts for more than 90% of human exposure to these toxins in most countries. Other potential pathways of exposure include inhalation, ingestion of contaminated water and soil, and skin contact with contaminated soil[1]. The environmental pollution has been related to extrusion from industry and waste incinerators.

PCBs and dioxin levels in the Netherlands and some other European countries are among the highest world-wide. These countries have had high levels of industrialization for many years. Hong Kong has experienced rapid economic development and urbanization during the past three or four decades and therefore environmental pollution has become an area of public health concern in Hong Kong as elsewhere[2-4]. Aquatic food products from more industrialized countries including Hong Kong, have been found to contain significant levels of PCBs[2]and heavy metal contamination[5]. A small survey of organochlorine contaminants in human breast milk in Hong Kong was reported in 1989 and compared with data from 1976[6]. It was noted that the concentrations of DDT, DDE and beta-hexachlorocyclohexane in Hong Kong breast milk samples were amongst the highest reported in the literature. However concentrations of pesticides (DDT,dieldrin and hexachlorobenzene) significantly lower than those samples taken a decade earlier although levels of gamma-hexachlorocyclohexane were higher.

A recent proposal to build a waste incinerator in Hong Kong has raised public awareness of dioxins and PCBs in the environment and in breast milk. Such concerns reported in the media may have a negative impact on the promotion of breastfeeding. Since nothing is known about PCB/dioxin levels in breast milk in Hong Kong, this study compared dioxin and PCB levels in human milk samples from Hong Kong Chinese mothers with levels from Dutch mothers using DR-CALUX® bioassay.

Methods

Subjects

Hong Kong

Pregnant women were recruited in the postnatal wards of the Obstetrics and Gynaecology Department in the Prince of Wales Hospital, Hong Kong. They were healthy Hong Kong Chinese women who delivered a full term healthy singleton infant (gestation age $\geq$37 weeks) and who were still breastfeeding fully or partially between 2 and 6 weeks postpartum. Information collected included the baby's sex, date of birth, birth weight and gestational age and mother's date and place of birth, length of residence in Hong Kong and details of other places of residence. Information about the mother's diet before pregnancy was collected by means of food frequency questionnaires and data on current diet was collected by a 24-h recall.

The Netherlands

In the northern part of the Netherlands a project investigated the effect of endocrine disrupters like PCBs and dioxins on the neuro-endocrine development of newborn infants. From this study, pregnant women were asked to collect breast milk in the sixth week postpartum. They were healthy Caucasian Dutch-speaking women who delivered a healthy infant at term. Obstetrical and socio-economic data were collected by means of a standardised questionnaire (the optimality concept according to Touwen and Prechtl). Dietary information was not collected in this group of mothers. To compare Hong Kong dietary habits and Dutch dietary habits information collected in previous studies was used.

To both Hong Kong Chinese women and Dutch women the purpose and nature of the study was explained and informed written consent was obtained. The Ethics Committee of the Faculty of Medicine from the Chinese University of Hong Kong and the University Hospital Groningen approved the study protocols.

Breast milk sample analysis

Hong Kong

Approximately 10 to 15 ml breast milk were collected for each subject during a home visit. Breast milk samples were stored at -80^{o}C in glass bottles with screw tops including a Teflon ring until analysis.

The Netherlands

*E*ach mother collected 40-60 ml breast milk over 24h, according to a protocol they received from the research staff. The samples were stored at -20^{o}C in glass bottles.

Both Hong Kong and Dutch samples were transported on dry ice to the Institute for Environmental Studies in Amsterdam (The Netherlands) for the determination of total dioxin and PCBs. The total toxic equivalence (TEQ) of dioxin and PCBs were determined using the DR-CALUX® bioassay (BioDetection Systems bv, Amsterdam, The Netherlands) essentially as described by Murk et al. (1997)[7]. This bioassay, which was developed in 1993, specifically detects the total dioxin-like response in genetically engineered cells, containing a reporter gene (luciferase) under transcriptional control of the aryl hydrocarbon (Ah) receptor, e.g. the dioxin receptor. Overall, excellent correlations between DR-CALUX® -TEQs and high-resolution gas chromatography-high-resolution mass spectrometry (HRGC-HRNS) TEQs for mixtures of PCBs and PCBs with dioxins/furans have been demonstrated. Although PCB 188 is lower, TEF values of PCBs of 126, 77, 169 showed good correlation between the WHO list and DR-CALUX®. Aliquots of breast milk were mixed with n-hexane and milk lipids were extracted. Thereafter the lipid extract was cleaned up via a sulphuric acid-silica column. The fraction containing dioxins and dioxin-like PCBs was collected and mixed with culture media of the DR-CALUX® cells. After 24 h of exposure, the CALUX® cells were lysed and the luciferase activity measured after substrate addition in a 96-well luminometer plate reader. TEQ was calculated from a standard curve of the most toxic congener 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) and expressed as pg TEQ/g lipid. The extraction and clean-up method for DR-CALUX® assay applies to both blood and breast milk samples, and in the extraction no separation between dioxins/furans and PCBs was made.

Statistical analysis

Data were analysed with SPSS Version 8.0 for Windows (SPSS Inc, Chicago, IL, USA). Descriptive data and dietary data are presented as the mean and range. The independent sample *t*-test was used to analyse the difference between the TEQ levels from Hong Kong and the Netherlands.

Results

Ten mothers in Hong Kong and 10 in the Netherlands were included. There were no differences in the general characteristics of the Hong Kong and Dutch mothers and their infants with the exception of birth weight, which was higher in the Netherlands (Table 3.1). The TEQ levels in the Hong Kong group ranged from 3.1 to 27.9 pg TEQ/g lipid, with a mean value of 12.5 pg TEQ/g lipid. In the Netherlands, the levels range

from 8.9 to 89.5 pg TEQ/g lipid, mean 48.7 pg TEQ/g lipid (Table 3.2). The levels in Hong Kong were four times lower than the levels in the Netherlands (*P*=0.004).

Table 3.1 General characteristics of the study population.

	Hong Kong (n=10)	The Netherlands (n=10)
Mothers age (years)	31 (23-26)	32 (26-39)
Parity (gravida/para)	-	g2p1 (g1p0-g3p2)
Gestational age (weeks)	39 (36-41)	40 (39-41)
Birthweight child (grams)	3270 (2700-3800)	3677 (2840-4330)
Male/female	3/7	7/3

Data are means (range)

Table 3.2 Total dioxin and PCB levels in breast milk (pg TEQ/g lipid).

	Collection date (weeks)	PCB and dioxin level
Hong Kong (n=10)	4 (4-6)	12.5 (3.1-27.9)
The Netherlands (n=10)	6 (5-8)	48.7 (8.9-89.5)

Data are means (range)

Discussion

PCB/dioxin levels in breast milk of Hong Kong mothers were 4 fold lower than levels in the Netherlands, despite Hong Kong's high level of urbanisation and industrialisation and the high rate of fish consumption by the study mothers and the general Hong Kong population. The levels (mean 48.7 pg TEQ/g lipid) found in the present Dutch samples were almost the same as the levels (mean 46.9 pg TEQ/g lipid)[8,9] found in a previous Dutch PCB/dioxin study from 10 years ago. Although different analytic techniques were used in these two studies, it appears unlikely that there has been a major change in these toxic congeners in Dutch breast milk samples over these 10 years. Levels of dioxin and Furans (expressed as pg TEQ/g fat) reported elsewhere have also shown levels that were generally lower in Asian and South American countries (2.1 for Hanoi, Vietnam, in 1988; 2.6 for China in 1990; 8.1 for Rio de Janeiro, Brazil, in 1992) than in European countries (31 for Germany in 1990; 16 for Germany in 1995; and 20.1 for Paris, France, in 1990)[10].

PCB and dioxin levels in human milk are dependent on the body burden. In turn, the body burden of these substances is related to the level of pollution in the environment, although this is not a direct relationship. These pollutants are concentrated in food products and the extent to which the population is exposed to the pollution of the environment depends on the extent to which the pollution enters

the food chain consumed by the population. There are a number of explanations for the difference in the PCB/dioxin level between Hong Kong and the Netherlands. There could be a lower level of background pollution in Hong Kong, which is reflected in low levels in the local food chain. The industrialization and use of waste incinerators in the Netherlands started many years ago, whereas the industrialization in Hong Kong started three to four decades ago. Although it might be speculated that environmental pollution might be less severe in Hong Kong, it is equally plausible that high levels of environmental pollution are not reflected in the food consumed as in Hong Kong most food is imported. In 2001, the leading sources by value of food imports were: the Chinese mainland (25%), USA (16%) and Thailand (7%), and for milk, butter, cheese and eggs, the leading sources were: Ireland (21%), New Zeeland (18%) and the Chinese mainland (16%). Corresponding leading rankings for fish and fish products were: the Chinese mainland (13%), Australia (13%) and Japan (10%) (data from the Hong Kong Census and Statistics Department). However, note that the source of current food imports may not reflect past imports when PCBs/dioxin were being accumulated in the Hong Kong subjects.

The body burden of PCb/dioxin is also dependent on the age of the mother as an indicator of duration of food intake, food habits and the amount of breast milk given[11,12]. The ages of the mothers were not different between the Hong Kong and Dutch population (Table 3.1) and therefore cannot explain the higher levels in the Netherlands. Details of the parity of the Hong Kong mothers were not collected but they are not thought to differ significantly from the Dutch mothers.

Differences in dietary habits and levels of contamination of the diet might also explain differences in body burden and hence differences in breast milk levels. Before pregnancy, the Hong Kong mothers consumed fish more than three times a week and dairy products less than once a week. Based on 24-h recall postpartum, the Hong Kong mothers consumed 101 gm/d (SD 119) of fish and 141 gm/d (SD 162) of dairy products (Table 3.3). The diet of the Hong Kong pregnant women in this study (Table 3.3) is comparable with the diet of the general population of Hong Kong[13,14]. Food habits in the Netherlands are rather different from food habits in the Hong Kong general population (Table 3.4)[14-16]. These differences in diet, especially of fish and dairy product intake, could explain differences in body burden and thus differences in toxic congeners in breast milk. Although Hong Kong's fish intake is higher than in European countries, the types and origins of fish consumed may be important. Fish from the ocean are less contaminated than those caught in rivers and coastal seas. In Hong Kong the ratio of marine fish and fresh water fish consumed is 3:1.Thus, despite the high rate of fish consumption in Hong Kong, the proportion of contaminated fish intake may be relatively less.

Table 3.3 Frequency of different food consumption before pregnancy (food frequency questionnaire) and daily food intake after delivery (24-h recall) in the Hong Kong study population (n=10).

	Mean consumption	Gram/day Mean (SD)
Fish	More than 3 times a week	101 (119)
Chicken	1-3 times a week	67 (117)
Meat		291 (256)
Beef	Less than 1 time a week	
Pork	More than 3 times a week	
Offal	Less than 1 time a week	
Dairy products	Less than 1 time a week	141 (162)
Eggs		23 (33)

Table 3.4 Comparison of average amounts of food consumption in Hong Kong general population and other countries[15,16,17].

Country	Daily intake of different food items (gram/day)			
	Meat	Fish	Eggs	Milk and cheese
Hong Kong	151	93	19	42
Finland	105-107	7-58	11-35	1108-1211
Italy	85-226	22-35	25-54	17-337
Yugoslavia	70-212	0-96	19-51	200-437
The Netherlands	138	12	27	478
USA	273	3	40	249
Greece	35	18-60	5-25	84-248
Spain	181	72	32	383
Japan	8	93-207	19-39	23-28

Finally, and possibly most important, the lower consumption of dairy products in Hong Kong in contrast to the Dutch population might explain the lower levels of the total PCB/dioxin levels in the breast milk samples. Culturally, dairy products have been a very minor part of the Hong Kong Chinese diet. Only in recent years has the consumption of milk been actively promoted for children and mothers during pregnancy and lactation for its perceived health benefits. In the Netherlands, it has been estimated that 43-50% of PCB/dixion intake is through the consumption of dairy products[9].

In conclusion, the total PCB/dioxin levels in breast milk are lower in Hong Kong compared with the Netherlands. These lower levels may be due to lower levels of background pollution and therefore a lower level of these contaminants in food or to a different pattern of diet resulting in a different body burden of the mothers. The lower consumption of dairy products in Hong Kong may be of particular importance as these foods may account for up to 50% of PCB/dioxin intake. Our results should be reassuring to breastfeeding mothers in Hong Kong, although in the longer term it will be important to continue to address this issue. In areas with both high and low levels of PCBs and dioxins, public health focus should be primarily directed to the improvement of environmental protection.

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Chapter 4

SERUM LEVELS OF POLYCHLORINATED BIPHENYLS AND HYDROXYLATED POLYCHLORINATED BIPHENYLS IN PREGNANT WOMEN OVER A TEN-YEAR PERIOD IN ONE GEOGRAPHICAL AREA IN THE NETHERLANDS

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Abstract

Polychlorinated biphenyls (PCBs) and their metabolites hydroxylated PCBs (OH-PCBs) are still present in the environment and the human body despite a production ban thirty years ago. PCBs and OH-PCBs negatively affect human health, especially that of the developing fetus. We present the trend in four PCB and three OH-PCB levels in pregnant women from three cohorts, founded in 1991-1992, 1998-2000 and 2001-2002. All women were of comparable age and recruited in the same geographical area in The Netherlands. Levels of three out of four analysed PCBs and two out of three analysed OH-PCBs decreased over the years. Over the ten-year period the main congener, 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153), showed a decrease of 50% in serum of pregnant women, from 830 pg/g serum to 444 pg/g serum.

Introduction

Polychlorinated biphenyls (PCBs) were widely used in several industrial products as coolants and lubricants, until they were banned thirty years ago due to adverse effects on animal and human health, especially on the developing foetus[1]. PCBs are lipophilic and accumulate in the environment, animals and humans. Human exposure is mainly through dietary intake of animal fat[2]. Due to slow degradation in the environment, exposure to PCBs still exists and negative influence on human health may be ongoing.
In the human body PCBs are slowly metabolised and mainly stored in adipose tissue[3]. After metabolization hydroxylated PCBs (OH-PCBs) are formed with less lipophilic properties and therefore a higher excretion rate. Transplacental transfer of PCBs and OH-PCBs occurs during pregnancy leading to prenatal exposure of the foetus[4,5].
To investigate the trend of PCB and OH-PCB levels in serum of pregnant women over a ten-year period, the results of three cohorts founded in the northern provinces of The Netherlands are compared.

Material and methods

The first cohort was founded between 1991 and 1992, the second between 1998 and 2000 and the third cohort between 2001 and 2002. All three cohorts had the same inclusion and exclusion criteria: healthy women delivering a single, term, healthy child. Written informed consent was obtained after fully explaining the study protocol. All protocols were approved by the Medical Ethical Committee of the University Medical Centre Groningen.
At 35th week of pregnancy 30 ml blood was taken from all participating women. After centrifugation at 3600 rpm for 10 minutes the serum was separated and stored into acetone prewashed glass tubes at -20º Celsius until analysis. The method of analysis was comparable[6,7]. In the first cohort PCB-118 (2,3',4,4',5-pentaCB), PCB-138 (2,2',3,4,4',5'-hexaCB), PCB-153 (2,2',4,4',5,5'-hexaCB), and PCB-180 (2,2',3,4,4',5,5'-heptaCB) were analysed in 209 samples. In the second cohort these PCBs were analysed in 97 samples together with 4OH-CB-107 (2,3,3',4',5-pentaCB), 4OH-CB-146 (2,2',3,4',5,5'-hexaCB) and 4OH-CB-187 (2,2',3,4',5,5',6-heptaCB) in 96 samples. Analyses were performed at The Department of Environmental Chemistry, University of Stockholm, Sweden. In the third cohort the same hydroxylated metabolites were analysed together with CB-153 in 90 samples. Analyses were performed at the Institute for Environmental Studies, Vrije Universiteit, Amsterdam, The Netherlands. All values are presented as medians with range due to skewed distribution, in pg/g serum. Statistical significance was considered with a *P*-value <0.05. Missing data were replaced by mean value. All analyses were performed in SPSS 11.0 for Windows (Chicago, IL, U.S.A.). The Wilcoxon signed rank test was used to calculate statistical

significant difference between two cohorts. The Friedman test was used to calculate statistical significant difference in CB-153 level between the three cohorts.

Results and discussion

In Table 4.1 the clinical details of the cohorts are presented. No significant difference in maternal age, height and weight between the three cohorts was observed.
In Table 4.2 an overview of the levels of PCBs and OH-PCBs in serum of the participating women taken at 35th week of pregnancy is presented.

Table 4.1 Clinical details of the three cohorts. Data are median and range.

	1991-1992 Median (range)	1998-2000 Median (range)	2001-2002 Median (range)
Age (yrs)	29 (19-38)	32 (21-42)	32 (24-42)
Height (cm)	170 (150-193)	172 (155-186)	171 (154-183)
Weight (kg)	64 (48-109)	69 (48-105)	68 (49-109)

Table 4.2 Comparison of PCBs and OH-PCBs levels between the three cohorts in maternal serum on fresh weight basis (pg/g serum). Data are median and range.

	1991-1992 Median (Range)	1998-2000 Median (Range)	2001-2002 Median (Range)
CB-118	140 (40-580)	141 (20-1012)	
CB-138	560 (130-1600)	439* (30-1340)	
CB-153	830 (180-2300)	588* (4-1773)	444* (113-1468)
CB-180	500 (80-1300)	308* (50-1010)	
4OH-CB-107		71 (14-333)	26* (nd-120)
4OH-CB-146		72 (32-265)	103* (36-696)
4OH-CB-187		142 (51-339)	80* (36-478)

1992: n=209, 1998: n=97 for the PCBs and n=96 for the hydroxylated-PCBs, 2002: n=90. * P<0.01.

CB-153 was the main PCB-congener in the first and second cohort, followed by CB-138, 180 and 118. 4OH-CB-187 was the main OH-PCB-congener in the second and 4OH-CB-146 was the main OH-PCB-congener in the third cohort.
PCB and OH-PCB level decreased over time between the first, second and third cohort, except for CB-118 level, which remained the same between the first and second cohort, and 4OH-CB-146 level, which increased between the second and third cohort. Decrease in PCB level between first and second cohort was highest for CB-180 (39%, highest chlorinated PCB analysed). CB-153 level decreased with 29% between the first and second cohort and 47% between the first and third cohort. Decrease in OH-PCB between the second and third cohort was highest for 4OH-CB-107 (63%, lowest chlorinated OH-PCB analysed).
The decrease in level of most PCBs found in this study is in accordance with other studies. According to a study conducted in the Baltic area the mean estimated half-life

of CB-118, CB-138, CB-153 and CB-180 taken together was 0.7 years in air, 15.4 years in water, 20.5 years in soil and 20.6 years in sediments. Half-life of higher chlorinated biphenyls was longer than half-life of lower chlorinated congeners[8].

Limited data is present regarding trend over time of PCBs in human blood. To our knowledge no data are available on the trend over time of OH-PCBs in human blood. According to a study by Masuda in Yucheng and Yusho patients the half-life of PCB congeners in human blood varied between 1.6 and 17.6 years, measured over a thirty-years period[9]. PCB level was higher in Yucheng patients compared to Yusho patients, leading to the conclusion that PCB congener half-life was longer when initial PCB level was lower. This observation was also made by Shirai[10], in his paper describing the uncertainties of estimated half-lives of PCBs in humans. In a study conducted in human serum samples from a blood bank in Memphis, CB-153 level decreased with 50% over a ten years period[11]. Median CB-153 level in those subjects decreased from 66 ng/g lipid in 1990-1994 to 35 ng/g lipid in 2000-2002.

A variety of reasons could explain the absence of change in CB-118 level and increase in 4OH-CB-146 level over time, observed in our study. Lack of change in CB-118 level over time could be due to a constant amount of exposure, lack of excretion from the human body due to its stability, or chance finding. A constant level of exposure can be explained by a high stability of CB-118 in the environment or dietary products. Another possibility is the conversion of higher chlorinated PCBs to lower chlorinated PCBs in environment or dietary products. The increase in level of 4OH-CB-146 may be a chance finding, or could be explained by higher exposure to the metabolite over time. OH-PCBs are also present in animals and therefore exposure to these compounds through dietary intake is also possible[12]. Further studies are needed to objectify the results obtained for CB-118 and 4OH-CB-146.

In this study we present a decrease in serum levels of PCBs and OH-PCBs over a ten-year period in pregnant women from three cohorts living in the same geographical area, due to the ban in the production and usage. This decrease will diminish their influence on human health which is especially important for the developing fetus.

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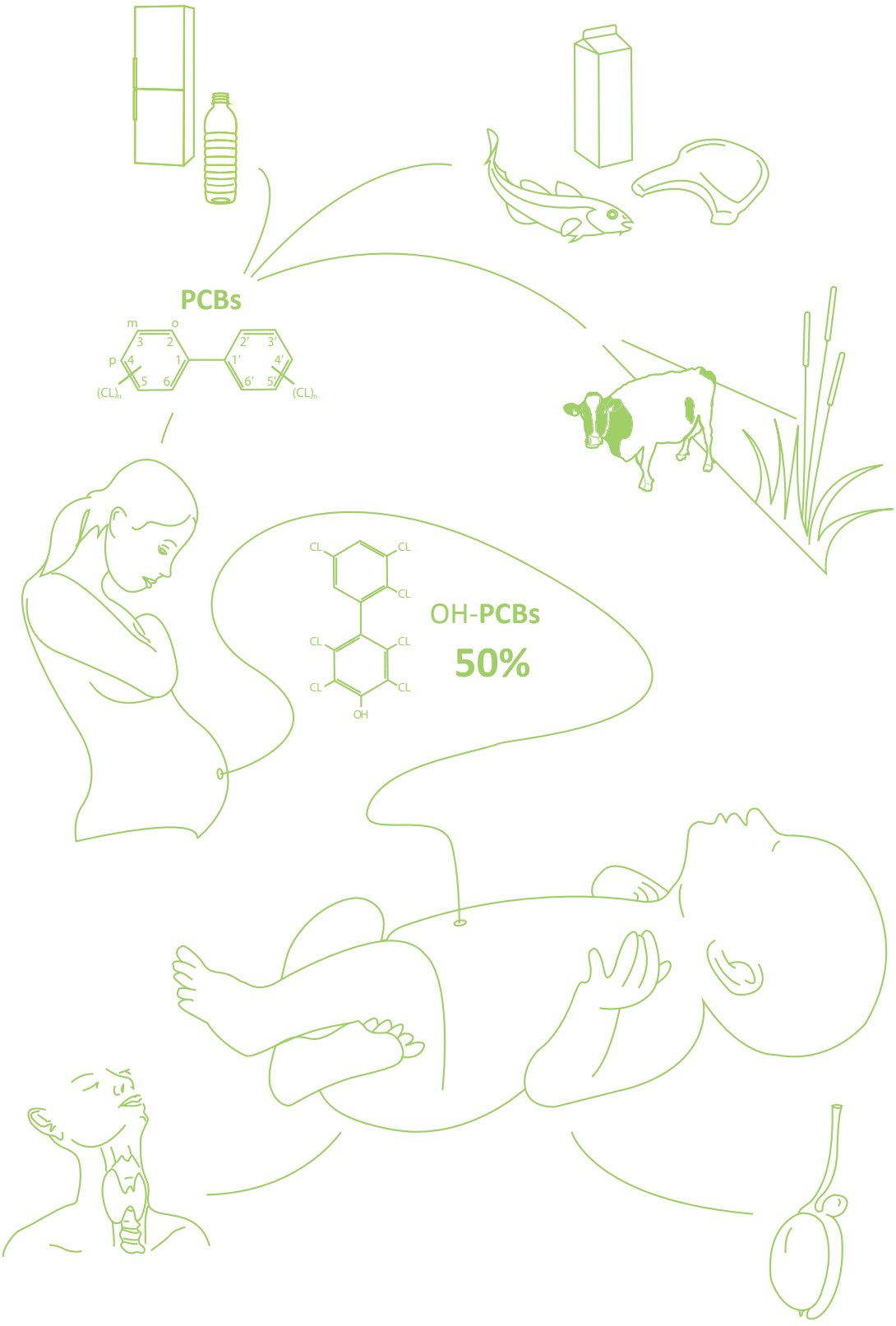
Endocrine disruptors



Chapter 5

TESTICULAR VOLUME OF NEWBORN INFANTS IN THE NETHERLANDS IN RELATION TO POLYCHLORINATED AROMATIC HYDROCARBONS (PCAH'S)

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Submitted

Abstract

Polychlorinated aromatic hydrocarbons are known to have a negative effect on human health, especially during fetal development. Some studies indicate a negative effect of these compounds on male sexual development.

The objective of this study is to evaluate if PCBs and their degradation products, OH-PCBs at background levels influence testicular volume at 3 and 18 months. Fifty-five boys, as part of a larger study on effects of PCBs and OH-PCBs on fetal development, were studied. No relation between PCBs and OH-PCBs with testicular volume has been found except for OH-PCB-153.

We therefore conclude that the current background levels of PCBs and OH-PCBs in general do not have an important influence on testicular growth.

Introduction

Environmental pollutants are man-made chemicals, which are present in the environment and might have a negative effect on human health. Two of the most well known environmental pollutants are dioxins and PCBs. Although their production has been banned since several decades, they are still present in the environment and can be found in almost all human tissues. Their persistence is related to the fat-solubility and low rate of degradation in the environment[1]. Studies have shown negative effects of dioxins and PCBs on human health. Antenatal exposure is related to a lower birth weight[2,3], effects on thyroid hormones[4-6] and negative effects on neurodevelopment[7,8] and the immune system[9,10]. A delayed sexual maturation in boys and girls[11] and a lower sperm production[12] can be related to exposure at a later age. Also a change in sex ratio in offspring of fathers exposed to PCBs has been seen[13].

PCBs are converted to OH-PCB in the body before excretion and OH-PCBs are more water-soluble than PCBs[14]. Therefore they might pass barriers like the blood brain barrier. A study in rats showed that OH-PCBs accumulate in the fetal brain[15]. OH-PCBs therefore might have a more negative effect on human health than the parent PCBs itself.

An important part of the development of sexual organs takes place during fetal development. Exposure to environmental pollutants during this period might have an effect on these organs. Recently we described a correlation between two OH-PCBs (4-OH-CB-107 and 4-OH-CB-187) and two brominated compounds (BDE-154 and HBCDD) with sex hormones in boys at 3 months of age[16]. No correlation between the compounds and testis volume has been found. In that study only PCB-153 and 3 OH-PCBs were investigated. All polychlorinated aromatic hydrocarbons (PCAHs) are considered to have their effect via activation of the Aryl-hydrocarbon (Ah) receptor. Using the CALUX assay it is possible to estimate the effect of all PCAHs together on the Ah receptor. The CALUX assay might be an indicator of anti-estrogenic activity[17]. Studies investigating other PCBs than PCB-153, as well as several other OH-PCBs, and the CALUX assay on testis growth are missing.

In this study, as part of a larger study on the effects of prenatal exposure to PCBs and OH-PCBs on growth and development, we evaluated the effects of these compounds on testicular volume at 3 and 18 months of age.

Methods

Cohort

The study design and details on the measurement of the different congeners have been published before[12]. In short, from September 1998 to December 2000, pregnant women from the northern part of The Netherlands were invited by their midwife or obstetrician to participate in a study on the exposure to PCBs and OH-PCBs and their potential effect on the development of the newborn infant. Mothers had to be of Western European origin, and Dutch had to be their native language. The pregnancy and delivery had to be without complications. Only infants born at term age (37-42 weeks of gestation) were included. The admission of an infant at the hospital for more than one day after birth was an exclusion criterion. The Medical Ethical Committee of the Groningen University has approved the study.

Study design

After inclusion into the study, a blood sample was taken from the pregnant women in the third trimester of the pregnancy for the measurement of PCBs and OH-PCB levels and the CALUX assay.

Testicular volumes

The testicular volume (Figure5.1) was measured by ultrasound (7.0 MHz linear transducer) using an Antares Ultrasound system (Siemens, Erlangen, Germany) at 3 and 18 months of age, calculated from the date of birth. 2 pediatric radiologists trained for this examination have performed the measurements. Testicular volume was calculated using the formula for an ellipsoid (1/6 x π x L x B x H) in mm^3 and the mean was calculated from three measurements each for the left and right testis[16].

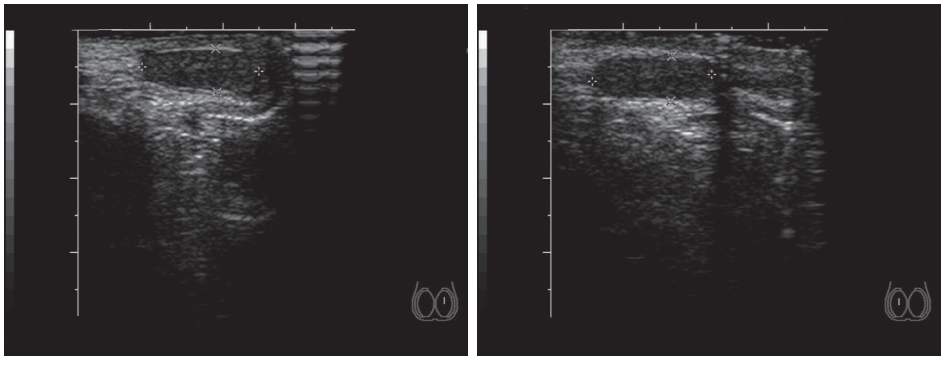

Figure 5.1 Example of testicular volume measured by ultrasound at 18 months of age.

Measurement of compounds

Compounds were measured using a gas chromatograph (Varian 3400 GC) equipped with an electron capture detector, a Varian 822\00 autosampler and a split/splitless injector operated in a splitless mode. The fused silica-capillary column used was a non-polar column, CP-SIL 8CB (25 m x 0.15 mm x 0.12 microm) from Chrompack (Middelburg, The Netherlands). Further details of the measurements have been described before (Soechitram, 2004). The PCBs and OH-PCB levels measured are shown in Table 5.2. The chemical activated luciferase gene expression (CALUX) assay was used for measuring the total TEQ level in the maternal serum.

Statistical analyses

The results are presented as mean and standard error. Testicular volume is presented as mean volume (left + right/2). Levels of polychlorinated biphenyls are expressed on lipid weight basis (ng/g lipid weight) and hydroxylated biphenyls are expressed on fresh weight basis (ng/g fresh weight). Bivariate correlations using Spearmen's rho were calculated.

Results

104 mother/infant pairs were included in the study. Fifty-five boys were born within this cohort and form the basis for this study. Characteristics of the mothers and newborn boys are shown in Table 5.1. All boys were born after an uncomplicated pregnancy at a gestational age of 40±1.2 weeks. The birth weight was 3735±550 g. Seventy-two percent of the boys were breastfed.

The levels of the PCB and OH-PCB congeners in maternal serum are shown in Table 5.2. The levels of PCBs ranged from 89.4±38.9 ng/g lipid for PCB 153 till 8.0±5.8 for PCB-183. The levels of the OH-PCBs were much lower and ranged from 0.08 ng/ng serum for OH-PCB-146 till 0.01 ng/g serum for OH-PCB -172. The total TEQ as measured by the CALUX showed a value of 29±26 pgTEQ/g lipid.

Table 5.1 Characteristics of the mothers and the boys.

		Number of cases
Feeding type	Breast feeding	40
	Formula feeding	15
Smoking of mother	No smoking	43
	<10 per day	9
	>10 per day	3
Parity	Primipara /first child	22
	Second child	25
	Multipara (>2)	8
		Mean (SD)
Mothers age (years)		31.4 (4)
Birth weight (gram)		3735 (550)
Weight at 3 months (gram)		6314 (666)
Weight at 18 months (gram)		11898 (1477)
Testicular volume at 3 months (mm^3)		353.1 (122)
Testicular volume at 18 months (mm^3)		414.4 (163)

Table 5.2 Levels of PCBs and OH-PCBs.

PCB congeners (ng/g lipid)	Mean (SD)	OH-PCB congeners (ng/g serum)	Mean (SD)
118	27.0 (19.9)	107	0.08 (0.055)
146	9.9 (6.4)	153	0.04 (0.032)
153	94.3 (42.9)	146	0.09 (0.043)
105	7.8 (8.7)	138	0.06 (0.043)
138	69.8 (31.5)	187	0.14 (0.052)
187	13.5 (6.4)	172	0.02 (0.010)
183	8.4 (5.8)	sum	0.42 (0.190)
156	11.2 (4.9)		
180	46.2 (21.2)		
170	19.9 (8.7)		
sum	308.9 (133.9)		

In 49 out of the 55 boys the testicular volume could be measured by ultrasound at 3 and 18 months. The total testis volume, the combined results of right and left testis increased from 353 mm3 at 3 months to 414.4 mm3 at 18 months (Table 5.1). The relation between the different PCBs and OH-PCBs versus testicular volume at 3 and 18 months is shown in Table 5.3. There were no significant correlations except for OH-PCB 153, which showed a significant positive correlation with testis volume at 3 months. No correlation between the CALUX values and testis volume has been found.

Table 5.3 Spearman's rho correlation between PCBs/OH-PCBs versus testicular volume at 3 and 18 months of age.

PCB congeners (ng/g lipid)	Corr coeff for testicular volume at 3 months	Corr coeff for testicular volume at 18 months
118	0.098	0.113
146	0.065	0.125
153	0.113	0.212
105	0.001	0.105
138	0.143	0.220
187	0.011	0.089
183	0.007	0.315
156	0.052	0.207
180	0.109	0.221
170	0.072	0.228
sum	0.088	0.203
OH-PCB congeners (ng/g serum)		
107	0.029	0.198
153	0.287*	0.079
146	0.097	0.105
138	0.129	-0.096
187	-0.068	-0.006
172	0.161	0.112
sum	0.113	0.072

*P<0.05

Discussion

In this study we did not find a relation between the testicular volume in newborn infants and the levels of PCBs and OH-PCBs except for OH-PCB 153 at 3 months.
Studies conducted over the past decades have shown a decrease in sperm count, an increase in testicular germ cell cancer as well as an increase in hypospadia and undescended testis. A data analysis on the semen quality since 1930 showed a significant decrease in the sperm count from 113 ml in 1940 to 66 million per ml in 1990, and a decline in seminal volume of 3.40 to 2.75 ml[12,18,19]. The rate of cryptorchism doubled in the USA between 1970 and 1993 according to Paulozzi[20]. Hypospadia also doubled between 1970 and 1993 in the USA as well as in the UK[21,22]. Skakkebak et al., stated in 2001 that undescended testis, hypospadia, poor semen quality and testicular cancer are all components of one syndrome, the testicular dysgenesis syndrome[23]. Although, not completely proven, it is suggested that the syndrome is related to environmental pollutants that have an oestrogenic or anti-androgenic effect. A number of studies indicated an effect of PCBs on male testicular development. A recent study showed an association between PCB-153 and cryptorchism[24]. PCB-153 levels in controls were 59ng/g fat compared to 73 ng/g fat in boys with cryptorchism. Mocarelli showed a change in sex ratio of fathers exposed to higher levels of PCBs[13]. A study from Sweden found evidence of negative effects of exposure to PCB-135 on sperm motility and free testosterone levels in young men[25].

A study in Belgium found a delay in sexual maturation in adolescent boys exposed to higher levels of PCBs as well as a lower testicular volume[11]. All these studies described effects of PCB levels measured at a later age. Few studies evaluated the potential effect of antenatal PCBs on the sexual development of infants. The first indication that antenatal exposure to PCBs and PCDFs influences sexual development in humans, came from the Yucheng incidence in 1997. In utero exposure to PCBs and PCDFs resulted in a decrease in sperm mobility at an adult age[26]. A recent study from Germany found a lower testosterone level in cord blood from girls and estrogen levels in boys in relation to exposure to PCBs[27]. We recently found out that OH-PCB-107 and OH-PCB-187 next to the brominated compound DDE 154 are related to sex hormones in boys at 3 months of age[16]. Vreugdenhil et al showed a significant feminization of rated play behavior in boys with increasing cord PCB levels. In girls a non-significant opposite trend was found[28].

We did not find an effect of antenatal exposure to PCB and OH-PCB testis volume, except for a negative relation between OH-PCB 153 and testis volume at 3 month. No relation with PCB 153 itself was found, while OH-PCB 153 is a degradation product of PCB 153. PCB-153 has a strong estrogenic effect[25]. Interestingly, PCB-153 is related to sperm chromatin integrity[29] and reduced sperm motility[30]. Giwercman[31] found a statistical significant interaction between PCB-153 and the CAG gene, as part of the AhR receptor. Adults with a high PCB-153 exposure and low number of CAG repeats showed a lower total sperm count. The study of Den Hond from Belgium also indicates that PCB 153 might be one of the PCBs influencing sexual development[11]. That we observed a negative correlation between OH-PCB-153 but not with the parent compound PCB-153 might be caused by a higher affinity of OH-PCBs than PCBs itself to the Ah receptor or another stronger anti-androgenic effect. Another explanation could be a different distribution in the body. A study in animals showed higher levels of OH-PCBs in the brain, compared to plasma. The contrary was found for the parent PCB compound. It might therefore be speculated that the levels of PCB-153 in this cohort were too low in order to show a correlation with testis volume, but OH-PCB-153 due to its higher binding to the AhR receptor and /or higher brain level did show a relation.

There might be different reasons for which we did not find a relation between all other PCB and OH-PCB levels and testis volume in newborn boys. PCB levels in The Netherlands have been declining over the past years and might at present be too low to show an effect. Still, in a cohort founded 10 years prior to the cohort described in this paper, we found a negative correlation between antenatal exposure to PCBs and thyroid hormones and neurological development, as well as all markers of immune development[5,7,9]. In that cohort a feminization of play behavior in boys at a school age was also found[28]

Next to the levels of individual PCBs we also used the CALUX method as an indicator of the presence of environmental pollutants. The CALUX assay used in this study provides an indication of a range of compounds activating the AhR receptor, and therefore is an indicator of overall anti-estrogenic activity. The growth of the testis is mainly dependent on testosterone, which could explain why no relation between testis volume and the CALUX results was found. This is in accordance with the study in adolescent boys, where a relation was found between individual PCBs and testis volume and sexual maturation, but not with CALUX results[11].

Conclusion

In summary: in this study we show that the levels of PCBs present in The Netherlands do not have an important influence on testis growth in boys. Our study does not support the hypothesis, that PCBs at present levels are inducers of the testicular dysgenesis syndrome.

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Chapter 6

THYROID HORMONE LEVELS IN NEWBORN INFANTS ARE RELATED TO PCB EXPOSURE

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Submitted

Abstract

A number of studies have shown an effect of PCBs on serum thyroid hormone levels in pregnant woman and their infants, while other studies could not confirm this effect. How PCBs might affect thyroid hormones is not well known, nor whether the effect is caused by PCBs themselves or by their hydroxylated metabolites (OH-PCBs).

We measured in a cohort of mothers and their infants, exposed to background PCB levels in The Netherlands, the correlation between the concentrations of 10 PCB congeners and 6 OH-PCBs in maternal blood and the serum T4, T4 sulfate (T4S), T3, rT3, TSH and the TBG levels in cord blood at 3 and 18 months of age.

No correlation was found between any of the PCBs or the sum of PCBs, and OH-PCBs with T4, TSH and TBG at any point in time. Six of the PCBs showed a positive correlation with cord serum T3 and a negative correlation with cord serum rT3. The cord serum T3/rT3 ratio, which may reflect tissue type 3-deiodinase activities, was correlated with 6 PCB's. No correlation was seen with OH-PCBs.

In conclusion: Our results suggest that PCBs have a negative effect on type 3-deiodinase activity. We have identified a potential mechanism through which PCBs may affect thyroid hormones during human development.

Introduction

Despite the ban on their production, which happened already decades ago, polychlorinated biphenyls (PCBs) are still present in the environment and in human tissues[1,2]. Humans are mainly exposed to these compounds through the food chain. Different studies found negative effects of prenatal exposure to these compounds on neurodevelopment, thyroid hormones (TH's) and the development of the immune system in infants. In a cohort studied in the early nineties we found negative effects of PCB on TH's as well as on neurodevelopment and development of the immune system[2-9]. The levels of PCBs are particularly high in inhabitants of The Netherlands and Belgium[2,5]. A more recent study from Germany did not show an effect of background PCB levels on the TH status and neurodevelopment in newborn infants[10]. This might be related to a gradual decrease in PCB levels, as well as lower levels in Germany compared to The Netherlands.

Studies with humans mainly investigated the effects of PCBs themselves on human development. PCBs are metabolized by hydroxylation to OH-PCBs, which are more water-soluble and therefore more rapidly excreted from the body[2]. Some OH-PCBs have a higher affinity for transthyretin (TTR) than thyroxin (T4) itself. OH-PCBs accumulate in the brain of a fetal rat, thereby reducing the T4 levels in the fetal brain, which are compensated in part by the increased type II iodothyronine deiodinase (D2) activity, a well known response of the rat brain to maintain brain 3,3',5-triiodothyronine (T3) levels when circulating T4 concentrations are decreased[11-13]. The development of the central nervous system depends on the regulation of TH's and therefore may be at risk for PCB-induced alterations in brain TH concentrations[13]. A recent study showed a negative correlation between cord blood levels of OH-PCB 107 and mental and psychomotor development in children of 16 months of age[14].

Studies have shown lower levels of T4 and T3 in pregnant women in relation to PCB exposure[8,14,15]. Maternal T4 is an important source of TH for the fetus. Decreased maternal levels of T4 therefore might have negative effects on the fetus and newborn infant.

Not much is known about how Persistent Organic Pollutants (POPs) might affect thyroid function in the human fetus and newborn infant. In animals the most likely mechanism is the binding of POPs to TTR. This, however, is not likely to be a mechanism in humans, as thyroid-binding-globulin (TBG) is the most prominent TH-binding protein and POPs do not bind to TBG. Other potential mechanisms influencing TH levels in humans are changes in the activities of deiodinases and sulfotransferases involved in TH metabolism. Schuur et al.[16] studied different iodothyronine sulfotransferases catalyzing the sulfation of TH's in humans and in rats. They also found out that various POPs potently inhibit TH and estrogen sulfation. The

possible interference of PCBs with these enzyme activities in newborn infants has not been investigated yet. The aim of the present study therefore was to evaluate the effect of PCBs and OH-PCBs on serum TH metabolites in human infants, which may reflect changes in tissue deiodinase and sulfotransferase activities.

Methods

Cohort

The study design and details on the measurement of the different congeners have been published before[2]. In short, from September 1998 to December 2000, pregnant women from the Northern part of The Netherlands were invited by their midwife or obstetrician to participate in a study on the exposure to PCBs and OH-PCBs and their potential effect on the development of the newborn infant. Mothers had to be of Western European origin, and Dutch had to be their native language. The pregnancy and delivery had to be without complications. Only infants born at term (37-42 weeks of gestation) were included. The admission of an infant at the hospital for more than one day after birth was an exclusion criterion. The Medical Ethical Committee of the University Medical Centre Groningen has approved the study.

Study design

After the inclusion into the study, a blood sample was taken from the pregnant women in the 3rd trimester of pregnancy for measurement of PCB and OH-PCB levels. Cord blood as well as blood taken by venipuncture at 3 and 18 months was collected for the determination of the T4, thyroxin sulfontransferase (T4S), thyroid stimulating hormone (TSH), T3, inactive T3 metabolite/ reverse T3 (rT3) and TBG.

Measurement of PCB and OH-PCB

Compounds were measured using a gas chromotograph (Varian 3400 GC) equipped with an electron capture detector, a Varian 822\00 autosampler and a split/splitless injector operated in a splitless mode. The fused silica-capillary column used was a non-polar column, CP-SIL 8CB (25 m x 0.15 mm x 0.12 µm) from Chrompack (Middelburg, The Netherlands). Further details of the measurements have been described before[2]. The PCB and OH-PCB levels measured are shown in Table 6.1. The chemical activated luciferase gene expression (Calux) essay was used for measuring total Toxic Equivalent Quotient (TEQ) levels in maternal serum.

Table 6.1 Maternal PCB and OH-PCB levels.

Congener	Mean ± SD	(n)
PCB118	26.1 ± 17.8	(100)
PCB146	9.7 ± 5.9	(100)
PCB153	96.4 ± 43.3	(100)
PCB105	8.9 ± 14.8	(100)
PCB138	70.5 ± 31.8	(100)
PCB187	13.4 ± 6.4	(100)
PCB183	15.4 ± 56.0	(100)
PCB156	11.2 ± 4.8	(98)
PCB180	47.1 ± 20.0	(98)
PCB170	20.1 ± 8.2	(98)
ΣPCB	310.5 ± 128.2	(98)
OH-PCB107	.077 ± .053	(98)
OH-PCB153	.044 ± .028	(98)
OH-PCB146	.083 ± .044	(98)
OH-PCB138	.055 ± .036	(98)
OH-PCB187	.147 ± .055	(98)
OH-PCB172	.017 ± .008	(78)
ΣOH-PCB	.421 ± .178	(98)
Calux	33.9 ± 28.8	(102)

PCB levels are presented in ng/g lipid weight basis; OH–PCB levels are presented in ng/g fresh weight basis; Calux levels in pg TEQ/g lipid

Measurement of thyroid parameters

Serum T4, T3 and rT3 were measured by in-house radioimmunoassay's; FT4 by Vitros ECI technology (Ortho-Clinical Diagnostics, Amersham, UK); TSH by Dynotest IRMA; and TBG by Dynotest RIA (Brahms, Berlin, Germany). The measurements of serum T4S were done by radioimmunoassay[17]. Within-assay coefficients of variation were calculated as 2-8% for T4, 3-7% for FT4, 2-6% for T3, 3-4% for rT3, 6-17% for T4S, 2-5% for TSH and 2-4% for TBG. Between-assay coefficients of variation were 5-10% for T4, 5-10% for FT4, 8% for T3, 9-16% for rT3, 4-19% for T4S, 2-14% for TSH, and 2-3% for TBG.

Statistical analysis

The results are presented as mean and standard deviation when appropriate. PCB levels are expressed on lipid weight basis (ng/g lipid) and OH-PCB levels on fresh weight basis (pg/g serum). Bivariate non-parametric correlations were calculated using Spearman's rho. Differences were considered statistically significant at $P<0.05$ levels. All analyses were performed in SPSS IBM 19 for Windows.

Results

104 mother-infant pairs were included in the study. Clinical details of the cohort as well as levels of PCBs and OH-PCBs have been published before[8] and are shown in Tables 6.1 and 6.2. Serum TH levels are shown in Table 6.3. In this study population none of the children had a hyper- or hypothyroidism. Normal values for T4S, rT3 and TBG are not available for newborns. No correlation between any of the PCBs or OH-PCBs and T4, TSH, T4S or TBG was found. Also no correlation between any of the TH levels and the Calux results was observed. A significant positive correlation was found between PCB 118, 146, 153, 138, 187, 183 as well as the sum of PCB levels (ΣPCB) with cord serum T3. A significant negative correlation was found between 4 of these 6 PCB's and ΣPCB and cord serum rT3 (Table 6.4). The T3/rT3 ratio showed a positive correlation with PCBs 118, 146, 153, 138 and 187 (all $P<0.01$). No consistent effects of any of the PCBs or OH-PCBs on any of the TH levels were observed at 3 and 18 months.

Table 6.2 Characteristics of the mothers and children.

	N	Mean	Sd
Sex of child			
Male	55		
Female	49		
Type of feeding			
Breastfeeding	73		
Formula feeding	30		
Birth weight (gram)		3635	498
Gestational age (weeks)		40	1
Smoking mother			
no smoking	80		
<5 sig/day	14		
>6 sig/day	10		
Age of mother (years)		32	4

Table 6.3 Plasma thyroid parameters (mean ± SD) in umbilical cord and in infants at 3 and 18 months of age.

Thyroid hormone parameter	Umbilical cord (n=86)	Infant third month (n=90)	Infant eighteen month (n=86)
T4 nmol/l	126.4 ± 26.6	131.7 ± 21.9	114.5 ± 18.1
T4s pmol/l	484.0 ± 176.3	49.8 ± 20.14	21.6 ± 12.5
TSH mU/l	14.17 ± 8.45	3.36 ± 1.53	3.28 ± 1.42
T3 nmol/l	0.71 ± 0.23	2.79 ± 0.39	2.41 ± 0.32
rT3 nmol/l	3.7 ± 1.22	0.56 ± 0.14	0.32 ± 0.06
TBG mg/l	30.3 ± 6.08	29.9 ± 6.11	25.6 ± 4.31
T4-TBG ratio	4.19	4.50	4.52

Table 6.4 Spearman's rho correlations between maternal PCBs and plasma T3, rT3 and T3/rT3 ratio in umbilical cord blood.

PCB (ng/g lipid weight)	Correlation coefficient (P)		
	T3 (nmol/l)	rT3 (nmol/l)	T3/rT3 ratio
118	0.279 (0.010)	-0.237 (0.029)	0.307 (0.004)
146	0.343 (0.001)	-0.271 (0.012)	0.356 (0.001)
153	0.263 (0.015)	-0.210 (0.054)	0.284 (0.008)
138	0.259 (0.017)	-0.235 (0.030)	0.301 (0.005)
187	0.320 (0.003)	-0.223 (0.040)	0.321 (0.003)
183	0.224 (0.040)	-0.037 (0.734)	0.156 (0.153)
Sum	0.279 (0.011)	-0.224 (0.042)	0.300 (0.006)

Discussion

In this study we have not observed any effect of the background levels of PCBs or OH-PCBs on T4, TSH and TBG in the cord blood of newborn infants. However, a positive correlation was found between 6 PCBs in maternal serum and cord serum T3, as well as a negative correlation between 4 of these 6 PCBs and cord serum rT3. The T3/rT3 ratio showed a significant positive correlation with 5 PCBs and ΣPCBs.

Studies with animal models have shown effects of POPs on thyroid functions. A number of mechanisms have been identified that might play a role: direct negative effects on the thyroid gland; competition with TH binding to TTR, the main TH-binding protein in rodents; increased biliary excretion of TH's; interaction with T3 receptors; interaction with the pituitary-thyroid axis; and finally changes in the activities of deiodinases, enzymes regulating the levels of active TH's[11,18-20]. It is unclear which of these mechanisms might play a role in the human fetus and newborn. Although the main binding protein in humans is not TTR, but TBG, TTR still plays a role in mediating the delivery of T4 across the blood-brain barrier, transporting T4 into the cerebrospinal fluid and transferring maternal-to-fetal T4 over the placenta[21,22]. Most of the hydroxylated PCB metabolites in human plasma were found to be associated (>85%) with human TTR. Therefore, it is reasonable to suggest that fetal accumulation of hydroxylated PHAHs may also occur in human and animal species[11].

During the first half of gestation, fetal TH levels are dependent on maternal T4 supply. Also after the onset of fetal thyroid production, maternal T4 supply to the fetus continues to represent an important proportion of the TH available to the fetus[23]. Different studies have showed a negative correlation between POPs and TH levels in pregnant women[8,15,24]. Lower maternal TH levels might result in lower TH levels in the fetus.

In the present study we did not find an effect of any of the PCBs or OH-PCBs on either T4, TSH or TBG, which might be due to the presently lower levels of PCBs in pregnant mothers compared to the levels in our first cohort published in 1994[8]. A number of

studies have evaluated the effect of POPs on TH levels in newborns. An overview of these studies is presented in Table 6.5. Mostly cord samples were used to measure the thyroid status and exposure levels. In Table 6.5, studies are summed up with their effects on TH and TSH levels. Two European studies with a relatively high background exposure found a negative correlation between T4, FT4 and PCBs[3,8]. Most European studies found a positive correlation between PCBs and TSH's. No effect was observed in studies from Canada and the Faroe Islands. This latter finding might be related to the high fish consumption in Canada and Faroe Islands, an important source of both PCBs and iodine.

Table 6.5 Overview of Thyroid and PCB studies.

Reference	N	PCB	Sample	Level	TT3	fT3	TT4	fT4	TSH	Sample	Country
(8)	78	∑PCB	Breast milk	72.4 pg/g fat			↓		↑	2 wks	The Netherlands
(32)	173	∑PCB	Serum	1.12 µg/g					=	Cord	Faroe Islands
(33)	30	∑PCB	Cord	1.5-843 pg/g plasma	=			=	=	Cord	Canada[a]
(34)	70	∑PCB	Cord	0.14 ng/ml					↑	Cord	Spain
(15)	101	∑PCB	Maternal serum	0.35 µg/l	↓			=	=	Cord	Canada
(35)	118	∑PCB+ dioxin	Cord serum	15.1 pg/g lipid	=			=	=	Cord	Taiwan
(3)	198	∑PCB	Cord serum	106 ng/g fat		↓		↓	=	Cord	Belgium
(36)	410 260	PCB153	Cord	95 µg/kg	=			=	=	Cord	Canada
(37)	120	PCB153	Maternal plasma	107 µg/kg	=		=		=	Cord	Canada[b]
			Cord	83 µg/kg							
(38)	453	PCB153	Cord	131 ng/g lipid					=	Cord	Spain
(10)	182	∑PCB-TEQ	Maternal serum	5.71pg/g fat	=	=	=	=	=	Cord	Germany
(39)	382	PCB153	Cord serum	0.14-0.32 ng/ml					↑	Cord	Spain
(40)	23	PCB153	Cord	102 ng/g fat				=	=	4-6 day p.n.	Japan[c]
(24)	160	PCB153	milk	65 ng/g fat	↓				↑	3 wks	Sweden

[a] Negative correlation FT4 with ΣOH-PCB; [b] Negative correlation PCB with TBG; [c] Positive correlation T4 with OH-PCB187 ; p.n.=postnatal

No study in humans so far evaluated if POPs might have an effect on the activity of deiodinases in human infants. A study in rats shows that PCBs competitively inhibit T4 binding to TTR and D1 activity[16]. Another study by Morse et al.[13], shows that reductions in brain T4 levels were most severe in the fetus, and the induction of D2 activity in the fetal forebrain can account for the maintenance of forebrain T3 levels in the fetus. It has been suggested that type III iodothyronine 5-deiodinase (D3) regulates cerebellar T3 levels by regulating the deiodiniation of T3 to T2[25].

In the present study we show a positive correlation between a number of PCBs with T3 in umbilical cord blood and a negative correlation with rT3. Also, the serum T3/rT3 ratio showed a significant positive correlation with PCBs. TH homeostasis in humans is regulated by the activities of the iodothyronine deiodinases D1, D2 and D3, glucoronidation and sulfation[23]. D1 is present in the liver, kidney and thyroid gland, and cleaves iodine from the inner and outer ring. D1 in adults is considered to be an important source of T3 and responsible for the clearance of rT3. D2 is present in the brain, anterior pituitary and thyroid gland. It plays an important role in the production of T3 in the brain. D3 is present in particular in the fetal brain, placental and fetal tissues. It catalyzes the inner ring deiodination, resulting in the conversion of T4 into rT3 and T3 into 3,3'-T2[23].

D3 in tissues such as the brain is thought to play a role in the regulation of intracellular T3 levels, while its presence in placenta and fetal tissues may protect developing tissues against exposure to high levels of active TH[26].
The pattern of activity of the deiodinases is different between the fetus and the adult. D2 is found to be hardly active in fetal life[27,28]. D3, however, is found to be very active during fetal life, while the activity rapidly decreases after birth[27-30]. Due to the high D3 activity, especially in placental tissues, fetal rT3 levels are much higher compared to the postnatal period, while the opposite is true for T3. A high D3 activity is not only found in placental tissue, but also in human fetal liver and fetal brain tissue[31]. The high D3 activities in fetal tissues and placenta are thought to prevent growing tissues against exposure to undue T3 levels. Our results of a positive correlation between the T3/rT3 ratio and PCB 118, 146, 153, 138 or 187, as well as the sum PCBs suggest a negative effect of these compounds on D3 activity. The possible effect of this lower D3 activity on the human fetus is still unclear. At the same time it is consistent with most studies in human infants, where either no effect of PCBs on T3 or an increased level was found, Table 6.5.

We did not observe a correlation between any of the OH-PCBs with TH levels. Whether OH-PCBs, in contrast to animal studies, have no effect on human TH levels or are, like PCBs themselves, presently too low to have an effect, is not clear. We also did not find an effect of PCBs on levels of T4S. This might indicate that the prevailing PCB concentrations do not influence the sulfation of T4 in infants. This also suggests that POPs do not influence D1 activity, as D1 is important for the clearance of T4S.

In conclusion, we did not find an effect on T4, T4S and TSH in newborn infants exposed to background levels of PCBs in The Netherlands. At the same time, we observed indications of reduced fetal or placental D3 activity in relation to PCB congeners. This might also be of influence on the regulation of T3 in fetal brain and therefore on brain development.

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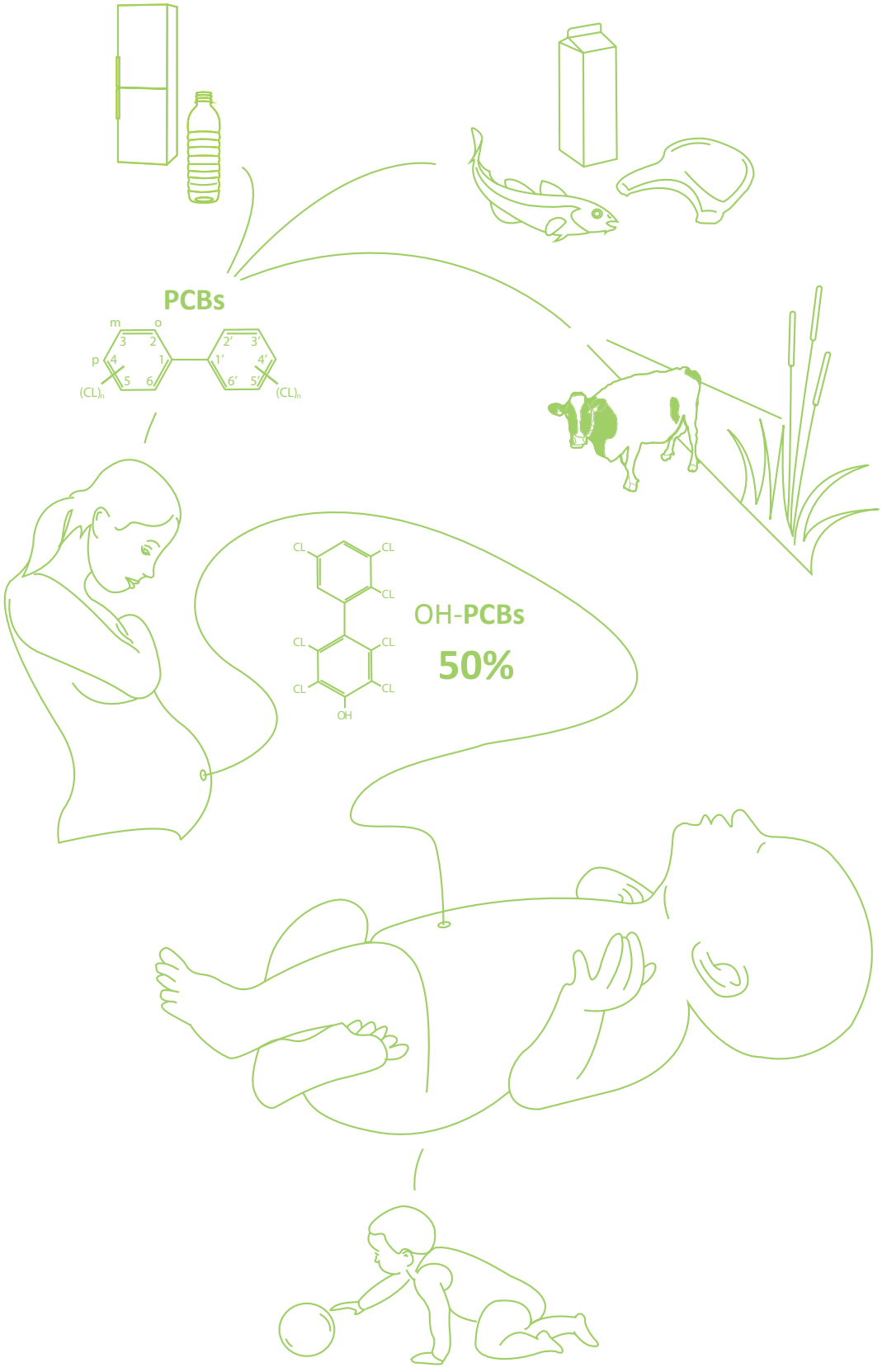
Neurological development



Chapter 7

PRENATAL EXPOSURE TO POLYCHLORINATED BIPHENYLS AND THEIR HYDROXYLATED METABOLITES IS ASSOCIATED WITH QUALITY OF MOTOR REPERTOIRE OF THREE-MONTH-OLD INFANTS

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Abstract

Background

Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants that are potentially toxic to the developing brain. Moreover, it has been suggested that the hydroxylated metabolites of PCBs (OH-PCBs) are even more toxic due to active transplacental transfer to the fetus as well as hydroxylation by the fetus itself. Apart from these facts about PCBs and OH-PCBs little else is known about their short-term effects on human health.

Objectives

To determine whether prenatal background exposure to PCBs and OH-PCBs was associated with the neurological conditions of three-month-old infants.

Methods

Ninety-seven mother-infant pairs participated in this Dutch, observational cohort study. We determined the concentrations of PCBs and OH-PCBs in cord blood samples. When the infants were three months old we evaluated their neurological conditions by assessing the quality of their motor repertoires from video and calculating a Motor Optimality Score (MOS). The score in three-month-old infants could range from low (5) to high (28) optimality. We explored the correlations between PCB and OH-PCB levels and MOS. Subsequently, we tested whether the levels differed between infants with a low (<26) or high (≥26) MOS and whether the levels associated with detailed aspects of their motor repertoires.

Results

We found several associations between PCB and OH-PCB levels and MOS, including detailed aspects of the infants' motor repertoires. High 4-OH-PCB-107 levels were associated with a low MOS (P=0.013). High PCB-187 levels were associated with reduced midline arm and leg movements (P=0.047 and P=0.043, respectively). High 4'-OH-PCB-172 levels were associated with more manipulation (P=0.033).

Conclusion

Prenatal exposure to high background 4-OH-PCB-107 levels was associated with a non-optimal quality of the motor repertoire of three-month-old infants. Higher levels of PCB-187 were associated with reduced midline movements and 4'-OH-PCB-172 with more manipulation. Possibly, these effects were mediated by reduced concentrations of thyroid hormone in the brain.

Introduction

Polychlorinated biphenyls (PCBs), a significantly toxic group of industrial chemical compounds, were widely used commercially in, for example, hydraulic fluids, adhesives, inks, lubricants, and as coolants in heat transfer systems. Although the production and use of PCBs has been banned by law since 1985, humans and animals alike continue to be exposed to the contaminating effects of PCBs still remaining in the environment. PCBs persist over long periods of time because of their resistance to chemical and biological degradation. They are lipophilic compounds that bioaccumulate and biomagnify in the food chain. Considerable concentrations of PCBs are found in fish taken from contaminated waters. Studies of populations with high exposures to PCBs caused by consuming contaminated fish, show neurobehavioral alterations in newborn and older infants[1,2]. Especially during the fetal period, PCBs may affect the development of the central nervous system, the reproductive system, and the immunological system.

PCBs are metabolized by hepatic microsomal oxidase to form hydroxylated metabolites (OH-PCBs). This process is mediated by the cytochrome P450 enzymatic system. In contrast to the relatively stable and highly lipophilic PCBs, OH-PCBs are readily conjugated and excreted. During the last decade, techniques have become available to detect these hydroxylated metabolites in human serum, allowing us to determine the effect of these metabolites on human health. Previously, we reported a correlation between the PCB and OH-PCB levels in maternal and umbilical cord plasma, indicating considerable placental transfer of these compounds[3]. The OH-PCB levels in umbilical cord plasma were approximately 50% of maternal levels, whereas the PCB levels were approximately 30% of maternal levels. We suggested that this difference may be explained by the different sources of the compounds and their solubility. PCBs reach the fetus by transplacental transfer only, whereas OH-PCBs can also be formed by the fetuses themselves through hydroxylation.

OH-PCBs are considered endocrine disruptors in animals, with effects on thyroid hormones, estrogens, and testosterone. Animal studies have shown a significant reduction of thyroid hormones in the brain after exposure to OH-PCBs[4,5]. Meerts et al. concluded that maternal exposure to 4-OH-PCB-107 could exert adverse effects on neurotransmitter levels and brain development in rat offspring. In humans an association was found between exposure to OH-PCBs and neurodevelopment at the age of sixteen months[6]. In a study of children at school age, prenatal exposure to OH-PCBs correlates with worse fine manipulative abilities, better attention, and better visual perception[7]. This study indicated that even low background levels of OH-PCBs may interfere with developmental processes.

Little is known about the toxicological impact of PCBs and especially of OH-PCBs on humans and very young infants in particular. A reliable, non-invasive method to evaluate the brain function of young infants is to assess the quality of their spontaneous motor repertoire[8]. The age of approximately three months after term is particularly revealing. At this age the infant's motor repertoire is characterized by so-called fidgety movements (FMs). These are spontaneous movements of small amplitude, moderate speed, and variable acceleration in all directions. FMs are highly predictive of subsequent neurological outcome: most infants (96%) with normal FMs have normal neurological outcomes, while most infants (95%) in whom FMs were absent during this specific period develop cerebral palsy[8]. Recent studies reported that detailed aspects of FMs and the concurrent motor repertoire, as expressed in the Motor Optimality Score (MOS), are also predictive of mild motor abnormalities and cognitive impairments later on[9,10].

The aim of this study was to determine whether prenatal background exposure to OH-PCBs and PCBs was associated with the quality of the motor repertoire in three-month-old infants. Since previous human and animal studies showed impaired developmental outcomes after prenatal exposure to OH-PCBs and PCBs, we hypothesized that exposure to higher levels of these compounds would be associated with poorer quality of the early motor repertoire.

Methods

Cohort

Ninety-seven mother-infant pairs were included in this observational study between September 1998 and December 2000. They were members of a cohort participating in prospective studies on exposure to PCBs and OH-PCBs and their potential effects on the development of the newborn infant[3]. Women from the northern part of the Netherlands were invited to participate by their midwife or obstetrician. Only women of Western European origin, who spoke Dutch as their native language, were included. Women whose pregnancy or delivery had involved serious illness or complications were excluded. Only infants born at term (between 37 to 42 weeks' gestation), who had no congenital anomalies or diseases, were included. Infants who had been admitted to a hospital more than one day after birth were not included. All parents had given their informed consent before videotaping commenced. The study was approved by the medical ethics committee of the University of Groningen.

Measurement of PCBs and OH-PCBs

Umbilical cord blood samples were taken immediately after delivery. A detailed description of the analysis of the blood samples is provided by Soechitram et al.[3]. We

used the same extraction and clean-up procedure as described by Hovander et al.[11]. The samples were analyzed at the analytical laboratory participating in the study. We numbered the PCBs according to Ballschmiter et al.[12] and we numbered the OH-PCB congeners according to Letcher et al.[13]. Concentrations of PCBs are given in ng/g lipid in cord blood. Because they are hydrophilic, concentrations of OH-PCBs are expressed per gram fresh weight.

Videotaping

Video recordings were made at approximately three months of age during an outpatient visit to the clinic. A ten-minute optimal recording is sufficient to reliably assess the quality of an infant's motor repertoire[14]. All recordings were made with the infants lying supine and dressed lightly and comfortably. The infants' arms and legs were bare and they could move their limbs and trunks freely. The recordings were made during a period of active wakefulness. Interferences were avoided during recording. We also took care that the infants were not fussing, crying or sucking on a pacifier, as it is impossible to assess the quality of the motor repertoire under such circumstances.

Assessment of the motor repertoire

The motor repertoire of the three-month-old infants were assessed off-line by at least two observers according to the method described by Einspieler and Prechtl and Bruggink et al.[9,14].

First, we assessed the quality of the FMs by Gestalt perception, a powerful tool for analyzing complex phenomena. Normally, FMs first occur at six to nine weeks post-term, and remain present until fifteen to twenty weeks, at which age intentional and antigravity movements appear and start to dominate the repertoire. We classified FMs as normal (N) or abnormal (A). Normal FMs are movements of small amplitude, moderate speed, and variable acceleration and involve movements of the neck, trunk, and limbs, in all directions. They are continually present, except during fussing and crying. Various other movements may occur concurrent with FMs, such as wiggling-oscillating and saccadic arm movements, swipes, fiddling, and trunk rotation. Abnormal FMs resemble normal FMs but are exaggerated the regard to amplitude, speed, and jerkiness. If FMs were not observed we noted this as 'absence of fidgety movements' (classified as abnormal FMs).

Second, we performed a more detailed analysis of the motor repertoire by combining the quality of FMs with the concurrent motor repertoire. We used the Motor Optimality List compiled by Bruggink et al.[9] to analyze the quality of FMs and to determine the infant's MOS at three months post-term. The list distinguishes three aspects of the motor repertoire: movement patterns, postural patterns, and movement character. The Motor Optimality List consists of five subcategories covering these three aspects:

1) Fidgety movements (FMs)
2) Repertoire of co-existent other movements
3) Presence and normality of individual movement patterns
4) Presence and normality of individual postural patterns
5) Quality of the concurrent motor repertoire.

Each subcategory, except FMs, is assigned a score of either 4, 2, or 1, in descending order of normality. FMs are assigned a score of either 12 (normal), 4 (abnormal), or 1 (absent). The sum of the scores of these subcategories is the Motor Optimality Score. For three-month-old infants MOS can range from low optimality with a minimum of 5 points to high optimality with a maximum of 28 points. In addition to MOS, we also dichotomized the score into a low (<26) or high (≥26) MOS. A low MOS is characterized by an abnormal score on more than one subcategory of the Motor Optimality List.

Data and statistical analyses

First, we used the Mann-Whitney-U test to investigate whether there was an association between PCB and OH-PCB levels and the quality of FMs.

Second, we used the Spearman rank correlation test to investigate whether there was an association between PCB and OH-PCB levels and MOS. Subsequently, we compared the PCB and OH-PCB levels of the group with a high MOS (≥26) to the group with a low MOS (<26) with the Mann-Whitney-U test.

In addition, we used the Mann-Whitney-U test to investigate whether there was an association between the PCB and OH-PCB levels and the scores on the five subcategories of MOS. Next, we clustered several movement patterns into four categories, i.e. midline movements of arms, midline movements of legs, manipulation, and antigravity movements, and investigated whether the PCB and OH-PCB levels were associated with these movement patterns, again using the Mann-Whitney-U test. We also used the Mann-Whitney-U test to investigate whether the PCB and OH-PCB levels were associated with movement character.

To determine whether the relation between PCBs and OH-PCBs and the quality of the motor repertoire was confounded by other characteristics (gender, gestational age, birth weight, age at recording, maternal education level, and maternal smoking and alcohol consumption during pregnancy), we performed univariate logistic regression analyses. These characteristics were considered as possible confounders if they had a *P* value of less than .2 in the univariate analysis model. We performed multivariate logistic regression analyses (method: enter) to assess whether these confounders had influenced our results. A *P* value of less than .05 we considered statistically significant and a *P* value of more than .05 but less than .10, marginally significant. We used the Statistical Package for the Social Sciences, version 18.0.3 (SPSS Inc, Chicago, Ill.) for the statistical analyses.

Results

Of the 104 infants initially included in the study, seven were later excluded for various reasons: for three infants no PCB or OH-PCB values were available, two infants had not been videotaped at three months due to logistic reasons, and two infants were excluded because their motor repertoire could not be evaluated from videotape because the recordings were too short. The final study group consisted of 97 mother-infant pairs.

Characteristics of the study group

In Table 7.1 we present the characteristics of the mothers and infants who participated. The median age of the infants at the time of videotaping was fourteen weeks (range eleven to seventeen weeks).

Table 7.1 Characteristics of the study group (n=97 mother-infant pairs). Data are given as frequencies (n/n), median (min-max), or mean ± SD.

Characteristic	Value
Gender, male/female	51/46
Gestational age (weeks)	40 ± 1
Apgar score 3 min	10 (6-10)
Birth weight (grams)	3607 ± 494
Maternal education level	
Below average (≤11 years education)	13
Average (12-13 years education)	40
Above average (≥14 years education)	44
Maternal smoking during pregnancy yes/no	22/75
Maternal alcohol consumption during pregnancy yes/no	24/73

Concentrations of PCBs and OH-PCBs

The concentrations of the ten PCBs and six OH-PCBs measured are given in Table 7.2a.

Table 7.2a Placental concentrations of PCBs and OH-PCBs. Data are given as median (range).

Compound	Concentration (ng/g lipid weight)	Compound	Concentration (ng/g fresh weight)
PCB-118	20.9 (5.2-128.9)	4-OH-PCB-107	0.067 (0.014-0.333)
PCB-146	8.0 (1.9-29.2)	3-OH-PCB-153	0.038 (0.008-0.152)
PCB-153	89.8 (28.7-223.1)	4-OH-PCB-146	0.070 (0.022-0.265)
PCB-105	4.0 (0.5-131.1)	3'-OH-PCB-138	0.046 (0.013-0.235)
PCB-138	67.9 (6.8-174.3)	4-OH-PCB-187	0.137 (0.051-0.339)
PCB-187	11.8 (4.5-38.9)	4'-OH-PCB-172	0.017 (0.005-0.045)
PCB-183	8.1 (2.6-548.0)	Sum OH-PCBs	0.389 (0.180-1.045)
PCB-156	11.1 (3.2-22.1)		
PCB-180	44.9 (14.5-124.5)		
PCB-170	19.0 (4.3-47.6)		
Sum PCBs	290.0 (108.9-698.7)		

Quality of fidgety movements

Out of the 97 infants, 96 showed normal FMs. One infant had abnormal FMs.

Motor optimality score

MOS total score

MOS in our study group ranged from 18 to 28. Out of the 97 infants, seventeen infants had a low MOS (<26) and eighty infants had a high MOS (≥26). Table 7.2b illustrates that a relatively large proportion of the infants had abnormal scores on the second and fifth subcategories. We examined these subcategories in more detail.

Table 7.2b Scores on the Motor Optimality List at the age of three months (n=97).

Subcategories of Motor Optimality List		Normal	Reduced/abnormal
Fidgety movements		96	1
Repertoire of co-existent other movements		77	20
	Movement clusters	Present	Absent
	Midline arm movements	61	36
	Midline leg movements	91	6
	Manipulation [a]	86	11
	Antigravity movements	94	3
Presence and normality of individual movement patterns		95	2
Presence and normality of individual postural patterns		96	1
Quality of the concurrent motor repertoire		50	47
	Character (global score)		Present, abnormal
	Jerky		13
	Monotonous		25
	Cramped		10
	Cramped-synchronized		1
	Tremulous		15
	Stiff		18
	Predominantly fast speed		6
	Predominantly large amplitude		1

[a] Manipulation includes the following movement patterns: hand-hand manipulation, foot-foot manipulation, and fiddling at cloths or blanket.

MOS and repertoire of co-existent other movements

The second subcategory on the Motor Optimality List assesses the age-adequacy of the repertoire of co-existent other movements. Twenty infants had abnormal scores on this subcategory. As shown in Table 7.2b, we clustered several movement patterns into one of four categories to provide more detail. Most infants showed midline leg movements, manipulation movements, and antigravity movements, while approximately a third of the infants showed no midline arm movements.

MOS and quality of the concurrent motor repertoire

The fifth subcategory on the Motor Optimality List scores the character of the concurrent movements and represents a global score of movement character. Fifty infants showed smooth and fluent movement character, whereas 47 infants showed abnormal movement character. The frequencies of abnormal movement characters are given in Table 7.2b. Several infants scored more than one abnormal movement character.

PCB and OH-PCB levels and Fidgety Movements

We found no associations between PCB and OH-PCB levels and the quality of FMs.

PCB and OH-PCB levels and the motor optimality score

We found no correlations between PCB and OH-PCB levels and MOS. After dichotomizing MOS into a low and high MOS, higher levels of OH-PCB-107 were found to relate to low MOS (P=0.04; Table 7.3). Exposure to higher OH-PCB-107 was associated with a reduced repertoire of co-existent other movements (P=0.08).
Next, we investigated whether exposure to PCBs and OH-PCBs was associated with the movement clusters within the repertoire of co-existent other movements (Table 7.3). High levels of PCB-187 were associated with reduced midline arm movements and reduced midline leg movements, P=0.04 and P=0.06, respectively. High levels of PCB-183 were associated with reduced midline leg movements and reduced manipulation, P=0.01 and P=0.05, respectively. High levels of 4'-OH-CB172 were associated with more midline leg movements and more manipulation, P=0.04 and P=0.02, respectively. We also found an association between exposure to some PCB levels and the presence of antigravity movements. High levels of the following compounds were associated with reduced antigravity movements: PCB-118, PCB-146, PCB-105, PCB-138 (P<0.05). The associations between high levels of PCB-153, PCB-156, and the sum of the PCBs measured, were nearly significant with reduced antigravity movements (P<0.10).
We then determined whether exposure to PCBs and OH-PCBs was associated with movement character. Regarding the scores on the fifth subcategory, quality of the concurrent motor repertoire, we found that high levels of PCB-118 and PCB-138 were associated more frequently with a cramped movement character (P=0.05 and P=0.09, respectively). The sum of the PCB levels measured was also more frequently associated with a cramped movement character (P=0.09).
Finally, we performed univariate logistic regression analyses to determine the odds ratios (ORs) for the effect of exposure to PCBs and OH-PCB compounds on the motor repertoire. In Table 7.4 we present those ORs that almost reached significance at P<0.10. We determined whether other factors might have confounded our findings. Univariate logistic regression analyses revealed that only maternal smoking during pregnancy, a higher gestational age, and female gender were associated with a low

MOS at *P*<0.20. We repeated the logistic regression analyses adjusting for these potential confounders and provide the adjusted ORs in Table 7.4.

Table 7.3 Prenatal exposure to PCBs and OH-PCBs related to abnormal movement patterns and character at the age of three months. [a]

			Clusters of movement patterns				Movement character		
Compound	MOS< 26 (n=17)	Reduced repertoire of co-existent movements (n=20)	Absent midline arm movements (n=36)	Absent midline leg movements (n=6)	Absent manipulation (n=11)	Absent antigravity movements (n=3)	Jerky (n=13)	Monotonous (n=25)	Cramped (n=10)
PCB-118						++			++
PCB-146						++			
PCB-153						+			
PCB-105				+		++			
PCB-138						++			+
PCB-187			++	+					
PCB-183				++	+				
PCB-156						+			
PCB-180									
PCB-170			(+)						
Sum PCBs						+			+
4-OH-PCB-107	++	+							
3-OH-PCB-153									
4-OH-PCB-146					-				
3'-OH-PCB-138									
4-OH-PCB-187					-				
4'-OH-PCB-172				- -	- -				
Sum OH-PCBs									

[a] Calculated by the Mann-Whitney-U-test; MOS= Motor Optimality Score; ++ *P*<0.05 and + *P*<0.10, (+) *P*=0.101, indicating that exposure to higher levels is associated with less optimal outcomes; - - *P*<0.05 and - *P*<0.10 , indicating that exposure to higher levels is associated with more optimal outcomes.

Table 7.4 Associations between (OH-)PCB levels and aspects of the motor repertoire.

Compound	Observed movement patterns or character	OR (95% CI)	*P*-value	OR adjusted for confounders (95% CI)	*P*-value
4-OH-PCB-107	MOS < 26	3.56 (1.30-9.73) [a]	0.013**	2.34 (0.67-8.24) [a]	0.066*
	Reduced repertoire of co-existent other movements	3.32 (1.26-8.74) [a]	0.015**	2.79 (0.99-7.83) [a]	0.052*
4'-OH-PCB-172	Absent midline leg movements	0.17 (0.02-1.38) [b]	0.098*	0.17 (0.02-1.42) [b]	0.102
	Absent manipulation	0.24 (0.06-0.89) [b]	0.033**	0.19 (0.05-0.79) [b]	0.022**
PCB-118	Absent antigravity movements	1.41 (0.98-2.04) [c]	0.065*	1.84 (1.02-3.32) [c]	0.043**
PCB-146	Absent antigravity movements	4.09 (0.81-20.64) [c]	0.088*	3.80 (0.65-22.26) [c]	0.138
PCB-138	Absent antigravity movements	1.27 (0.96-1.68) [c]	0.096*	1.21 (0.90-1.62) [c]	0.202
PCB-187	Absent midline arm movements	1.96 (1.01-3.79) [c]	0.047**	1.81 (0.89-3.67) [c]	0.102
	Absent midline leg movements	2.80 (1.03-7.57) [c]	0.043**	2.74 (0.96-7.87) [c]	0.061*
PCB-156	Absent antigravity movements	11.06 (0.73-168.85) [c]	0.084*	8.47 (0.48-148.96) [c]	0.144
PCB-170	Absent midline arm movements	1.57 (0.94-2.64) [c]	0.088*	1.46 (0.85-2.53) [c]	0.174
Sum PCBs	Absent antigravity movements	1.07 (0.99-1.15) [c]	0.086*	1.06 (0.98-1.15) [c]	0.160

[a] Per 0.10 ng/g fresh weight; [b] per 0.01 ng/g fresh weight; [c] per 10 ng/g lipid weight; **P<.05; *P<0.10.

Discussion

This observational cohort study demonstrated that prenatal background exposure to PCBs and OH-PCBs is associated with the quality of the motor repertoire of three-month-old infants. We found no associations between PCB and OH-PCB levels and the quality of FMs, but associations did exist between several PCBs and OH-PCB levels and aspects of the infants' motor repertoire. Firstly, prenatal exposure to higher levels of 4-OH-PCB-107 was associated with a poorer quality of the motor repertoire as reflected by a lower MOS. Secondly, some PCBs and OH-PCBs were associated with the acquisition of developmental milestones, such as the presence of midline movements, manipulation, and antigravity movements. Most associations indicated delayed development of age-adequate movements after exposure to higher levels of the compounds, except for some associations where we found the opposite. Thirdly, exposure to higher levels of OH-PCB-118 was associated more frequently with a cramped movement character. The results partially confirmed our hypothesis that prenatal exposure to higher PCB and OH-PCB levels was associated with a poorer quality of the early motor repertoire.

Our most important finding was that exposure to high 4-OH-PCB-107 levels was associated with a low MOS (<26). No other PCBs or OH-PCBs correlated with MOS. Higher levels of 4-OH-PCB-107 were associated almost significantly with a reduced repertoire of co-existent other movements. The 4-OH-PCB-107 metabolite is one of the major PCB metabolites in humans, formed predominantly by hydroxylation of PCB-105 and PCB-118. This metabolite was found to induce long-term adverse effects on behaviour and neurodevelopment in rats[5]. In human studies, Park et al. found that 4-OH-PCB-107 is the only metabolite that associates significantly with a decrease in the Mental Development Index of sixteen-month-old infants[6]. Roze et al. found both positive and negative correlations of 4-OH-PCB-107 with the neuro-developmental outcomes of five to six-year-old infants - it correlates with worse fine manipulative abilities, better attention, and better visual perception[7].

A second important finding of our study was that prenatal exposure to higher PCB and OH-PCB levels was associated with the development of age-adequate movement patterns. Exposure to higher levels of 4'-OH-PCB-172 was associated with more manipulation and more midline leg movements. We found a near-significant association of exposure to higher levels of 4-OH-PCB-146 and 4-OH-PCB-187 and more manipulation. The study by Roze et al.[7] also suggests negative effects of hydroxylated metabolites on manipulation, while we found predominantly positive effects on manipulation.
Unlike exposure to OH-PCBs, exposure to higher levels of PCBs was negatively associated with the development of age-adequate movements. Exposure to higher levels of PCB-105, PCB-187, PCB-183, and PCB-170 was associated with reduced midline movements of arms or legs or of arms and legs. Exposure to higher levels of PCB-183 was also associated with reduced manipulation. Although only three infants had no antigravity movements, we would like to emphasize that we did find an association between exposure to higher levels of six out of ten PCBs and absent antigravity movements. Our findings suggested a delay in motor development after prenatal exposure to higher levels of PCBs. These results of adverse neurobehavioral effects of PCBs were consistent with findings in animal studies. For a review see Faroon et al.[15]. Two studies suggested that OH-PCBs might be even more neurotoxic than PCBs[16,17]. In contrast, our findings in three-month-old infants suggested that exposure to OH-PCBs might be less neurotoxic compared to exposure to PCBs. A possible explanation for this difference might be the difference in exposure levels: the exposure to PCBs was higher compared to the exposure to OH-PCBs. This observation requires more detailed study.

Our last finding was that exposure to higher levels of PCB-118 was associated more frequently with cramped movements. The association of exposure to PCB-138 and the sum of the PCBs measured nearly reached significance with cramped movements. Exposure to PCB and OH-PCB levels was not associated with other abnormal

movement characters. In infants at high risk of cerebral palsy, Hamer et al. found that the presence of predominantly cramped movements at three months of age was associated with impaired gross motor development at eighteen months[18]. In infants who had developed spastic cerebral palsy, presence of cramped movements around three months of age was associated with a lower level of self-mobility at school age[19,20]. In contrast, Bruggink et al. were unable to demonstrate worse outcomes at school age in non-spastic preterm infants who had cramped movements at three months[21]. Further study is required to evaluate the relation between prenatal exposure to environmental toxins and the presence of cramped movements in three-month-old infants given the suggestion that these movements are prognostic of outcomes in later life.

Our findings can be explained in several ways. Although there are many theories on the way PCBs and their hydroxylated metabolites may affect neurodevelopment, the main hypothesis involves disruption of thyroid hormone homeostasis[22]. Thyroid hormones regulate neuronal proliferation and cell migration and differentiation, as well as the timing of these processes. Studies in rats showed that the transport of thyroid hormone to the brain requires thyroxine (T4) to pass through the blood-brain barrier by binding to the thyroid hormone transport protein transthyretin (TTR)[23]. A study on rats found that the metabolite 4-OH-PCB-107 binds to TTR in maternal and fetal plasma, suggesting that the binding of a compound to TTR *in vivo* could lead to facilitated maternal to fetal transfer, decreased maternal and fetal plasma T4 levels, and decreased fetal brain T4 levels[24]. Hydroxylated metabolites of PCBs show *in vitro* binding affinities as high as twelve times the binding affinity of the natural ligand T4[25]. According to these findings the binding of OH-PCBs to TTR may lead to brain thyroid hormone deficiencies *in utero,* possibly affecting brain development.
Our study confirmed that PCBs and OH-PCBs can influence development even though our population was merely exposed to background levels of PCBs. The PCB and OH-PCB levels measured in our study were lower than those found in cord plasma in Québec[26]. This could be explained by the higher level of contamination due to the greater consumption of fish by the people of Québec.

We point out several limitations of our study. We included the mother-infant pairs on a voluntary basis. Although we expected to have drawn a random sample of mothers from our region, a certain amount of bias on the part of the mothers cannot be ruled out. On the one hand, some mothers may have been aware of the concerns about these environmental pollutants and would perhaps have been selective about what they ate. On the other hand, other mothers may have been equally aware of the concerns but may have lacked the means of being selective about their food-intake and worried about what they had eaten as a consequence. Nevertheless, we believe that on the whole our sample is a valid representation of the population in our region.

A second limitation is that since the threshold levels of the toxicity of PCBs and OH-PCBs are unknown, we did not statistically test low versus high PCB and OH-PCB levels in relation to neurodevelopmental outcomes.
A final limitation is that we cannot exclude the possibility of confounding by other persistent organic pollutants. Nevertheless, it seems rather unlikely that these pollutants associated with only one PCB metabolite, i.e. 4- OH-PCB-107, to the exclusion of other metabolites in our study. Because we tested PCBs and OH-PCBs in relation to specific aspects of the quality of infants' motor repertoire, some results could be due to chance. Nevertheless, we believe that exploring different PCBs and OH-PCBs was justified as part of a careful evaluation of a rich data set in the context of hypothesis-driven research[27].

Conclusion

Prenatal exposure to PCBs, and to a lesser extent prenatal exposure to OH-PCBs, was associated with poorer quality of the motor repertoire of three-month-old infants. Some OH-PCBs were associated with a more optimal quality of the infants' motor repertoire. Our results suggested that prenatal exposure to background levels of PCBs and their metabolites, possibly mediated by disruption of the thyroid hormone homeostasis, may influence neurodevelopment in three-month-old infants both favourably and adversely.

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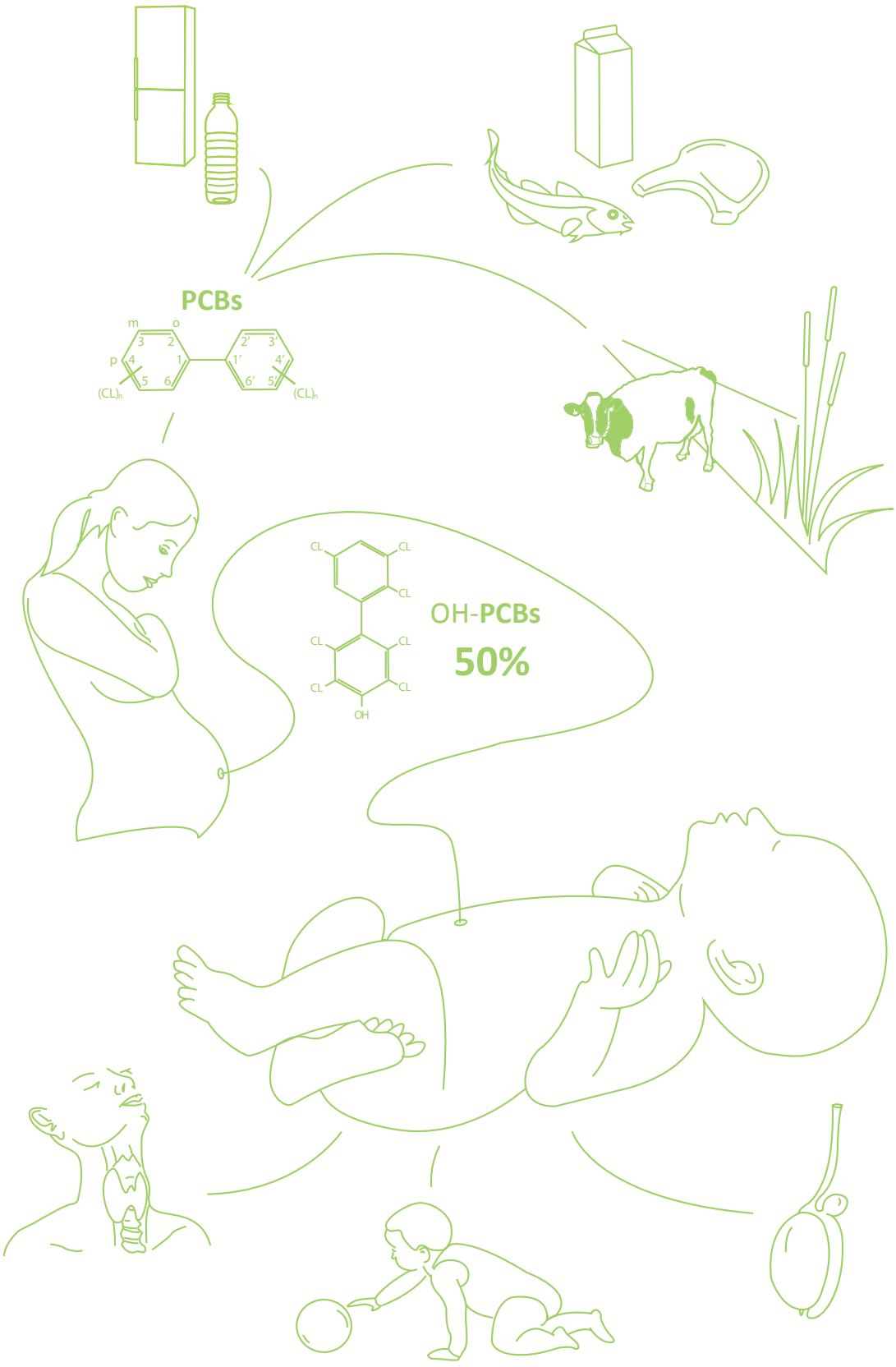
Discussion



Chapter 8

DISCUSSION

Discussion

The studies described in this thesis are part of a larger study, the Risk of Endocrine Contaminants on human health study (RENCO study, ENV4-CT96-0170). The European Community funded the RENCO study. It is a collaborative study including studies on human prenatal exposure to polychlorinated aromatic hydrocarbons (PCAH's) and animal experimental studies. The theses of Andreas Sjödin and Ilonka Meerts were part of this program. Andreas Sjödin conducted toxicological research especially on the identification and on the quantification of polybrominated diphenyl ethers. He also conducted research on the influence of the consumption of fatty Baltic Sea fish on the plasma levels of OH-PCBs in Latvian and Swedish men. Ilonka Meerts performed animal experiments on rats exposed to PCBs and OH-PCBs. In the present thesis, the levels of polychlorinated biphenyls (PCBs) and their metabolites, hydroxylated polychlorinated biphenyls (OH-PCBs), in pregnant women and in umbilical cord blood, are presented. Furthermore, possible endocrinological and neurological effects of these pollutants on early child development have been investigated.

The human infant part of the study was done at the Beatrix Children's Hospital, at the department of Pediatrics of the University Medical Center Groningen. The cohort was founded in and around the province of Groningen (the north eastern part of The Netherlands). From September 1998 through December 2000 pregnant women were invited to participate in this study by their midwife or obstetrician. The mothers had to be of Western European origin, and Dutch had to be their native language. In the second or third trimester of the pregnancy blood samples were taken from the mothers to screen the total TEQ level in maternal serum with the Chemical Acitvated LUciferase gene eXpression essay (CALUX). In total 215 pregnant women agreed to participate in the study. Based on the results of the Calux measurements, the 52 mothers with the highest levels (TEQ above 55 pgram/gram fat) as well as the 52 mothers with the lowest levels (below 21 pgram/gram fat) were selected and included in this study. At the Department of Environmental Chemistry of the Stockholm University, Sweden, ten PCBs and six OH-PCBs were measured in the 104 maternal and umbilical cord samples. All included infants had to be healthy, term born, not have been admitted to a hospital directly after birth for more than one day and had to be without neonatal complications or serious illnesses. All parents signed the informed consent and the Medical Ethical Committee of the University Medical Center Groningen approved the study.

According to the study protocol, a prospective longitudinal follow-up study has been done till the age of 18 months after birth. Growth, endocrine parameters and neurological development were measured in order to answer whether PCBs and OH-PCBs influence these outcome parameters or not.

Chapter 2

Fetal exposure to PCBs and their hydroxylated metabolites in a Dutch cohort. In chapter 2 we described the levels of 6 PCBs and 6 OH-PCBs in a first sample of 51 maternal and umbilical cord samples. In total 104 mother-infant pairs participated in the study. No significant differences were found between the first 51 mother-infant pairs and the whole group (104 mother-infant pairs) for population characteristics and for the levels of PCBs and OH-PCBs. For the analysis of the outcome data, a total of 10 PCBs and 6 OH-PCBs were analyzed in all samples. We found a strong correlation between the parent PCB and its OH-PCB metabolite in maternal blood as well as in umbilical cord blood. Transplacental transfer was investigated for PCBs and OH-PCBs. Both PCBs and OH-PCBs cross the placenta. The levels of OH-PCBs in cord plasma were approximately 50% of the levels found in the mothers. The placental transfer of OH-PCBs may be explained through their strong binding to the transport protein transthyretin (TTR). Secondly, as OH-PCBs are more water-soluble then the parent compounds, which are mainly fat-soluble, they might be transported easier over the placenta. PCBs are neutral lipophilic compounds strongly bound to lipids and therefore do not cross very well the over placenta. When they were expressed on a fat basis, almost the same levels were found in mothers and newborn infants.

Chapter 3

The comparison between the dioxin and PCB concentrations in human breast milk samples from Hong Kong and The Netherlands show, that the PCB levels in breast milk in The Netherlands (8.9-89.5ng/g fat) are fourfold higher than they are in Hong Kong (3.1-27.9mg/g fat). Higher, or at least equal, levels compared to The Netherlands were expected in the Hong Kong cohort. This would be due to the high urbanization and industrialization in Hong Kong and the high rate of fish consumption, as fish is known to be polluted by these compounds.

The Dutch levels are comparable to the data collected ten years prior to this cohort by Patandin et al. There might be several explanations for the difference between The Netherlands and Hong Kong. Hong Kong could for instance have a lower level of background pollution. The industrialization in The Netherlands started about a hundred years ago; while in Hong Kong it started only three to four decades ago. Any differences in dietary habits between both populations might explain the differences in exposure levels. Daily intake of fish and dairy products could explain the differences in body burden and thus in breast milk. The fish intake in Hong Kong is higher than in The Netherlands, but comes from the ocean and is less contaminated than the fish from rivers and coastal seas eaten in The Netherlands. The Dutch population also has a higher intake of dairy products (478 g/day) compared to 42 gram/day in Hong Kong. It has been estimated that 43-50% of PCB/dioxin intake is through the consumption of dairy products in The Netherlands.

Chapter 4

The serum levels of polychlorinated biphenyls and hydroxylated polychlorinated biphenyls in pregnant women over a 10-year period in one geographical area in The Netherlands illustrates that, although the use of PCBs in industrial products as coolants and lubricants was banned thirty years ago, the PCBs are still present in maternal plasma. In the last decade OH-PCBs are more and more in the picture, because of their potentially higher toxicity, which was discovered through animal studies. Dutch data are only available during a 3-4 years period. In this period there was almost a 50% decline in OH-PCB 107 and 187. However there was an increase of 60% in OH-PCB 146. This could be explained by the conversion of the parent compound PCB 153 into OH-PCB 146. A decline of 50% of PCB 153 was shown in a 10 years period. It is credible to assume that part of this decline in levels is related to the conversion of PCB 153 into OH-PCB 146 in animals, which are included in our food chain, and therefore could explain the increase of OH-PCB 146 with 60%.
In general there was a decrease of PCB levels in this 10 years period, but there is still concern about the impact of these pollutants on human health. In these 10 years (1991-2002) two European accidents have occurred with regard to PCB and dioxin pollution. In Belgium, in 1999, contamination appeared in animal products, mainly eggs and chickens, where especially PCB 153 and PCB 138 levels were detected. This was caused by the contamination of the feed. In Germany, in 2010, animal feed was contaminated with dioxin, which entered the human food chain via pork, eggs and chicken. Although in both accidents the level of pollution did not exceed the European Community standard, the effect on our food chain and the presence of health risks still needs to be investigated.

Chapter 5

No significant correlation between polychlorinated aromatic hydrocarbons (PCAHs), at three and eighteen months, and testicular volume of newborn infants in The Netherlands has been discoverded. In this study we did not find an effect of exposure to either PCBs or OH-PCBs on testicular volume or penile length in boys at three and eighteen month. A study by Den Hond et al.[1], conducted in Belgium, showed a lower testicular volume in adolescent boys exposed to higher levels of PCBs. In Belgium the serum concentration of summated PCB congeners 138, 153 and 180 averaged 107 ng per gram of blood fat. In our study the concentration of the summated PCBs (138,153 and 180) was 71 ng per gram lipid. Compared to the levels in Belgium and Germany (181 ng/g lipid)[2] the levels measured in this study in The Netherlands were lower than found in an earlier period (study of Koopman et al.).
In other studies correlations with PCBs are described for semen quality, hypospadia and crytorchidism at pubertal or adolescent age. Skakkebaek[3] states that the testicular dysgenesis syndrome, involving undescended testis, poor semen quality, hypospadias and testicular cancer, has increased in the last decades due to estrogenic

or anti androgenic hormonal pollution in the environment, such as PCBs and dioxins. The mild form of the syndrome may be as common as 20% of male population. The medium form was is being found by 5% of the males in Denmark and the most severe form (all four components) is extremely rare[3]. Cryptorchidism, infertility, breast-, prostate-, ovarian- and testicular cancer are reported in Spain[4] and Brazil[5] in relation to exposure to pesticides. These relations between environmental pollution and the effects on sexual development have also been described in animal studies. Meerts et al. described the differences in estrous cyclicity and plasma estradiol concentrations in female rats exposed to 4-OH-PCB 107. In the same study[6] no effects on sex organs were observed in male rats. The results of Meerts indicate that in utero exposure to OH-PCB 107 leads to reproductive changes that may reflect signs of reproductive senescence in female offspring at a relative early stage in life (11 month).
The fetus might be more vulnerable to environmental pollutants than adolescents or adults, as the fetus is in a phase of rapid development and adolescents or adults are not. Due to transplacental transfer the fetus is exposed to almost the same levels of pollutants as an adult. In our study we found a significant negative correlation between OH-PCB 153 and testicular volume at 3 months of age. Primarily, this can be explained by the fact that OH-PCB 153 has a higher affinity for the Ah receptor than its parent compound (PCB153) does. In other studies PCB 153 itself showed correlations with sexual development parameters due to its interaction with the CAG gene (part of the Ah receptor). Secondly, in our study the children are exposed to background levels of PCBs and OH-PCBs that presently might be too low to show an effect of the other compounds on testicular volume. Thirdly, the measurement of testicular volume at this young age might not be accurate enough to detect an effect of these compounds on sexual development. Although we only found a relation between OH-PCB 153 and testicular volume, other studies have shown correlations between endocrine disrupters and sexual development. The meta-analysis by Carlsen contributed to the impact of the theory that human male reproductive disorders may be related to the exposure to endocrine disruptors. A decrease in human male sperm count, a decline in seminal volume, a rise in cryptorchidism and hypospadias and an increased infertility can be seen from the nineteen fifties till recent years in The USA and Europe (Spain, Great Britain, Belgium, Sweden, Germany and Denmark).
There are different kinds of endocrine disruptors. All of them are manmade chemicals (such as DDT, PCB, Methoxychlor and phenolic derivatives). Although several endocrine disruptors are no longer being used in the developed countries, persistence in the environment cannot be excluded and others are still ubiquitous.

Chapter 6

Thyroid hormone levels in newborn infants are related to PCB exposure. Three-iodothyronine (T3) and rT3 levels in newborn infants are related to PCB exposure, which might cause a decreased activity of deiodinase type III (D3). D3 is mainly found in the placenta and in the fetal brain. D3 metabolizes thyroxin (T4) into rT3 and T3

into T2. In this study we show that PCBs have a negative correlation with the ratio T3-rT3, meaning that the activity of D3 has decreased. D3 in tissues, such as the brain, is thought to play a role in the regulation of intracellular T3 levels, while its presence in the placenta and fetal tissue may protect developing tissues against exposure to high levels of the active thyroid hormone[7]. The lower activity of D3 might be a compensation for a lower T4 supply from the mother to the fetus as result of lower maternal T4 levels, which is induced by POPs.

In our study we did not find a relation between PCBs and T4 levels in the umbilical cord. Deficiency in thyroid hormones can explain neurological developmental defects in children. Brain development depends on appropriate levels of thyroid hormones during critical developmental periods. Thyroid hormones regulate glial cell proliferation and differentiation. Thyroid hormones cross the placenta and enter the brain primarily as T4. With humans the thyroid hormone secretion starts at approximately 10-12 weeks of gestation, in a rat at 17 days of gestation. In the fetal brain T4 is converted into T3 by the enzyme D3. T3 is the predominant form of the hormone binding to the thyroid receptor. PCBs show a structural resemblance to thyroid hormones. PCBs have an effect through the Aryl Hydrocarbon receptor, can compete with the thyroxin binding protein transthyretin (TTR) and can have an effect on the thyroid transport system. PCBs can suppress serum T4 in pregnant women. T3 is the predominant form in which thyroid hormones have their activity in the brain; therefore low T3 levels potentially harm neurological function without impairing metabolism (Porterfield 1994). The neuro-toxic effects of PCBs are associated with a fairly specific profile of cognitive impairment, especially for the executive functions (planning, working memory and response inhibition). Also negative effects have been observed for speed of information processing, verbal abilities and visual recognition memory. Areas of the brain sensitive to developmental actions of thyroid hormone are also: the basal forebrain, the cerebral cortex, the hippocampus and the cerebellum. All these brain functions are located in the prefrontal cortex and it is suggested that PCB exposure may influences the development of this part of the brain.

Chapter 7

Prenatal exposure to polychlorinated biphenyls and their hydroxylated metabolites is associated with the quality of the motor repertoire in three-month-old infants.

The most important outcome of this part of the study is that exposure to higher 4-OH-PCB-107 levels is associated with a significantly reduced movement repertoire. These outcomes, that prenatal exposure to higher 4-OH-PCB-107 levels is associated with a deterioration of the motor repertoire, whereas exposure to other OH-PCBs is not, confirmed the results of previous studies with animals and humans. This metabolite has proven to induce long-term effects on behavior- and neuro-development in rats, as shown in a study with rats by Meerts et al.[8].

The second important outcome is that exposure to higher levels OH-PCBs and PCBs are associated with several aspects in the subcategory 'repertoire of co-existent other movements'. These outcomes suggest a delay in motor development after prenatal exposure to higher levels of PCB´s. These results on neurobehavioral effects of PCB´s are consistent with the outcomes in animal studies[9].
Our third point is that exposure to higher levels of PCB 118 is associated with a more frequent presence of a cramped character.
Although there are many theories about how PCBs and OH-PCBs affect neurodevelopment, the main hypothesis involves the disruption of thyroid hormone homeostasis[10]. Thyroid hormones regulate neuronal proliferation, cell migration and differentiation, including the timing of these processes. Studies with rats showed us that the transport of the thyroid hormone to the brain requires thyroxine (T4) to pass through the blood-brain barrier by binding itself to the thyroid hormone transport protein transthyretin (TTR)[11]. Meerts et al. detected in rats that the metabolite 4-OH-PCB-107 was bound to the TTR in maternal and fetal plasma, which suggests that binding of a compound to the TTR *in vivo* could lead to facilitated maternal to fetal transfer of that compound, decreased maternal and fetal plasma T4 levels and decreased fetal brain T4 levels[12]. OH-PCBs have shown high *in vitro* binding affinities to TTR, which can be as high as 12 times the binding affinity of the natural ligand T4[13]. According to these outcomes, the binding of OH-PCBs to TTR might lead to brain thyroid hormone deficiencies *in utero,* which could possibly affect brain development.

Conclusion

Studies performed after accidental high exposure during pregnancy to PCBs have shown negative effects of this exposure on newborn infants. Moreover, studies have also discovered effects in newborn infants due to background exposure during pregnancy. Negative effects were seen on thyroid and sex hormones, birth weight, neurodevelopment and the immune system. The transplacental transfer of the compounds, as shown in different studies, causes these effects. Those negative effects have especially been observed in newborn infants and are related to the interference of these pollutants with rapidly developing organs in the fetus. Not all studies have shown effects of antenatal exposure to PCBs in newborn infants. This could –partly- be explained by the variation in exposure of the mothers to different PCB congeners. Different PCBs might have other, sometimes opposing effects. Moreover, it is still unclear whether the effects discovered in infants are due to the PCBs themselves or to their degradation products, OH-PCBs.
In the present study we have proven that levels of PCBs in maternal blood have been decreasing over the past 10 years, which is a result of the ban on the production of these compounds 40 years ago. Still, despite this ban on the production long ago, almost all congeners studied were at detection level in maternal blood. Moreover, we

also found a number of OH-PCBs not only in maternal but also in cord blood, indicating the presence of these products in the fetal plasma and the transfer to the fetus.

In this study we did not find any effects of PCBs, at the levels present in 2000, on thyroid hormones. Yet, we did find that these compounds might be related to the activity of type III deiodinase, an enzyme, which converts T3 in the inactive hormone rT3. Whether or not this might have clinical implications still needs further investigation.

Contrary to previous studies, we did not find effects of PCBs on testicular volume as an indicator of sexual development in newborn boys. We did however find a negative correlation between OH-PCB 153 and testicular volume at three months. Whether or not OH-PCBs might have a lasting effect on sexual development at a later age needs to be investigated further.

We observed correlations between PCBs and OH-PCBs and general movements in newborn infants. This outcome causes concern, because abnormal general movements at an early age are related to non-optimal development at a later age and therefore needs further follow-up.

Although the background PCB levels in The Netherlands might have been too low in 2000 (and can be expected to be lower today) to cause evident negative health effects in newborn infants, concerns remain. Studies in earlier cohorts have shown effects at 6-9 years of age. Subtle effects of these compounds might not be visible at a very young age, but may become detectable at later ages. A follow-up of the cohort described in this thesis at a later age therefore is needed to ascertain that those background levels in the Netherlands are presently so low that they do not cause negative health effects. Moreover, PCBs are –partly- replaced by compounds like brominated flame retardants, which might have similar negative health effects. Efforts to decrease the contamination of this planet by manmade compounds, which might have negative health effects on humans and animals, therefore remain very important.

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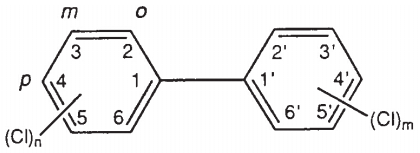
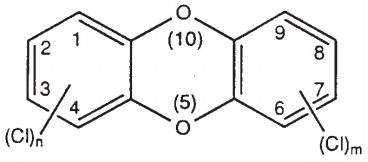
SAMENVATTING

Samenvatting

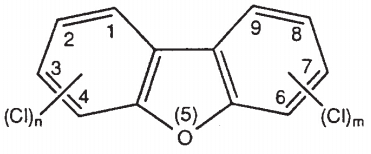
PCBs, polychloorbiphenylen zijn lipofiele toxische verbindingen die op grote schaal geproduceerd werden in de jaren '30-'70. Ze werden gebruikt in hydraulische vloeistoffen, plastics, warmte geleidingstransformatoren, vlam vertragers etc. Deze PCB bronnen worden open systemen genoemd omdat de mens er direct aan blootsgesteld kan zijn. PCBs komen ook voor in gesloten systemen zoals diëlectrische vloeistoffen in koeltransformatoren. Bij het vernietigen van deze apparaten lekken de PCBs in het milieu.
Dioxinen worden gevormd als bijproducten tijdens verbrandingsprocessen van organochloorverbindingen. Een van de belangrijkste bronnen van dioxine vervuiling in Nederland is de verbranding van afval. Door installatie van beter absorberende filters in de vuilverbrandingfabrieken is deze bron van vervuiling sinds enkele decenia afgenomen. Niet alleen tijdens vuilverbranding komen deze stoffen vrij, maar ook in het verkeer en b.v. ook tijdens roken.
Het vaststellen van deze stabiele componenten in het milieu leidde in de tachtiger jaren van de vorige eeuw tot een wereldwijd verbod op het gebruik van deze stoffen. Ondanks dit verbod zijn er toch nog steeds landen die deze stoffen produceren voor gebruik in transformatoren.
Net als PCBs zijn dioxinen zeer stabiele stoffen met een half-waarde tijd van 2-7 jaar afhankelijk van de congeneer (PCB variant). Deze stoffen worden in het lichaam omgezet tot andere stoffen (meer of minder toxisch).



Polychlorinated biphenyls (PCBs)



Polychlorinated dibenzo-p-dioxins (PCDDs)

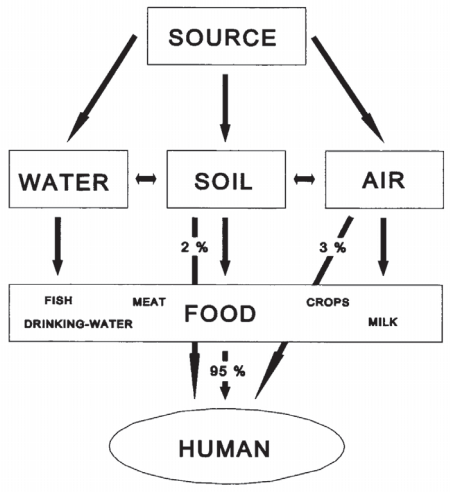


Polychlorinated dibenzofurans (PCDFs)

O= Ortho position M= Meta position P=Para position

PCBs en dioxinen zijn aromatische verbindingen met verschillende mogelijkheden voor de substitutie van het chlooratoom. Er zijn 209 verschillende PCB-congeneren en 210 verschillende dioxine congeneren. Vanwege de dubbele binding zijn dioxinen planaire tricyclische aromatische componenten welke toxischer zijn dan de PCBs die een enkele binding hebben tussen de fenylringen en zijn dus ook minder planair.

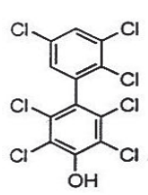
PCBs en dioxinen zijn stoffen die vanuit het milieu bij de mens terecht komen. Via water, bodem en lucht vervuiling komt het in de voeding terecht en via de voeding bij de mens, waar ze opgeslagen worden in het vetweefsel. Tijdens de zwangerschap bereiken deze stoffen via de placenta het nog ongeboren kind. Omdat het lipofiele stoffen zijn is de overdracht over de placenta lager dan bij water oplosbare stoffen. De gehaltes zijn een factor 4 lager dan bij de moeder.



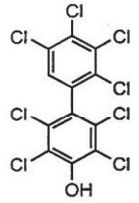
In dierexperimentele studies zijn vele effecten gevonden m.n. van pre- en vroeg postnatale belasting die sterk overeenkomen met de effecten gevonden bij humane studies.
In humane studies is m.n. de prenatale belasting gerelateerd aan negatieve effecten. Dit blijkt zowel uit studies waarbij door ongelukken blootstelling plaats vond aan grote hoeveelheden van deze stoffen als uit longitudinale studies met een achtergrond belasting van deze stoffen.

Uit dierstudies van de laatste 10 jaar is naar voren gekomen dat PCBs en dioxinen omgezet worden tot OH-PCBs welke een meer toxische invloed hebben op de ontwikkeling van de foetus, met name de endocriene ontwikkeling.
OH-PCBs lijken sterk op het schildklierhormoon. Ze zijn beide meervoudig gehalogeneerde componenten met 2 phenylringen. Vanwege deze sterke gelijkenis

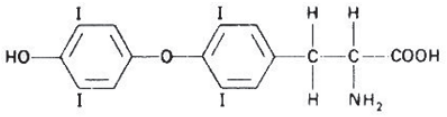
zullen afhankelijk van de diersoort, expositie en de congeneer deze stoffen functioneren als agonist, antagonist of partiele agonist van het schildklierhormoon.



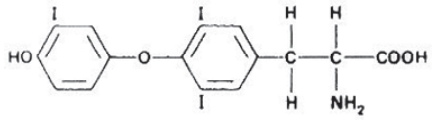
4-OH-178



4-OH-199



L-Thyroxine (T_4)
(3, 5, 3′, 5′-tetraiodothyronine)



L-Triiodothyronine (T_3)
(3, 5, 3′-triiodothyronine)

De studies beschreven in dit proefschrift zijn onderdeel van een grotere studie, een studie die vanuit breder perspectief heeft gekeken naar het risico van milieu verontreinigingen op endocriene effecten bij de mens (RENCO studie, ENV4-CT96-0170). De RENCO studie werd gefinancierd door de Europese Gemeenschap. In deze studies is onderzocht wat het effect is van menselijke prenatale blootstelling aan gechloreerde aromatische koolwaterstoffen (PCAH's). Tevens zijn de toxische effecten van deze stoffen onderzocht en aangetoond in dierexperimentele studies. Het proefschrift van Andreas Sjödin en Ilonka Meerts maakten deel uit van deze RENCO studie. Andreas Sjödin verrichte toxicologisch onderzoek vooral naar de identificatie en kwantificering van de polybroomdifenylethers en ook de invloed van de consumptie van vette Baltische zee vis op de plasmaspiegels van OH-PCBs bij Letse en Zweedse mannen. Ilonka Meerts heeft dierproeven met ratten gedaan die blootgesteld waren aan PCBs en OH-PCBs.
In dit proefschrift is onderzoek gedaan naar de spiegels van polychloorbifenylen (PCBs) en hun metabolieten, gehydroxyleerde polychloorbifenylen (OH-PCBs), bij zwangere vrouwen en in navelstrengbloed, de resultaten van dit onderzoek worden alhier gepresenteerd. Daarnaast zijn de mogelijk endocrinologische en neurologische effecten van deze verontreinigende stoffen op de vroege ontwikkeling van het kind onderzocht.

Het onderzoek bij zwangere vrouwen en de pasgeborenen werd uitgevoerd vanuit het Beatrix Kinderziekenhuis van het Universitair Medisch Centrum Groningen. Het cohort

werd samengesteld in en rond de provincie Groningen. Vanaf september 1998 tot en met december 2000 werden zwangere vrouwen uitgenodigd door hun verloskundige of gynaecoloog om deel te nemen aan dit onderzoek. De moeders moesten van West-Europese afkomst zijn, en het Nederlands moest hun moedertaal zijn. In het tweede of derde trimester van de zwangerschap werden bloedmonsters genomen van de moeders om het totale TEQ-gehalte (toxiciteit van een mengsel van PCBs) in serum van de moeder te screenen met de Chemical Acitvated luciferasegenexpressie essay (CALUX). In totaal hebben 215 zwangere vrouwen ingestemd met deelname aan het onderzoek. Gebaseerd op de resultaten van de metingen met de CALUX, zijn 52 moeders met het hoogste niveau (TEQ >55 pgram / gram vet) en 52 moeders met de laagste TEQ(<21 pgram/gram vet) geselecteerd voor deze studie. In het Department of Environmental Chemistry van de Universiteit van Stockholm, Zweden, zijn tien PCBs en zes OH-PCBs gemeten in de 104 moeders en navelstreng monsters. Alle pasgeborenen moesten gezond zijn, a-term geboren, niet opgenomen te zijn geweest in een ziekenhuis direct na de geboorte voor meer dan een dag en tevens mocht er geen sprake zijn neonatale complicaties of ernstige ziekte. Alle ouders ondertekenden een informed consent en de studie werd goedgekeurd door de Medisch Ethische Commissie van het Universitair Medisch Centrum Groningen.

Volgens het studie protocol, werd een prospectieve longitudinale follow-up studie gedaan tot de leeftijd van 18 maanden na de geboorte. Groei en endocriene parameters en de neurologische ontwikkeling werd gemeten om de vraag te beantwoorden of PCBs en OH-PCBs invloed hebben op deze uitkomst parameters.

Hoofdstuk 2 beschrijft de foetale blootstelling aan 6 PCBs en 6 gehydroxyleerde metabolieten (OH-PCBs) in een Nederlands cohort in een eerste serie monsters van 51 moeders en gekoppelde navelstreng monsters. Aan de studie namen in totaal 104 moeder-kind paren deel. Er werden geen significante verschillen gevonden tussen de eerste 51 moeder-kind paren en de hele groep (n=104) voor bevolkingskenmerken en voor de concentraties van PCBs en OH-PCBs. De resultaten zijn gebaseerd op een totaal van 10 geanalyseerde PCBs en 6 OH-PCBs in alle samples. We vonden een sterke correlatie tussen de PCBs en de OH-PCB metabolieten in maternale in relatie met navelstreng samples. Transplacentair overdracht werd onderzocht voor PCBs en OH-PCBs. Zowel PCBs en OH-PCBs passeren de placenta. De spiegels van de OH-PCBs en PCBs in foetaal plasma was respectievelijk 50% en 30% van de spiegels die in het maternaal plasma werd gevonden. De hogere placenta overdracht van OH-PCBs kan worden verklaard uit hun sterke binding aan het transporteiwit transthyretine (TTR). Ten tweede zijn OH-PCB meer wateroplosbaar dan de oorspronkelijke verbindingen (PCBs) die hoofdzakelijk vet oplosbaar zijn, waardoor OH-PCBs gemakkelijker getransporteerd worden over de placenta. PCBs zijn neutrale lipofiele stoffen die sterk binden aan lipiden(vetten) en dus minder makkelijk de placenta barrière passeren.

Uitgedrukt op basis van het vetgehalte, werden bijna dezelfde spiegels gevonden in moeders en hun pasgeboren baby's.

In **hoofdstuk 3** worden dioxine- en PCB concentraties vergeleken in moedermelk monsters uit Hong Kong en Nederland. Dit onderzoek laat zien dat de PCB spiegels in moedermelk bij Nederlandse vrouwen (8.9-89.5 ng/g vet) vier keer hoger is dan de spiegels van moedermelk monsters uit Hong Kong (3.1-27.9 mg/g vet). Hogere of ten minste een niveau gelijk aan spiegels in Nederland werd verwacht in de Hong Kong cohort, vanwege de hoge verstedelijking en industrialisatie in Hong Kong en de hoge visconsumptie, waarvan bekend is dat deze verontreinigd kan zijn door PCBs en dioxinen.
De Nederlandse PCB/dioxine spiegels zijn vergelijkbaar met gegevens die tien jaar eerder verzameld zijn in het cohort van Patandin et al.. Er kunnen verschillende redenen zijn om het verschil tussen Nederland en Hong Kong te verklaren. De industrialisatie in Nederland begon ongeveer honderd jaar geleden, in tegenstelling tot Hong Kong, waar het drie tot vier decennia geleden begon. Dit kan maken dat Hong Kong een lager niveau van achtergrond vervuiling heeft. Verschillen in voedingsgewoonten tussen beide populaties kunnen ook de verschillen in de blootstelling verklaren. Dagelijkse inname van vis en zuivelproducten kunnen de verschillen in belasting van het lichaam en dus in moedermelk verklaren. De vis inname in Hong Kong is hoger dan in Nederland, maar komt uit de oceaan en is minder vervuild dan vis uit rivieren en kustwateren zoals gegeten in Nederland. De Nederlandse bevolking heeft ook een hogere inname van zuivelproducten (478 g/dag) vergeleken met 42 gram/dag in Hong Kong. Er wordt geschat dat 43 tot 50% van het PCB/dioxine-inname komt door de consumptie van zuivelproducten in Nederland.

Hoofdstuk 4 geeft een overzicht van serumspiegels van polychloorbifenylen en gehydroxyleerde polychloorbifenylen bij zwangere vrouwen over een periode van 10 jaar in Nederland weer. Deze studie illustreert dat, hoewel het gebruik van PCBs in industriële producten als koel- en smeermiddelen dertig jaar geleden werd verboden, de PCBs nog steeds aanwezig zijn in plasma van de moeder. In het laatste decennium zijn OH-PCBs meer en meer in de belangstelling gekomen, vanwege hun potentieel hogere toxiciteit, die werd gevonden in dierstudies. Nederlandse OH-PCB gegevens zijn alleen beschikbaar over een periode van 3-4 jaar. In deze periode is er bijna een 50% daling van de OH-PCB 107 en 187. OH-PCB 146 toont echter een stijging van 60%. Dit kan verklaard worden door de omzetting van de oorspronkelijke stof PCB 153 in OH-PCB 146. PCB 153 toont een 50% daling in een periode van 10 jaar. Het is aannemelijk dat een deel van deze daling in spiegels gerelateerd is aan de omzetting van PCB 153 in OH-PCB 146 in dieren die betrokken zijn in onze voedselketen, en zou dus de toename van OH-PCB 146 met 60% kunnen verklaren. In het algemeen is er een daling van de PCB spiegels in deze periode van 10 jaar, maar de bezorgdheid over de gevolgen van deze verontreinigende stoffen op de menselijke gezondheid blijft

aanwezig. In deze 10 jaar (1991-2002) hebben zich twee Europese ongevallen voorgedaan met betrekking tot verontreinigingen met PCBs en dioxines. In België heeft in 1999 PCB vervuiling plaatsgevonden van dierlijke producten, voornamelijk eieren en kippen, waarbij met name PCB 153 en PCB 138 werden gedetecteerd. Dit werd veroorzaakt door verontreiniging in diervoedersbedrijven. In Duitsland werd in 2010 diervoedsel besmet met dioxine, die in de voedselketen kwam via varkensvlees, eieren en kip. Hoewel in beide ongevallen het niveau van de vervuiling niet boven de door de Europese Gemeenschap gestelde norm kwam, moet het effect op onze voedselketen en de aanwezigheid van risico voor de gezondheid nog onderzocht worden.

In **hoofdstuk 5** wordt geen significante correlatie gevonden van blootstelling aan zowel PCBs of OH-PCBs op het testiculaire volume of penislengte bij jongens op drie en achttien maand. In onze studie vonden we een significante negatieve correlatie tussen OH-PCB 153 en testiculaire volume op 3 maanden. Ten eerste zou dit verklaard kunnen worden door het feit dat OH-PCB 153 een hogere affiniteit voor de Ah-receptor heeft dan de moederverbinding (PCB153). Ten tweede zijn de kinderen in onze studie blootgesteld aan achtergrondniveaus van PCBs en OH-PCB die momenteel te laag zouden kunnen zijn om een effect op het testiculaire volume te kunnen geven. Ten derde kan de meting van testiculaire volume op jonge leeftijd niet nauwkeurig genoeg zijn om een effect van deze verbindingen te detecteren ten aanzien van de seksuele ontwikkeling. Hoewel we alleen een relatie tussen OH-PCB 153 en testiculaire volume vonden zijn in andere studies correlaties aangetoond tussen PCBs en stoffen zoals ftalaten en gebromeerde verbindingen en seksuele ontwikkeling.

Een studie van Den Hond et al.[1], uitgevoerd in België, toonde een lagere testisvolume bij adolescente jongens blootgesteld aan hogere niveaus van PCBs. In België is de serumconcentratie van gesommeerd PCB-congeneren 138, 153 en 180 gemiddeld 107 ng per gram vet in het bloed. In onze studie is de concentratie van de gesommeerde PCB (138,153 en 180) 71 ng gram per gram vet. Vergeleken met spiegels in België en Duitsland (181 ng/g lipide)[2] zijn in deze studies de gemeten spiegels in Nederland lager dan in een eerdere periode (studie Koopman-Esseboom et al.).
In andere studies is aangetoond dat PCB 153 een correlatie heeft met parameters voor seksuele ontwikkeling door interactie met de CAG gen (deel van de Ah receptor). In die studies zijn correlaties met PCBs beschreven voor sperma kwaliteit, hypospadie en cryptorchisme op puberale en adolescente leeftijd. Skakkebaek[3] heeft het testiculaire dysgenesis syndroom beschreven, waarbij niet-ingedaalde testis, slechte kwaliteit van het sperma, hypospadie en teelbalkanker als diagnose samen kunnen voorkomen. De laatste decennia is dit syndroom toegenomen mogelijk als gevolg van oestrogene of anti androgene hormonale verontreinigingen van het milieu, zoals PCBs en dioxines. In ongeveer 20% komt de milde vorm van het syndroom voor. De matig

ernstige vorm werd gevonden bij 5% van de mannen in Denemarken en de meest ernstige vorm (alle vier de onderdelen) is uiterst zeldzaam[3]. Cryptorchisme, onvruchtbaarheid, borst-, prostaat-, ovarium-en testiculaire kanker zijn in Spanje[4] en Brazilië[5] aangetoond met betrekking tot blootstelling aan pesticiden. Deze relaties tussen milieuverontreiniging en effecten op seksuele ontwikkeling zijn ook beschreven in dierstudies. Meerts et al. beschreven verschillen in oestrogeencyclus en plasma oestradiol concentraties in vrouwelijke ratten blootgesteld aan 4-OH-PCB 107. In dezelfde studie[6] zijn geen effecten op geslachtsorganen bij mannelijke ratten waargenomen. De resultaten van Meerts beschrijven dat in utero blootstelling aan 4-OH-PCB 107 leidt tot voortplantingsveranderingen waarbij sprake is van voortplantingsveroudering bij vrouwelijke nakomelingen in een relatief vroege stadium (11 maand).
De foetus is kwetsbaarder voor milieuverontreinigende stoffen dan adolescenten of volwassenen, omdat de foetus zich in een fase van snelle ontwikkeling bevindt in tegenstelling tot adolescenten of volwassenen. Door transplacentair overdracht wordt de foetus blootgesteld aan bijna dezelfde spiegels van verontreinigende stoffen als een volwassene.
De meta-analyse van Carlsen heeft bijgedragen aan de impact van de theorie dat de menselijke mannelijke reproductieve stoornissen verband kunnen houden met blootstelling aan hormoon ontregelaars. Een daling van de menselijke mannelijke zaadcellen, daling van het rudimentaire volume, een stijging van cryptorchisme en hypospadie en een verhoogde onvruchtbaarheid werd gevonden in de jaren vijftig tot en met de laatste jaren in de VS en Europa (Spanje, Groot-Brittannië, België, Zweden, Duitsland en Denemarken).
Er zijn verschillende soorten van hormoon verstorende stoffen. Het zijn allemaal door de mens gemaakte chemicaliën (zoals DDT, PCB, Methoxychlor en fenolderivaten). Hoewel verscheidene hormoonontregelende stoffen niet meer worden gebruikt in de ontwikkelde landen, toch kan persisteren in het milieu niet worden uitgesloten. Andere toxische stoffen zijn nog steeds alom vertegenwoordigd.

In **hoofdstuk 6** zijn verschillende schildklierhormonen bepaalt in relatie tot PCB en OH-PCB. In diverse onderzoeken komt naar voren dat schildklierhormoon spiegels bij pasgeborenen gerelateerd zijn aan PCB blootstelling. In ons onderzoek hebben we geen verband gevonden tussen PCBs en T4 spiegels in navelstreng. Daarentegen zien we dat three-iodothyronine (T3) en rT3 spiegels bij pasgeborenen zijn gerelateerd aan PCBs wat samen kan hangen met een verlaagde activiteit van deiodinase type III (D3). D3 komt voornamelijk voor in de placenta en de foetale hersenen. D3 metaboliseert thyroxine (T4) in rT3 en T3 in T2. Van D3 in weefsels, zoals de hersenen, wordt verondersteld dat het een rol speelt in de regulatie van intracellulaire niveaus van T3, terwijl de aanwezigheid in placenta en foetaal weefsel bescherming zou bieden om ontwikkelende weefsels te beschermen tegen blootstelling aan hoge schildklier-hormoon (T4) gehaltes[7]. De lagere activiteit van D3 kan een compensatiemechanisme

zijn voor een lagere T4 levering vanuit de moeder naar de foetus als een mogelijk gevolg van lagere maternale T4 spiegels veroorzaakt door POP's.

Tekort aan schildklierhormonen kan neurologische ontwikkelingsstoornissen geven bij kinderen. Ontwikkeling van de hersenen is afhankelijk van de juiste hoeveelheden schildklierhormonen tijdens kritieke ontwikkelingsperiodes. Het schildklierhormoon reguleert de gliacel proliferatie en differentiatie. Schildklierhormonen passeren de placenta en de hersenen voornamelijk als T4. Bij de mens begint de schildklier-hormoon secretie bij ongeveer 10-12 weken zwangerschapsduur, in een rat is dit vanaf 17 dagen van de dracht. In de foetale hersenen wordt T4 omgezet in T3 door het enzym D3. T3 is de belangrijkste vorm van het hormoon wat bindt aan de schildklierreceptor. PCBs vertonen een structurele gelijkenis met schildklierhormonen. PCBs hebben een effect via arylkoolwaterstofreceptor en concurreren met het thyroxine bindend eiwit, transthyretine (TTR) wat een effect heeft op het schildklier transport systeem. PCBs kunnen serum T4 onderdrukken bij zwangere vrouwen. T3 is de belangrijkste schildklierhormonen die actief is in de hersenen, daardoor zijn lagere intracellulaire T3 spiegels schadelijk voor de neurologische functie zonder dat dit in de schildklierstofwisseling zichtbaar is (Porterfield 1994).
De neuro-toxische effecten van PCBs worden geassocieerd met een vrij specifiek profiel van cognitieve stoornissen, in het bijzonder voor de uitvoerende functies (plannen, werkgeheugen en respons inhibitie). Ook zijn negatieve effecten waargenomen voor de snelheid van informatieverwerking, verbale vaardigheden en visuele herkenning. Al deze hersenfuncties bevinden zich in de prefrontale cortex en er wordt gesuggereerd dat PCB blootstelling dit deel van de hersenenontwikkeling kan beïnvloeden. Gebieden van de hersenen die het schildklierhormoon nodig hebben voor hun ontwikkeling zijn ook: de basale voorhersenen, cerebrale cortex, hippocampus en cerebellum. De impact die PCBs hebben op D3, zoals gevonden in ons onderzoek kan een eerste verklaring zijn voor bovenstaande cognitieve problemen.

In **hoofdstuk 7** is onderzocht wat de invloed is van prenatale blootstelling aan PCBs en de OH-PCBs op de kwaliteit van motorische bewegingen (motor repertoire) bij drie maanden oude baby's. Het belangrijkste resultaat in dit deel van de studie is dat blootstelling aan hogere 4-OH-PCB-107 spiegels geassocieerd is met een significant verminderde motor repertoire. Deze resultaten, dat prenatale blootstelling aan hogere 4-OH-PCB-107 spiegels geassocieerd is met een achteruitgang van de motor repertoire, terwijl blootstelling aan andere OH-PCBs niet dit resultaat tonen. Dit bevestigt de resultaten gevonden in eerdere studies bij dieren en mensen. Deze metabolieten hebben aangetoond van invloed te zijn op lange termijn effecten van gedrags- en neurologische ontwikkelingen bij ratten, zoals in een studie met ratten door Meerts et al.[8].

De tweede belangrijke uitkomst is dat blootstelling aan hogere spiegels van OH-PCBs en PCBs zijn geassocieerd met een aantal aspecten in de subcategorie “repertoire of co-existent other movement”. Deze uitkomsten suggereren een vertraging in de motorische ontwikkeling na prenatale blootstelling aan hogere spiegels van PCBs. Ook deze resultaten van neurologische effecten van PCBs komen overeen met resultaten in dierstudies[9].
Het derde resultaat is dat blootstelling aan hogere PCB118 spiegels is geassocieerd met een meer frequente aanwezigheid van een verkrampte beweging.

Hoewel er veel theorieën zijn over hoe PCBs en OH-PCBs die de neurologische ontwikkeling beïnvloeden, betreft de belangrijkste hypothese verstoring van de schildklierhormoon homeostase[10]. Schildklierhormonen regelen neuronale proliferatie en cel migratie en differentiatie, met inbegrip van de timing van deze processen. Studies met ratten toonden aan dat transport van het schildklierhormoon thyroxine (T4) naar de hersenen het schildklierhormoon transporteiwit transthyretine (TTR)vereist om de bloed-hersenbarrière te passeren[11]. Meerts et al. stelde bij ratten vast dat de metaboliet 4-OH-PCB-107 zich bindt aan TTR van maternale en foetale plasma, wat suggereert dat binding van een hormoon ontregelaar aan TTR in vivo, kan leiden tot een vergemakkelijkte materno-foetale overdracht van die hormoon ontregelaar, een verminderde maternale en foetale plasmaspiegels van T4 en verminderde T4 spiegels in foetale hersenen[12]. OH-PCBs blijken in vitro een hoge bindingsaffiniteit te hebben voor TTR, die kan oplopen tot 12 keer de bindingsaffiniteit voor het natuurlijke ligand T4[13]. Volgens deze uitkomsten, kan de binding van OH-PCBs aan TTR leiden tot tekortkomingen in schildklierhormoonhuishouding in de hersenen, in utero, wat de ontwikkeling van de hersenen zou kunnen beïnvloeden.

Conclusie

Studies uitgevoerd na accidentele hoge blootstelling tijdens de zwangerschap aan PCBs hebben negatieve effecten aangetoond van deze blootstelling op pasgeboren baby's. Daarna tonen studies ook effecten aan bij pasgeboren baby's als gevolg van achtergrond blootstelling aan PCBs tijdens de zwangerschap. Negatieve effecten werden gezien op de schildklier en geslachtshormonen, geboortegewicht, neurologische ontwikkeling en het immuunsysteem. Deze effecten worden veroorzaakt door transplacentair transport van de verbindingen, zoals in verschillende studies aangetoond. Deze ongunstige gevolgen zijn waargenomen bij pasgeborenen en hebben betrekking op de interferentie van deze stoffen met snel ontwikkelende organen in de foetus. Niet alle studies hebben effecten aangetoond van prenatale blootstelling aan PCBs bij pasgeborenen. Dit kan deels worden verklaard door de variatie in blootstelling van de moeder aan verschillende PCB-congeneren. Verschillende PCBs kunnen andere en soms tegengestelde effecten hebben.

Bovendien is nog onduidelijk of de gevonden effecten bij zuigelingen komen door PCBs zelf of hun afbraakproducten, de OH-PCBs.
In deze studie hebben we aangetoond dat PCB spiegels in maternaal bloed zijn afgenomen in de afgelopen 10 jaar, wat een gevolg is van het verbod op de productie van deze verbindingen 40 jaar geleden. Ondanks dat dit verbod op de productie zo lang geleden is, zijn bijna alle onderzochte congeneren nog meetbaar in maternaal bloed. Ten tweede vonden we een aantal OH-PCBs niet alleen in maternaal bloed, maar ook in navelstrengbloed, wat wijst op de aanwezigheid van deze stoffen in foetale plasma en overdracht van deze stoffen naar de foetus.
In deze studie hebben we geen effecten gevonden van PCBs op schildklierhormonen, bij spiegels zoals gevonden in 2000. Wel vonden we dat deze verbindingen mogelijk een relatie toonden met de activiteit van type III deiodinase, een enzym wat T3 omzet in het inactieve hormoon rT3. Of dit klinische implicaties zou kunnen hebben vergt verder onderzoek.
In tegenstelling tot eerdere studies hebben we geen effecten gevonden van PCBs op testisvolume als indicator van de seksuele ontwikkeling bij pasgeboren jongens. Er was echter een negatieve correlatie tussen OH-PCB 153 en testisvolume op drie maanden. Of OH-PCBs een blijvend effect kunnen hebben op de seksuele ontwikkeling op latere leeftijd, behoefte meer onderzoek.
We vonden correlaties tussen PCBs en OH-PCBs en motor repertoire bij pasgeborenen. Abnormale motor repertoire is gerelateerd aan een niet-optimale ontwikkeling op latere leeftijd. Deze uitkomsten geven aanleiding tot bezorgdheid en follow-up onderzoek moet ook hier volgen.
Hoewel achtergrond gehalten aan PCBs in Nederland waarschijnlijk te laag zijn geweest in 2000 (en naar verwachting momenteel nog lager is) om evident negatieve effecten op de gezondheid bij pasgeborenen te veroorzaken, blijft de bezorgdheid voor de gezondheid bestaan. Studies in eerdere cohorten toonden negatieve effecten op de leeftijd van 6-9 jaar. Subtiele effecten van deze stoffen zijn misschien niet zichtbaar op zeer jonge leeftijd, maar kan aantoonbaar worden op latere leeftijd. Een follow-up van het beschreven cohort in dit proefschrift op latere leeftijd is dan ook nodig om na te gaan of de achtergrondbelasting in Nederland op dit moment zo laag is dat ze geen negatieve gevolgen veroorzaken voor de gezondheid. Bovendien worden PCBs deels vervangen door verbindingen zoals broomhoudende vlamvertragers, die vergelijkbare negatieve effecten op de gezondheid kunnen hebben. De inspanningen om de besmetting van deze planeet door mensen gemaakte verbindingen, die negatieve effecten kunnen hebben op de gezondheid van de mens te verminderen, blijft derhalve van groot belang.

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