Effects of Environmental Exposure to Polychlorinated Biphenyls and Dioxins on Growth and Development in Young Children

A prospective follow-up study of breast-fed and formula-fed infants from birth until 42 months of age

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EFFECTS OF ENVIRONMENTAL EXPOSURE TO POLYCHLORINATED BIPHENYLS AND DIOXINS ON GROWTH AND DEVELOPMENT IN YOUNG CHILDREN

A PROSPECTIVE FOLLOW-UP STUDY OF BREAST-FED AND FORMULA-FED INFANTS FROM BIRTH UNTIL 42 MONTHS OF AGE

Effecten van Achtergrond Blootstelling aan Polychloorbiphenylen
en Dioxinen op de Groei en Ontwikkeling van Jonge Kinderen

Een prospectieve follow-up studie van borstgevoede en flesgevoede kinderen
onderzocht vanaf de geboorte tot en met de leeftijd van 42 maanden

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Voor Talin, Jesse en onze ouders

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CHAPTER 1
GENERAL INTRODUCTION

1.1 Context of the study

1.2 Sources of PCBs and Dioxins

1.3 Environmental fate of PCBs and Dioxins

1.4 Toxic and biochemical effects of PCBs and Dioxins

1.5 Impact of PCBs and Dioxins on health and development

1.6 The Dutch PCB/Dioxin study

1.7 Objectives and structure of the thesis

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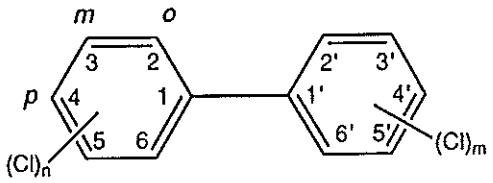
1.1 CONTEXT OF THE STUDY

Polychlorinated biphenyls (PCBs) as well as dioxins, polychlorinated dibenzo-p-dioxins (PCDDs) and -dibenzo-furans (PCDFs) are potentially hazardous compounds in the environment. As in other industrialized countries, contamination of breast milk with PCBs and dioxins (PCDDs/PCDFs) in the Netherlands has led to considerable public concern. The Dutch government launched a prospective follow-up study in 1989 to investigate the potential adverse effects of environmental exposure to PCBs and dioxins on growth and development of the human foetus and newborn. The observation period was expanded until 42 months of age in an EC-funded collaborative project entitled: 'Neonatal PCB exposure and neurodevelopmental deficit', financed by the ENVIRONMENTAL and CLIMATE research programme, DGXII of the European Commission, Contract-No EV5V-CT92-0207. The study was designed to look into a spectrum of PCB-related effects in order to decide if neurological and cognitive development are affected by perinatal exposure to PCBs and related compounds, and if European background concentrations represent levels of concern. In this multi center cohort study, our Dutch cohort, a German and a Danish cohort are included. The work in this thesis describes the results from the Dutch cohort. In this prospective longitudinal study, breast-fed and formula-fed infants were studied from birth until 42 months of age. Results on growth, health and development until 42 months of age are presented.

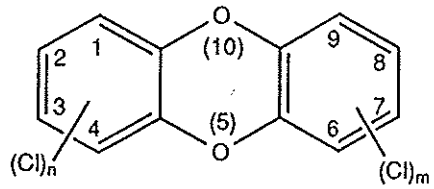
1.2 SOURCES OF PCBS AND DIOXINS

Polychlorinated biphenyls (PCBs) are members of the polyhalogenated aromatic hydrocarbon (PAH) group of environmental pollutants that are lipophilic and bioaccumulating. Søren Jensen first detected PCBs in environmental samples as a series of complex peaks observed in a gas chromatic screening of environmental samples for DDT(dichloro diphenyl-trichloroethane) and related compounds.[1] Subsequent studies in several laboratories have identified PCBs in almost every component of the global ecosystem including air, water, sediments, fish, wildlife and human tissues.[2-4]

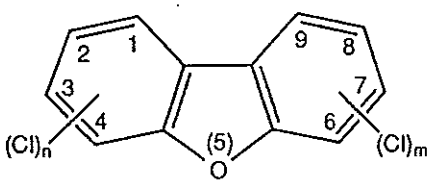
PCBs consist of a biphenyl ring which may be substituted with chlorine on the *ortho*, *meta* and *para* positions, yielding 209 different congeners, see Figure 1.1. Most of these congeners have been shown to be present in commercial PCB mixtures. About half of these congeners have been found in the environment.[5] Mono-ortho PCBs have one chlorine atom on the *ortho* position. Di-ortho PCBs have two chlorine atoms on the *ortho* position. Planar PCBs resemble dioxins, because of their planar structure and have chlorine atoms in the *para* and *meta* position of the biphenyl ring structure.



Polychlorinated biphenyls (PCBs)



Polychlorinated dibenzo-p-dioxins (PCDDs)



Polychlorinated dibenzofurans (PCDFs)

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

Figure 1.1 Molecular structures of PCBs, PCDDs and PCFs

PCBs were produced by the chlorination of the biphenyl and resulting products were marketed according to their percentage of chlorine content. For example, Aroclor 1221, 1232, 1248, 1254 are commercially produced PCBs and contain 21, 32, 48 and 54% chlorine (by weight).[3] The production of PCBs started in the late 1920s, and has been abandoned now. It was still taking place on a large scale until the 1980s. Under trade names such as Aroclor, Clophen, Phenoclor, Fenclor, Pyralenes and Kaneclor, PCBs have been produced and utilized over the years in different industrialized countries as well as the former USSR and Czechoslovakia. Commercial PCBs exhibit a broad range of physicochemical properties that are dependent, in part, on their degree of chlorination. These properties contributed to the diverse applications of PCBs in numerous products. For example, PCBs have been used as organic diluents, plasticizers, pesticide extenders, adhesives, dust-reducing agents, cutting oils, flame retardants, heat transfer fluids, dielectric fluids for transformers and capacitors, hydraulic lubricants, sealants, and in carbonless copy paper.[4] Some of the uses of PCBs have resulted in their direct introduction into the environment; however, a significant portion of the environmental burden of these compounds has resulted from careless disposal practices, accidents, leakage from various industrial facilities, and from chemical waste disposal sites (Table 1.1). The total amount of PCBs produced worldwide has been estimated at approximately 1.5 million metric tons. Up to this moment at least a third of this amount is believed to have found its way into the environment.[6]

Table 1.1 Sources of Polychlorinated Biphenyls

- Leakage from old closed systems: capacitors and transformers
- Disposal of materials contaminated with PCBs
- Old paints, painted construction materials, recycling of paper, lubricant oils ,sealing material and fire retardants in old fire extinguishers.
- De Novo synthesis of PCBs during combustion processes
- Transport from river sediments
- Leakage from dump sites, dumping from sewage sludge
- Long range atmospheric transport.

From Ahlborg et al 1992[9]

Dioxins, a term often referred to polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzo-furans (PCDFs) also belong to the group of PAHs. Like PCBs, variations in the degree and site of chlorination result in 75 PCDD and 135 PCDF congeners (Figure 1.1). They are all more or less lipid-soluble and bioaccumulating, but many of them degrade comparatively readily. In living organisms only limited numbers of dioxins are usually found, primarily those with chlorine atoms at positions 2,3,7 and 8 (and possibly also at other positions). These congeners can remain for many years in fatty tissue of different species. PCDDs and PCDFs are formed as undesirable byproducts in a variety of chemical and thermal processes and, except for scientific research, they are of no economical importance.[7] It has been well established that PCDDs and PCDFs are formed during the synthesis of a wide array of commercial chemical products, especially those based on chlorinated aromatics, precursors, and intermediates. These products have a wide range of applications, ranging from herbicides and fungicides for chlorinated phenoxybenzenes and phenols, to heat transfer fluids and fire retardants for chlorinated and brominated biphenyls (PCBs and PBBs). In addition a variety of combustion processes, e.g. burning of solid waste from muncipal incinerators, lead to continous formation and partial release of PCDDs and PCDFs in the environment.[7,8] (see Table 1.2)

Table 1.2 Sources of Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo-furans

Thermal Processes

- Incineration of various types of wastes (muncipal, hospital, hazardous)
- Production of iron, steel and copper
- Combustion of leaded gasoline
- Combustion processes involving chlorinated organic or inorganic compounds.
- Accidental burning of PCB-containing electrical equipment
- Incineration of coal, peat and wood
- Cigarette smoke

Chemical Processes

- Chlorine bleaching of pulp and paper products
- Intermediates in production of chlorophenol-based products
- Biochemical processes in sewage sludge and compost

Non-anthropogenic sources

- Forest fires or burning of sea-weeds containing NaCl, their contribution are relatively low
- Photochemical reactions under atmospheric conditions

Adapted from Rappe[10]

1.3 ENVIRONMENTAL FATE OF PCBS AND DIOXINS

The chemical properties primarily responsible for many of the industrial applications of PCBs contribute to their environmental problems that is, their inflammability, chemical stability, and miscibility with organic compounds (i.e. lipohilicity).[4] Once emitted into the abiotic environment major fractions of these pollutants bind to surfaces of air dust, soil and sediment particulate matter. After deposition these particulates act as an environmental sink and form a reservoir for exposure of aquatic, terrestrial and avian organisms.[11] The stable PCBs degrade relatively slowly and undergo cycling and transport within various components of the global ecosystem. Because of their similar chemical properties to PCBs, dioxins have also been found as wide-spread environmental pollutants, and undergo similar long-range atmospheric transport.[12] PCBs and dioxins are extremely stable towards physical (heat), chemical (acids, bases, hydrolysis) and biological (metabolic transformation)

degradation. Especially the higher chlorinated PCBs and some PCDDs and PCDFs undergo extremely slow biodegradation, and consequently bioaccumulate and biomagnify at higher tropic levels of the food chain. [4, 11, 13]

Humans are at the end of the food chain eating the meat and dairy products of herbivores as well as fish and plants.[14] More than 90% of the total daily human exposure to PCBs and dioxins is from food intake, whereas other routes e.g. water, air and soil contribute to less than 10% of total exposure.[15, 16] (Figure 1.2) The average daily dose for a 65-kg adult is about 1-3 pg/kg body weight (BW) of toxic equivalents (TEQ) of dioxin-like compounds considered equivalent in toxicity to 2,3,7,8-tetra chloro-dibenzo-dioxin (TCDD).[15] Different living conditions or consumption habits may result in lower or higher intakes for specific populations. Nursing infants have drawn attention for many years. It has been estimated that exposure to dioxins and related compounds is on average of 40-50 fold higher in breast-fed infants than that for adults.[17]

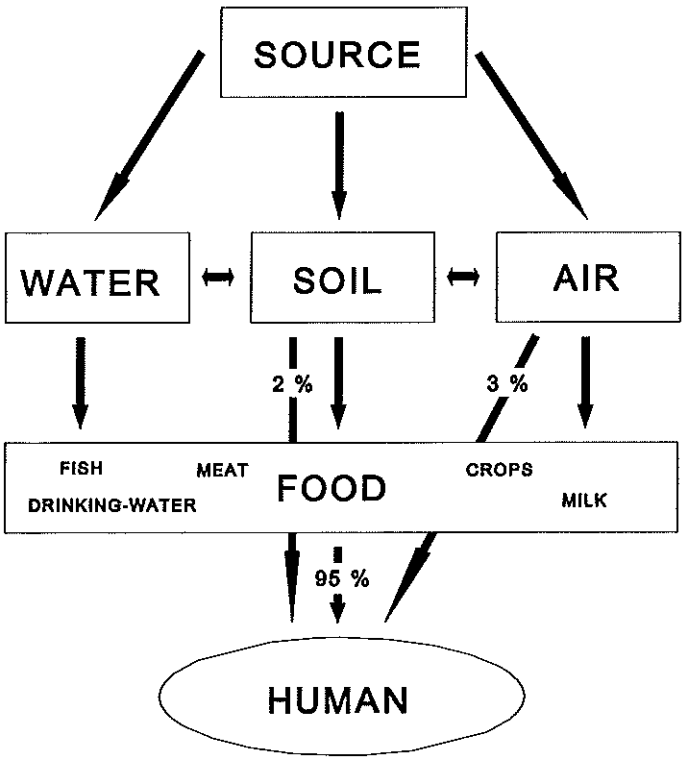


Figure 1.2 Human PCB and dioxin exposure through different sources.

These compounds are readily absorbed from the gastrointestinal tract, and depending on their chlorine substitution pattern may be preferentially retained in the liver and adipose tissue. Combined with the low metabolic degradation and rate of excretion, the half-life is very long, for higher chlorinated PCDDs and PCDFs ranging between 4 to 12 years[18] and for PCBs ranging between 5 to 15 years.[19] In humans the body distribution

of PCBs and dioxins differs considerably from that of most laboratory species, since most of the body burden is sequestered in adipose tissue.[7] Elimination of these compounds is slower than in any other known mammal species, resulting in much higher tissue concentrations at a given exposure.

In human milk fat of women from the general population all over the world, PCBs and dioxins have been found.[20] Global data from the World Health Organization on human milk are used to describe the world-wide distribution of these compounds in humans.[21, 22] Although the local sources of contamination might influence the typical congener profile, the background pattern is remarkably similar in most areas of the world. In Figure 1.3, the sum of TEQs of seventeen 2,3,7,8-substituted PCDDs and PCDFs, as well as dioxin-like PCBs, International Union of Pure and Applied Chemistry (IUPAC) nos 105, 118, 77, 126 and 169 are given for several countries. The highest TEQ levels are found in Belgium, Canada, Finland, Spain and the Netherlands, the lowest levels are found in Albania, Hungary, Pakistan. The dioxin-like PCBs are high in Canada and Lithuania.

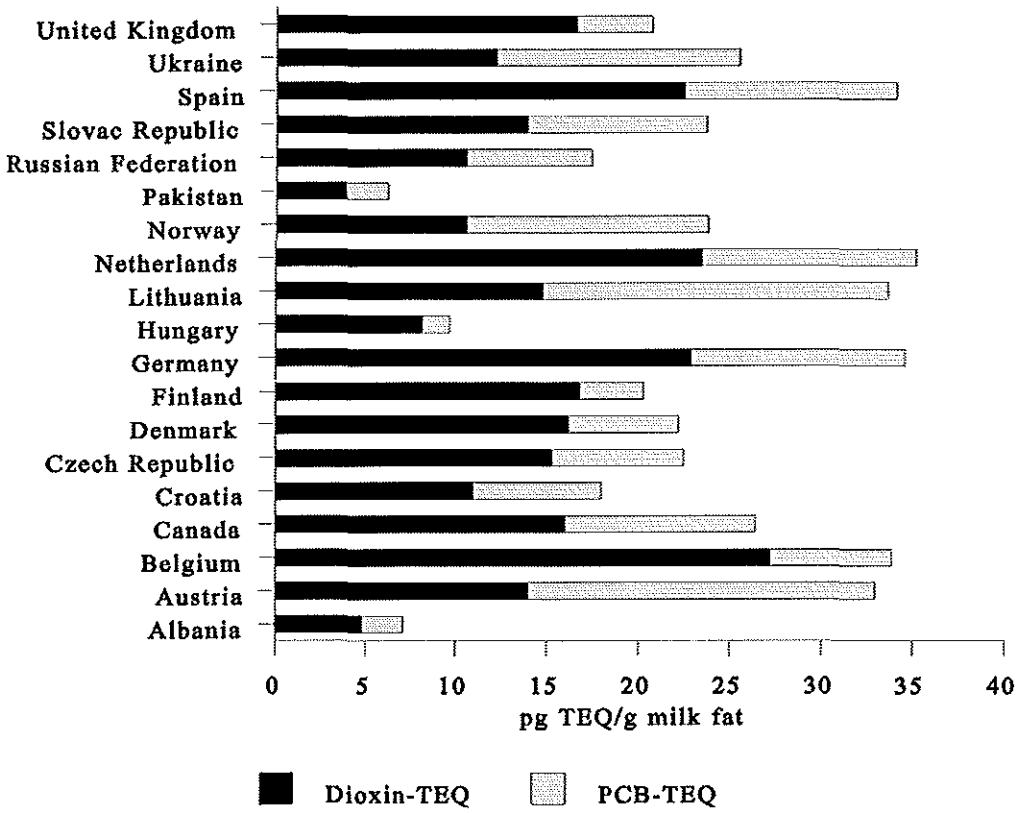


Figure 1.3 *Concentrations of dioxin-TEQ and dioxin-like PCB-TEQ in human milk from different countries adapted from the second round WHO-coordinated exposure study*[22]

1.4 TOXIC AND BIOCHEMICAL EFFECTS OF PCBS AND DIOXINS

Analysis of environmental samples, for example adipose tissue of mammals, clearly demonstrate that the PCB composition is highly variable and does not resemble the composition of commercial mixtures.[4] Individual PCB congeners exhibit a variety of physico-chemical properties, that influence their rates of partitioning, uptake and retention in environmental matrices, and their rate of breakdown by various environmental pathways (e.g. photolysis, microbial degradation, and metabolism) which alters the composition of PCB mixtures present in the environment. The kinetics and metabolism of PCBs, PCDDs, and PCDFs in adult animals have been reviewed in detail.[4, 7, 23]

A broad spectrum of toxic and biochemical effects have been reported for individual and complex mixtures of PCBs, PCDDs and PCDFs in experimental animals.[4, 9, 23-26] In addition, there is a considerable body of human toxicity data on these compounds derived from accidental or occupational exposures that occurred during the last 30 years.[2, 7, 28-34] Striking aspects of PCBs/PCDDs/PCDFs toxicity are the pleiotropic nature. In Table 1.3 several toxic effects are summarized from animal as well as human studies. The species differ in sensitivity and spectrum of toxic and biochemical responses, and the wide range of congener-specific toxic potencies.[9, 24]

Table 1.3 Toxic effects of PCBs and dioxins

Animal Studies	*Human Studies*
Lethality	Higher mortality
Dermal lesions	Lower Birth weight
Neurotoxicity	Growth delay
Immunotoxicity	Chloracne/Hyper pigmentation
Hepatotoxicity	Nail/teeth deformities
Enzyme Induction	Neuropathy
Reproductive effects	Hypotonia
Endocrine effects	Psychomotor delay
Carcinogenic effects	Lower IQ scores
Mutagenic effects	Behavior effects

1.4.1 Mechanisms of action

Ah Receptor mediated effects

Many laboratory animal studies and mammalian cell cultures have indicated the role of cytosolic receptor protein, aryl hydrocarbon (Ah), mediating the majority of toxic and biochemical effects induced by dioxins and coplanar PCBs.[35] PCDDs, PCDFs and PCBs are Ah receptor agonists, i.e. they bind competitively to the Ah receptor and elicit induction of specific cytochrome P450 gene expression (CYP1A1 and CYP1A2) and associated enzyme activities, aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-O-deethylase (EROD). In addition to Ah receptor affinity these compounds consequently elicit dioxin-specific biochemical and toxic responses, including body weight reduction, hepatotoxicity and thymus atrophy. Based on structure activity relationships (SAR) and bioassays, a toxic equivalent factor (TEF) concept has been developed, which allows the expression of a complex mixture of individual toxic congeners into one sum parameter, the toxic equivalency (TEQ) value. The TEQ of the mixture corresponds to the potency of the most toxic congener 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) with a TEF value of one.[4, 23] Based on the TEF approach analytical results can be transformed into toxic equivalents (TEQs) where $\Sigma([\text{Congener i}] \times \text{TEF i})=\text{TEQ}$.[4] Summation is only valid when the individual congeners exhibit an additive interactive response.

Limitations of this concept are that it only takes into account additive but not synergistic or nonadditive antagonistic interactions between various PCBs alone, and PCBs with PCDDs and PCDFs.[36] Only Ah receptor mediated effects are taken into consideration. In particular the nonplanar, di-ortho PCBs elicit a diverse spectrum of non-Ah receptor mediated effects such as induction of certain cytochrome p450 isozymes, hypovitaminosis A, hypothyroidism, neurotoxicity, and carcinogenicity.

International recommendations for the TEF values of PCDDs/PCDFs and PCBs have been reported. A recent WHO meeting on TEFs in Stockholm 1997[37] has proposed an additional series of TEFs for mammals, birds and fish. Di-ortho PCBs, IUPAC nos. 170 and 180, are considered to be weak Ah receptor agonists and therefore excluded from this list of TEF values.[37] The TEF approach is nowadays widely used for risk management of dioxins and dioxin-like PCBs and to assess adverse health effects observed in humans. A fundamental question remains however, whether the TEQs derived from analytical and toxicological data accurately predict the risk for adverse health effects in humans, particularly the human infant.

Non-Ah receptor-mediated effects

Exposure to nonplanar, di- and mono-ortho PCBs as well as their metabolites may elicit several other important toxic responses in animals,[9] including *neurobehavioral,*[38-42] *neurochemical,*[43-45] *carcinogenic*[9] and *endocrinological*[46] changes. Di-ortho PCBs, chlorine substitution in either the 2 or 6 position (Figure 1.1), may be *neurotoxic* and play an important role in the induction of psychomotor, cognitive, and movement disorders in both perinatally and adult exposed animals and humans.[44]

However the direction and qualitative nature of the effect appear to depend on the dose, time of exposure, and species.[43] Seegal and colleagues have reported a number of studies[43] designed to characterize the effects of PCBs on concentrations of brain neurotransmitters. Repeated exposure to PCBs has been reported to affect the dopaminergic neurochemical system to a greater extent than other neurotransmitter systems.[43] These alterations in neurotransmitter function may underlie PCB-induced neurobehavioral effects. Dopamine metabolism can be affected by PCB exposure, and the decrease in concentrations can be in more than one brain region.[43] Morse et al.[47] reported that gestational and lactational exposure to the commercial PCB mixture, Aroclor 1254 results in long-term alterations in neuronal and glial cell markers in specific brain regions of rats. These marker proteins may be useful for determining the structure activity relationship in PCB-induced neurotoxicity. It may be that the nervous system especially of the developing organism, is very sensitive to PCBs and related compounds, which affect the neurochemical functions rather than Ah receptor mechanisms.[48]

1.5 IMPACT OF PCBs AND DIOXINS ON HEALTH AND DEVELOPMENT

1.5.1 In utero transfer of PCBs and dioxins

In utero exposure to PCBs and dioxins has been documented in rodents, monkeys and humans.[49, 50] Data on the total body burden of fetal non-human primates or human fetuses and the degree of transplacental transfer in higher mammals are scarce. Levels of PCB congeners in umbilical cord plasma show high correlations with maternal plasma PCB levels.[51] There is also evidence that PCDDs and PCDFs cross the placenta and are present in fetal human liver.[52] Maternal PCBs particularly accumulate in fetal tissues that contain high levels of storage lipids (e.g. triglycerides).[53] Transplacental transfer of PCBs and dioxins means that the fetus is exposed to these toxic and teratogenic compounds during a critical period of organ growth and development.

1.5.2 Transfer of PCBs and dioxins during lactation

Human milk is the major source for PCBs and dioxins in the nursing infant. PCBs and dioxins are stored in the mother's body fat, which is in equilibrium with blood fat. These lipophilic compounds easily pass through the membranes that form a barrier between blood and mammary gland cells, and establish an equilibrium between blood fat and milk fat.[54] During breast-feeding maternal PCBs and dioxins are excreted in breast milk and transferred to the infant.[50, 55] Literature data indicate that PCBs and dioxins are almost completely absorbed in the digestive tract of the breast-fed infant.[56, 57] Milk lipids in formula are replaced by lipids mainly of vegetable origin, therefore the exposure to PCBs and dioxins of formula-fed infants is negligible.

Certain populations such as Inuit women from the Arctic Quebec[58-60] and from the Faroes Islands[61] have higher PCB levels in milk than other populations due to high consumption of fish and marine mammals, e.g whale blubber. In the Faroes Islands, seafood constitutes a considerable part of the average diet. Median PCB concentrations were 9 to 12 times higher than found in other countries, however levels of dioxins were similar to those found in other countries.[61]

1.5.3 Adverse effects of PCBs and dioxins

Neurodevelopmental effects

Animal studies

In animal studies developmental neurotoxicity of PCBs and related compounds was studied with several neurobehavioral tasks. Only limited information is available on the biochemical effects of PCBs on the developing brain and the mechanisms involved are not clear.[43] Many effects: hyperactivity, stereotyping circling, and decreased motor coordination are described in PCB exposed mice.[62-64] Hypoactivity was reported in studies of pre- as well as postnatal PCB exposed rats.[39, 65] In monkeys, PCB-induced hyperactivity changed into hypoactivity with ageing.[38] In general, pre- and postnatal PCB exposure results in increased activity in offspring. Cognitive effects have also been reported in the offspring of PCB-exposed monkeys. Gestational and lactational exposure to PCB mixtures produced deficits and delayed spatial alternation learning at the age of 3-4 years.[66] Higher PCB body burden in infant monkeys was correlated with more errors in learning tasks up to 2 years of age.[67, 68]

Accidental exposure in humans

Birth defects as well as neurotoxic effects in human beings were reported for the first time in poisoning studies. In Asia, there have been two large outbreaks of poisoning due to ingestion of cooking oil contaminated by thermally degraded PCBs and PCDFs; the Yusho accident in Japan in 1968[30, 31, 69] and the Yu-Cheng accident in Taiwan in 1979.[32, 34, 70] In the Taiwan incident more than 2000 people who had consumed the contaminated oil for up to about 9 months between 1978 and 1979 were included in the Yu-Cheng registry. Studies performed in these two accidental exposed populations, showed several adverse health and developmental effects. The victims showed severe dermal lesions, sensory neuropathy, weakness, headache, gastrointestinal and immunological symptoms. Yusho exposed children were described as apathetic and dull.[69] In the first three years after the outbreak in Taiwan, babies born to Yu-Cheng mothers had a high infant mortality rate.[34] Infants with congenital PCB poisoning were characterized by intrauterine growth retardation, brown staining of the skin and mucous membranes, widely open fontanelles, spotty calcification of the skull, natal teeth and delayed developmental milestones.[32, 34, 69-72] Surviving children were found to have ectodermal defects[73] developmental delay, more behavioral problems and higher activity levels.[33, 74] The Yu-Cheng children were smaller, had a developmental and psychomotor delay. Followed from 1985 to 1991 with cognitive and neurobehavioral tests, these children were also found to have poorer cognitive development up to 7 years of

age.[33, 70] Mean P300 latencies were significantly longer in exposed children at school age suggesting that higher cortical function rather than a sensory pathway was affect by prenatal PCB exposure.[75] After 11 years of follow-up the dermatological manifestations were still more frequent in the exposed group, especially nail changes were the most persistent, indicating that the fetal nailmatrix developed abnormal after PCB intoxication.[76]

In 1976, an explosion occurred, during the production of chlorophenol in a plant situated in the area round Seveso, Italy (20 km from Milan).[77] A cloud of toxic material was released into an area of 1800 densely populated hectares referred to as the Seveso accident.[78] The most toxic compound released was TCDD. Three areas of decreasing contamination levels were identified (Zone A, B and R). A study on frequency of birth defects was conducted, but the number of exposed pregnancies was not big enough to show specific teratogenic risk increase.[78] Long-term effects from Seveso accident were chloracne.[79] Reversible effects were peripheral neuropathy and liver enzyme induction. An unusual cardiovascular mortality pattern was also reported in the exposed population.[80, 81]

Background exposure in human infants

In USA, Michigan, Jacobson et al[55, 82, 83] investigated a group of 313 children, known as the Michigan cohort. From these children, 242 were born from mothers who had consumed Lake Michigan fish, which was contaminated by PCBs. Prenatal PCB exposure was associated with a negative effect on the autonomic maturity, reflexes and a range of state cluster scores on the Brazelton Neonatal Assessment Scales,[82] less preference for a novel stimulus in infancy,[84] and defects in short term memory and processing efficiency at 4 years of age.[83, 85] Four-year PCB body burden was associated with reduced activity level.[86] The majority of these neurodevelopmental effects are subtle and within the normal range. However, long-term implications of PCB exposure to later school performance at 11 years has been found.[87]

In another US study, known as the North Carolina cohort, 930 children from volunteer families of the general population with no accidental exposure to PCBs were followed from birth until schoolage.[88, 89] Prenatal exposure was associated with hypotonia and hyporeflexia at birth.[88] They also described an association between the prenatal PCB exposure and a poorer performance on the psychomotor developmental index of the Bayley Scales of Infant Development at 6, 12, 18 and 24 months.[90, 91] These findings were no longer apparent at a later age.[92]

In the Netherlands two prospective follow-up studies were undertaken to study the possible adverse effects of background exposure to PCBs and dioxins. The first, was a study of 34 breast-fed babies, who were neurologically examined in the first week after birth and at 6 months of age.[93] In this study only dioxins were measured in breast milk. They reported no adverse effects of perinatal PCDD and PCDF exposure on the neurological outcome during infancy. At 2.5 years of age an enhanced neuromotor maturation and higher reflexes with the neurological examination for toddlers according to Hempel[94] is found in the high-exposure group.[95] The other study, known as the Dutch PCB/dioxin study, will be extensively described in this thesis.

Growth and reproductive health

Prenatal PCB and dioxin exposure may influence growth and development. In the Yu-Cheng accident thirty-nine babies showing Hyper pigmentation were born from PCB-poisoned mothers, eight of them died.[34] The in utero exposed surviving children had 500 g lower gestational age-adjusted birth weights than controls and were still 3.1 cm shorter and had less total lean mass and soft tissue mass at schoolage as compared to matched control subjects.[96] Boys who were born in the earliest years after their mothers intoxication had reduced penis length compared to those of their non-exposed controls.[97] In Inuit infants birth size among male infants was inversely related to the PCB concentration.[98] In the live offspring of women occupationally exposed to PCBs during the manufacture of capacitors in Upstate New York, a significant effect was seen for birth weight as well as gestational age. When gestational age was accounted for in addition to other variables related to birth weight, estimated serum PCB was no longer a significant predictor of birth weight.[99] In the Lake Michigan cohort, offspring of the highest exposed group were 160 to 190 grams lighter at births than controls, and their head circumferences were 0.6 to 0.7 cm smaller.[100] At 4 years of age the highest prenatally exposed children still weighted 1.8 kg less on the average than the least exposed.[86] In the North Carolina cohort, lower birth weight was not associated with background PCB exposure.[88]

Immunological effects

A wide variety of immunological effects of especially TCDD in experimental animals has been documented.[101] Data regarding the potential toxic effects of in utero and lactational PCB and dioxin exposure in human beings, however are scarce. In vitro studies suggest that the CD8+ cells are generated in substantially increased numbers in TetraCB-exposed fetal thymus.[102] In the mouse, dioxin immunotoxicity is an Ah receptor-dependent process.[103] The biochemical and molecular alterations that occur subsequent to Ah receptor activation that lead to altered immune reactivity remains to be elucidated.

In the Yu-Cheng incident, prenatal exposed children had histories of more frequent bronchitis, upper respiratory infections, and ear infections, as reported by the parents.[32] In Inuit infants from Northern Quebec, whose mothers have levels of PCBs and dioxins in their breast-milk at least twice the level of the Dutch samples, the CD4+ (helper) : CD8+ (cytotoxic) T-cell ratio was decreased at 6 and 12 months of age and they experienced more episodes of acute otitis media at 3 to 6 months of age.[98] In the North Carolina cohort there were no adverse effect on the frequency of physician visits for various illnesses.[104]

1.6 THE DUTCH PCB/DIOXIN STUDY

The 'Dutch PCB/Dioxin study' started in 1989 as a cooperative effort between animal (experimental) and human (observational) studies to investigate adverse health effects of perinatal exposure to PCBs and dioxins. This study was supported by the Dutch Toxicology Research Promotion Programme and the Health Research Stimulation Programme. The human study was performed at the Department of Pediatrics, Sophia Children's Hospital and Erasmus University Rotterdam, and at the Department of Obstetrics and Gynecology from the University of Groningen. From 1990-1992, healthy pregnant women living in Rotterdam and Groningen were asked by their obstetrician or midwife to participate in a prospective, longitudinal neurodevelopmental study. The Rotterdam area is a highly industrialized and densely populated region situated in the west, and the Groningen area is a semi-urban region in the north of the Netherlands.

1.6.1 Inclusion criteria and subjects

To study the effects of in utero as well as postnatal PCB and dioxin exposure, women were included who intended to breast-feed their child for at least 6 weeks (breast-fed group) in addition to women who intended to use formula-feeding (formula-fed group). All formula-fed infants received formula from a single batch (Almiron M2, Nutricia NV, the Netherlands) from birth until 7 months of age. In this formula, concentrations of both PCBs and dioxins were not detectable. Further inclusion criteria were: 1) Pregnancy and delivery had to be without complications or serious illnesses. Instrumental deliveries or caesarian sections were excluded. 2) First or second born infants. 3) Born at term, 37-42 weeks of gestation. 4) No congenital anomalies or diseases. 5) Caucasian race. The Medical Ethics Committees of both University Hospitals approved the study protocol and informed consent was given by participating parents. Four hundred and eighteen mother-infant pairs were included in the study, 209 infants were breast-fed for at least six weeks and 209 were formula-fed as babies. Two hundred and seven subjects are living in the Rotterdam area and 211 subjects are living in the Groningen area.

1.6.2 Exposure measures

Maternal plasma samples obtained in the last month of pregnancy, and umbilical cord plasma samples collected shortly after delivery were analyzed for 4 PCB congeners, IUPAC nos 118, 138, 153, and 180 by gas chromatography with electron capture detection (GC-ECD).[51] In the second and sixth week after delivery, 24-hour representative breast milk samples were collected and analyzed for 17 most abundant PCDD and PCDF congeners and three planar PCB congeners, IUPAC nos 77, 126 and 169, by gas chromatography-high-resolution mass spectrometry (GC-HRMS).[51] Twenty-three non-planar PCBs, IUPAC nos 28, 52, 66, 70, 99, 101, 105, 118, 128, 137, 138, 141, 151, 153, 156, 170, 177, 180, 183, 187, 194, 195 and 202, were measured by GC-ECD.[51]

1.6.3 Outcome measures

Participating mothers in Groningen as well as Rotterdam were asked to complete a semi-quantitative food questionnaire about their food habits during pregnancy in order to assess the daily PCB and dioxin dietary intake. All 418 infants underwent an age-adequate neurological examination at 2 weeks after birth, according to Prechtl[105] and at 18 months of age according to Hempel and Touwen.[94] In the Rotterdam cohort (n=207) additional outcome variables were assessed based on results from animal as well as human studies described in Chapter 1.5. The psychomotor and mental development at 3, 7 and 18 months of age was assessed with the Bayley Scales of Infant Development, as well as the child's short term memory with the Fagan test of infant intelligence at the age of 3 and 7 months. Thyroid hormone status from the 207 mother-infant pairs in Rotterdam were measured as well as the childs' health and immunological status. Maternal, cord and 3 month child plasma samples were analyzed for thyroid functions (TT3, TT4, TSH, FT4). The child's health status was assessed with a questionnaire and antibody levels to mumps, measles and rubella were measured in 18 month plasma samples. In a subgroup (n=55) of the Rotterdam cohort, immunological marker analysis and white blood cell counts were analyzed in cord blood, and 3 and 18 month blood samples respectively.

1.6.4 Other variables

Data on socio-economic background, obstetrical and neonatal history, maternal age, parents' education level, parity, gender, fetal exposure to alcohol and cigarette smoking, type of feeding during infancy and the breast-feeding period were assessed by means of a questionnaire. The neurological examinations were performed by two trained researchers, one in each study center. To correct for inter-observer and between study differences, study center was included as an explanatory variable. Examiners were unaware of the pre- and postnatal PCB and dioxin exposure. The child's home environment was assessed by the Dutch version of the Home Observation for Measurement of the Environment (HOME).[106]

1.6.5 Results up to 18 months

PCB and dioxin concentrations in the Dutch population belongs to highest measured in the world and is comparable to those measured in other industrialized countries such as Germany and Belgium. Mean dioxin TEQ and planar PCB TEQ concentration is 30 respectively 16 pg TEQ/g milk fat. Maternal and cord blood as well as breast milk PCB concentrations are highly correlated with each other, Spearman rank correlations (rs) range from 0.70 to 0.79 between individual congeners. Plasma PCB concentrations also correlate highly with breast milk dioxin-TEQ and dioxin-like PCB-TEQ (rs = 0.67-0.71).[51]

The levels of PCB and dioxins were compared in the 418 mother-infant pairs according to their living area, Rotterdam in the west and Groningen in the north of the Netherlands. After adjustment for covariates, the total dioxin level in human milk and

PCB IUPAC no 118 in maternal plasma of the Rotterdam population are slightly but significantly higher compared to those in Groningen.[107] No significant differences between planar, mono-ortho and di-ortho PCBs in breast milk, as well as other congeners measured in plasma, were found between the two study areas.

The dietary PCB/dioxin intake of pregnant women was studied. Dairy products account for about half and processed foods/industrial oils for about a quarter of the daily PCB/dioxin intake. Since PCBs and dioxins accumulate for years in adipose tissue and are slowly metabolized, no clear relationship was found with dietary intake measurements during pregnancy and the concentrations measured in human milk, maternal and cord plasma. Decrease of fetal and infant exposure to PCBs and dioxins requires a long-term reduction of the intake of these pollutants, rather than short-term dietary regiments.[108]

PCBs, PCDDs and PCDFs in breast milk which are a measure of prenatal exposure, are related to poorer neonatal neurological condition. Planar PCBs are related to a higher incidence of hypotonia.[109] After adjustment for covariates, transplacental exposure to PCBs, measured from cord and maternal plasma PCB concentrations, is negatively related to the neurological optimality at 18 months.[110] No effects of lactational exposure to PCBs and dioxins were found. Moreover, a beneficial effect of breast-feeding on the fluency of movements at 18 months is found.[110]

The psychomotor and mental development was assessed with the Bayley Scales of Infant Development at 3, 7 and 18 months of age. At 3 months of age, in utero exposure was significantly associated with lower psychomotor scores, a doubling of exposure results in a decrease of 3 points. At 7 months of age breast-fed infants scored significantly higher on the psychomotor and mental scale of the Bayley Scales compared with the formula-fed infants. However the psychomotor scale of the 66% highest exposed breast-fed infants was negatively influenced by postnatal PCB/dioxin TEQ exposure, and comparable to the psychomotor score of their formula-fed counterparts. The mental scale at 3 and 7 months of age is not negatively influenced by perinatal PCB and dioxin exposure. At 18 months of age no negative associations between PCB and dioxin exposure and psychomotor and mental development are found.[111]

Maternal and infants' thyroid hormone status were related to perinatal PCB and dioxin exposure. PCB and dioxin concentrations in breast milk are related to lower maternal plasma TT3 and TT4 levels, and to higher plasma TSH measured in infants at 2 weeks and 3 months of age.[112] Infants exposed to higher dioxin-TEQ levels had also lower plasma free thyroxin (FT4) and TT4 levels in the second week after birth. In addition, the infants' thyroid hormone status was related to their neonatal neurological examination. Overt neurological abnormalities found in the neonatal period are not caused by either direct effects of PCB or dioxin exposure or lowered thyroid hormone levels induced by these pollutants.[113]

Immunological effects in relation to perinatal exposure to PCBs and dioxins were described in the Rotterdam cohort. The child's health status and antibody level to mumps, measles and rubella after vaccination at 18 months of age was related to perinatal PCB and dioxin exposure. T-cell marker analysis as well as white cell blood counts were

assessed in cord, 3 and 18 month plasma samples. No significant effects were found between upper/lower respiratory tract symptoms or altered humoral antibody levels and perinatal PCB and dioxin exposure. Prenatal exposure to PCBs and dioxins was associated with an increase in the number of TcR$\gamma\delta$+ T cells in cord blood, and an increase with the number of CD8+, TcR$\alpha\beta$+ and TcR$\gamma\delta$+ T cells at 18 months of age. Prenatal as well as postnatal PCB and dioxin exposure was associated with lower monocyte and granulocyte counts at 3 months of age. This study suggests that background levels of PCB/dioxin exposure influences the human fetal and neonatal immune system.[114]

1.6.6 Conclusions

At background concentrations measured in The Netherlands, subtle signs of neurological dysfunctioning, small delay in psychomotor development, alterations in thyroid hormone status and immunologic functions are found in relation to perinatal exposure to PCBs and dioxins in the Netherlands. The mechanisms by which these contaminants can cause such effects are not clear. Breast feeding itself has a positive effect on the mental outcome at 7 months of age as well as the fluency of movements at 18 months of age. To investigate whether these adverse effects persist at later age, follow-up studies are needed, with special attention to higher cortical functions at (pre-)school age, e.g. the development of more complex cognitive functions.

1.7 OBJECTIVES AND STRUCTURE OF THE THESIS

1.7.1 Introduction to the study at 42 months

The development of the child is a continuous process and not all of the more subtle neurodevelopmental effects might be detected at an early age. Therefore a detailed neurodevelopmental follow-up, designed for (pre)-school age is an essential element in the detection of possible adverse effects of the long-term background exposure to PCBs and dioxins. On the basis of results from animal studies as well as findings from our and other human studies with accidental and background PCB/dioxin exposure, the follow-up study until 42 months of age was designed.

At pre-school age (42 months), the Dutch PCB/dioxin study was financed by the European Commission as part of a multi center cohort study, two Dutch cohorts (Rotterdam, n=207 and Groningen, n=211) recruited in 1990-1992, a German (Düsseldorf, n=170) and a Danish cohort (Faroer Islands, n=192) recruited in 1994-1995. All cohorts have the same inclusion criteria, however the German and Danish cohort held no restrictions in the inclusion of the number of breast feeding mothers. Both cohorts have mainly breast-feeding mothers in contrast to the Dutch cohort which included 50% formula-fed and 50% breast-fed children. The major developmental assessments were similar. Each study center included additional outcome variables of own particular interest. In the final stage of the project results from the three study areas

will be combined and analyzed for the overall effects of perinatal background PCB exposure on the major developmental endpoints: neurological evaluation at birth, 18 and 42 months, and the cognitive evaluation at 7, 18 and 42 months of age.

1.7.2 Objectives

Based on our study and other human and animal studies it was hypothesized that exposure to PCBs and dioxins may be responsible for several toxic effects, especially developmental neurotoxicity. The aim of the present study was to investigate the adverse effects of environmental exposure to PCBs and dioxins through transplacental transfer as well as during lactation, on growth, health and neurodevelopmental outcome measures in healthy term born infants studied from birth until 42 months of age.

In this 42-month follow-up study the following questions will be answered.

- What are the PCB levels at 42 months of age in children either breast-fed or formula-fed during infancy.
- What are the major predictors for the child's PCB body burden at 42 months of age and what is the contribution of the diet during the toddler age.
- Which food products are the most important contributors for PCB and dioxin intake at toddler age.
- What is the impact of lactational PCB and dioxin exposure when compared to toddler and long-term dietary exposure until reproduction age, especially in women.
- Is environmental exposure to PCBs and dioxins as found in the Netherlands adversely related to any of the following outcome variables:
  - Birth weight, length and head circumference.
  - Postnatal growth at 3, 7, 18 and 42 months of age
  - Cognitive abilities at 42 months of age
  - Neurological condition at 42 months of age
  - Attentional processes and activity at 42 months of age
  - Behavioral measurements at 42 months of age
  - Health and immunological status at 42 months of age

1.7.3 Structure of the thesis

Prenatal PCB exposure was measured from PCB concentrations IUPAC nos. 118, 138, 153, and 180 in maternal and cord plasma. Seventeen PCDD/PCDF congeners and 26 PCB congeners were measured in human milk. Maternal milk PCB and dioxin concentrations are an indirect measure of prenatal exposure. PCB and

dioxin concentrations in maternal milk multiplied by breast feeding period are a good measure for lactational exposure. In Chapter 2.1 these exposure variables as well the toxic equivalents (TEQs) are presented according to the latest WHO TEF values.[37]

The PCB concentrations of congeners IUPAC nos. 118, 138, 153, and 180, measured in 42-month-old plasma samples, are a measure for current PCB body burden. The predictors for plasma PCB levels in Dutch preschool children either breast-fed or formula-fed during infancy are summarized in Chapter 2.2.

In Chapter 3, the dietary exposure to PCBs and dioxins during infancy, childhood and adulthood are compared, especially the influence of *lactational and long-term dietary* exposure until the reproductive age. The PCB/Dioxin *dietary intake at toddler age* is measured by a validated food questionnaire (FQ) designed by the Division of Human Nutrition and Epidemiology, Agricultural University Wageningen, The Netherlands. A model is described for the contribution of breast feeding and diet after weaning until adulthood for the total dietary intake. This is particularly important for the body burden of young women in the child baring age.

In Chapter 4, the effects of environmental exposure to PCBs and Dioxins on *birth size* and *growth* in Dutch children is described. Weight at birth was measured by the obstetrician or midwife. Weight, length or height and head circumference were measured at the ages of 10 days, 3, 7, 18 and 42 months. Anthropometric measurements at each time point were converted into standard deviation scores (SDS) using weight, height and head circumference standards of healthy Dutch children as reference data.[115]

In Chapter 5, the effects of environmental exposure to PCBs and Dioxins on *cognitive abilities* in Dutch children at 42 months of age is described. *Cognitive functioning* is measured by the Dutch version of the Kaufman Assessment Battery for Children (K-ABC).[116] The overall cognitive scale is based on a neuropsychological model of sequential and simultaneous processing confirmed by factor analysis of 11 subtests that are combined to form the Sequen tial and Simultaneous Processing scales. *Comprehensive language* is measured by the Dutch version of the Reynell Developmental Language Scales (RDLS).[117]

In Chapter 6, the *neurological condition* in 42-month-old children is related to pre- and postnatal exposure to PCBs and dioxins. A standardized *neurological examination* for toddlers, adapted from Hempel[94] is performed. Findings are classified as normal, minor neurological dysfunction (MND) or abnormal. In addition a *neurological optimality score* (NOS) and a *fluency* cluster score is calculated.

In Chapter 7, *attentional processes* and *activity* in 42-month-old Dutch children with environmental exposure to PCBs and Dioxins is described. *Attentional processes* are measured by a structured behavioral observation on the basis of a videotape of a *free play* session.[118] *Reaction Time* and *sustained attention* is measured by a computer game, especially designed for preschool children.[118] *Inattention* and *activity* were assessed from a Groninger Behavior Observation Scale, a parent questionnaire.

In Chapter 8, *problem behavior* in Dutch preschool children in relation to

environmental PCB and dioxin exposure is described. *Behavior* is measured by the Dutch version of the Child Behavior Checklist (CBCL).[119] This parent questionnaire contains 99 items for problem behavior. *Six syndromes*, were derived from factor analyses and labeled Oppositional, Aggressive, and Overactive, which constituted a broadband Externalizing grouping; Withdrawn/Depressed and Anxious, which constituted a broadband Internalizing grouping; and Sleep Problems. A *Total Problem Score* (TPS) is obtained by summing up all items.[119]

In **Chapter 9**, *immunological* and *health* status of Dutch toddlers are related to PCB and Dioxin exposure. The child's *health* status over the period from birth until 4 years of age was assessed with a health questionnaire. The prevalence of *infectious and allergic diseases* was assessed by parent questionnaire. *Humoral immunity* was measured by detecting antibody levels to mumps, measles and rubella after primary vaccination. *Immunological marker analyses* of lymphocytes were performed in a subgroup of 89 children.

In **Chapter 10** all relevant findings will be summarized according to type of effect, age and time of exposure (prenatal, lactational and current exposure). In addition a few methodological remarks will be mentioned and results are discussed for possible mechanisms of action in connection with animal and human literature. The contribution of prenatal versus postnatal exposure, and the role of type of feeding on neurobehavioral and cognitive outcome measures will be discussed.

In **Chapter 11** a summary of the thesis, conclusions as well as future research recommendations will be given.

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CHAPTER 2
LEVELS OF EXPOSURE

2.1 PCB and Dioxin levels in breast milk according to the 1997 TEF values

2.2 Plasma Polychlorinated Biphenyl levels in Dutch preschool children either breast-fed or formula-fed during infancy

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2.1 PCB AND DIOXIN LEVELS IN BREAST MILK ACCORDING TO THE 1997 TEF VALUES

2.1.1 Introduction

In this chapter levels of exposure will be summarized from previous published results[1]. The study group consist of 418 healthy mother-infant pairs recruited between June 1990 and June 1992. Two hundred and seven pairs have been living in Rotterdam and the surrounding area, which is a highly industrialized region in the western part of the Netherlands. The other 211 pairs have been living in Groningen and the surrounding area, which is a more rural region in the north. Women were asked by their obstetrician or midwife to volunteer for the study. During the last month of pregnancy, mothers were visited at home for an explanation of the study protocol. The study protocol had been approved by the medical ethical committee of the University Hospital Rotterdam/Sophia Children's Hospital and the University Hospital of Groningen. Informed consent was given by the parents.

2.1.2 Exposure assessment

Maternal plasma samples were obtained in the last month of pregnancy, and umbilical cord plasma samples were collected shortly after delivery. Maternal and cord plasma samples were analyzed for 4 PCB congeners, International Union of Pure and Applied Chemistry (IUPAC) nos. 118, 138, 153, and 180 by gas chromatography with electron capture detection (GC-ECD).[1] Plasma was denaturated with methanol and the chlorinated biphenyl congeners were isolated from the plasma with hexane-ethyl ether. The extract was dried over sodium sulphate, concentrated and purified by chromatography over basic alumina deactivated with 10% water. The analysis was carried out on two capillary columns of different polarity. The limit of determination for the four congeners was 0.01 µg/L plasma. The recovery of the chlorinated biphenyl congeners added to the plasma before extraction and determined as described above, was >95%. In each series a chemical blank and a control sample have been analyzed to assure the quality.

In the second and sixth week after delivery, 24-hour representative breast milk samples were collected and analyzed for the 17 most abundant 2,3,7,8-substituted PCDD and PCDF congeners and three planar PCB congeners (3,3',4,4'-tetrachlorobiphenyl (PCB 77), 3,3',4,4',5-pentachlorobiphenyl (PCB 126), and 3,3',4,4',5,5'-hexachlorobiphenyl (PCB 169)) by gas chromatography-high-resolution mass spectrometry (GC-HRMS).[1] Twenty-three non-planar PCB congeners, IUPAC nos 28, 52, 66, 70, 99, 101, 105, 118, 128, 137, 138, 141, 151, 153, 156, 170, 177, 180, 183, 187, 194, 195, and 202, were measured by gas chromatography with electron capture detection (GC-ECD). To express the toxic potency of the mixture of dioxins and PCBs in breast milk samples, the toxic equivalence factor (TEF) approach was used according to Safe[2] and the WHO 1993.[3] Recently the WHO revised the TEF values[4] and based on an evaluation of present database, the di-ortho PCBs, IUPAC nos 170 and 180 are no longer considered dioxin related and therefore deleted from the list.

A TEF value was assigned to the dioxin and dioxin-like PCB congeners which represents their relative toxic potency towards 2,3,7,8-TCDD, the most toxic dioxin congener which TEF value is one. By multiplying the concentration (pg/g milk fat) and TEF value, the toxic equivalent (TEQ) of each congener was calculated (pg TEQ/g milk fat). By adding up the TEQs of all congeners the total-TEQ value was obtained. The ΣTEQ of the 17 dioxin congeners yielded a Σdioxin-TEQ, the three planar PCBs nos. 77, 126 and 169, three mono-ortho PCBs nos 105, 118 and 156, revealed a planar and mono-ortho PCB-TEQ respectively. The other PCB congeners have no TEF value because they have negligible dioxin-like activity.

In Table 2.1 the levels of PCB congeners 118, 138, 153, and 180 of these 4 congeners are presented for cord and maternal plasma. In Table 2.1 the median TEQ levels are presented according to the new TEF values. The changed TEF values are given in italics. PCDD no 54 had a TEF of 0.5 and is now considered as toxic as TCDD with a TEF of 1.0. The other TEF value are lowered, for OCDD, no 75 and OCDF no 135 the TEF is 0.0001 instead of 0.001, for planar PCB no 77 the TEF is 0.001 and that was 0.005.

Compared to the previous results the Dioxin TEQ values is somewhat higher. Previously the median value was 30.2, now this is 33.5 pg TEQ/g fat. The median Planar PCB-TEQ is somewhat lower, instead of 16 pg TEQ/g fat, this value is 14.5 pg TEQ/g fat. Mono-ortho PCB-TEQs are the same, with a median value of 14.2 pg TEQ/ g fat.

Table 2.1.1 Mean, median and range of PCB 118, 138, 153 and 180 levels (μg/L plasma) in maternal and cord plasma.

	PCB 118	PCB 138	PCB 153	PCB 180
Maternal plasma				
n	415	415	415	415
mean	0.16	0.60	0.91	0.54
median	0.15	0.56	0.84	0.50
range	0.02-0.60	0.13-1.60	0.18-2.50	0.08-3.10
Cord plasma				
n	373	382	382	382
mean	0.04	0.13	0.18	0.10
median	0.04	0.11	0.15	0.08
range	0.01-0.16	0.01-0.59	0.02-0.85	0.01-0.76

Adapted from C.Koopman-Esseboom et al with permission[1]

Table 2.1.2 Median TEQ values (pg/g fat) of TEQ (pg TEQ/g fat) of PCDDs and PCDFs (N = 176), planar and mono-ortho PCBs (N = 194) in human milk samples two weeks after birth, according to the new TEF values of the WHO[4]

PCDDs	IUPAC	TEF	P50
2,3,7,8-TCDD	48	1.0	3.6
1,2,3,7,8-PECDD	54	*1.0*	10.2
1,2,3,4,7,8-HXCDD	66	0.1	1.5
1,2,3,6,7,8-HXCDD	67	0.1	4.6
1,2,3,7,8,9-HXCDD	70	0.1	0.67
1,2,3,4,6,7,8-HPCDD	73	0.01	0.58
1,2,3,4,6,7,8,9-OCDD	75	*0.0001*	0.07
PCDFs	IUPAC	TEF	P50
2,3,7,8-TCDF	83	0.1	0.073
1,2,3,7,8-PECDF	94	0.05	0.005
2,3,4,7,8-PECDF	114	0.5	10.8
1,2,3,4,7,8-HXCDF	118	0.1	0.65
1,2,3,6,7,8-HXCDF	121	0.1	0.55
2,3,4,6,7,8-HXCDF	130	0.1	0.30
1,2,3,7,8,9-HXCDF	124	0.1	0.008
1,2,3,4,6,7,8-HPCDF	131	0.01	0.063
1,2,3,4,7,8,9-HPCDF	134	0.01	0.002
1,2,3,4,6,7,8,9-OCDF	135	*0.0001*	0.000
ΣDIOXIN-TEQ			33.5
Planar PCBs	IUPAC	TEF	TEQ
3,3',4,4'-PCB	77	*0.0001*	0.001
3,3',4,4',5-PCB	126	0.1	13.8
3,3',4,4',5,5'-PCB	169	0.01	0.8
ΣPlanar PCB-TEQs			14.47
Mono-ortho PCBs	IUPAC	TEF	TEQ
2,3,3',4,4'-PCB	105	0.0001	0.87
2,3',4,4',5-PCB	118	0.0001	3.28
2,3,3',4,4',5-PCB	156	0.0005	10.0
ΣMono-ortho-PCB TEQ			14.22
ΣTotal-TEQ			63.52

IUPAC = International Union of Pure and Applied Chemistry

TEF = Toxic Equivalence Factor TEQ= Toxic Equivalent

2.1.3 References

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2.2 PLASMA POLYCHLORINATED BIPHENYL LEVELS IN DUTCH PRESCHOOL CHILDREN EITHER BREAST-FED OR FORMULA-FED DURING INFANCY

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2.2.1 Abstract

Objectives: This study examined the influence of lactational and in utero exposure to Polychlorinated biphenyls (PCBs) on plasma PCB levels in children.

Methods: Plasma PCB levels were measured in 173 Dutch children at 3.5 years, of whom 91 were breast-fed and 82 were formula-fed in infancy.

Results: Median plasma PCB levels were 3.6 times higher in breast-fed children (0.75 µg/L) than in their formula-fed peers (0.21 µg/l). Breast-feeding period and breast milk PCB levels were important predictors for PCB levels in the breast-fed group. For children in the formula-fed group, PCB levels were significantly related to their maternal plasma PCB levels.

Conclusions: PCB levels in Dutch preschool children are related to transfer of maternal PCBs; therefore, strategies should be aimed at reducing maternal PCB body burden.

2.2.2 Introduction

Polychlorinated biphenyls (PCBs), polychlorinated dibenzo-para-dioxins (PCDDs), and polychlorinated dibenzo-furans (PCDFs) are widespread environmental contaminants.[1] In Dutch infants subtle signs of neurological dysfunctioning,[2] small delays in psychomotor development[3] and alterations in thyroid hormone[4] and immunological status[5] during infancy are associated with perinatal exposure to PCBs and PCDDs/PCDFs.

Human exposure to PCBs and PCDDs/PCDFs is mainly through the food chain, for example, dairy products, fish and meat.[6, 7] The Netherlands is among the countries with the highest environmental levels of PCBs and PCDDs/PCDFs as measured in breast milk.[8] In breast-fed infants, daily PCB and PCDD/PCDF intake is 20 times higher than the Tolerable Daily Intake (TDI) of 10 pg toxic equivalent per kilogram per day.[6, 9]

We report plasma PCB levels measured in Dutch children at 3.5 years and the contribution of in utero and lactational exposure to PCBs. Furthermore, we relate plasma PCB levels in these children to their dietary intake of PCBs and PCDDs/PCDFs and to their body fat.

2.2.3 Methods

From 1990 to 1992 this study enrolled 207 children, of whom 105 were breast-fed and 102 were formula-fed during infancy. Subjects were living in Rotterdam or its surroundings, an industrialized and densely populated area in the Netherlands. The formula consumed in the formula-fed group was from one batch (Almiron M2, Nutricia NV, The Netherlands) and had negligible concentrations of PCBs and PCDDs/PCDFs. The study was approved by the Medical Ethical Committee of the University Hospital Rotterdam. The study design and chemical analysis methods are described elsewhere.[3, 10]

In 1994, at 3.5 years of age, children were visited at home for a parental interview, a food questionnaire, and their developmental follow-up. Blood collection for PCB analysis and measurement of weight, height and skinfold thickness took place at the Children's Hospital.

Analysis of four PCB congeners International Union of Pure and Applied Chemistry (IUPAC) numbers 118, 138, 153 and 180, was performed at the Nutrition and Food Research Institute, Zeist, the Netherlands. The same method was used for PCB analysis in maternal and cord plasma.[10,11] PCB values in plasma are reported on a volume basis (µg/L) and the PCB sum (ΣPCB) was calculated by adding up the four congeners in each plasma sample.

Prenatal PCB exposure was estimated from the ΣPCB in maternal plasma collected during the last trimester of pregnancy and in cord plasma.[10] Postnatal PCB exposure was estimated by the ΣPCB of the same four congeners in breast milk and number of weeks of breast-feeding. Total toxic equivalent (TEQ) in breast milk was calculated from 8 dioxin-like PCBs and 17 PCDDs/PCDFs according to the World Health Organization and S.H. Safe.[10,12,13]

Dietary intake of PCBs and PCDDs/PCDFs at preschool age was assessed by a validated food questionnaire developed by the Agricultural University Wageningen, the Netherlands. Comparison of the results of the questionnaire with the dietary history as a reference showed a good agreement between the mean intakes (r= 0.58 for PCDDs/PCDFs and r= 0.40 for PCBs). The total TEQ intake was calculated in each food item by means of reference data for food products provided by the National Institute of Public Health and the Environment (RIVM).[6,14]

Weight (kg), height (m) and skinfold thickness (mm) at 4 sites- bicipital, tricipital, subscapular and suprailiacal skinfolds-were measured according to Tanner.[15] Total body fat percentage was calculated from 4 skinfolds as described by Weststrate and Deurenberg.[16]

Plasma PCB levels were normalized by natural logarithmic transformation. Median and range of the ΣPCB are reported. The Student's t-test and chi-squared test were used to compare differences between the breast-fed and the formula-fed group. Multiple linear regression analysis was performed separately for the breast-fed and the formula-fed group. Plasma ΣPCB levels at 3.5 years of age were entered as dependent variable. The PCB levels in breast milk and duration of breast-feeding in weeks were entered as exposure variables for the breast-fed group. The ΣPCB levels in maternal plasma were entered as prenatal exposure in both groups. Results were statistically significant at a p-value $\leq$ 0.05. Data analysis was performed with SPSS.[17]

2.2.4 Results

Of the original 207 infants, 193 were examined at 3.5 years (100 breast-fed and 93 formula-fed). Fourteen infants were lost to follow-up, owing to lack of interest (n = 10) and emigration (n=4). Polychlorinated biphenyl levels were measured in plasma of 173 children (91 breast-fed and 82 formula-fed).

In the breast-fed group there were slightly more boys (p=0.04) and the total body fat was lower (p=0.007) compared with the formula-fed group. Children in the breast-fed group had significantly higher median PCB levels in plasma (p<0.0001) than children in the formula-fed group. Dietary intake of total TEQs did not differ in the two groups (Table 2.2.1 and Table 2.2.2).

Table 2.2.1 Characteristics and PCB levels of formula-fed and breast-fed children at 3.5 years of age

	Formula-fed group	Breast-fed group	P-value[a]
Number	93	100	
Gender			
male	43 (46%)	61 (61%)	0.04
Parity			
Firstborn	44 (47%)	53 (53%)	ns
Age of child (yr)	3.54 ± 0.10	3.53 ± 0.08	ns
Weight of child (kg)	17 ± 2.1	16.5 ± 2.0	ns
Body fat of child (%)	20.4 ± 3.5	19 ± 3.0	0.007
Maternal age (yr)	28.9 ± 4.2	28.8 ± 3.2	ns
Daily dietary intake[b]			
TEQs (pg/day)	104 ± 33.4	107 ± 38.8	ns

a: student t-test or chi-square test.

b: TEQ = toxic equivalents of dioxin like PCBs and 2,3,7,8 TCDD consumed per day.

Values are numbers (percentages), means ± standard deviation and median (range)

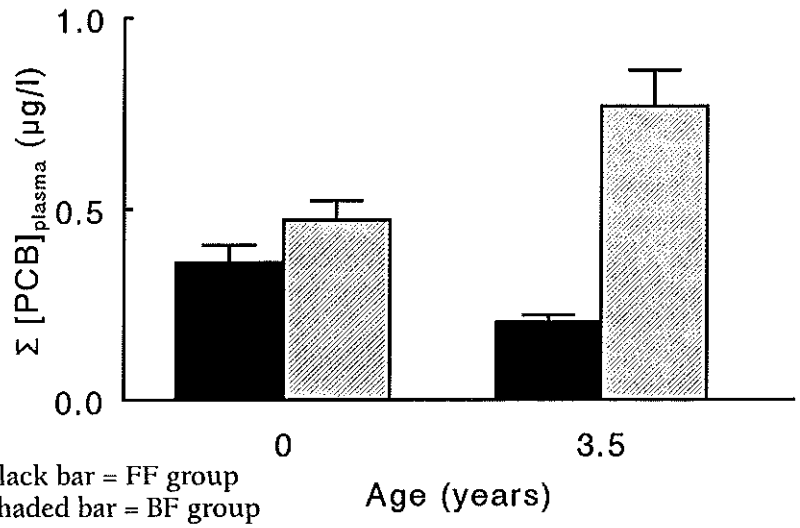
Table 2.2.2 Median, and range of PCB 118, 138, 153, 180 and ΣPCB, in μg/L plasma of children at 3.5 years of age either breast- or formula-fed during infancy

	PCB 118	PCB138	PCB 153	PCB 180	ΣPCB[a]
breast-fed group					
n=91					
Median	0.060	0.200	0.330	0.160	0.750
Range	0.02-0.22	0.06-1.30	0.10-2.40	0.04-2.10	0.23-5.90
formula-fed group					
n=82					
Median	0.030	0.060	0.080	0.040	0.210
Range	0.0-0.06	0.02-0.140	0.03-0.20	0.01-0.09	0.08-0.46

a: sum of PCB 118, 138, 153 and 180. Comparison for all individual PCB congeners and ΣPCB between the formula-fed and breast-fed group was significant Student t-test P<0.0001.

Levels of PCBs measured in cord plasma and in 3.5 year-old plasma, were available for 155 children (81 breast-fed and 74 formula-fed). Plasma ΣPCB levels at 3.5 years of age in the breast-fed group were significantly higher than their levels in cord plasma (median = 0.75 vs. 0.45 µg/L, p=0.001), whereas in the formula-fed group, plasma ΣPCB levels were significantly lower than their levels in cord plasma (median = 0.21 vs 0.35 µg/L, p=0.003), see Figure 2.2.1.

Figure 2.2.1 Comparison between mean PCB values at birth and preschool age in children breast-fed or formula-fed in infancy



Mean and 95% confidence interval is given by error bars for plasma ΣPCB levels at birth (0 years) and at preschool age (3.5 years). Black bars represents the formula-fed group (n=74) and shaded bars represents the breast-fed group (n=81). Plasma PCB levels are significantly different between 0 and 3.5 years of age (paired t-test, p<0.01).

PCB levels in maternal and cord plasma were significantly correlated with the PCB levels at 3.5 years in the breast-fed group (r=0.40 for maternal plasma; r=0.37 for cord plasma) as well as in the formula-fed group (r=0.44; r=0.35). In the breast-fed group, PCB levels were significantly correlated with the period of breast-feeding (r=0.63), milk PCB levels (r=0.39), and the total TEQ in breast milk (r=0.36).

The breast-feeding period (p<0.0001) and ΣPCB level in breast milk (p=0.02) were important predictors for plasma PCB levels at 3.5 years in the breast-fed group. In the formula-fed group, PCB levels at 3.5 years were significantly associated with their maternal ΣPCB levels (p=0.009) and daily total TEQ intake through diet (p=0.04). A higher body weight of the child was significantly associated with lower plasma PCB levels at 3.5 years in both groups (Table 2.2.3a and 2.2.3b). The same was true for body fat when this was entered in the regression analysis. The standardized regression coefficient increased to -0.51 and -0.31 for the breast-fed and formula-fed groups, respectively.

Table 2.2.3a Multiple linear regression analysis with plasma SPCBa at 3.5 years of age as dependent variable in the breast-fed group.

	regression coefficient	(se)	Standardized regression coefficient	P-value
Constant	-0.938	(0.572)		0.10
Weeks of breast feeding	0.030	(0.003)	0.64	<0.0001
ΣPCB breast milk (ng/g fat)	1.031	(0.448)	0.30	0.02
ΣPCB maternal plasma (µg/L)	0.088	(0.081)	0.15	0.28
Maternal age (years)	0.003	(0.015)	0.02	0.85
Weight of child (kg)	-0.055	(0.024)	-0.17	0.02
Dietary intake of Total TEQ (pg/day)	0.002	(0.001)	0.13	0.09

N=81, R^2 = 0.63 Multipele R = 0.79 a: Sum of Polychlorinated Biphenyls, IUPAC nrs 118, 138, 153 and 180

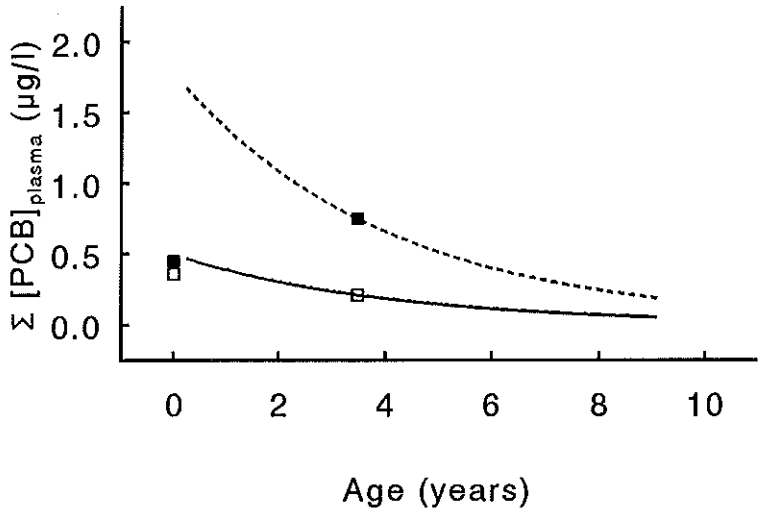
Table 2.2.3b Multiple linear regression analysis with plasma ΣPCB[a] at 3.5 years of age as dependent variable in the formula-fed group.

	regression coefficient	(se)	Standardized regression coefficient	P-value
Constant	-1.434	(0.461)		0.003
ΣPCB maternal plasma (µg/L)	0.121	(0.045)	0.29	0.009
Maternal age (years)	0.020	(0.011)	0.20	0.07
Weight of child (kg)	-0.07	(0.02)	-0.38	0.0002
Dietary intake of Total TEQ (pg/day)	0.003	(0.001)	0.20	0.04

N=79, R^2 = 0.36 Multipele R = 0.60 a: Sum of Polychlorinated Biphenyls, IUPAC nrs 118, 138, 153 and 180

Figure 2.2.2 gives an estimate of plasma PCB levels from birth to school age based on the assumption that the half-life for plasma PCBs is 2.8 years in children[18] and that the dietary intake of PCBs after weaning is negligible compared with prenatal and lactational exposure. On the basis of these assumptions and the measured PCB levels at 3.5 years, PCB levels in infants during breast-feeding must have reached levels as high as their mother's

Figure 2.2.2 Estimated PCB levels from infancy to school age for children breast-fed or formula-fed during infancy.



Estimated PCB values are calculated from measured PCB values at 3.5 years of age and the assumption that the half-life of PCBs is 2.8 years. The dashed line represents estimated values for children from the breast-fed group, and the solid line, estimated values for children from the formula-fed group. Measured values have been marked with closed squares (breast-fed group) and open squares (formula-fed group).

2.2.5 Discussion

Our study demonstrates that plasma PCB levels of preschool children breast-fed in infancy are 3.6 times higher than those of their formula-fed peers. Plasma PCB levels in the breast-fed group are 167 % of their cord plasma levels, compared with 60% in the formula-fed group. To our knowledge, no other study has measured plasma PCB levels in children -either formula-fed or breast-fed during infancy- in relation to environmental exposure to PCBs.

In agreement with studies performed in children concerning populations either accidentally or occupationally exposed[18,19] to PCBs, or populations living in farms with PCB-contaminated silos or consumption of contaminated fish,[20,21] PCB levels in Dutch breast-fed children were significantly related to the period of breast-feeding and PCB levels in breast milk. A German study measured PCBs in adipose tissue of infants

and toddlers, however, the quantity of breast milk consumed was estimated, not the PCB levels in breast milk.[22] In contrast to our study, comparison with a fomula-fed group was not performed in these studies.

Jacobson et al, could detect PCB levels in half of the samples tested in 4 year-old children whose mothers were exposed either by contaminated fish or farm products.[20] In our study, PCB levels were detectable in all children. This might be due to higher exposure in our group, however, it is more likely due to a more accurate laboratory technique[10,11] used in our study.

We were not able to estimate the dietary intake of the four PCB congeners measured in plasma. Instead the total TEQ intake was measured. There is, however, a good correlation between total TEQ and individual congeners.[10] The dietary TEQ intake had a small but significant effect on the plasma PCB levels in the formula-fed group, but not in the breast-fed group. This difference can be explained by the high intake of PCBs and total TEQs through breast milk.

The observed negative association between total body fat (body weight) and plasma PCB levels is in agreement with two other studies.[18,19] PCBs are distributed over all fat-containing components in the body. Lower PCB levels in children with a higher total body fat most likely concerns a dilutional effect. The total body burden of infants with a high fat content might even be higher compared to that of leaner children. To calculate total body burden, PCBs should be expressed per gram of fat in plasma or adipose tissue, and total body fat should be estimated.

We conclude that plasma PCB levels in Dutch preschool children are the result of exposure through breast milk and in utero exposure. The influence of dietary intake of PCBs after weaning is small compared to the intake during breast-feeding. To lower PCB levels in childhood, future mothers will have to reduce their long-term intake of PCBs years before pregnancy in order to lower both in utero and lactational transfer. Strategies should be aimed at reducing PCB accumulation in the food chain.

2.2.6 Acknowledgments

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CHAPTER 3

DIETARY EXPOSURE TO POLYCHLORINATED BIPHENYLS AND DIOXINS FROM INFANCY UNTIL ADULTHOOD: A COMPARISON BETWEEN BREAST-FEEDING, TODDLER AND LONG-TERM EXPOSURE.

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3.1 ABSTRACT

Background: Food is the major source for polychlorinated biphenyl (PCB) and dioxin accumulation in the human body. Therefore, investigating food habits from early ages until reproduction age (25 years) is important in order to assess exposure risk for the next generation.

Objectives: To assess the PCB/dioxin exposure and the relative contribution of different foods to total exposure during preschool age. Particularly, the importance of lactational PCB/dioxin exposure vs. dietary exposure until adulthood was investigated.

Design: A cohort of 207 children was studied from birth until preschool age. Based on three planar PCBs and seventeen 2,3,7,8 substituted -dibenzo-*para*-dioxins (PCDDs) and -dibenzo-furans (PCDFs) measured in breast milk, a model was developed to calculate the cumulative toxic equivalent (TEQ) intake during breast-feeding (0-1 year). In 3.5 year-old children, daily dietary intake of planar PCB-TEQ and dioxin-TEQ was measured with a validated food questionnaire (FQ). Cumulative TEQ intake from 1-5 years was estimated using the PCB- and dioxin-TEQ intake measured with the FQ. Cumulative TEQ intake from 6-25 years was estimated using national food consumption and contamination data of PCB- and dioxin-TEQ intake.

Results: In toddlers, dairy products contributed 43 % to PCB-TEQ and 50 % to dioxin-TEQ intake. Meat and meat products contributed to 14 % and 19 %, respectively and processed foods to 23 % and 15 %, respectively. Breast-feeding for 6 months, contributed 12% in boys and 14 % in girls, to the cumulative PCB/dioxin TEQ intake until 25 years of age. The daily TEQ intake per kg body weight in breast-fed infants is 50 times higher, and toddlers is three times higher than in adults.

Conclusions: Long-term dietary exposure to PCBs and dioxins in men and women, is partly due to breast-feeding (12 respectively 14 %). After weaning, dairy products, processed foods and meat are major contributors of PCB and dioxin accumulation until reproduction age. Instead of discouraging breast-feeding, maternal transfer of PCBs and dioxins to the next generation must be avoided by enforcement of strict regulations of PCB and dioxin discharge, and by reducing consumption of animal products and processed foods, starting at all ages.

3.2 INTRODUCTION

Polychlorinated Biphenyls (PCBs) and polychlorinated dibenzo-*para*-dioxins (PCDDs) and dibenzofurans (PCDFs) are halogenated aromatic compounds that are widespread and persistent environmental pollutants.[1] The highly lipophilic and hydrophobic PCBs, dioxins (PCDDs and PCDFs) and related compounds, tend to partition into soil and sediment, bioconcentrate from water to aquatic animal and biomagnify up the multi step food chain.[1] Humans are also high on the food chain, eating meat and dairy

products of herbivores as well as fish and plants.[1] More than 90% of the total daily human exposure to PCBs and dioxins is made up of oral intake from food, whereas other routes e.g. water, air and soil contribute to less than 10% of total exposure.[23] The average daily dose is about 1-3 pg/kg body weight (BW) of dioxin-like compounds considered equivalent in toxicity to 2,3,7,8-tetra chloro-dibenzo-dioxin (TCDD).[2]

The human fetus is exposed to PCBs and dioxins through placental transport[4-6] and higher quantities of these compounds are transferred to the infant during breast-feeding.[7,8] The concentration of these compounds in breast milk or cord blood is dependent on the maternal PCB and dioxin body burden. This body burden is the result of accumulation of PCBs and dioxins over many years in especially fat tissue, combined with the low metabolic degradation and rate of excretion.[1] As a result the half-life is very long, for higher chlorinated PCDDs and PCDFs ranging between 4 to 12 years[9] and for PCBs ranging between 5 to 15 years.[10] Maternal age is positively related and the period of previous breast-feeding is negatively related to maternal PCB and dioxin concentrations.[11] Results from previous studies showed that, the contribution of pregnancy related diet to PCB and dioxin concentrations in human milk and in maternal and cord plasma was very low.[12] The same was concluded when dietary intake of PCBs and dioxins in preschool children were related to plasma PCB levels measured at preschool age.[8] Short term dietary regiments with low dioxins during the lactation period showed no reduction in dioxin concentrations in breast milk.[13]

Follow-up studies performed in children of women accidentally exposed to high levels of PCBs and related compounds (Japanese and Taiwanese rice oil incidents) have demonstrated a variety of health effects, e.g. lower birth weight, hyper pigmentation, conjunctivitis, nail changes and developmental delay,[14,15] similar to toxic effects reported in animal studies.[16] Newborns of mothers who reported consumption of PCB contaminated fish from Lake Michigan USA showed lower birth weight[17] and lower IQ scores at school age.[18] The PCB levels in the Michigan cohort were at or slightly above background levels.

As in other industrialized countries in Western Europe, contamination of breast milk with PCBs and dioxins in the Netherlands has led to considerable public concern. The Dutch government launched a longitudinal neurodevelopmental study in 1989 aimed at investigating the adverse effects of background exposure to PCBs and dioxins on growth and development of healthy full-term infants. The period of observation was expanded to preschool age in an European Community funded European collaborative project. Previously reported results showed that, in Dutch infants, lower birth weights and decreased postnatal growth rate[19] delay in psychomotor development[20] and neurodevelopment,[21] alterations in thyroid hormone[22] and immunological status[23] were associated with prenatal PCB and dioxin exposure rather than with lactational exposure.

Given the results from above mentioned studies, and the bioaccumulation of PCBs and dioxins in the food chain, an estimate of dietary exposure to PCBs and dioxins from early ages until reproduction age is important, in order to assess exposure risk for the next generation. In the present study, dietary intake of PCBs and dioxins and the relative

contribution of different foods to total PCB/dioxin exposure in Dutch preschool children as assessed with a validated food questionnaire are reported. We describe a model that represent the cumulative intake of PCBs and dioxins during breast-feeding versus the cumulative intake after breast-feeding, e.g. until 25 years of age.

3.3 METHODS

Context of the study, subjects and design

From 1990-1992, 207 mother-infant pairs were recruited in Rotterdam and surroundings, a highly industrialized area in the Netherlands. Infants included in the study were first or second children, born at full-term, Caucasian, without perinatal complications. To study the effects of prenatal and postnatal exposure to PCBs and dioxins, two groups of women were included: a group of women who intended to breast-feed their child for at least 6 weeks (the breast-fed group) and a group of women who intended to give formula (the formula-fed group) to their newborn infants. The formula (Almiron M2, Nutricia NV, The Netherlands) given to the infants had no detectable concentrations of PCBs and dioxins. Children were examined at 2 weeks; 3, 7 and 18 months; and 3.5 years of age for their growth and neurodevelopmental follow-up. At 3.5 years of age, 14 children (7%) were lost to follow-up: 10 were lost due to lack of interest and 4 due to inability to cooperate (illness in the family, separation and emigration). Informed consent was given by participating parents, and the study protocol was approved by the medical ethics committee of the University Hospital Rotterdam, The Netherlands.

Four PCB congeners, International Union for Pure and Applied Chemistry (IUPAC) nos 118, 138, 153 and 180, were measured in maternal plasma collected during the last month of pregnancy. These four PCB congeners were also measured in cord plasma collected directly after birth and in plasma samples collected from children at 3.5 years of age. All plasma samples were analyzed at the Nutrition and Food Research Institute, Zeist, the Netherlands by gas chromatography with electron capture detection (GC-ECD). Methods of determination, laboratory validation and quality control have already been published.[8,24] Breast milk samples were analyzed for PCB and PCDD/PCDF congeners as described below.

TEQ intake of PCBs and dioxins during breast-feeding. (0-1 year)

In the second week after delivery, a 24 hour representative sample of breast milk was collected from breast-feeding mothers. Breast milk samples were analyzed for the seventeen most abundant 2,3,7,8 substituted PCDD and PCDF congeners and three planar PCB congeners, IUPAC nos 77, 126 and 169, by gas chromatography-high-resolution mass spectrometry (GC-HRMS).[25] Twenty-three non-planar PCBs, IUPAC nos 28, 52, 66, 70, 99, 101, 105, 118, 128, 137, 138, 141, 151, 153, 156, 170, 177, 180, 183, 187, 194, 195 and 202, were measured by GC-ECD at the State Institute for Quality Control of Agricultural Products (RIKILT-DLO), Wageningen, The

Netherlands.[25] To express the total toxic potency of dioxins and planar PCBs, the toxic equivalent factor (TEF) approach was used according to Safe[26] and Ahlborg et al.[27] A TEF value was assigned to the dioxin and planar PCB congeners which represents their relative toxic potency towards 2,3,7,8-tetra chloro dibenzo-p-dioxin (TCDD), the most toxic congener with a TEF value of one. The toxic equivalents (TEQ) were calculated by multiplying the concentration (pg/g milk fat) of each congener by its TEF value. Representative dioxin-TEQ and planar PCB-TEQ levels could be calculated for 83 and 95 milk samples, respectively. The remaining human milk samples were missing or not analyzed due to organizational failure. Levels and methods of determination of PCBs and dioxins in breast milk have been reported previously.[24,25]

We used the planar PCB-TEQ and dioxin-TEQ values measured in breast milk to calculate the cumulative TEQ intake during breast-feeding in order to compare the TEQ intake during breast-feeding with the TEQ intake measured by the food questionnaire (FQ) during preschool age and national food consumption and contamination data. The cumulative TEQ intake during breast-feeding was calculated based on the following assumptions:

(1) From birth until 6 months of age (26 weeks) an infant will drink an average of 800 ml/day,[28-30] from 6 to 9 months (27-39 weeks), 500 ml/day,[30] and from 9 months (> 39 weeks) until cessation of breast feeding 400 ml/day. Breast-feeding period was reported by the mother as the number of weeks during which the infant was predominantly breast-fed.

(2) Literature data indicate that 95% of all PCBs and dioxins are absorbed in the digestive tract,[31,32]

(3) Several studies have shown that PCB and dioxin body burden of a mother during breast-feeding decreases by 20% per three months and therefore we used this percentage to calculate a weekly decrease of 1.7% in PCB and dioxin concentration in breast milk of each breast-feeding mother.[33,34]

The following equation was used to calculate the cumulative planar PCB- and dioxin-TEQ intake (I) during the whole breast-feeding period:

$$I = 0.95 \times V \times [BMF] \times [TEQ]_{breast\text{-}milk} \times {}_0\int^{T} e^{-0.017t}\, dt$$

I = Cumulative intake of planar PCB-TEQ and dioxin-TEQ from for each breast-fed infant during the period of breast-feeding (pg).

0.95 = Fraction of PCBs/dioxins absorption from breast-milk in the intestinal tract.

T= Period of breast-feeding in weeks.

V= Volume of breast milk consumed in ml per week.

BMF= Breast milk fat concentration in g/ml.

TEQ= Toxic equivalents of planar PCBs (IUPAC nos 77, 126 and 169) and 17 dioxin congeners measured in pg/g milk fat.

TEQ intake of PCBs and dioxins during the preschool period (1-5 years)

At the age of 3.5 years, the primary care giver of the child, usually the mother, was asked to fill out a semi-quantitative food questionnaire (FQ) developed by the Division of Human Nutrition and Epidemiology, Agricultural University Wageningen, the Netherlands. The FQ, which reflected the usual diet of the child over the last month, was validated against the dietary history method in a subgroup of the study population (n=47).[8] The FQ was designed to assess dietary TEQ intake of three planar PCBs, IUPAC nos 77, 126 and 169, and seventeen 2,3,7,8 chlorine substituted PCDDs and PCDFs, as well as energy, fat, carbohydrate and protein intake in preschool children. The daily intake of PCB-TEQ and dioxin-TEQ was determined by calculating the TEQs of three planar PCBs and seventeen 2,3,7,8-chlorine substituted dioxins, in each food item[26,27] using reference data for food products provided by the National Institute of Public Health and the Environment (RIVM).[3,35] Intake of energy, fat, protein and carbohydrate were calculated using the Dutch Food Database 1993.[36] Food intake data were converted into energy (kilojoules), nutrients (grams) and TEQs (picograms) using the software package Komeet.®[37] Daily total-TEQ intake was defined as the sum of planar PCB-TEQ and dioxin-TEQ intake in picograms per day (pg/d). From 193 children studied at 3.5 years, 183 FQs were available for analysis: five FQs were not returned or filled out by the parents and five were excluded because of incomplete or unreliable answers.

The cumulative intake of dioxin-TEQ and planar PCB-TEQ from 1-5 years for boys and girls was calculated from the mean total-TEQ and mean fat intake per day estimated from the FQ. The cumulative TEQ intake from 1-5 years was calculated for boys and girls separately according to the following equation, assuming an intestinal PCB/dioxin absorption of 95%:

I = 0.95 x Fat x [TEQ] x 365.25 x number of years.

I = Cumulative intake of planar PCB-TEQ and dioxin-TEQ (pg) from 1-5 years of age for boys and girls

0.95 = Fraction of PCBs/dioxins absorption in the intestinal tract.

Fat = Mean daily fat intake in grams (g), for boys or girls, derived from the FQ.

TEQ= Daily dietary intake of toxic equivalents of planar PCBs (IUPAC nos 77,126 and 169) and 17 dioxin congeners measured in pg per g fat intake, derived from the FQ.

TEQ intake from childhood until adulthood (6-25 years)

The cumulative TEQ intake from 6-25 years of age was calculated based on data from the RIVM[35] and the National Food Consumption Survey (FCS, 1992).[38] The FCS includes comprehensive description of intake of foods, energy and nutrients of 6,218 persons in 1992, by age group and gender.[38] Median intake, estimated from RIVM data in the Dutch population, of planar PCB-TEQ, was 70 pg/day; for dioxin-

TEQ, this was 65 pg/day yielding a total TEQ intake of 135 pg/day.[39] This value in combination with a mean daily fat consumption of 92 g in the Dutch population derived from the FCS[38] yields a mean daily TEQ consumption of 1.47 pg/g fat. Using this value and values for mean daily fat consumption per age group and gender derived from the FCS 1992, daily TEQ intake by gender and age group (6-10 y, 11-15 y, 16-20 y and 21-25 y) was calculated. The cumulative TEQ intake for men and women was calculated for each 5-year age-group using the following equation, again assuming an intestinal absorption of 95%:

I = 0.95 x Fat x 1.47 pg TEQ/g fat x 365.25 x number of years.

I = Cumulative intake of planar PCB-TEQ and dioxin-TEQ (pg) from 6-25 years of age for men and women.

0.95 = Fraction of PCBs/dioxins absorption in the intestinal tract.

Fat = Mean daily fat intake in grams (g), for men and women per age group derived from the FCS, 1992.[38]

Other variables

Information regarding socio-economic background, obstetrical and neonatal history, maternal age, parents' education level, parity and gender were obtained at birth. At 3.5 years of age weight, height and skinfolds-thicknesses (mm) at 4 sites (bicipital, tricipital, subscapular and supra-iliacal) were measured according to Tanner.[40] Total body fat percentage was calculated from 4 skinfolds as described by Weststrate and Deurenberg.[41]

Data analysis and statistical methods

The student t-test, chi-square test and Mann-Whitney test were used to compare differences between groups. The cumulative intake of PCB-TEQ and dioxin-TEQ during breast-feeding was calculated with the method of integration by pieces according to the volume of breast milk consumption varying over time (6-26 weeks, 27-39 weeks, > 39 weeks). The predictive value of various variables for daily dietary TEQ exposure at 3.5 years was estimated by means of multiple linear regression analyses, after adjustment for confounders. Results were considered statistically significant at a p-value ≤ 0.05. Data analysis was performed by the statistical software package SPSS for Windows.[42]

3.4 RESULTS

In Table 3.1 general characteristics of the population are presented. The original study population consisted of 207 mother-infant pairs. Fifty-one percent (n=105) was breast-fed and 49 % (n=102) was formula-fed in infancy. The median (range) of the breast-feeding period was 16 (6-72) weeks. The mean maternal age at birth was 29 years, 19 % of the mothers was lower educated (primary school finished, secondary school not finished), 40 % was middle-educated (secondary school finished) and 41 % was higher educated (high school finished or professional/ university training). The median sum of four PCB congeners measured in maternal plasma during pregnancy and in cord plasma were 2.04 respectively 0.40 µg/L. At 3.5 years, children in the breast-fed group had sum PCB levels that were nearly 4 times higher compared to sum PCB levels measured in the formula-fed group (0.75 vs 0.21 µg/L, Table 3.1). Median planar PCB-TEQ, dioxin-TEQ and total TEQ concentrations in breast milk were 14.8, 30.6 and 46.9 pg TEQ/g fat, respectively (Table 3.1).

The mean daily intake of selected foods, energy and nutrients at 3.5 years for boys and girls are presented in Table 3.2. Milk and milk products, industrial products, cheese, meat and meat products were consumed by nearly all preschoolers, whereas fish was consumed by a smaller group (29%) of children. Both energy and carbohydrate intake were significantly lower in girls when compared with boys (t-test, $p<0.05$, Table 3.2). Multiple linear regression analyses showed that energy, fat, carbohydrate and protein intake at 3.5 years were significantly lower in the group with higher educated mothers (all p-values <0.01). Furthermore, body weight and body fat percentage at 3.5 years were positively associated with energy, fat, protein and carbohydrate intake (all p-values <0.05).

At 3.5 years, the mean daily PCB-TEQ intake was 60 pg in the previously breast-fed group and 57 pg in the previously formula-fed group (not significant). The mean daily dioxin-TEQ intake was 46 and 47 pg, respectively (not significant). No differences in mean daily PCB-TEQ and dioxin-TEQ was found between boys and girls (Table 3.3).

The mean daily TEQ intake in boys and girls was 6.5 respectively 6.3 pg TEQ/kg body weight (BW) per day. Four percent of children at 3.5 years exceed the Tolerable Daily Intake (TDI) according to the WHO of 10 pg TEQ/kg BW[43] and 100% of all 3.5 year olds exceed the recommended TDI of 1 pg TEQ/kg BW/day, by The Netherlands Health Council's Committee on Risk Evaluation of Substances/Dioxins 1996.[44] Multiple linear regression analyses showed that after adjustment for gender and body weight, daily intake of planar PCB-TEQ and dioxin-TEQ at 3.5 years were both significantly lower in children whose mothers had a high education level compared to children from mothers with a low and middle level of education (all p-values <0.01).

Table 3.1: Characteristics of the study group from birth until 3.5 years of age

At birth (n=207)	
Formula-fed	102 (49%)
Breast-fed	105 (51%)
-Breast feeding period (weeks)	16 (6-72)
Gender	
-male	109 (53%)
Parity	
-firstborn	102 (49%)
Maternal age (yr)	29 ± 4
Maternal education [1]	
-low	40 (19%)
-middle	82 (40%)
-high	85 (41%)
At 3.5 years (n=193)	
Age (months)	42 (41-47)
Weight (kg)	
-Boys	17.0 ± 2.1
-Girls	16.4 ± 2.0
Body fat[2] (%) (n=163)	
-Boys	18.6 ± 3.2
-Girls	20.9 ± 3.1
Toxic compounds	
Measured in plasma	
-ΣPCB[3] maternal (µg/L) (n=206)	2.04 (0.59-7.35)
-ΣPCB[3] cord (µg/L) (n=182)	0.40 (0.08-2.08)
-ΣPCB[3] at 3.5 years (µg/L) (n=173)	0.35 (0.08-5.90)
Formula-fed group (n=82)	0.21 (0.08-0.46)
Breast-fed group (n=91)	0.75 (0.23-5.90)
Measured in breast milk (breast-fed group, n=105)	
-Fat percentage in breast milk (n=105)	2.98 ± 0.71
-Planar PCB-TEQ[4] (pg/g milk fat) (n=95)	14.8 (4.4-45.7)
-Dioxin-TEQ[4](pg/g milk fat) (n=83)	30.6 (11.1-76.4)
-Total TEQ[4] (pg/g milk fat) (n=83)	46.9 (19.1-102.6)

1: Low ;Primary school finished and secondary school not finished. Medium; secondary school finished High; high school finished/professional and university training 2: body fat percentage was calculated from 4 skinfolds as described by Weststrate and Deurenberg (41). 3: sum of PCBs IUPAC nos. 118, 138, 153 and 180 measured in maternal-, cord- and 3.5-year-old plasma samples 4: TEQ = toxic equivalents of planar PCBs (IUPAC nos. 77, 126 and 169) and seventeen substituted 2,3,7,8 PCDDs and PCDFs measured in breast milk samples. Total TEQ= the sum of PCB-TEQ and dioxin-TEQ in breast milk. Values are numbers (percentages), means ± standard deviation and median (range)

Table 3.2: Daily intake of selected foods, energy and macro nutrients at 3.5 years of age according to gender.

	Boys (n=96) mean±SD	Girls (n=87) mean±SD
Foods:		
1. Meat and fish		
-Cow meat (g)	15±12	12±11
-Pork meat (g)	12±11	13±10
-Lean fish (g)	2±6	2±4
-Fatty Fish (g)	0.2±1.0	0.3±1.0
2. Dairy products		
-Milk and milk products (g)	555±251	533±186
-Cheese (g)	12±11	15±10
-Butter (g)	2±5	1±4
3. Chicken and eggs		
-Egg (g)	10±8	10±7
-Chicken (g)	8±8	8±7
4. Vegetables and oils		
-Vegetables (g)	42±25	37±22
-Vegetable oil (g)	31±17	30±18
-Nuts and seeds (g)	1±2	1±3
5. Industrial products		
-Processed foods[b] (g)	115±38	107±34
-Meat products (g)	31±21	32±19
Energy and macro nutrients:		
Carbohydrate (g)	208±44	192±35[a]
Protein (g)	59±15	56±12
Total fat (g)	69±20	65±18
Total energy (MJ)	7.1±1.4	6.6 ±1.3[a]

a:T-test $p<0.05$ b: Processed foods except meat products

Table 3.3: Daily dietary intake of toxic compounds at 3.5 years of age by gender.

	Boys (n=96)		Girls (n=87)	
	TEQ	TEQ/kg BW	TEQ	TEQ/kg BW
Toxic compounds:				
TCDD (pg)	8.0±3.2	0.5±0.2	7.5±2.5	0.5±0.2
Planar PCB-TEQ (pg)	61±23	3.6±1.5	57±21	3.5±1.4
Dioxin-TEQ (pg)	48±16	2.9±1.0	45±14	2.8±0.9
Total TEQ (pg)	110±38	6.5±2.4	102±33	6.3±2.3
Total TEQ per g fat[a] (pg/g fat)	1.60±0.47		1.60±0.43	

TEQ: Toxic equivalent. BW= body weight. Planar PCB-TEQ: TEQs from planar PCB congeners, IUPAC Nos 77, 126 and 169, TCDD; 2,3,7,8 tetra chloro dioxin; dioxin-TEQ: TEQs from seventeen substituted 2,3,7,8 PCDDs/ PCDFs (dioxin) congeners, Total-TEQ; the sum of planar PCB-TEQ and dioxin-TEQ
a: Total TEQ per g fat: Total TEQ divided by grams of fat consumed by boys respectively girls (see Table 3.2).
Values are means ± standard deviations.

Figure 3.1.a

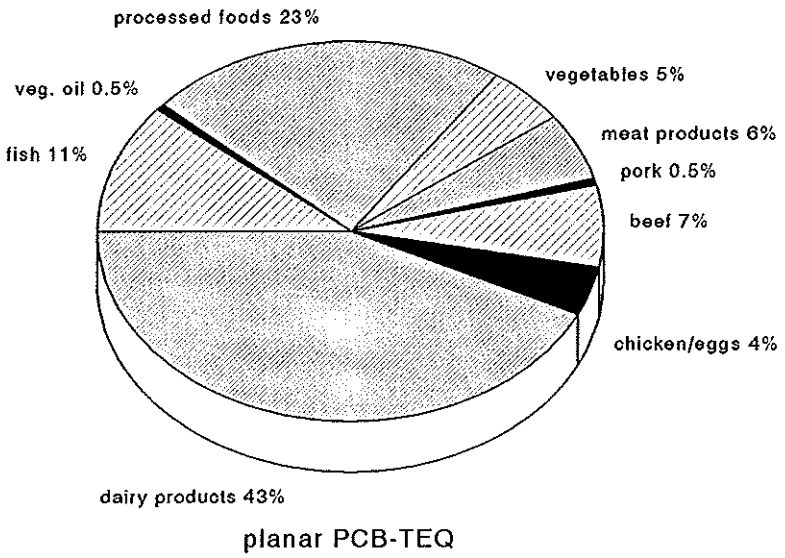

Figure 3.1.b

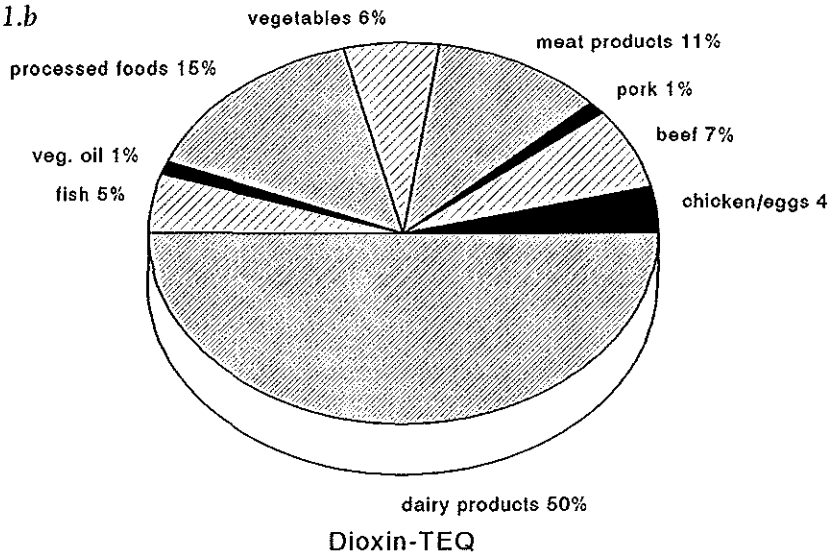

Figure 3.1.a and 3.1.b Contribution of different food groups (percentages) to dietary intake of planar PCB-TEQ (a) dioxin-TEQ (b) in preschool children.

Figure 3.1 a-b shows the contributions of different groups of food items to dietary intake of planar PCB-TEQ and dioxin-TEQ in preschool children is given. Dairy products, meat and meat products, and processed foods were the major contributors to dietary intake of PCB-TEQ and dioxin-TEQ. Dairy products contributed 43 % to PCB-TEQ intake and 50 % to dioxin-TEQ intake. Meat and meat products contributed 14 % respectively 19 % to PCB-TEQ and dioxin-TEQ intake, and processed foods 23 % and 15 %, respectively. The contribution of fish was much lower (11% and 5 %, respectively). Consumption of processed foods and fish contributed relatively more to PCB-TEQ intake than dioxin-TEQ intake.

In Figure 3.2, the cumulative intake of PCB- and dioxin-TEQ is plotted as a function of breast-feeding period in weeks. Three different curves are shown, based on the observed mean fat percentage in breast milk of 3 % and total-TEQ concentrations of 27.2 (P10), 46.9 (P50) and 69.6 pg TEQ/g fat (P90) (P10, P50, P90 in Figure 3.2). Contaminant levels in breast milk and breast-feeding period are important determinants of the cumulative contaminant intake during breast feeding. For instance, an infant breast-fed for 26 weeks with a relatively low TEQ concentration (P10) in breast milk would have a similar cumulative intake as an infant breast-fed for 8 weeks by a mother with a relatively high TEQ concentration (P90) in breast milk.

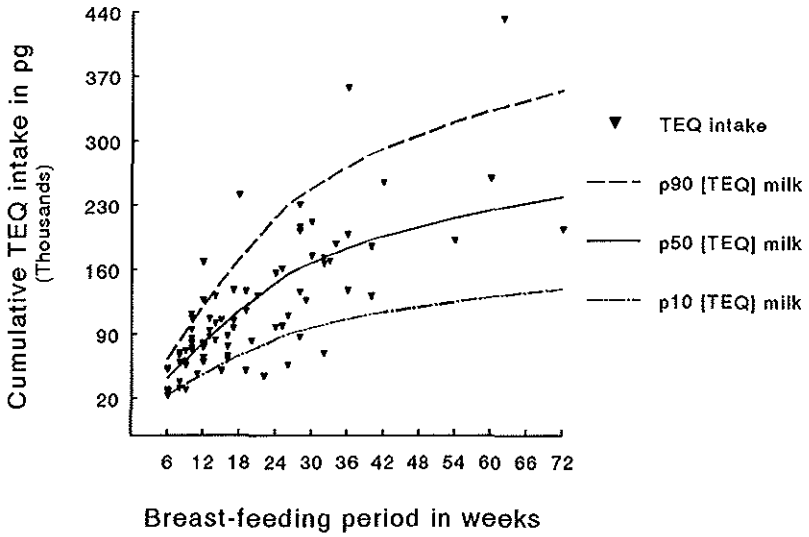

Figure 3.2 *Cumulative intake of PCB and dioxin toxic equivalents (TEQs) as a function of breast-feeding period. Dotted lines are percentiles based on the observed mean fat percentage in breast milk of 3 % and total-TEQ concentrations of 27.2 (P10), 46.9 (P50) and 69.6 pg TEQ/g fat (P90) in breast milk.*

Table 3.4 represents the mean daily TEQ intake for males and females and the mean daily TEQ intake per kg body weight (BW) at different ages. Mean body weight standards of healthy Dutch children were used as reference data.[45] The mean daily TEQ intake per kg BW per day for infants receiving breast milk for 6 months was calculated according to the formula presented in the methods. For boys this was 112 pg TEQ/kg BW and for girls this was 118 pg TEQ/kg BW, which is about 50 times higher than the mean daily TEQ/ kg BW from 20-25 years (2.3 and 2.0 pg TEQ/kg BW, respectively). The mean daily TEQ intake/kg BW during childhood (1-5 and 6-10 years) is 2 to 3 times higher than in adults (20-25 years).

Table 3.4: Estimated mean daily intake of PCB and dioxin toxic equivalents (TEQ) from birth until 25 years for males and females.

Age group	Males				Females			
	daily fat intake[d]	total daily TEQ intake	mean body weight[e]	daily TEQ intake per kg	daily fat intake[d]	total daily TEQ intake	mean body weight[e]	daily TEQ intake per kg
	(g)	(pg)	(kg)	(pg/kg BW)	(g)	(pg)	(kg)	(pg/kg BW)
Birth-6 months[a]	-	852	7.6	112	-	852	7.2	118
1-5 years[b]	69	110	17	6.5	65	102	16.4	6.3
6-10 years[c]	74	109	28.1	3.9	66	97	27.8	3.5
10-15 years[c]	98	144	47.4	3.0	88	129	48.2	2.7
16-20 years[c]	117	172	68.2	2.5	85	125	58.2	2.1
20-25 years[c]	116	171	70.8	2.4	86	126	58.6	2.2

TEQ: Toxic equivalents, sum of TEQs of 17 dioxin and three planar PCB congeners

a: estimated mean total-TEQ consumed per day, derived from cumulative intake during 6 months (26 weeks) of breast-feeding according to the formula described in the methods.

b: Daily total-TEQ consumption is 1.60 pg TEQ/g fat according to the FQ.

c: Daily total-TEQ consumption is 1.47 pg TEQ/g fat derived from the National Food consumption survey (FCS 1992) and the National Institute of Public Health and the Environment (RIVM)

d: Daily fat intake per age group and gender derived from the food questionnaire (FQ) for 1-5 years and from the FCS 1992 for 6-25 years.

e: Mean body weight (BW) derived from the weight standards of Dutch reference data for 6-25 years and for 1-5 year olds mean BW was derived from this study population. Note: daily fat intake (g) multiplied by TEQ/g fat gives the total daily TEQ intake.

Table 3.5 gives the estimated cumulative TEQ intake for infants who were either formula-fed, or breast-fed for 3 respectively 6 months, both in µg and as a proportion of total dietary TEQ exposure until 25 years. The total cumulative TEQ intake over a 25 year period is 1.38 µg in men and 1.16 µg in women who were breast-fed for 6 months during infancy. Total cumulative TEQ intake in men and women at 25 years of age who were formula-fed in infancy is 1.22 µg and 1.01 µg, respectively. The TEQ intake during 6 months of breast-feeding accounts for 12 % of the cumulative TEQ intake during 25 years in males, and 14 % in females.

Table 3.5: Mean cumulative intake of dioxin-TEQ and planar PCB-TEQ presented from birth to 25 years of age according to gender. Numbers are given for persons either formula-fed in infancy or breast-fed for 3 respectively 6 months in infancy.

	Mean cumulative TEQ intake in µg (% of total)[b]					
	Male			Female		
	Formula-fed	Breast-fed for:		Formula-fed	Breast-fed for:	
		3 months	6 months		3 months	6 months
0-3 months[a]	-	0.09 (7%)		-	0.09 (8 %)	
0-6 months[a]	-	-	0.16 (12 %)	-	-	0.16 (14 %)
0-5 years	0.19 (16 %)	0.27 (21 %)	0.35 (25 %)	0.18 (18 %)	0.27 (25 %)	0.34 (29 %)
0-10 years	0.38 (31 %)	0.47 (36 %)	0.53 (38 %)	0.35 (35 %)	0.43 (39 %)	0.50 (43 %)
0-15 years	0.63 (52 %)	0.71 (54 %)	0.78 (57 %)	0.57 (56 %)	0.66 (61 %)	0.73 (63 %)
0-20 years	0.93 (76 %)	1.01 (77 %)	1.08 (78 %)	0.79 (78 %)	0.88 (81 %)	0.95 (82 %)
0-25 years	1.22 (100 %)	1.31 (100%)	1.38 (100%)	1.01 (100%)	1.09 (100%)	1.16 (100%)

Values in brackets are percentages of the total cumulative TEQ intake until 25 years of age. TEQ: toxic equivalent for 17 dioxin congeners and 3 planar PCB congeners. a: Cumulative TEQ intake calculated from TEQ levels measured in breast milk and breast feeding until 3, respectively 6 months, according to the formula described in the methods section. b: Cumulative TEQ intake from 1 to 5 years was calculated from the mean daily TEQ/g fat intake (1.60 pg TEQ/g fat) measured from the food questionnaire (FQ). Cumulated TEQ intake from 6 to 25 years was calculated from the mean daily TEQ/g fat consumption (1.47 pg TEQ/g fat) derived from the combined data of the National Food Consumption Survey (FCS 1992) and National Institute of Public Health and Environmental Protection (RIVM) (see methods).

3.5 DISCUSSION

In this study we assessed the dietary PCB and dioxin intake, and the relative contribution of different foods to PCB and dioxin exposure in preschool children. The mean daily intake of planar PCB-TEQ and dioxin-TEQ in this population of Dutch preschool children is 59 and 47 pg, respectively. The mean daily intake of total TEQs for boys and girls is 6.5 respectively 6.3 pg/kg BW. The main contributors of PCB-TEQs and dioxin-TEQs in Dutch preschool children are dairy products, followed by

processed foods, and meat and meat products. Fish plays only a marginal role with respect to PCB/dioxin exposure in this population.

Some limitations to the methodology of this study should be noted. The intake of PCB- and dioxin-TEQ at preschool age was estimated from a semi-quantitative FQ combined with mean figures for TEQ content of foods in the Netherlands.[35] Our numbers are somewhat higher for fat and energy intake (19 % and 3 %, respectively) when compared with the results from 3 and 4 year olds of the national Food Consumption Survey (FCS, 1992).[38] We know of only one other study in children (n=14; from 22 months to 5 years of age) in which dietary intake of dioxin-TEQ was measured by the duplicate method[46] Schrey et al.[46] measured a mean daily fat intake of 64 g (range 37-91 g) and a mean daily dioxin-TEQ intake of 44 pg (range 19-140 pg). We report a daily fat intake of 67 g (range 26-117 g) and a daily dioxin-TEQ intake of 47 pg (range 14-98 pg) measured with the FQ (n=183) in the present study. The similarity of our results to those derived from this duplicate portion method underline the validity of our data.

Four percent of the preschool children exceed the Tolerable Daily Intake (TDI) of 10 pg TEQ/kg BW per day. The TDI of 10 pg TEQ/kg BW, as defined by the WHO[43] was recently challenged based on new experimental data: The Netherlands Health Council's Committee on Risk Evaluation of Substances/Dioxins 1996[44] has derived a recommended limit of human exposure to dioxin-like compounds of 1 pg TEQ/ kg BW day, which is 10 times lower than previously recommended by the WHO.[43] A TDI set at 1 pg TEQ/ kg BW day would be exceeded by 100% of 1- to 5-year-old children and almost all adults. The U.S. Environmental Protection Agency (EPA) uses a safe dose of 0.006 pg TEQ/kg BW per day, producing an upper-limit cancer risk of 10-6.[47] The amount of daily intake from birth until adulthood markedly exceeds these safety margins.

In 1991, the RIVM determined the dietary intake of planar PCB-TEQ and dioxin-TEQ in a representative sample of the Dutch population. Three planar PCBs and seventeen 2,3,7,8, chlorine substituted dioxins and furans in different food samples were analyzed and combined with results from the National Food Consumption Survey, 1988.[3] Using these combined data, a statistical model was developed to calculate the median daily TEQ intake in relation to age.[3] The median daily intake for adults was 1.4 pg PCB-TEQ/ kg BW and 1 pg dioxin-TEQ/kg BW, resulting in a median daily intake of dioxin-TEQ and planar PCB-TEQ in Dutch adults of 65 and 70 pg, respectively.[3,39]

Several other studies have assessed the dietary dioxin-TEQ intake in adults. Calculations from other industrialized countries (USA, Canada, Germany) revealed that daily exposure to dioxins and related compounds from food is in the order of 1-3 pg dioxin-TEQ per kg BW per day,[2] which is comparable to that found by the RIVM in the Netherlands. Schecter et al, measured levels of dioxins in US food.[48] Average daily food intake for adults was 0.3-3 pg TEQ/kg BW. A nursing infant may consume an average of 35-53 pg TEQ/kg BW per day during the first year of life. Daily dioxin TEQ intake in boys from 1-4 years ranged from 1.4-32 pg/kg BW. Most studies did not estimate the dioxin-like (planar) PCBs, which contributes to a substantial amount of the dioxin-like compounds. We report a mean daily intake of 112 to 118 pg TEQ/kg BW

in breast-fed infants, and 6.3 to 6.5 pg TEQ/kg BW in 1 to 5 year old children. Accounting for PCB-TEQs would at least double the total TEQ intake, which is in accordance with the US EPA[47] and the study of Schecter and colleagues.[48]

We estimated the cumulative PCB- and dioxin-TEQ intake from birth until 25 years of age. According to our model, breast-feeding for 6 months accounts for 12 to 14 % of the dietary exposure until 25 years of age. The daily TEQ intake per kg BW for infants breast-fed during 6 months is approximately 50 times higher than for adults, which about the same as reported by the US EPA.[47] For children until 5 years of age the daily intake per kg BW is three times as high as in adults. The numbers presented should be regarded as an indication rather than as exact values, because of the following limitations in the available data. Because we had no exact data on how much a breast-fed infant drinks per day, mean values from literature were used.[28-30] Since the RIVM provides only mean values for PCB and dioxin concentrations from different foods, it was not possible to give a range or distribution of cumulative intakes.[35] Furthermore, food preparation and or cooking method could have altered PCB and dioxin levels in the final product. Finally, it was not possible to account for additional PCB-TEQ intake of mono-ortho PCBs, IUPAC nos 105, 118 and 156, since these PCB congeners were not included in the RIVM food analyses.

Although some model calculations of PCB and dioxin body burden and infant exposure through breast milk have been published[34,49] the cumulated PCB and dioxin intake from infancy until adulthood has not been quantitatively assessed previously. It should be noted that the cumulative intake as estimated in this study is not identical to body burden, since losses by excretion and the long half-lives of different PCB and dioxin congeners are not taken into account. In the present paper we report that the relative contribution of cumulative intake of PCB/dioxin TEQ until 25 years is 7 % for males and 8 % for females when breast-fed for 3 months, and 12 % respectively 14 %, when breast-fed for 6 months. These values are in the order of magnitude as model calculations by other authors.[34,49]

Given that more than 10% of the cumulative TEQ intake until 25 years is due to 6 months of breast-feeding, limiting the nursing period might be considered. However, next to this disadvantage of PCB/dioxin accumulation in the infants' body during breast-feeding, there are numerous advantages of nursing it self on the general development of young children. Reports from our study[20,50] and others[51] showed that children breast-fed during infancy, performed better on neurological- and cognitive outcome measurements, when compared with their formula-fed counterparts. Since the positive influences of nursing on child development outweighs the negative ones, we do not encourage shortening of the lactation period to achieve a lower PCB/dioxin body burden.

In conclusion, our results show that breast-feeding for 6 months makes up for a reasonable proportion (12-14 %) of the cumulative dietary PCB and dioxin exposure until reproduction age. The main food sources of PCBs and dioxins after weaning in young children are dairy products, processed foods, and meat and meat products. Given that disturbances in growth and development in children are mainly related to in

utero exposure, rather than lactational exposure to PCBs and dioxins, we conclude that strict regulations and enforcement of these regulations could reduce the maternal PCB/dioxin body burden, and thereby the in utero and lactational exposure. Strategies should be directed towards reducing PCB and dioxin intake through the food chain at all ages, by lowering the consumption of animal products and processed foods, and not at discouraging breast-feeding.

3.6 ACKNOWLEDGMENTS

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CHAPTER 4

EFFECTS OF ENVIRONMENTAL EXPOSURE TO POLYCHLORINATED BIPHENYLS AND DIOXINS ON BIRTH SIZE AND GROWTH IN DUTCH CHILDREN.

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4.1 ABSTRACT

Background: Lower birth weight and growth retardation has been found in studies with laboratory animals, in children born of mothers exposed to accidental high levels of Polychlorinated Biphenyls (PCBs) and related compounds, and in children born of mothers who consumed PCB-contaminated fish. The effect of background exposure to PCBs and dioxins on birth size and growth in human newborns however, is still unknown. This study examined birth size and postnatal growth of term newborns in relation to their background PCB and dioxin exposure.

Methods: Birth weight and weight, length and head circumference were measured at 10 days, 3, 7, 18 and 42 months of age from 207 children, of whom 105 were breast-fed and 102 were formula-fed during infancy. The effect of in utero exposure to PCBs on birth size, assessed by cord and maternal plasma PCB levels, was investigated in the whole group. The effect of prenatal PCB exposure on postnatal growth was studied in the formula-fed group, whereas the effect of prenatal as well as lactational exposure to PCBs and dioxins on postnatal growth was studied in the breast-fed group.

Results: After adjustment for covariates, cord and maternal plasma PCB levels where both negatively associated with birth weight. Infants with high cord plasma PCB levels (P90=0.80 μg/L) weighed 165 grammes less compared to infants with low cord plasma PCB levels (P10=0.20 μg/L). Cord and maternal plasma PCB levels where both significantly associated with lower growth rate, defined as change in standard deviation score (SDS) of weight, length and head circumference from birth to 3 months in the formula-fed group (all p-values<0.05). No negative effects of prenatal PCB exposure on growth rate were found from 3 to 42 months of age. Postnatal PCB and dioxin exposure was not negatively associated with growth rate in the breast-fed group.

Conclusions: In utero exposure to environmental levels of PCBs is negatively associated with birth weight and postnatal growth until 3 months of age. Although this growth delay was described in healthy term born infants, intrauterine and postnatal growth retardation are potentially harmful to the developing human and should be avoided by reducing maternal PCB and dioxin body burden, and consequently fetal exposure to these pollutants.

4.2 INTRODUCTION

Polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-p-dioxins (PCDDs) and -dibenzo-furans (PCDFs), summarized as dioxins, are widespread environmental contaminants that are lipophilic and resistant to either chemical and biologic degradation. PCBs and dioxins frequently occur simultaneously in the environment and accumulate especially in fat tissue.[1,2] The half-lives of the congeners are fairly long, for instance 7 y for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).[3] In western industrial countries, e.g. The Netherlands, considerable amounts of PCBs and

dioxins have been found in fish, meat, dairy products, and, in particular, in human milk.[4] Human exposure to PCBs and dioxins is mainly through the food chain.[5,6] The human fetus is exposed to PCBs and dioxins through placental transport[7,8] and large quantities of PCBs and dioxins are transferred to the infant during breast-feeding.[8,9]

Studies in rhesus monkeys[10] and rats[11,12] showed that prenatal exposure to PCBs was associated with reduced birth weight. The negative effect of prenatal PCB exposure may persist beyond the newborn period; exposed rats gained weight more slowly than controls during the first four months of life.[12] Intrauterine growth retardation was reported in two incidents in Asia (Japan and Taiwan) in which pregnant women consumed cooking oil accidentally contaminated with PCBs and related compounds, referred as the Yusho and Yu-Cheng incidents.[13,14] Yu-Cheng children were smaller and weighed less compared with their matched controls at school age.[14,15] In the United States, reduced birth weight and a shorter gestation was also observed in infants with elevated cord PCB levels whose mothers consumed contaminated fish from Lake Michigan.[16]

We reported previously that in Dutch infants subtle signs of neurological dysfunctioning,[17] small delay in psychomotor development.[18] alterations in thyroid hormone[19] and immunological status[20] are associated with perinatal PCB and dioxin exposure. In this report we describe the effect of pre- and postnatal exposure to PCBs and dioxins on birth size and growth in children from birth until 42 months of age. This study is part of the Dutch PCB/dioxin project and an EC funded European collaborative study, a prospective follow-up study on possible adverse effects of environmental exposure to PCBs and dioxins on growth and development of young children.

4.3 METHODS

Subjects

From 1990-1992, infants born at term (37-42 wk of gestation), without congenital anomalies or diseases were recruited for this study. Pregnancy and delivery had to be without complications or serious illnesses. All mother-infant pairs included were Caucasian. To study the effects of prenatal and postnatal exposure to PCBs and dioxins, women were included who intended to give formula-feeding (formula-fed group) next to women who intended to breast-feed their child for at least 6 wk (breast-fed group). All formula-fed infants received formula from a single batch (Almiron M2, Nutricia NV, The Netherlands) from birth until 7 months of age. In this formula, concentrations of both PCBs and dioxins were negligible. Participants lived in Rotterdam or its immediate surroundings, a highly industrialized and densely populated area in the western part of The Netherlands. The study design and chemical analysis methods are described elsewhere.[9]

This study was approved by the Medical Ethics Committee of the Sophia Children's Hospital Rotterdam. During the last month of pregnancy, mothers were visited at home for an explanation of the study protocol. Informed consent was given by the parents.

Outcome measures

Weight at birth was measured by the obstetrician or midwife. Weight, length or height and head circumference were measured at the ages of 10 days, 3, 7, 18 and 42 months. Since length and head circumference at birth were not measured in most newborns, length and head circumference measured at 10 days and birth weight were used as measurements for birth size. Until 7 months of age, infants were weighed naked on a baby scale up to the nearest 5 gram, and length was measured on a length board up to the nearest mm. At 18 and 42 months of age, children were weighed on a beam scale in kilograms, and their height was measured with a Harpenden stadiometer in centimeters. Head circumference was measured in centimeters with a standard tape at all ages.

Weight, length or height, and head circumference at each time point were converted into standard deviation scores (SDS) using weight, height and head circumference standards of healthy Dutch children as reference data.[21] Growth rate per time interval, e.g. 0-3 months, was defined as change in SD score of weight (ΔwSDS), length (ΔhSDS) and head circumference (ΔhcSDS) between 3 months and birth/10 days. Growth rate from 3-7 months was defined as change in SD score of weight, height and head circumference between 7 and 3 months. The same definition was used for growth rate from 7 to 18 months and 18 to 42 months of age.

Exposure variables

Analysis of four PCB congeners, International Union for Pure and Applied Chemistry (IUPAC) nos. 118, 138, 153 and 180 in maternal plasma samples, collected during the last month of pregnancy and in cord plasma samples was performed at the Nutrition and Food Research Institute, Zeist, the Netherlands.[9] The PCB sum (ΣPCB) was calculated by adding up the four congeners in each plasma sample and were reported on a volume basis (μg/L).

A 24-h representative breast milk sample was collected from nursing mothers in the second week after delivery and was analysed for the 17 most abundant 2,3,7,8-substituted PCDD and PCDF congeners, three planar PCBs IUPAC nos. 77, 126 and 169, three mono-ortho PCBs IUPAC nos. 105, 118, 156, two di-ortho PCBs IUPAC nos. 170, 180 and 18 other non-planar PCBs IUPAC no's 28, 52, 66, 70, 99, 101, 128, 137, 138, 141, 151, 153, 177, 183, 187, 194, 195 and 202.[9,22] To express the total toxic potency of dioxins and planar, mono-ortho and di-ortho PCBs (dioxin-like PCBs), the toxic equivalent factor (TEF) approach was used according to Safe and the WHO.[23,24] By multiplying the concentration in breast milk (nanogram/kg of milk fat) and its TEF value, the TEQ of each congener was calculated (nanogram TEQs/kg of milk fat). By adding up the TEQs of all congeners the total TEQ value was obtained. Levels of all exposure variables have been published elsewhere.[9]

Prenatal PCB exposure was assessed from the ΣPCB in maternal and cord plasma. Postnatal PCB and dioxin exposure was calculated by multiplying the total TEQ levels measured in breast milk with the number of weeks of breast-feeding as reported by the mother.

Other variables

Potential confounding variables for birth size and growth were selected from a list pertaining data on socio-economic background, maternal (health) history, pregnancy and delivery, gestational age, parity, gender and fetal exposure to alcohol and cigarette smoking. Parent's height served to calculate the target height (TH in centimeters) as predicting variable for birth size and growth. TH was calculated according to the formulas: (father's height + mother's height + 12)/2 + 3 cm for boys and (father's height + mother's height - 12) /2 + 3 cm for girls.[25] TH in centimeters was converted into target hSDS, using Dutch standards for final height.[21]

Statistics and data analysis

Maternal and cord plasma PCB levels were normalized by natural logarithmic transformation. The student t-test, chi-square and Mann Whitney U test were used to compare differences between the breast-fed and formula-fed group.

The influence of prenatal PCB exposure on birth size was studied in the whole group. Multiple linear regression analyses were carried out to study the influence of prenatal PCB exposure on birth weight, length and head circumference at 10 days (SDS). Potential confounding variables were considered to be included in the regression model if they were related to the outcome variable, known from literature and clinical knowledge. Furthermore, variables were also included in the final model when they influenced the regression coefficient of the exposure variable in such a way that confounding exists. If the crude and adjusted estimates were meaningfully different, then confounding was present and one or more control variables were included in the regression model.[26]

The influence of PCB and dioxin exposure through formula-feeding is negligible, therefore the formula-fed (FF) group represents infants prenatally exposed to PCBs and dioxins. The influence of cord and maternal plasma PCB levels on changes in SDS of weight, length, and head circumference per time interval, e.g. growth rate from 0-3, 3-7, 7-18 and 18-42 months was studied in the FF group. The breast-fed (BF) group represents infants who were also postnatally exposed to PCBs and dioxins. The influence of PCB and dioxin exposure through breast milk on growth rate from 0-3, 3-7, 7-18 and 18-42 months was therefore studied in the BF group. Multiple linear regression analyses were also carried out in both groups as described above. Furthermore, the effect of feeding type in relation to growth rate was investigated in the whole group in the same manner. Results are considered statistically significant at a p-value ≤ 0.05.

4.4 RESULTS

In this study 207 mother-infant pairs were enrolled, of whom 105 were in BF group and 102 in the FF group. Table 4.1 represents the general characteristics of all mother-infant pairs. There were no differences between both feeding groups except a smaller number of boys ($p<0.05$) and a lower level of maternal education in the FF group ($p<0.001$). In Table 4.2 levels of PCBs and dioxins measured in plasma and maternal milk samples are given. The sum of four PCB congeners could be calculated in 206 maternal and 182 cord plasma samples. In human milk, representative dioxin, planar, mono- and di-ortho PCB congeners were available in 83, 95, 100, and 100 milk samples, respectively. The remaining plasma and human milk samples missed or were not analysed due to organizational failure. The sum of PCB- and dioxin-TEQ (total TEQ) could be calculated for 80 breast milk samples.

Table 4.1 Characteristics of the study population; the formula-fed group and the breast-fed group

Characteristic	Formula-fed group n=102	Breast-fed group n=105	P-value[a]
Gender, male	47 (46%)	63 (59%)	0.05
Parity, firstborn	49 (48%)	53 (50%)	ns
Gestational age (wk)	40.1 ± 1.3	40.1 ± 1.2	ns
Maternal age (y)	28.9 ± 4.4	28.8 ± 3.2	ns
Maternal height (cm)	168.9 ± 6.0	169.0 ± 5.6	ns
Paternal height (cm)	181.3 ± 6.9	181.2 ± 6.9	ns
Maternal education[b]			<0.001
Low	39 (38%)	14 (13%)	
Medium	34 (33%)	35 (33%)	
High	29 (29%)	56 (54%)	
Smoking during pregnancy			
yes	29 (28%)	19 (18%)	ns
Alcohol use during pregnancy			
yes	17 (17%)	19 (18%)	ns
Breast feeding period (wk)	0	16 (6-72)	

a: t-test, chi-square test or Mann-Whitney U test Values are numbers and percentages in parenthesis, means ± SD or median (range)
b: Low, Primary school finished and secondary school not finished. Medium, secondary school finished High, high school finished or professional and university training.

Table 4.2 Exposure variables measured in plasma and in breast milk samples

Measured in plasma (formula-fed and breast-fed group n=207)	
ΣPCB maternal (μg/L) (n=206)	2.04 (0.59-7.35)
ΣPCB cord (μg/L (n=182)	0.40 (0.08-2.08)
Measured in breast milk (breast-fed group n=105)	
ΣPCB (μg/kg fat) (n=100)	391.5 (173.7-1226.4)
Mono-ortho PCB-TEQ (ng/kg fat) (n=100)	13.9 (3.2-44.4)
Di-ortho PCB-TEQ (ng/kg fat) (n=100)	3.8 (1.6-26.2)
Planar PCB-TEQ(ng/kg fat) (n=95)	14.8 (4.4-45.7)
Dioxin-TEQ(ng/kg fat) (n=83)	30.6 (11.1-76.4)
Total TEQ (ng/kg fat) (n=80)	64.8 (28.0-155.0)

ΣPCB= sum of polychlorinated biphenyls, nos. 118, 138, 153, and 180 measured in plasma and breast milk. TEQ = toxic equivalents of mono-ortho PCBs, nos. 105, 118, 156, di-ortho PCBs nos. 170, 180 and planar PCBs nos. 77, 126, and 169 and seventeen 2,3,7,8 substituted poly chlorinated di-benzo dioxins (PCDDs) and furans (PCDFs) measured in breast milk samples. Total TEQ= the sum of mono-ortho-, di-ortho- and planar PCB-TEQ, and dioxin-TEQ in breast milk. Values are medians (range)

Anthropometric data for both feeding groups are given in Table 4.3. All children were in the normal range when compared with the standards of Dutch children as reference data.[21] There were no differences in birth weight between the two feeding groups. At 10 days a significantly higher mean body length in BF girls was found compared to the FF counterparts (p=0.02). Significantly higher mean body weights at 7, 18, and 42 months of age were found in FF boys compared with BF boys (all p values <0.05). A smaller number of children at 42 months are presented; 14 children were lost to follow-up due to lack of interest (n=10) and migration (n=4).

Table 4.3 *Anthropometric data for the formula-fed and breast-fed group from birth until 42 months of age*

	Males				Females			
	Formula-fed		Breast-fed		Formula-fed		Breast-fed	
Age	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
Birth								
W (g)	47	3606 (444)	63	3446 (440)	55	3353 (405)	42	3488 (465)
10 days								
L	47	51.9 (1.8)	63	52.2 (1.9)	55	50.7 (1.6)	42	51.6 (2.0)*
HC	47	36.0 (1.1)	63	36.0 (1.2)	55	35.1 (1.1)	42	35.3 (1.5)
3 months								
W (g)	47	6219 (709)	63	6069 (636)	53	5560 (456)	42	5782 (676)
L	47	61.1(2.1)	63	61.6 (1.9)	55	59.7 (1.9)	42	60.1 (1.9)
HC	45	41.2 (1.1)	61	41.2 (1.1)	53	40.2 (1.1)	42	40.3 (1.3)
7 months								
W (g)	47	8566 (912)†	61	8080 (722)	55	7713 (608)	41	7817 (820)
H	47	69.8 (2.2)	59	69.3 (2.1)	55	67.9 (1.9)	41	67.9 (2.0)
HC	46	44.9 (1.1)	56	44.5 (1.1)	54	43.8 (1.1)	40	43.7 (1.4)
18 months								
W (kg)	44	12.5 (1.4)†	62	11.8 (1.3)	54	11.2 (1.1)	39	11.5 (1.1)
H	45	82.5 (2.8)	62	82.1 (2.7)	54	80.6 (2.5)	40	81.0 (3.0)
HC	46	49.0 (1.3)	61	48.7 (1.3)	55	47.7 (1.3)	40	47.7 (1.5)
42 months								
W (kg)	43	17.6 (2.2)†	61	16.6 (2.0)	50	16.4 (1.9)	38	16.4 (2.1)
H	43	103.2 (3.1)	61	102.1 (3.7)	50	101.2 (3.6)	38	102.0 (4.6)
HC	43	51.1 (1.2)	60	51.2 (1.4)	50	50.2 (1.3)	37	50.2 (1.6)

Values are numbers and means (standard deviation)
†: t-test, significantly heavier than breast-fed boys ($p<0.05$ at 7, 18 and 42 months of age)
*: t-test, significantly taller than formula-fed girls ($p=0.02$)
W: Weight in g or kg, L: Length in cm, H: Height in cm, HC: Head circumference in cm

The effect of prenatal PCB exposure on birth size was analyzed by means of multiple linear regression analyses with birth weight, length at 10 days and head circumference at 10 days as dependent variables and LnΣPCB in cord plasma as exposure variable. Table 4.4 shows that, after adjustment for parity, gestational age, target height, smoking, and alcohol use during pregnancy, LnΣPCB in cord plasma is negatively associated with birth weight ($p=0.03$). An infant with a median PCB level (P50=0.41 µg/L) in cord plasma has a birth weight of 86 g less than an infant with a cord PCB level at P10 (0.20 µg/L). An infant with a cord PCB level at P90 (=0.80 µg/L) has a birth

weight of 165 g less than an infant with a cord PCB level at the P10 level. Similar results were found with LnΣPCB in maternal plasma as a measure for prenatal PCB exposure, Table 4.5. Parity, gestational age, target height, smoking and alcohol use during pregnancy have a significant effect on birth weight as well. Infants exposed to maternal smoking and alcohol use during pregnancy have predicted birth weights of 144 grams respectively 138 grams lower compared to infants not exposed to maternal smoking and alcohol use, Table 4.4. There were no significant negative effects of LnΣPCB in cord and LnΣPCB in maternal plasma on length/hSDS and head circumference/ hcSDS measured at 10 days. According to the regression model presented in Table 4.4, a prediction was made for birth weight in infants exposed to different levels of ΣPCB in cord plasma with or without exposure to cigarette and alcohol use during pregnancy (see Figure 4.1). Prediction for birth weight is given when gestational age is 40.1 wk (mean value), target height is 178.8 cm (mean value) and parity is zero (first born).

Table 4.4 The influence of prenatal PCB exposure, measured from cord plasma PCB levels, on birth weight (grams)

	regression coefficient	(SE)[b]	p-value
Constant	-5782	(1215)	<0.0001
Ln(ΣPCB)cord[a]	-119.4	(53.7)	0.027
Parity 0= first 1= second	163.8	(57.7)	0.005
Gestational Age (weeks)	150.1	(24.4)	<0.0001
Smoking mother 0 = no 1= yes	-144.1	(68.2)	0.036
Alcohol use mother 0= no 1= yes	-138.4	(80.7)	0.088
Target Height (cm)	17.3	(3.5)	<0.0001

N = 179, R^2 whole model = 0.32 Multiple R = 0.56

a: natural logarithm of the sum of PCBs, IUPAC nos. 118, 138, 153, and 180 in cord blood (µg/L)

b: Regression coefficient (standard error)= grams change in birth weight per unit change in variable.

Y (birth weight in g) = -5789 - 119.4*(LnΣPCBcord) - 144.1* (smoking) - 138.4* (alcohol) + 150.1*(gestational age) + 17.3*(target height) + 163.8*(parity)

Table 4.5 The influence of prenatal PCB exposure, measured from maternal plasma PCB levels during pregnancy, on birth weight (grams)

	regression coefficient	(SE)[b]	p-value
Constant	-4986	(1128)	<0.0001
Ln(ΣPCB)maternal[a]	-123.1	(64.4)	0.057
Parity 0= first 1= second	167.7	(53.6)	0.002
Gestational Age (weeks)	143.6	(22.7)	<0.0001
Smoking mother 0 = no 1= yes	-127.1	(63.2)	0.045
Alcohol use mother 0 = no 1= yes	-164.6	(72.1)	0.023
Target Height (cm)	15.5	(3.2)	<0.0001

N = 203, R^2 whole model = 0.30 Multiple R = 0.55

a:natural logarithm of the sum PCBs IUPAC nos. 118, 138, 153, and 180 in maternal plasma measured during pregnancy(µg/L)

b: Regression coefficient (standard error)= grams change in birth weight per unit change in variable.

Y (birth weight in g) = -4986 - 123.1*(LnΣPCBmaternal) - 127.1* (smoking) - 164.6* (alcohol) + 143.6*(gestational age) + 15.5*(target height) + 167.7*(parity)

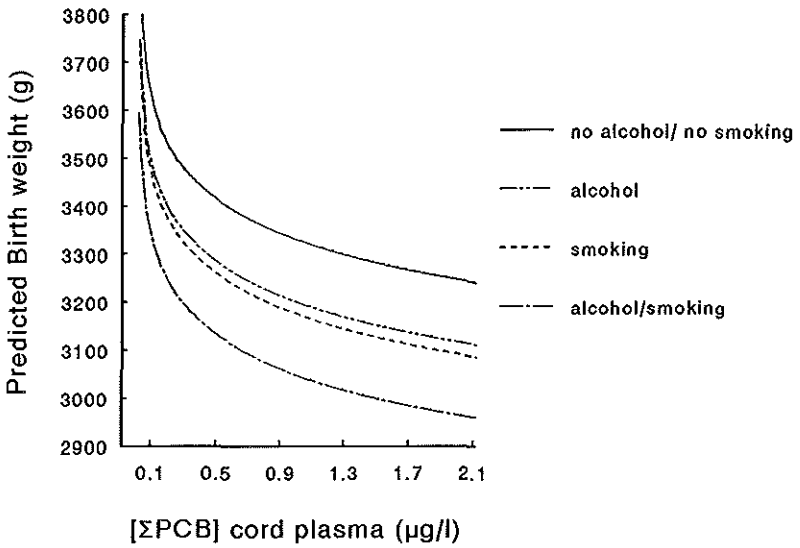

Figure 4.1 The prediction of birth weight in relation to cord plasma PCB levels.

The relation of fetal exposure to cigarette smoking and alcohol during pregnancy and birth weight is given. Prediction is given for birth weight when gestational age is 40.1 weeks (mean value), target height is 178.8 cm (mean value) parity is zero (first born).

Predicted birth weight (g) = -5789 - 119.4*(LnΣPCBcord) - 144.1* (smoking) - 138.4* (alcohol) + 150.1*(40.1) + 17.3*(178.8) + 163.8*(0)

ΣPCBcord = the sum of PCB congeners 118, 138, 153 and 180 in cord blood

Smoking, 0= no 1= yes

Alcohol use; 0= no 1=yes

The effect of prenatal PCB exposure on growth rate between 0-3, 3-7, 7-18 and 18-42 months was studied by multiple linear regression analysis in the FF group. After adjustment for covariates, ln ΣPCB in cord plasma was negatively associated with ΔwSDS, ΔhSDS and ΔhcSDS between 0-3 months (all p-values <0.05, Table 4.6). This means that there is a significant negative effect of cord plasma PCB levels on growth rate of weight, length and head circumference from 0 to 3 months. Similar results were found when ln ΣPCB in maternal plasma was entered in the regression analyses as a measure for prenatal PCB exposure. After adjustment for the same covariates as presented in Table 4.6, regression coefficients (standard errors) for ln ΣPCB in maternal plasma on ΔwSDS, ΔhSDS and ΔhcSDS between 0 to 3 months are -0.51 (0.18), -0.40 (0.14) and -0.30 (0.14), respectively (all P-values < 0.05). There were no negative associations found between prenatal PCB exposure and growth rate between 3-7, 7-18 and 18-42 months of age in the FF group.

Table 4.6 The effect of prenatal PCB exposure measured from cord plasma PCB levels on growth rate from 0-3 months in the formula-fed group.

	Δ wSDS		Δ hSDS		Δ hcSDS	
	regr.coeff (SE)[a]	p-value	regr.coeff (SE)	p-value	regr.coeff (SE)	p-value
Constant	3.76 (2.77)	0.18	-0.67 (2.39)	0.78	6.6 (2.3)	0.006
Ln(ΣPCB)cord [b]	-0.37 (0.16)	0.02	-0.28 (0.12)	0.03	-0.26 (0.12)	0.04
Gestational Age (weeks)	-0.10 (0.07)	0.14	0.012 (0.06)	0.83	-0.17 (0.06)	0.004
Smoking mother 0= no 1= yes	0.28 (0.17)	0.11	-0.17 (0.15)	0.25	0.014 (0.14)	0.91
Alcohol use 0= no 1= yes	0.63 (0.23)	0.009	-0.12 (0.20)	0.56	0.30 (0.19)	0.12
wSDS at birth	-0.37 (0.09)	0.0001				
hSDS at 10 days			-0.36 (0.08)	<0.0001		
hcSDS at 10 days					-0.25 (0.07)	0.001

Growth rate is expressed as change in standard deviation score of body weight (ΔwSDS), body length (ΔhSDS) and head circumference (ΔhcSDS) from birth/10 days till 3 months. ΔwSDS= growth rate of body weight; N = 87 ΔhSDS= growth rate of body length; N=83 ΔhcSDS= growth rate of head circumference; N= 81
a: Regression coefficient (standard error)= change in ΔSD score per unit change in variable.
b: Sum of PCBs, IUPAC nos. 118, 138, 153, and 180 in cord blood(μg/L).

The effect of pre- as well as postnatal PCB and dioxin exposure on growth rate between 0-3, 3-7, 7-18 and 18-42 months was studied in the BF group. Neither an effect of prenatal PCB exposure nor an effect of total TEQs in breast milk multiplied by the number of weeks of breast-feeding (lactational exposure), was found on growth rate between 0-3 months in the BF group (Table 4.7). After adjustment for covariates, postnatal PCB and dioxin exposure was negatively associated with change in length SD score (ΔhSDS) (regression coefficient = -0.21, p=0.04) but not with change in SD of weight (ΔwSDS) and head circumference (ΔhcSDS) from 3 to 7 months. Growth rate studied from 7-18 and 18-42 months, were neither influenced by pre- nor postnatal PCB and dioxin exposure in the BF group.

Table 4.7 The effect of lactational exposure to PCBs and dioxins on growth rate from 0-3 months in the breast-fed group.

	Δ wSDS		Δ hSDS		Δ hcSDS	
	regr.coeff (SE)[a]	p-value	regr.coeff (SE)	p-value	regr.coeff (SE)	p-value
Constant	4.9 (4.3)	0.26	-0.60 (3.0)	0.84	4.9 (3.7)	0.19
Ln(Σ PCB)cord[b]	0.08 (0.25)	0.76	0.002 (0.19)	0.99	0.12 (0.21)	0.57
Total TEQ X breast-feeding period[c]	-0.72 (0.46)	0.12	-0.05 (0.31)	0.88	-0.49 (0.37)	0.19
Gestational Age (weeks)	-0.10 (0.10)	0.33	0.02 (0.07)	0.79	-0.11 (0.09)	0.22
Smoking mother 0= no 1=yes	-0.02 (0.26)	0.93	-0.15 (0.17)	0.38	0.03 (0.19)	0.89
Alcohol use 0= no 1=yes	0.02 (0.29)	0.94	0.18 (0.21)	0.40	0.09 (0.25)	0.73
wSDS at birth	-0.30 (0.14)	0.03				
hSDS at 10 days			-0.28 (0.09)	0.002		
hcSDS at 10 days					-0.19 (0.09)	0.05

Growth rate is expressed as change in standard deviation score of body weight (Δ wSDS), body length (Δ hSDS) and head circumference (Δ hcSDS) from birth/10 days till 3 months. Δ wSDS= growth rate of body weight; N = 72 Δ hSDS= growth rate of body length; N= 59 Δ hcSDS= growth rate of head circumference N= 59

a: Regression coefficient (standard error)= change in Δ SD score per unit change in variable.

b: Sum of PCBs, IUPAC nos. 118, 138, 153, and 180 in cord blood (μg/L)

c: Lactational exposure; sum of toxic equivalents (TEQs) of 8 dioxin like PCB and 17 dioxin congeners measured in breast milk, multiplied by the number of weeks of breast-feeding until 3 months (ng/g fat).

In addition, the effect of feeding type during infancy on growth rate was studied in the whole group. No significant effect of feeding type on growth rate was found between 0-3, 7-18 and 18-42 months of age. Growth rate between 3 and 7 months however, was significantly influenced by feeding type; infants from the BF group showed a lower ΔwSDS (regression coefficient (SE) = -0.16 (0.08), p=0.05) and ΔhSDS (regression coefficient (SE) = -0.16 (0.07), p=0.04) from 3 to 7 months compared with infants from the FF group, after adjustment for maternal education, gender, TH and SDS of weight and length at 3 months. There was no effect of feeding type on growth rate of head circumference from 3 to 7 months.

4.5 DISCUSSION

We report that in utero exposure to environmental levels of PCBs negatively influences birth weight. Furthermore, prenatal PCB exposure was negatively associated with growth rate of weight, length and head circumference from birth to 3 months of age.

These results are partly consistent with studies in laboratory animals[10,12] and human studies concerning exposure by accident, the Yusho[13] and Yu-Cheng incidents.[14] In the Yu-Cheng incident reduced growth was found until school age[14,15] and in the

Yusho incident female children with lower birth weights remained smaller and lighter at later ages e.g. school age, these differences correlated with PCB concentration in breast milk of the Japanese mothers. This phenomenon, however, was not observed in male children.[27] Taylor et al.[28] studied the relation between birth weight and gestational age among live offspring of women occupationally exposed to PCBs and reported a significant association between increased serum PCB levels and lower birth weight and gestational age. Jacobson et al.,[29] reported a decrease in birth weight of 160-190 g as well as a 0.6-0.7-cm smaller head circumference in infants of mothers who consumed contaminated fish from Lake Michigan. A weight deficit of 1.8 kg at 4 years of age was described in the same cohort, associated with prenatal PCB exposure.[16,29]

Rogan et al.,[30] studied the effects of environmental exposure to PCBs in North Carolina USA, but did not find an effect of prenatal PCB exposure on birth weight, whereas we report that prenatal PCB exposure is associated with reduced birth weight and growth rate until 3 months of age. In North Carolina, as well as in Michigan, total PCB levels were measured in cord and maternal blood with the Webb McCall method, using packed column gas chromatography,[8,30] whereas in our study PCB levels were measured by gas chromatography with electron capture detection which provides information of individual PCB congeners.[9] Although state-of-the-art at the time of the study, the Webb McCall method is a crude measure of PCB levels and gives no information of individual congeners. Differences between results from the North Carolina and Dutch cohort can be explained by the following reasons. Environmental levels of PCBs and dioxins are probably lower in the USA compared to background levels in the Netherlands,[4] This might explain why effects on birth weight in the Michigan cohort are similar to what we have found, but not with the findings in the North Carolina cohort. Probably levels of exposure in the Michigan cohort, which are slightly above background PCB levels, are comparable to levels measured in the Dutch cohort. On the other hand, levels could be similar in North Carolina and the Netherlands, but due to a presumably more accurate measure of exposure in the Netherlands effects on birth weight and growth were found in the Dutch cohort and not in the North Carolina cohort. Differences in analytical measurements of exposure data still makes it difficult to compare PCB levels from different regions.[4]

Both cord and maternal plasma PCB levels were negatively associated with birth weight and postnatal growth rate until 3 months of age. Although PCB levels in cord plasma are a direct measure of fetal exposure to PCBs when compared with maternal plasma PCB levels measured during pregnancy, the analytical precision of PCB determination in cord plasma is lower than for maternal plasma, due to a lower lipid content in cord plasma. However, our results for cord as well as maternal plasma PCB levels are similar and therefore support the hypothesis that in utero exposure to PCBs affects fetal growth (birth weight) and growth during early infancy.

Growth retardation as described in this paper might be due to the transplacental transfer of the four PCB congeners measured, but could also be due to other contaminants, e.g. PCB, dioxins, and PCB metabolites. At the time of this study, it was not possible to determine dioxins and dioxin-like PCB congeners in plasma, as these

measurements required a large volume of blood (100-200 ml). Only the four most prevalent PCB congeners could be analyzed. There is a good correlation between PCB congeners measured by us in maternal and cord plasma and other PCB and dioxin congeners in human milk.[9] It is not possible to rule out the effect of other toxic substances, nor is it possible to determine the mechanism by which prenatal exposure to PCBs and related compounds causes a reduction in birth weight and postnatal growth. Additional studies in which more PCB and dioxin congeners, as well as their metabolites are measured need to clarify which congeners are responsible for diminished growth. The biochemical effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds including dioxin-like PCBs, can be grouped in three classes; altered metabolism, resulting from changes in enzyme levels; altered homeostasis resulting from changes in hormones and their receptors; and altered growth and differentiation resulting from changes in growth factors and their receptors.[31]

In this study a lower growth rate in relation to prenatal PCB exposure was observed up to 3 months of age in the FF group. In the BF group however, this prenatal effect was not observed. Except a lower growth rate for body length between 3-7 months no effects of lactational exposure to PCBs and dioxins on growth rate from birth to 42 months of age was observed. This effect of lactational exposure on growth rate for body weight and head circumference from 3-7 months was not found. The results for postnatal PCB and dioxin exposure on growth rate are not consistent in time and therefore could be a chance finding. We presented results only from postnatal PCB and dioxin TEQ exposure on growth rate in BF infants, but results were similar when postnatal exposure was measured from the four PCB congeners (IUPAC nos. 118, 138, 153, and 180) in breast milk. This further supports our finding that lactational exposure to PCBs as well as dioxins and dioxin-like PCBs are not negatively associated with postnatal growth rate until 42 months of age. We could speculate on the positive influences of breast milk or breast-feeding, despite the high levels of PCBs and dioxins transferred to the infant. Numerous studies on infant nutrition show that breast-feeding has a beneficial effect on growth, morbidity and neurological and cognitive functioning later in life.[32] Breast milk contains certain hormones, e.g. thyroid hormone, prolactin, gonadotrophin hormone, adrenal gland hormones and long chain-poly unsatured fatty acids (LC-PUFA's), nucleotides, and epidermal growth factors (EGF),[33] which are not available in formula-feeding. Perhaps these factors, or factors associated with breast-feeding e.g. socio-economic environment overcome the diminished growth observed in the FF group.

We reported that growth in BF infants differs from that of FF infants. BF boys weighed significantly less than their FF counterparts at 7, 18 and 42 months of age, and BF infants had a lower growth rate for weight and length from 3-7 months of age compared to FF infants. These findings are partly in accordance to what others have found. Dewey et al.[34] described that weight patterns of BF infants differed from those of FF infants and from current reference data.

The following is more a thorough discussion of the significance of the reduced birth weight and postnatal growth rate in term infants. It is generally known that growth before birth is

vitally important to the child's future well-being. According to the studies of Barker and colleagues, a relationship exists between a lower birth weight and a number of diseases in adult life, such as a higher incidence of cardiovascular diseases and type II diabetes.[35,36] Barker and colleagues suggested that these diseases are programmed by an inadequate supply of nutrients or oxygen in utero or immediately after birth. The phenomenon of programming suggests that undernutrition in early life permanently changes body structure and function. This conclusion has been strengthened by animal studies which show that experimentally induced low birth weight is followed by raised systolic blood pressure in adult rats.[37] Furthermore, results from The Dutch Famine birth cohort study.[38] showed that birth weight from offspring born at the time of severe famine in 1944-1946 was 257 g lower than control subjects when famine exposure was in the third trimester of pregnancy, and 133 g elevated when famine exposure was in the first trimester. In another paper it was reported that women born during this period of acute famine had twice the risk to become schizophrenic.[39] Offspring from the second generation showed that first born infants of women prenatally exposed to acute famine during the first trimester showed 73-g higher birth weights compared with control subjects, and second borns showed 96-g lower birth weights compared to control subjects.[40] It was concluded that maternal undernutrition early in pregnancy may have had an effect in offspring birth weight and a moderate effect on birth weight in the second generation. Undernutrition might have led to a permanent effect on the uterus of the female newborn, and during pregnancy later this would have an effect on birth weight of her offspring in the second generation. Among male infants subjected to in utero famine exposure and examined at 18 years, mid-pregnancy exposure was associated with reduced body mass index, whereas an increased prevalence of obesity was observed among males exposed in the first trimester.[41] Results from the above-mentioned studies show that events during pregnancy can have consequences after birth and later in life.

In conclusion, it is not clear what is being programmed in utero when exposed to PCBs and dioxins and what the impact of a lower birth weight has for later development, but given the results of these studies and what we have reported, all factors that negatively influence fetal and postnatal growth should be regarded as harmful. Therefore, all efforts should be directed towards reduction of maternal body burden long before pregnancy in order to reduce prenatal PCB exposure and consequently avoid reduced fetal and infant growth.

4.6 ACKNOWLEDGMENTS

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CHAPTER 5

EFFECTS OF ENVIRONMENTAL EXPOSURE TO POLYCHLORINATED BIPHENYLS AND DIOXINS ON COGNITIVE ABILITIES IN DUTCH CHILDREN AT 42 MONTHS OF AGE

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5.1 ABSTRACT

Objective: To study possible adverse effects of environmental exposure to polychlorinated biphenyls (PCB) and dioxins on cognitive functioning in young children.

Methods: In a follow-up of the Dutch PCB/Dioxin study, cognitive abilities were assessed with the Kaufman Assessment Battery for Children (K-ABC) in 395 42-month-old children. In a subgroup (n=193) verbal comprehension was assessed with the Reynell Language Developmental Scales (RDLS). Prenatal PCB exposure was estimated from the sum of PCBs 118, 138, 153 and 180 (ΣPCB) in maternal plasma. Lactational exposure was assessed from breast milk PCB and dioxin concentrations, multiplied by the number of weeks of breast-feeding. Current PCB body burden was estimated from ΣPCB in 42-month-old plasma samples.

Results: After adjustment was done for covariables, maternal ΣPCB was associated with lower scores on the overall cognitive and sequential and simultaneous processing scales, of the K-ABC (all $p<0.05$). The highest exposed group (ΣPCB ≥3.0 μg/L) scored 4 points lower on all 3 scales of the K-ABC when compared with the lowest exposed group (ΣPCB <1.5 μg/L). Both lactational and current exposure to PCBs and dioxins were not related to 42-month cognitive performance.

Conclusions: In utero exposure to "background" PCB concentrations is associated with poorer cognitive functioning in preschool children. Children of mothers at the upper end of exposure are especially at risk. Therefore maternal PCB body burden should be reduced, and breast-feeding should not be discouraged.

5.2 INTRODUCTION

Polychlorinated biphenyls (PCBs), dibenzo-dioxins (PCDDs) and dibenzo-furans (PCDFs), environmentally persistent organic pollutants, are believed to be neurotoxic.[1,2] The highly lipophilic PCBs and dioxins (PCDDs and PCDFs) tend to partition into soil and sediment, bioconcentrate from water to aquatic animal and accumulate in the food chain.[3] Human exposure to PCBs and dioxins is mainly from meat, dairy products and fish.[2] After ingestion occurs, these compounds accumulate in adipose tissue. Their elimination depends on metabolic degradation, which is minimal for most congeners, and on the rate of excretion, which is primarily by way of the feces. As a result, the half life is very long, approximately a decade.[2] The human fetus is exposed to PCBs and dioxins through placental transport,[4] and larger quantities are transferred to the infant during breast-feeding.[5,6]

Rice oil contaminated with PCBs, PCDFs and a small amount of PCDDs was used in cooking and ingested by more than 1850 individuals in Japan in 1968 (Yusho)[7] and more than 2000 persons in Taiwan in 1979 (Yu-Cheng) in remarkably similar incidents.[8] These exposures caused illness similar to those documented in animal

studies.[2] Mothers poisoned with rice oil reported lower birth weight, hyper pigmentation, conjunctivitis, nail changes and natal teeth in their offspring and delay in developmental milestones.[8] Follow-up studies of Yu-Cheng children at school age showed lower IQs,[9] behavioral effects,[10] and growth delay.[11]

Two prospective longitudinal studies in Michigan and North Carolina examined the effects of prenatal PCB exposure on developmental outcome in children.[4,5] PCB levels measured in both studies were at or slightly above U.S. background levels.[12] In the Michigan cohort poorer visual recognition memory (Fagan test) at 7 months,[13] lower performance on verbal and memory scales of the McCarthy Scales of Children's Abilities at 4 years[14] and lower IQ scores at 11 years[15] were associated with prenatal PCB exposure. In the North Carolina cohort, in utero exposure to PCBs, was associated with lower psychomotor scores measured with the Bayley Scales of infant development from 6 to 24 months.[16,17] No deficits, were apparent at 3, 4, or 5 years of age on the McCarthy Scales of Children's abilities.[18]

Contamination of breast milk with PCBs and dioxins in the Netherlands belong to the highest measured in the world.[19] A prospective follow-up study was launched by the Dutch government in 1989, the "Dutch PCB/dioxin study", to investigate possible adverse effects of PCBs and dioxins on growth and development of healthy term born babies. The follow-up at preschool age was funded by an European collaborative study. Previous results showed that prenatal PCB exposure was related to a lower birth weight,[20] lower growth rate,[20] and lower psychomotor scores at 3 months of age,[21] and a poorer neurological condition at birth [22] and 18 months.[23] Postnatal PCB and dioxin exposure was related to lower psychomotor development at 7 months.[21] We examined the effects of environmental PCB and dioxin exposure on cognitive abilities assessed at 42 months of age.

5.3 METHODS

Subjects

From June 1990 until June 1992, healthy pregnant women living in Rotterdam and Groningen were asked by their obstetrician or midwife to participate in a prospective, longitudinal neuro-developmental study. The Rotterdam area is a highly industrialized and densely populated region situated in the west, and the Groningen area is a semi-urban region in the north of the Netherlands. To study the effects of pre- as well as postnatal PCB and dioxin exposure, women were included who intended to breast-feed their child for at least 6 weeks (BF group) in addition to women who intended to use formula-feeding (FF group). All infants in the FF group received formula from a single batch (Almiron M2, Nutricia NV, the Netherlands) from birth until 7 months of age. In this formula, concentrations of both PCBs and dioxins were not detectable. Further inclusion criteria were (1) Pregnancy and delivery had to be without complications or serious illnesses; instrumental deliveries or cesarian sections were excluded, (2) first- or second-born infants (3) born at term, 37 to 42 weeks of

gestation, (4) no congenital anomalies or diseases, (5) Caucasian race. The Medical Ethics Committees of both University Hospitals approved the study protocol. Informed consent was given by participating parents. Children were examined for their growth and neurodevelopment at the ages of 2 weeks and 3, 7, 18 and 42 months. Details of the study design, chemical analysis, PCB, dioxin concentrations, and results up to 18 months have been published elsewhere.[6, 20-24]

Exposure variables

Maternal plasma samples were obtained in the last month of pregnancy, and umbilical cord plasma samples were collected shortly after delivery. Plasma samples were collected from the children when they were 42 months old. Four non-planar PCB congeners, International Union of Pure and Applied Chemistry (IUPAC) nos. 118, 138, 153, and 180, were analyzed in maternal, cord and 42-month-old plasma samples at the Nutrition and Food Research Institute, Zeist, the Netherlands by gas chromatography with electron capture detection (GC-ECD).[24] These 4 congeners were measured, because they are the predominant PCB congeners found in human tissue and make up approximately 50% of the total PCB.[24] Plasma PCB concentrations are reported on a volume basis (μg/L). The sum of 4 PCB congeners (ΣPCB) was calculated in each plasma sample, by adding the 4 concentrations.

A 24-hour representative breast milk sample was collected 2 weeks after delivery from each breast-feeding mother and analysed for 17 dioxins (PCDDs and PCDFs), 6 dioxin-like PCBs (IUPAC nos. 77, 105, 118, 126, 156 and 169), and 20 non-dioxin like PCBs (IUPAC nos. 28, 52, 66, 70, 99, 101, 128, 137, 138, 141, 151, 153, 170, 177, 180, 183, 187, 194, 195 and 202). To express the toxic potency of the mixture of dioxins and dioxin-like PCBs, the toxic equivalent factor (TEF) approach was used according to the latest World Health Organization meeting.[25] The toxic equivalents (TEQ) were calculated by multiplying the concentration of each congener by its TEF value. PCBs and dioxins measured in breast milk shortly after birth are an index of the maternal PCB and dioxin body burden. Breast milk PCB and dioxin concentrations are therefore an indirect measure of prenatal exposure.[5]

Prenatal PCB exposure was estimated from ΣPCB concentrations in maternal plasma and cord plasma. In the BF group prenatal exposure to dioxin-TEQs, dioxin-like PCB-TEQs and nondioxin-like PCBs (sum of 20 PCB congeners) was assessed from breast milk concentrations. Three measures of lactational exposure were calculated by multiplying breast milk concentrations with the number of weeks of breast-feeding. 1) TEQ exposure, the sum of dioxin-TEQs and PCB-TEQs. 2) ΣPCB, and 3) the sum of 20 nondioxin-like PCBs. Current PCB body burden was estimated from ΣPCB measured in 42-months-old plasma samples.

Cognitive abilities at 42 months

The child's level of intellectual functioning at 42 months of age was measured with the Dutch version of the Kaufman Assessment Battery for Children (Dutch K-ABC).[26] The Dutch K-ABC consists of 11 subtests and is standardized for a large sample of normal preschool children in the 2.5 to 4.5 year range. Raw scores of each subtest are transformed into normalized standard scores with a mean (±SD) of 10 ± 3. Factor analysis of the 11 subtests yielded 2 scales, both considered to be equally vital to intellectual functioning: (1) the sequential processing scale, which is the average of the hand movements, number recall, arithmetic, gross and fine motor skills subtests and (2) the simultaneous processing scale, which is the average of the Magic Window, face recognition, Gestalt closure, vocabulary, faces and places, and riddles subtests. The standard scores were summed, and a scale score was calculated. The combined scale score of the sequential and simultaneous processing scale form the overall cognitive scale score. All 3 scale scores are normalized to a mean (±SD) of 100 ±15.[26]

The K-ABC is constructed to assess 2 types of mental functioning connoted by the terms sequential and simultaneous.[26] Solving problems sequentially is closely related to a variety of everyday, school-oriented skills. Each task in the sequential processing scale presents a problem, that must be solved by arranging the input in serial order, for instance, repeating numbers spoken by the examiner. The problems presented in the simultaneous processing scale are spatial, analogic, or organizational in nature. The input must be integrated and synthesized simultaneously to produce the appropriate solution, for example, identifying the object pictured in a partially completed drawing.

For logistic reasons verbal comprehension measured with the Dutch version of the Reynell Developmental Language Scales (Dutch RDLS) was assessed only in the Rotterdam cohort.[27] The RDLS is primarily a measure of language ability; however, this ability, particularly the verbal comprehension scale, is also a measure of general mental ability.[26,27] The Dutch RDLS was also standardized for a large sample of children in the 1.5- to 6-year range. The mean scale scores were also normalized to a mean (± SD) of 100 ± 15.

Covariables

The K-ABC was administered by 2 trained examiners (S.P. and C.I.L.), 1 in each study center, who were not aware of the prenatal, lactational, and current exposure values, nor were they aware of the feeding mode given during infancy. To adjust for inter-observer and between-center differences, the study center/examiner was included as a covariable. Covariables known to be related to child development were selected from a list containing data on socioeconomic background, obstetric and neonatal history, maternal age, parents' education level, parity, gender, fetal exposure to alcohol and cigarette smoking, type of feeding during infancy, and the breast-feeding period. Because small numbers of subjects were in the categories of smoking or alcohol use during pregnancy, dichomotizing these covariables into no or yes was justified. The child's home environment was assessed by the Dutch version of the Home Observation for Measurement of the Environment (HOME).[28] The verbal IQ of the parent, most often with the child (usually the mother), was assessed by 2 subtests, Information and

Vocabulary, from the Dutch Wechsler Adult Intelligence Scale (WAIS).[29] These two subtests exhibit good correlations with the verbal IQ scale.[29]

Statistical analysis

To compare groups for a single variable, we used the chi-square test, the Student's t-test, and the Mann-Whitney test. Plasma PCB values were positively skewed and therefore normalized by natural logarithm (lnΣPCB). The effects of prenatal, lactational and current exposure to PCBs and dioxins on cognitive abilities at 42 months of age were studied by multiple linear regression analyses adjusted for covariables. Dependent variables were scores on the overall cognitive scale, the sequential and simultaneous processing scale of the K-ABC, and the verbal comprehension scale of the RDLS. Each outcome variable was analyzed with each exposure variable separately in a regression analysis.

Covariables entered in the final regression analyses were selected from variables known from literature and clinical knowledge to have an effect on developmental outcome. Second, variables were also included in the regression model when the exposure parameter estimate changed after including this covariable (possible confounding variable).[30] Covariables included in the final regression model were maternal age at birth, parity (first or second born), gender, feeding type during infancy, breast-feeding period in weeks, HOME score, paternal and maternal education (3 levels; low-primary school finished/secondary school not finished, middle-secondary school finished, high-high school finished/professional and university training), parental verbal IQ score, smoking and alcohol use during pregnancy (yes or no), and study center/examiner (Groningen and Rotterdam).

The effect of prenatal PCB exposure measured from maternal LnΣPCB and cord LnΣPCB on outcome variables was examined in the whole group, the FF and BF group. The FF group represents children who were predominantly exposed to PCBs before birth. In addition, the effect of prenatal exposure to dioxin-TEQs, dioxin-like PCB-TEQs, and nondioxin-like PCBs measured from breast milk concentrations was studied in the BF group. The effect of lactational exposure to PCB- and dioxin-TEQs, ΣPCB in breast milk and nondioxin-like PCBs was also studied in the BF group. The effect of 42-month PCB body burden on cognitive abilities was investigated in the whole group as well as both feeding groups separately. Results were considered to be significant if $p \leq 0.05$.

5.4 RESULTS

From the original cohort of 418 children, 209 were BF and 209 were FF during infancy; 207 subjects were from the Rotterdam area, and 211 from the Groningen area. At 42 months of age 395 (94%) subjects were re-examined for their neurodevelopmental follow-up; 6% (n=23) were lost to follow up because of a lack of interest (n=19) and

emigration (n=4). Another 15 children failed to cooperate with the testing procedure, refusing to respond to some of the 11 subtests, and were excluded from the final analysis. The excluded children did not differ from the others in the sample in terms of PCB and dioxin concentrations. The RDLS was completed in all children from the Rotterdam cohort. Between the 2 study centers, Rotterdam (n=193) and Groningen (n=202), no differences were found with respect to PCB concentrations measured in maternal, cord and 42-month-old plasma nor in breast milk PCB and dioxin concentrations.

Exposure variables (Table 5.1)

Three maternal plasma samples were missing. In 382 cord plasma samples, concentrations of PCB IUPAC nos. 138, 153, and 180 were analyzed. Nine cord samples were missing for the analysis of PCB no. 118. In human milk dioxin-TEQ, mono-ortho PCB-TEQ and planar PCB-TEQ concentrations were available for 176, 195 and 194 milk samples, respectively. Blood samples for PCB analysis at 42 months were available in 299 (76%) children. Median ΣPCB concentration at 42 months of age in the BF group is nearly 4 times higher than median ΣPCB concentration in the FF group.

Table 5. 1 Concentrations of Polychlorinated Biphenyls and Dioxins measured in plasma and in breast milk samples

Concentration percentiles	P5	P50	P95
Plasma (µg/l)			
ΣPCB[a] maternal (n=415)	1.00	2.04	3.81
ΣPCB cord (n=373)	0.18	0.38	0.86
ΣPCB 42-months (n=299)	0.11	0.35	1.54
-breast-fed group (n=154)	0.29	0.78	1.90
-formula-fed group (n=145)	0.10	0.20	1.49
Measured in breast milk (breast-fed group, n=209)			
ΣPCB (µg/kg milk fat) (n=193)	205	405	723
Non-dioxin like PCBs[b] (µg/kg milk fat) (n=193)	269	545	914
Mono-ortho PCB-TEQ[c] (ng/kg fat) (n=195)	6.8	14.2	24.8
Planar PCB-TEQ(ng/kg fat) (n=194)	7.1	14.5	31.7
Dioxin-TEQ(ng/kg fat) (n=176)	17.2	33.5	59.5

a: ΣPCBs= sum of PCBs IUPAC nos. 118, 138, 153, and 180 measured in plasma and breast milk
b: Sum of 20 non dioxin-like PCBs in breast milk.
c: TEQ = toxic equivalents according to the 1997 WHO TEF values for mono-ortho PCBs, IUPAC nos. 105, 118, 156; planar PCBs IUPAC nos. 77, 126, and 169 and 17 2,3,7,8 substituted polychlorinated di-benzo dioxins (PCDDs) and furans (PCDFs).

In Table 5.2 covariables are presented according to prenatal PCB exposure estimated from maternal plasma ΣPCB concentrations, divided into 5 groups of exposure. Maternal age, parental education, verbal IQ and HOME score were higher in the highest exposed group. In Table 5.3 the mean scores ± SD on the 3 scales of the K-ABC and verbal comprehension scale of the RDLS are given for the whole group and both feeding groups. All scores are

within or higher than the mean ± SD population score of 100 ± 15. Children in the BF group had higher mean scores on the K-ABC and the RDLS when compared with the FF group (t-test, all p-values <0.01, Table 5.3). After adjustment was done for covariables, the outcome scores were not significantly different between the 2 feeding groups.

Table 5.2 Characteristics of study population according to PCB concentrations measured in maternal plasma during pregnancy.

	ΣPCB concentration in maternal plasma				
	<1.5 µg/L	1.5-1.99 µg/L	2.0-2.49 µg/L	2.5-2.99 µg/L	≥3.0 µg/L
Characteristics					
-At birth	*n=90*	*n=105*	*n=86*	*n=66*	*n=68*
Study center					
Rotterdam	47(52%)	46(44%)	42(59%)	31(47%)	40(59%)
Feeding type					
Breast-fed	28(31%)	47(45%)	49(57%)	41(62%)	42(62%)
Breast-feeding period (wk)	21.5 (6-78)	17 (6-56)	21.5 (9-54)	16 (6-56)	19.5 (6-62)
Maternal age (yr)	27 ± 4	28 ± 4	29 ±3	31±3	32±3
Maternal smoking					
during pregnancy, yes	30 (33%)	27 (26%)	18(21%)	18(27%)	15(22%)
Maternal alcohol use					
during pregnancy, yes	15(17%)	19(18%)	19(22%)	30(45%)	33(45%)
Gender, male	46(51%)	44(42%)	40(42%)	31(47%)	34(50%)
Birth order, firstborn	45(50%)	52(50%)	45(52%)	30(45%)	28(41%)
Gestational age (wk)	40.5 ±1.0	40.4 ± 1.2	40.4 ± 1.1	40.4 ± 1.2	39.9 ± 1.3
Birth Weight (g)	3567 ± 456	3533 ± 429	3519 ± 402	3468 ± 418	3470 ± 523
At 42 months examination	*n=83*	*n=103*	*n=82*	*n=61*	*n=65*
Maternal education					
low	39(46%)	25(24%)	11(13%)	6(10%)	5(8%)
medium	31(37%)	47(46%)	25(30%)	18(29%)	21(32%)
high	15(18%)	31(30%)	47(57%)	38(61%)	39(60%)
Paternal education					
low	42(50%)	31(31%)	13(16%)	11(18%)	10(15%)
medium	18 (21%)	30(30%)	23(28%)	14(22%)	10(15%)
high	24(29%)	40(39%)	47(57%)	37(60%)	45(70%)
HOME	38 ± 4	39 ± 3	39 ± 4	40 ± 2	40 ± 3
Parental Verbal IQ	111 ± 18	114 ± 15	123 ± 16	123 ± 12	125 ± 13

Values are numbers (percentages), means ± standard deviations or medians (range). Maternal and Paternal education: Low ;Primary school finished and secondary school not finished. Medium; secondary school finished High; high school finished or professional and university training. Parental verbal IQ: two subtests of the verbal scale of the Wechsler Adult Intelligence Scale assessed from the parent HOME: home observation for the measurement of the environment. ΣPCB= sum of Polychlorinated biphenyl (PCB) congeners IUPAC Nos 118, 138, 153 and 180 measured in maternal plasma during last month of pregnancy.

Table 5.3 *In utero exposure to polychlorinated Biphenyls in relation to cognitive abilities at 42 months.*

Outcome	n	Mean ±SD	LnΣPCBmaternal regression coefficient (SE)#	p-value	R^2 whole model	Residual SD
Whole group						
K-ABC[a]						
Overall Cognitive scale	373	111±14	-4.56 (1.62)	0.005	0.42	10.7
Sequential processing scale	373	109±14	-4.16 (1.79)	0.02	0.32	11.9
Simultaneous processing scale	384	109±14	-3.82 (1.60)	0.02	0.38	10.9
RDLS[b]						
Verbal comprehension scale	190	105±12	-3.36 (1.91)	0.08	0.40	9.4
Breast-fed group						
K-ABC						
Overall Cognitive scale	195	114±12*	-2.20 (2.14)	0.30	0.33	10.6
Sequential processing scale	195	111±13*	-1.49 (2.46)	0.54	0.25	12.1
Simultaneous processing scale	198	112±12*	-2.45 (2.18)	0.26	0.28	10.8
RDLS						
Verbal comprehension scale	100	108±11*	-0.20 (2.74)	0.94	0.38	9.3
Formula-fed group						
K-ABC						
Overall Cognitive scale	178	108±15	-8.69 (2.49)	0.0006	0.50	10.7
Sequential processing scale	178	107±14	-8.34 (2.68)	0.002	0.40	11.5
Simultaneous processing scale	186	106±14	-6.54 (2.39)	0.007	0.45	10.8
RDLS						
Verbal comprehension scale	90	101±12	-6.13 (2.79)	0.03	0.40	9.6

*: t-test, significantly higher in the breast-fed group lnΣPCB: Prenatal exposure was defined as the natural logarithm of 4 PCB congeners measured in maternal plasma during the last month of pregnancy.

a: Dutch version of the Kaufmann Assessment Battery for Children.

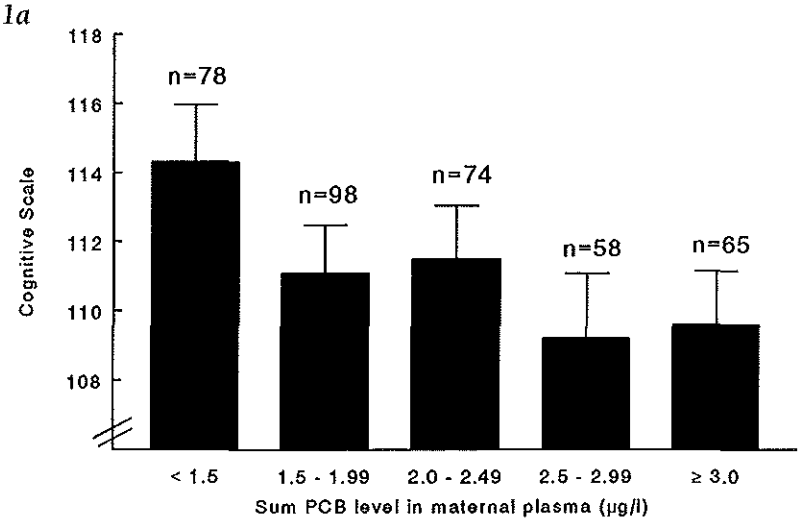
b: Dutch version of the Reynell Language developmental Scales only in the Rotterdam cohort.

#: regression coefficient and standard error (SE) for prenatal PCB exposure from multiple linear regression analyses. All the regressions are adjusted for study center (except RDLS), HOME, birth order, maternal age, parental verbal IQ, maternal and paternal education, gender, maternal cigarette and alcohol use during pregnancy. Feeding type and breast-feeding period were additionally entered as covariates in the whole group and period of breast-feeding was an additional covariate in the breast-fed group. Note, If the ΣPCB increases by 10 %, the expected additive change in outcome variable approximates 10 % of the regression coefficient of lnΣPCB.

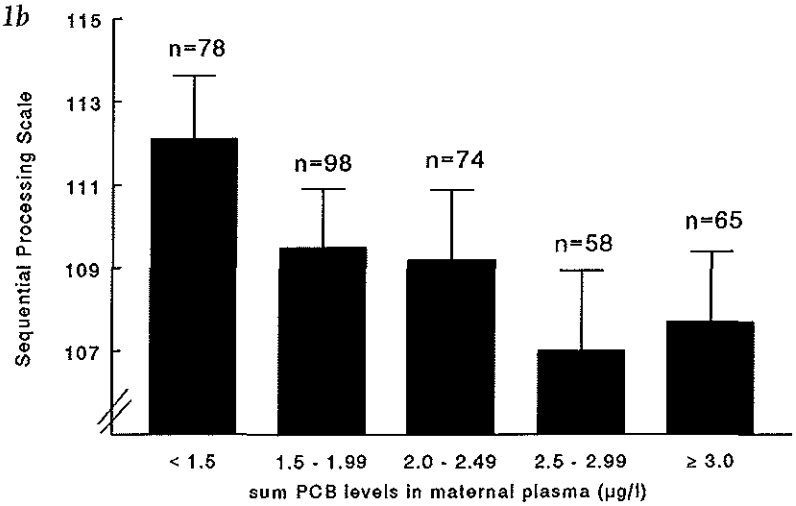
Multiple linear regression analyses showed that after controlling for covariables (maternal age, parity, gender, parental education and verbal IQ, HOME score, maternal alcohol use and cigarette smoking during pregnancy, feeding type in infancy, breast-feeding period and study center), prenatal PCB exposure measured from lnΣPCB concentration in maternal plasma was significantly associated with lower scores on the overall cognitive scale and the sequential and simultaneous processing scales of the K-ABC (Table 5.3). In addition, the 2 feeding groups (FF and BF group) were studied separately. LnΣPCB maternal was associated with lower scores on all 3 scales of the K-ABC and on the verbal comprehension scale of the RDLS in the FF group (all $p<0.05$, Table 5.3). Although negative associations between outcome variables and lnΣPCB maternal plasma were found in the BF group, this effect did not show statistical significance (Table 5.3). When lnΣPCB in cord plasma was entered as prenatal PCB exposure in the same regression model, results were significant for the simultaneous processing scale of the K-ABC (regression coefficient [standard error]= -3.26[1.34], p-value=0.02, n=345) in the whole group. In the FF group significant effects were found for the K-ABC simultaneous processing scale (-4.78 [1.98], p=0.02, n=167) and the RDLS verbal comprehension scale (-5.78[2.27], p=0.01, n=79).

Adjusted mean scores and SEM for the cognitive scale on the K-ABC at 42 months of age for the whole group according to prenatal PCB exposure are presented in Figure 5.1a. Five cut off points are given for maternal plasma ΣPCB based upon the range of plasma ΣPCB concentration. The mean score on the cognitive scale in the highest exposed group (ΣPCB maternal ≥3 µg/L) is 4 points lower compared with that in the lowest exposed group (ΣPCB maternal < 1.5 µg/L). In Figure 5.1b and 5.1c, similar dose-response relationships are presented for the sequential and simultaneous processing scale. A 4-point deficit on both processing scales is also calculated for the highest exposed group.

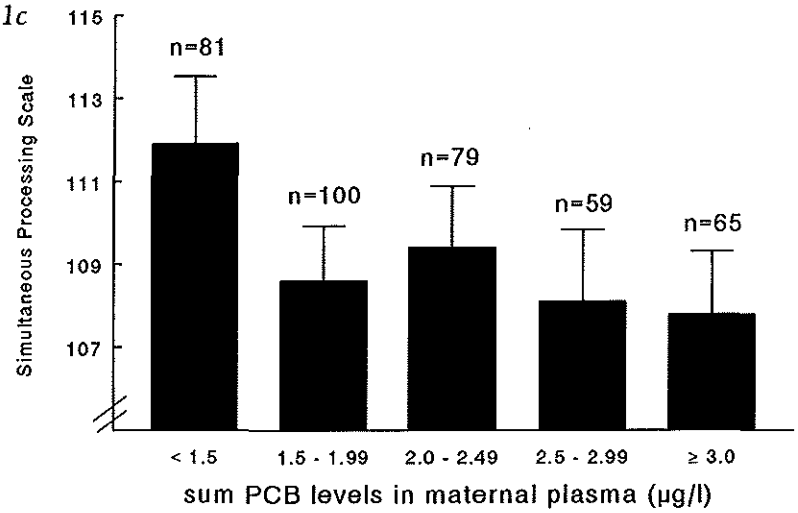
Figures 5.1a



Figures 5.1b



Figures 5.1c



Figures 5.1a-c

Five cut off points, are given for the ΣPCB levels measured in maternal plasma, based upon the range and distribution of the ΣPCB levels. The bars represent the dose-response relationship of the mean score and standard error of the mean (SEM) on the cognitive scale of the Dutch version of the Kaufman Assessment Battery for Children (K-ABC) and maternal ΣPCB concentrations, adjusted for covariables (maternal age, parity, gender, parental education and verbal IQ, HOME score, maternal alcohol use and cigarette smoking during pregnancy, feeding type in infancy, breast-feeding period and study center). The score on the cognitive scale in the highest exposed group (ΣPCB maternal ≥ 3 µg/l), is 4 points lower compared to the lowest exposed group (ΣPCB maternal < 1.5 µg/l), figure 5.1a. Similar dose-response relationships are given for the sequential and simultaneous processing scales of K-ABC, figure 5.1b respectively 5.1c.

In the BF group effects of prenatal exposure to dioxin-TEQs, dioxin-like PCB-TEQs, and nondioxin-like PCBs were examined. After adjustment was done for the same set of covariables, no negative effects of prenatal TEQ exposure or prenatal PCB exposure on performance of the K-ABC and the RDLS were found. Lactational exposure to nondioxin-like PCBs, dioxin-like PCB-TEQs and dioxin-TEQs were also not related to performance on the K-ABC and the RDLS (results not presented). The current PCB body burden measured from lnΣPCB in 42-month-old plasma samples was not related to cognitive abilities in the whole group (Table 5.4) and the two feeding groups separately.

Table 5.4 Current polychlorinated biphenyl body burden in relation to cognitive abilities at 42 months of age

Outcome	n	lnΣPCB 42 months regression coefficient (SE)#	P-value	R^2 whole model	Residual SD
Whole group					
K-ABC[a]					
Overall Cognitive scale	286	1.16 (1.56)	0.46	0.44	10.9
Sequential processing scale	286	1.82 (1.73)	0.30	0.34	12.1
Simultaneous processing scale	296	0.28 (1.48)	0.85	0.39	11.0
RDLS[b]					
Verbal comprehension scale	171	1.82 (1.55)	0.24	0.40	9.3

lnΣPCB42 months: Contemporary exposure, the sum of 4 PCB congeners measured in child's plasma at 42 months of age. #: Regression coefficient and standard error (SE) for lnΣPCB at 42 months from multiple linear regression analyses

a:Dutch version of the Kaufmann Assessment Battery for Children.

b: Dutch version of the Reynell Language developmental Scales only in the Rotterdam cohort. All the regressions are adjusted for study center (not for RDLS), feeding type, period of breast-feeding, HOME, birth order, maternal age, parental verbal IQ, parental education, gender, maternal cigarette and alcohol use during pregnancy. Note, If the ΣPCB level in plasma increases by 10 %, the expected additive change in outcome variable approximates 10 % of the regression coefficient of lnΣPCB.

5.5 Discussion

We report that prenatal exposure to "background" PCB concentrations is associated with poorer performance on cognitive tests in Dutch children at 42 months of age. No associations between lactational exposure to PCBs and dioxins nor current PCB body burden and cognitive abilities at 42 months of age are found, suggesting that the developing fetal brain is particularly sensitive to these compounds. Our results are in agreement with the reported cognitive deficits in the Yu-Cheng "poisoning" study [8, 9] and the Michigan "fish exposure" study.[14, 15] In the latter cohort adverse effects of prenatal PCB exposure were found on short-term memory on both verbal and numeric

tests at 4 years and on the full-scale and verbal IQ scores at 11 years of age. In our cohort effects are found on overall cognitive functioning and on the sequential and simultaneous processing scale including short- and long-term memory tasks.

Exposure data are difficult to compare because of differences in analytic methods used in the Yu-Cheng, U.S., and Dutch studies. In the Yu-Cheng study children from exposed mothers were compared with children in a matched control group.[9] In Michigan exposure was defined from total PCB levels determined in cord and maternal blood and breast milk by summing 10 Webb-McCall peaks. In North Carolina 2 Webb-McCall peaks were quantified with packed column gas chromatography.[12] Although the Webb-McCall method was state-of-the-art at that time, it is crude by today's standards and provides no information of individual PCB congeners.[12] In the Dutch study PCB concentrations were measured by gas chromatography with electron capture detection and provided information of 4 individual PCB congeners.[24] In the Michigan study pooled serum samples in 4-year-old children were also analyzed for individual PCB congeners, and it appears that the 4 PCBs (IUPAC nos. 118, 138, 153, and 180) constitute 46 % of the total PCBs.[31] Assuming that the PCB congener mix in the Netherlands is similar to that in Michigan, doubling the maternal plasma PCB values from the Dutch study (mean ΣPCB = 2.2 µg/L) gives PCB levels which are roughly comparable to those measured in Michigan (mean ΣPCB = 4.7 µg/L). In the North Carolina study developmental effects were found in the first 2 years of life and not at 3, 4 and 5 years of age. There is reason to suspect that exposure levels are lower in the North Carolina study. The Dutch cohort comes from a more industrialized and densely populated area which could result in higher background PCB levels in the Netherlands.

Whether the observed effects are due to PCBs or to other (related) contaminants is uncertain. In the Yu-Cheng incident the observed deficits might be due to other compounds than PCBs; the consumed rice oil was also contaminated with PCDFs and polychlorinated quarterphenyls.[8] In a subgroup (n=151) of the Dutch cohort lead and cadmium were measured in whole blood at 18 months of age, to study the influence of heavy metals as other possible neurotoxicants next to PCBs and dioxins. The concentrations of lead and cadmium were very low and not related to outcome variables. To measure dioxins and dioxin-like PCBs in blood at the time of the study, 100 to 200 mL of blood was needed; therefore these compounds were not measured in plasma. Seventeen dioxin congeners (PCDDs and PCDFs) and 26 PCB congeners were measured in maternal milk samples. Our results show that breast milk PCB and dioxin concentrations, defined as an index of prenatal exposure, are not related to cognitive outcome at 42 months of age. In studies with rats perinatal exposure to ortho-substituted PCBs (IUPAC nos. 28, 118 and 153) resulted in long-lasting deficits in learning,[32] and deficits in spatial learning and memory tasks were detected in monkeys at 4 to 6 years after perinatal exposure to a PCB mixture.[33] In the environment PCBs and dioxins are present as complex mixtures of various congeners. Questions raised by human studies can be resolved by studies in laboratory animals as well as other human studies in which more PCB and related compounds and their metabolites are measured and related to neurodevelopmental endpoints.

In contrast to other human studies, in which most of the included infants were breast-fed, our study included 50 % BF infants in addition to 50 % FF infants. The FF group represented children almost exclusively exposed to PCBs in utero, because in formula-feeding animal fats are replaced by vegetable oils with nondetectable PCB and dioxin concentrations. In the FF group the highest exposed children show a 6- to 8-point lower score on the K-ABC and the RDLS when compared with the lowest exposed group. In the BF group this cognitive deficit was 2 points on the K-ABC; however this result was not statistically significant. This lack of significance could be explained by a "power" problem caused by a smaller sample size or that these children are more advantaged. Children from the BF group have significantly higher HOME scores, higher parental education levels and parental verbal IQ. Moreover, substances in breast milk or factors associated with breast-feeding may have counteracted the negative influence of prenatal PCB exposure on cognitive development.

The development of the central nervous system both in utero and during childhood is a continuous process in which many morphological changes take place. Because of variability in the rate of development, especially in the younger age group, it is difficult to detect subtle neurodevelopmental deficits. Adverse effects of prenatal PCB exposure on the neurologic condition found at birth [22] and at 18 months of age [23], could not be detected at 42 months of age[34]; however, the 42-month cognitive abilities were negatively associated with in utero exposure. This difference can be explained by the different testing procedures. The K-ABC measures the child's cognitive abilities in a quantitative fashion, whereas the neurologic examination [34] is a qualitative measure of brain development.

Although lactational exposure to PCBs and dioxins is much higher compared to transplacental exposure, no negative effects were found in children breast-fed during infancy. Moreover, children who were breast-fed during infancy performed better on cognitive tests and were from a more advantaged socioeconomic environment than their formula-fed counterparts. Fetal exposure to PCBs and related compounds should be lowered by reducing maternal body burden rather than discouraging breast-feeding. Our data demonstrate the continuation of a toxic impact received in utero on cognitive functioning at toddler age. Studies at school age are required to investigate whether long-term implications for later intellectual functioning exist.

5.6 ACKNOWLEDGMENTS

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CHAPTER 6

NEUROLOGICAL CONDITION IN 42-MONTH-OLD CHILDREN IN RELATION TO PRE- AND POSTNATAL EXPOSURE TO POLYCHLORINATED BIPHENYLS AND DIOXINS.

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6.1 ABSTRACT

Adverse neurological effects of exposure to PCBs have been found up to 18 months of age. Now we report on the effect of pre- and postnatal exposure to PCBs and dioxins on the neurological condition at 42 months of age. For this purpose, PCB levels were determined in cord and maternal plasma, and used as a measure of prenatal exposure. Breast milk was analyzed for PCBs and dioxins. In addition, PCBs were determined in plasma sampled from the child at 42 months of age. We evaluated the neurological condition of 394 children using the Touwen/Hempel method. After adjustment for covariates, neither prenatal PCB exposure nor postnatal exposure to PCBs and dioxins was found to be related to the neurological condition at 42 months of age.

6.2 INTRODUCTION

Polychlorinated biphenyls (PCBs) and dioxins are polycyclic aromatic compounds, which are widespread environmental pollutants. PCBs are resistant to high temperatures, they can easily conduct heat, and have electrical insulating properties. They have been produced for use in plasticizers, fire retardants, dielectrical fluids in transformers and capacitators, and in hydraulic fluids. In the late 1970s their environmental persistence was recognized, and they were banned world-wide. Yet, in for example eastern Europe, PCBs have been produced until the mid-1980s.[1] Dioxins are derived from many sources, mainly thermal and industrial processes. The incineration of municipal and industrial waste has a major role in the environmental deposition.[2]

PCBs have a total number of 209 possible congeners which differ in the degree of chlorination and the position of the chlorine atoms. Dioxins are a mixture of 75 polychlorinated dibenzo-p-dioxins and 135 dibenzofurans. Both PCBs and dioxins are absorbed readily and, due to their very long half-life and high lipophilicity, they accumulate in the adipose tissue of animals and human beings. The uptake in plants is negligible. In organisms at the top of the food chain, including human beings, high concentrations have been detected in blood and adipose tissue.[3] PCBs and dioxins cross the placenta, and are transferred into human milk.[4] In formula milks, cow milk lipids are replaced by fats of vegetable origin having negligible contents of PCBs and dioxins.

Previously, we have studied the effects of prenatal and lactational exposure to PCBs and dioxins on the neurological condition in the second week after birth[5] and at 18 months of age.[6] For that purpose we followed a group of healthy term infants subjected to background levels of exposure from birth. In the second week after delivery, the combination of a high prenatal and a high lactational exposure was found to result in an adverse effect on the neurological condition and a higher incidence of hypotonia. When the children reached the age of 18 months, we found a negative impact of the prenatal PCB exposure on the neurological condition. No effect of lactational exposure to PCBs or dioxins could be detected. We now report on the effect of prenatal exposure to PCBs, PCB and dioxin exposure via breast-feeding, and the child's 42-month PCB body burden on the neurological condition at 42 months of age.

6.3 METHODS

Subjects

From June 1990 until June 1992, healthy pregnant women were asked to participate in the study in Groningen and Rotterdam. Groningen is a semi-urban area in the north of the Netherlands, whereas the Rotterdam area is a highly industrialized region in the Western parts of the Netherlands. It was intended to include 100 breast-feeding and 100 formula-feeding mothers in each study center. The women were approached by their midwives or obstetrician between the 32nd and 34th week of pregnancy and provisionally assigned to one of two feeding groups on the basis of their intention to breast- or formula-feed their infant. Only those mothers who formula-fed their child and those who were actually able to sustain breast-feeding for at least six weeks after delivery were included in the final study group. In addition, for inclusion in the final study group, the mother-infant pairs had to meet the following inclusion criteria: (1) Absence of serious illness and complications during pregnancy and delivery, (2) First or second born term infants, (3) A spontaneous vaginal delivery, (4) Caucasian origin. The medical ethics committees of both the University Hospital Groningen and the Rotterdam Sophia Children's Hospital approved the protocol.

Measures of exposure

PCB levels in cord and maternal plasma are considered to be a reflection of prenatal exposure. Maternal blood was collected in the last month of pregnancy and cord blood immediately after birth. In addition, child's blood was sampled at 42 months. Plasma was analyzed for the non-planar PCB congeners 118, 138, 153, and 180 (International Union of Pure and Applied Chemistry (IUPAC) nomenclature) by gas chromatography with electron capture detection (GC-ECD). The sum of the levels of these four PCB congeners in cord (SPCBcord), maternal (SPCBmaternal), and 42-month plasma (SPCB42mo) was calculated.

In order to assess the lactational exposure, human milk was collected as a 24-hour sample in the sixth week after delivery. Of the total amounts of breast milk, 10% aliquots were analyzed for the seventeen ubiquitous 2,3,7,8-substituted dioxin congeners by means of gas chromatography with high-resolution mass spectrometry.[7,8] Moreover, the levels of three planar PCB congeners, and 23 non-planar PCB congeners were measured by GC-ECD. In order to express the potential toxicity of the mixture of dioxins and dioxin-like PCBs (IUPAC no. 77, 126, 169, 105, 118, 156, 170, and 180) the toxic equivalence factor (TEF) approach was used. This factor expresses the relative toxicity towards the most toxic congener. For each congener, we calculated the toxic equivalent (TEQ) by multiplying its concentration with the TEF. All congener-specific TEQ values were added to form the total PCB/dioxin TEQ. Accordingly, a dioxin TEQ, a planar PCB TEQ (IUPAC no. 77, 126, and 169), a mono-ortho PCB TEQ (IUPAC no. 105, 118, and 156), and a di-ortho PCB TEQ (IUPAC no. 170 and 180) was calculated.

Outcome measures

All children underwent a neonatal neurological examination according to Prechtl in their second week after birth.[9] At 42 months, the children were neurologically examined according to Touwen/Hempel.[10] In contrast to other developmental tests (e.g. cognitive tests), which are a quantitative measure for the child's abilities, the neurological examination is used for the qualitative appraisal of brain function. The neurological examination at 42 months is an age-adequate technique which focuses on the observation of motor functions (prehension, sitting, crawling, standing, and walking) in a free-field situation. The neurological examination led to a clinical diagnosis: `normal', `mildly abnormal', or `abnormal'. The latter classification implies that a circumscript neurological syndrome was found. `Mildly abnormal' signifies the presence of mild signs which do not lead to a handicapping condition in daily life, such as slight asymmetries, or mild hypo- or hypertonia. In addition to the clinical diagnosis, we evaluated the neurological findings using a list of 56 pre-set criteria for optimality[11] (Appendix I). For each child a neurological optimality score (NOS) was established by counting the number of items considered optimal. It should be emphasized that optimality is not equal to normality, and that a reduced optimality does not necessarily mean that the clinical diagnosis `mildly abnormal' or `abnormal' is appropriate.[11] A point of special attention was the quality of movements in terms of fluency. Fluency of motility has been shown to be an indicator for the integrity of brain function.[6,12,13] The quality of movements was evaluated separately as a fluency cluster score (Appendix I).

Explanatory variables

The neurological examinations were performed by two researchers, one in each study center. They had been trained and were regularly supervised by BCLT. In order to correct for inter-observer differences, the study center was included as an explanatory variable. Both examiners were unaware of the pre- and postnatal PCB and dioxin exposure, and the type of feeding that had been given to the child during early life. The obstetrical, socioeconomic, pre-, intra-, and immediate postpartum conditions were recorded by means of a 72-item questionnaire. The number of items that fulfilled predefined optimality criteria[11] was used as an obstetrical optimality score.[14]

Data analysis

We used the chi-square, Student's t, and the Mann-Whitney U test to compare groups. In order to investigate the effect of pre- and postnatal exposure to PCBs and dioxins on the neurological condition at 42 months, we performed a linear regression analysis. The dependent variables were the NOS and the fluency cluster score at 42 months of age. The distribution of the NOS at 42 months is skew to the left, and has a highest possible score of 56. To achieve normality, the NOS was transformed into: -ln(56.5 - NOS). The independent variables were the logarithmically transformed PCB and dioxin levels in plasma and breast milk, the study center, the type of feeding during early life, the duration of breast-feeding, the socioeconomic-, the obstetrical-, and the perinatal conditions, and the obstetrical optimality score. Results of statistical tests were considered to be significant if p £ 0.05.

6.4 RESULTS

The study group consisted of 418 mother-infant pairs, of which 209 were in the breast-feeding group and 209 in the formula-feeding group. In the last month of pregnancy, 415 maternal blood samples were obtained. At birth, 382 cord blood samples were taken. Due to the limited volumes of blood, in nine children the PCB 118 level in cord plasma was not measurable. In the sixth week after delivery, 195 breast milk samples were collected. The dioxin, planar-, mono-ortho-, and di-ortho PCB TEQ values were determined in 176, 194, 195, and 195 milk samples, respectively. At 42 months, 299 plasma samples were analyzed for PCBs. The children whose blood could not be obtained at this age did not significantly differ from those whose blood was sampled in terms of PCB levels in cord plasma and PCB and dioxin levels in breast milk.

The 42-month neurological condition was assessed in 394 (94%) out of the 418 children. Twelve (3%) children were considered to be `mildly abnormal'. One child was diagnosed as `abnormal' because it showed signs of diplegia. The remaining 42-month-olds (n=381; 97%) were classified as neurologically `normal'. The children classified as `mildly abnormal' or `abnormal' did not significantly differ from the toddlers that were diagnosed as `normal' with respect to the PCB 118, 138, 153 and 180 levels in maternal, cord, and 42-month plasma, and the PCB and dioxin TEQ values in breast milk. The median NOS at 42 months was equal to 52 (range: 30 - 56). Table 6.1 presents the characteristics of the study group.

In a linear regression analysis, neither the ΣPCB_{cord} nor the $\Sigma PCB_{maternal}$ nor the ΣPCB_{42mo} was found to be significantly related to the NOS at 42 months of age. No significant effect of the plasma levels of the individual PCB congeners no. 118, 138, 153, and 180 was found. The final model consisted of the study center and the obstetrical optimality score. After adjustment for the study center and the obstetrical optimality score, no significant effect of the levels of dioxins, planar-, mono-ortho-, di-ortho PCBs, and total PCB/dioxin TEQ in breast milk on the NOS was found.

Neither the ΣPCB_{cord} nor the $\Sigma PCB_{maternal}$ nor the ΣPCB_{42mo} nor the levels of dioxins, planar-, mono-ortho-, di-ortho PCBs, and total PCB/dioxin TEQ in breast milk was significantly related to the fluency cluster score at 42 months of age. Adjustments were made for the study center and the type of early feeding.

Table 6.1 Characteristics of the study group.

Variables*	Outcome
Neonatal neurological diagnosis *(n=394)*	
normal	370 (94%)
mildly abnormal	20 (5%)
abnormal	4 (1%)
Neurological diagnosis at 42 months *(n=394)*	
normal	381 (97%)
mildly abnormal	12 (3%)
abnormal	1 (<1%)
Neurological optimality score at 42 months *(n=394)* p5, p50, p75	43, 52, 55
Fluency cluster score at 42 months *(n=394)* mean ± SD	13.2 ± 2.1
ΣPCB_{cord} *(μg/l; n=352)* p5, p50, p95	0.2, 0.4, 0.9
$\Sigma PCB_{maternal}$ *(μg/l; n=394)* p5, p50, p95	1.0, 2.0, 3.8
PCB/dioxin levels in breast milk (ng TEQ/kg fat) p5, p50, p95	
Dioxins *(n=170)*	14.9, 28.8, 51.5
Planar PCBs *(n=186)*	6.8, 14.5, 31.9
Mono-ortho PCBs *(n=186)*	6.9, 14.2, 24.8
Di-ortho PCBs *(n=186)*	2.1, 4.2, 7.8
ΣPCB_{42mo} *(μg/l; n=298)* p5, p50, p95	0.1, 0.4, 1.9
Obstetrical optimality score *(n=394)* p5, p50, p95	58, 64, 69
Maternal age *(years; n=394)* mean ± SD	29 ± 4
Maternal weight *(kg; n=394)* mean ± SD	65 ± 10
Maternal education *(n=394)* high	174 (44%)
Sex *(n=394)* male	211 (54%)
Birth order *(n=394)* first born	192 (49%)
Birth weight *(kg; n=394)* mean ± SD	3.5 ± 0.5
Duration of breast-feeding *(weeks; n=200)* p5, p50, p95	8, 19, 56

*ΣPCB_{cord}=sum of the levels of PCB congeners nr. 118, 138, 153, and 180 in cord plasma;
$\Sigma PCB_{maternal}$=sum of the levels of PCB congeners nr. 118, 138, 153, and 180 in maternal plasma;
Planar PCBs=congeners nr. 77, 126, and 169;
Mono-ortho PCBs=congeners nr. 105, 118, and 156;
Di-ortho PCBs=congeners nr. 170 and 180;
ΣPCB_{42mo}=sum of the levels of PCB congeners nr. 118, 138, 153, and 180 in 42-month plasma.

6.5 DISCUSSION

No adverse effects of prenatal PCB exposure on the neurological condition were found at 42 months of age. Although negative effects have been shown at neonatal age and at 18 months. [5,6] we found no relationship between the prenatal PCB exposure and the neurological condition at 42 months of age. In addition, neither the lactational exposure nor the child's current body burden was related to the neurological optimality at 42 months of age.

Our results are in accordance with the findings by Rogan and Gladen, who in the United States (US) studied the effects of prenatal and lactational exposure to PCBs on cognitive development. They found that the deficits seen through 2 years of age [15,16] were no longer apparent at 3, 4, or 5 years. [17] On the other hand, also in the US, Jacobson and co-workers [18] found that higher levels of prenatal exposure predicted poorer cognitive functioning at four years of age. Both US-studies used the McCarthy Scales of Children's Abilities as the measure of effect. Although differences in analytical methods make the PCB values not directly comparable, the reported levels in the two groups were in the same range, as are the levels in the Netherlands. It should be kept in mind that cognitive tests, such as the McCarthy Scales, measure the child's abilities in a quantitative fashion, whereas the neurological examination is a qualitative measure of brain function.

In Taiwan, between 1978 and 1985, a mass poisoning of heat-degraded PCBs took place, exposing mothers and their offspring. In children who were subjected to heat-degraded PCBs during the prenatal period, cognitive development was delayed up to the age of seven years as compared to a control group.[19] But, the levels of prenatal exposure were substantially higher than the 'background' levels as have been found in the aforementioned US-studies and in the present study.

In the present cohort, a negative effect of prenatal PCB exposure on the neurological condition has been found at 18 months. However, no effect was found at 42 months of age. It is difficult to relate the functions of the brain at 42 months to those at other ages as they are generated by 'different brains'. Maturation of the brain is an ongoing process from conception to many years after birth. During this period, large morphological changes occur. These maturational changes are reflected in the child's functional development. This also implies that, from the fact that we found no negative effects of prenatal PCB exposure at 42 months of age, it can not be concluded that there will be no effect of exposure to these substances on the child's neurological condition at later ages. In fact, in a US-study it has been found that higher levels of prenatal exposure to PCBs were associated with lower full-scale and verbal IQ scores in eleven-year-old children. [20] Moreover, other aspects of human development might be affected. From animal experiments, there are indications that exposure to PCBs and dioxins affects fertility in a negative manner. [21,22] Human exposure to PCBs and dioxins has been shown to be related to immunological changes. [23]

In conclusion, adverse effects of prenatal PCB exposure on the neurological condition, as have been found at birth and at 18 months of age, could not be detected at 42 months of age. In addition, postnatal exposure to PCBs and dioxins was not found to be related to the neurological condition at 42 months of age.

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6.8 APPENDIX

Criteria for the 56 items of the neurological optimality score at 42 months.

Items	Criteria for optimality
Prehension	
1. mode of grasping	pincer grasp present in left and right hand
2. handpreference	preference for either left or right hand
3. posture arm/shoulder	normal, variable posture
4. yoke movements	absent
5. quality of arm/shoulder movements*	smooth
6. posture hands/fingers	normal, variable posture
7. adjustment handopening	good
8. associated movements (hindering)*	absent or, if present, they do not hinder
9. quality of hand mobility*	smooth
Sitting	
10. sitting (up)	can sit (up) without help
11. posture head/trunk/legs/feet/toes	normal, variable, and well-adapted posture
12. trunk rotation, spontaneous*	trunk rotation >45°
13. trunk rotation, elicited*	trunk rotation >45°
14. fluency of trunk movements*	smooth
15. lateral supporting reactions	quick, adequate reactions
16. acceleration/deceleration*	smooth
Standing	
17. standing up/free	can stand up without help with object in hands/ stands free
18. variability in standing up	various ways of standing up
19. posture head/arms/trunk/legs/feet/toes	normal, variable, age-adequate, and well-adapted
20. distance between feet	medium
21. balance without movements	no correction movements visible
22. balance with movements	no correction movements visible
23. trunk rotation, elicited*	trunk rotation >45°
24. fluency of trunk movements*	smooth
25. reaction to push against shoulders	good balance
26. dyskinesia*	no dyskinesia

Walking	
27. ability to walk and toddling*	able to walk without help, and no toddling in the walking pattern at any speed
28. fluency of trunk movements*	smooth
29. fluency of leg movements*	smooth
30. reciprocal arm swing*	present
31. posture head/arms/trunk/legs/feet/toes	normal, variable, age-adequate, and well-adapted posture
32. gait width	medium
33. plantigrade walk	present
34. balance during walking	good balance, no correction movements needed
35. abduction shoulders	no abduction of the shoulders
36. walking on tip-toe*	no or sometimes walking on tiptoe involuntarily
37. variability of speed	variable speed
38. manoeuvrability	changing direction in sharp turns
39. ability to avoid objects	avoids obstacles adequately without interrupting walking
Head	
40. eyes, position	symmetrical and centred position
41. eyes, movements	smooth, symmetrical movements
42. nystagmus (spont./direct.)	no nystagmoid movements
43. optokinetic nystagmus	symmetrical present both horizontally and vertically
44. pupils size and shape/reaction to light, (in)direct	round, medium sized pupils/immediate reaction
45. visual fields	apparently intact
46. vision	apparently intact
47. hearing	quick and adequate reaction to sounds
48. facial mobility	normal, alert, and symmetrical
49. drooling	absent
50. speech/language	normal, age-adequate speech- and language development
Manipulative examination	
51. resistance against passive movements	moderate resistance
52. active muscle power	adequate for age
53. range of movements	medium range
54. tendon reflexes	normal intensity
55. reflex thresholds	medium threshold
56. footsole response	no movements or plantar flexion of big toe

* Included in the fluency cluster score.
For descriptive details of the items: see Hempel 1993. [10]

CHAPTER 7

ATTENTION AND ACTIVITY IN 42-MONTH-OLD DUTCH CHILDREN WITH ENVIRONMENTAL EXPOSURE TO POLYCHLORINATED BIPHENYLS AND DIOXINS

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Submitted

7.1 ABSTRACT

Introduction: At 42-months of age, 172 children either breast-fed or formula-fed during infancy, underwent neurobehavioral evaluation in an ongoing follow-up study for risk assessment of environmental exposure to polychlorinated biphenyls (PCBs) and dioxins.

Methods: Attentional processes during free play behavior observation were measured in terms of exploration, high level play, low level play, interaction and non-play behavior. Sustained attention and reaction time was measured in 153 children with a computerized vigilance task.

Results: After adjustment for covariates, cord and maternal plasma PCB levels, both measures for in utero exposure, are significantly associated with less time for high level play. In addition, cord PCB levels are associated with longer periods and more swifts of attention during non-play behavior. Plasma PCB levels in 42-month-old children, are significantly related to slower mean reaction times and more signs of hyperactive behavior reported by parents and with less sustained attention in the breast-fed group. Prenatal and lactational exposure to dioxin-like PCBs and dioxins assessed from breast milk PCB and dioxin concentrations were not related to attention and activity at preschool age.

Conclusions: Attention and activity in preschool children might be impaired by prenatal as well as postnatal PCB exposure. Whether these small but significant adverse effects will attenuate or increase in later life e.g. school age remains to be determined in a further follow-up.

7.2 INTRODUCTION

Polychlorinated biphenyls (PCBs), dibenzo-para-dioxins (PCDDs), and dibenzo-furans (PCDFs) are lipophilic and persistent organic pollutants, which are readily absorbed and accumulate in especially fat tissue.[1,2] PCBs have been utilized extensively in industry as dielectric and heat-exchange fluids between 1930s and 1970s. Although the production was banned in the 1980s, their distribution in nature is still ubiquitous. Dioxins, the general term for PCDDs and PCDFs, follow the same environmental fate as PCBs and are formed as unwanted byproducts, mainly during combustion processes.[2] The human fetus is exposed to maternal PCBs and dioxins through placental transport[3,4] and newborns are additionally exposed during breast-feeding. [4,5]

Birth defects in human beings were reported in two incidents with maternal consumption of rice oil, contaminated with heat-degraded PCBs and PCDFs, in Japan (Yusho, 1968)[6] and Taiwan (Yu-Cheng, 1979).[7] Children born to exposed women showed adverse effects on health, growth and cognitive development. [8,9] Hyperactivity, stereotyping circling, and decreased motor coordination are described in PCB exposed mice.[10-12] Hypoactivity was reported in studies of pre- as well as postnatal PCB exposed rats. [13,14] In monkeys, PCB-induced hyperactivity changed into hypoactivity with aging. [15]

The presence of PCBs and dioxins in mother's milk in most industrialized countries led to public concern because of possible health hazard to infants. The present study was conducted as part of an European collaborative and the Dutch PCB/dioxin study. It was aimed to investigate effects of PCB and dioxin exposure on growth and development in healthy term infants from birth up to school age. We previously described that in utero exposure to PCBs is associated with lower birth weight and lower postnatal growth up to 3 months.[16] Poorer neurological condition at birth[17] and 18 months of age, [18] and lower psychomotor development at 3 months are associated with prenatal exposure.[19] Recently we reported that prenatal PCB exposure resulted in a 4-point deficit on the overall cognitive scale as well as on the sequential and simultaneous processing scales in 42-month-olds, measured with the Kaufman Assessment Battery for Children (K-ABC). [20] In the US Michigan "fish exposure cohort study", deficits in processing efficiency and memory tasks were reported in 4-year-olds [21,22] and lower full scale and verbal IQ scores at 11 years in relation with prenatal PCB exposure.[23] Four-year PCB body burden was associated with reduced activity level.[24]

Attention is considered to be the foundation of most cognitive and neuropsychological functions. [25] Although attentional disorders have been studied primarily in children with attention deficit disorder with hyperactivity (ADHD), attentional deficits are also known to occur in children with neurological, medical or other psychiatric problems, and with exposure to toxins, such as lead[26] and alcohol.[27] Since relative little is known about the neurotoxicity of PCBs and related compounds, it was decided to study this more carefully. A detailed neurobehavior assessment was designed at 42 months of age and related to perinatal PCB and dioxin exposure. Attentional processes and activity level were measured during an observation of free play behavior, a computerized vigilance task, and with a parent questionnaire.

7.3 METHODS

Subjects and design

From 1990-1992, 207 mother-infant pairs were recruited in Rotterdam and surroundings, a densely populated and highly industrialized area in the Western part of the Netherlands. All mother-infant pairs were Caucasian. Mothers were included if their pregnancy and delivery was completed without overt signs of serious illness or complications. Only infants 1st or 2nd, born at term and without perinatal complications were included in the study. To study the effects of pre- and postnatal exposure to PCBs and dioxins, 2 groups of women were included: a group of women who intended to breast-feed their child for at least 6 weeks and a group of women who intended to give formula to their newborn infant. The formula (Almiron M2, Nutricia NV, The Netherlands) given to the infants had no detectable concentrations of PCBs and dioxins. Children were examined at 2 weeks, 3, 7, 18 and 42 months of age for their growth and development. Details of the survey methodology and recruitment process have been previously published.[19, 28] The

study protocol was approved by the medical ethics committee of the University Hospital Rotterdam, the Netherlands and informed consent was given by participating parents.

Measures of exposure

Plasma samples were collected from mothers during the last month of pregnancy and were analyzed for four PCB congeners, International Union for Pure and Applied Chemistry (IUPAC) nos. 118, 138, 153, and 180. Directly after birth cord plasma samples were collected and also analyzed for these 4 PCB congeners. At 42 months of age, plasma samples were collected from the children and analyzed for the same four PCB congeners. Two weeks after birth breast milk samples were collected and analyzed for seventeen 2,3,7,8 substituted PCDD and PCDF congeners, and 6 dioxin-like PCBs, 3 planar PCB congeners (IUPAC nos 77, 126, and 169) and 3 mono-ortho PCBs (IUPAC nos. 105, 118, 156), and 20 other PCBs (IUPAC nos 28, 52, 66, 70, 99, 101, 128, 137, 138, 141, 151, 153, 170, 177, 180, 183, 187, 194, 195, and 202). To express the total toxic potency of dioxins and dioxin-like PCBs, the toxic equivalent factor (TEF) according to the latest WHO meeting in 1997 was used.[29] Methods of determination as well as levels of PCBs, dioxins and TEQ levels have been published elsewhere.[28]

Prenatal PCB exposure was defined as the sum of the four PCB congeners measured in cord plasma (ΣPCBcord) directly after birth and in maternal plasma (ΣPCBmaternal) during the last month of pregnancy. In the breast-fed group, prenatal exposure to dioxin-TEQs, dioxin-like PCB-TEQs and non-dioxin like PCBs (sum of 20 PCB congeners) was assessed from breast milk concentrations. Lactational exposure is calculated by multiplying breast milk concentrations of dioxin-TEQ, dioxin-like PCB-TEQ and the sum of 20 nondioxin-like PCB congeners with the number of weeks of breast-feeding. Current body exposure to PCBs was measured from the sum of four PCB congeners measured in 42-month-old plasma samples (ΣPCB42months). Current PCB body burden in children breast-fed during infancy is mainly predicted by the breast-feeding period,[5] and is a measure of lactational exposure. Therefore, we investigated the breast-fed group separately.

Neurobehavioral Assessment

At 42 months of age each child was evaluated for its development in 2 visits by one trained examiner (SP) who was unaware of PCB and dioxin exposure levels and feeding type received during infancy. During the first visit, at home, cognitive abilities were assessed with the Dutch version of the Kaufman Assessment Battery for Children (Dutch K-ABC)[30] and children were neurologically examined according to Touwen/Hempel.[31] Results from the Dutch K-ABC [20] and neurological condition have been reported elsewhere.[32] During the second visit, at the Children's Hospital, children underwent neurobehavioral testing. Attentional processes and activity level were measured at 42 months of age during an observation of free play behavior and a vigilance task. Inattention and activity was assessed from a parent questionnaire. Free play observation as used in this study has been studied and validated in normal

children, aged 2 to 6 years by Veenstra.[33] The vigilance task was adapted from Veenstra[33] and Jacobson et al.[22]

Free play observation

Each child was accompanied by one of their parents during the free play observation, and this session took place in a one-way screen observation room at the Children's Hospital. Behavior was recorded with two hidden video cameras, each in one corner of the observation room and controlled by the experimenter in the registration room. Each child was observed during a fifteen minute period. A timecode was added on each videotape for later scoring. A Fisher Price^{R} main street was placed in the observation room. The toys consists of a small town with shops, a bank, a fire house, and a post office (l x w x h = 50 cm x 20 cm x 10 cm). Three small cars (a taxi, a postman car and a fire engine) can drive on two streets on two different levels. Five small puppets can be placed on the cars, behind the counter of the bank, or in the seats of the ice cream store etc. Furthermore there are objects like a traffic light, plastic postcards, a pillar box, a stop signal which can be placed at certain locations. This toy elicits exploratory behavior, because of some unknown features like working with traffic lights, a man hole, a push knob which lifts the counter of a bank, a bell, letter boxes of the shops, the back of the streets etc. The main street was placed in the corner of the observation room near the chair of the parent. There were no other objects in the room, except for two cameras hidden in cupboards, each in one corner of the room, and a microphone which was suspended from the ceiling. The parent was instructed not to play with the child, to give only short answers to questions and to ask the child to resume playing when the child was no longer interested in the toys.

Each videotape was scored independently by an extensively trained examiner (SP). The behavior of each child was scored in number of episodes and total time in terms of the following three categories:

Play behavior

1. Exploration: Manipulation of the toys, with the intention of getting information about their functioning. Exploration was scored when the child clearly investigated the toys and did not show behavior which could be categorized as high level or low level play.
2. High level play: Manipulation of the toys aimed mainly at using the toys appropriately, for example driving with a car, etc. Combination with other toys and imaginary play was also considered as high level play.
3. Low level play: Manipulation of the toys, where the use of the toys was defined as not appropriate. In low level play the child never combined two or more toys in a play situation. Throwing, destructive or purposeless manipulation of the toys is considered as low level play.

Non-play behavior

1. Manipulation: Manipulation of any object in the room that was not part of the set of toys.
2. Visual scanning: Visual fixation on a specific object in the room, other than the toys, for example the cameras, the one-way mirror, windows, curtains etc.
3. Doing nothing: Purposeless sitting or standing in the room, no intention for action.

Interaction

Communication of the child with the adult in both verbal and visual way.

A special keyboard was used to score the behavioral categories, by pressing the key presenting that behavior until the behavior stopped. Together with the behavior code the start time and release time were read by a computer program connected to the video recorder. The total observation period was 15 minutes, divided into three 5-minute blocks.

Outcome variables during free play observation are total play-time defined as the sum of exploration time, high level play-time and low level play-time. Total number of play-episodes is defined as the sum of exploration episodes, high level play episodes and low level play episodes. Total nonplay-time is defined as the sum of time for manipulation, visual scanning and doing nothing. Total number of nonplay-episodes is defined as the sum of episodes for manipulation, visual scanning and doing nothing. Interaction time and number of interaction episodes is categorized as a separate outcome variable. The different play and nonplay behaviors were also examined individually.

Vigilance task

Children underwent a 12 minute vigilance task for presentation on a Personal Computer. Stimuli were projected on a color monitor situated in front of the child. A hard-plastic response tableau with a response button in the middle and two hand-shaped buttons on both sides, was placed between the monitor screen and the child. An Event Data Multiplexer (EDM-PC) recorded all manipulations of the three buttons on real time basis. The time between stimulus presentation and pushing a button (reaction time, RT) together with codes for responses were simultaneously written to a computer file.

The child was asked to keep both hands on the hand-shaped buttons. A criterion stimulus, the picture of a small white puppy dog, and two distracter stimuli, a duck and a butterfly, appeared for 2,500 milliseconds on the screen at interstimuli intervals varying from 4 to 10 seconds, so that the timing was unpredictable. The task was repeated in three identical 4-minute periods in a row with 30 stimuli (puppy dog, butterfly and duck) each appearing 10 times per 4-minute period. The experiment was explained to the child as a small story: A mother-dog lost all of her puppies. The child was instructed to "catch the puppy dog" and bring it back home to the mother-dog, by pushing the middle red button as soon as, and only when, the puppy dog appeared on the screen, and than return with both hands on the hand-shaped buttons. If the child

pushed the red button within this 2,500 milliseconds the puppy dog ran to the right side of the monitor, coded as a correct response. If the red button was not pushed within 2,500 milliseconds the dog disappeared from the screen and this was coded as an error of omission. The child was instructed not to push the button when a distracter stimulus, a duck or a butterfly appeared on the screen. If the child responded to the distracter stimuli this was coded as an error of commission. Prior to the real experiment a 24-stimuli trial run was administered to make sure the child understood the directions and had encoded the puppy dog as correct response and the two distracter stimuli as wrong responses.

Outcome variables from the vigilance task are the reaction times of the criterion stimulus, the mean RT of the correct responses, the number of omission errors and the number of commission errors. The mean sequence number of omission errors was calculated as the average of those sequence numbers where an omission error was made in the row of 1 to 30. A lower mean sequence number of omission error is interpreted as less attentive to the criterion stimulus because more errors of omission are made in the beginning of the task. For example if a child makes omission errors at number 8, 10, 15, 18 and 24, the mean sequence number is (8+10+15+18+24)/5=15. Within each child a slope was calculated from a regression analysis of reaction time of the correct response on the sequence number (1,2,3,.....30) of this correct response, in course of the task. The slope is considered a summary measure for a child's sustained attention. A positive slope means that reaction times are increasing in course of time, which is interpreted as less sustained attention of a child. A negative slope means that reaction times are decreasing in course of time and is interpreted as better sustained attention in course of time, thus the ability of a child to react more efficient to a stimulus as the task continues.

Parent questionnaire

Parents were asked to fill out the Groninger Behavior Observation Scale (GBO; parent version)[34] which consists of 15 items covering behavior regarding activity, inattention, impulsivity, rapidly changing task orientation, and talkativeness. Each of the 15 items had a 4-point scale, ranging from applicable-1 to very applicable-4. This questionnaire was validated in normal, hyperactive and neurologically abnormal children in the age range of 6 to 10 years[34] and adapted for 4-year olds as a screening instrument of hyperactive behavior.[33] Factor analysis showed that two scales could be formed, activity and attention respectively.[34] A higher score was interpreted as more activity and less attention, respectively.

Covariate measures

Variables likely to confound the relation between exposure and child behavior or development, included socio-economic background, obstetrical and neonatal history, maternal age, parents' education level, birth order, gender, fetal exposure to alcohol and cigarette smoking, type of feeding and the duration of breast-feeding during infancy, the child's home environment assessed by the Home Observation for Measurement of the Environment (HOME)[35], and the verbal IQ of the

parent mostly with the child, usually the mother. The parental verbal IQ was measured with two subtests; Information and Vocabulary, from the Dutch version of the Wechsler Adult Intelligence Scale (WAIS).[36]

Statistical analysis

To compare groups for a single variable we used the chi-square test for categorical nominal variables, Student's t-test for variables with a Gaussian-shaped distribution, and the Mann-Whitney test for categorical ordinal variables, or variables with skewed distributions. Paired t-tests or Wilcoxon matched paired tests were used to study differences within groups of paired observations, depending on whether the within-subject difference was Gaussian shaped or non-Gaussian shaped. PCB concentrations measured in maternal, cord and 42-month-old plasma samples were skewed to the right and transformed by natural logarithm.

If outcome variables showed Gaussian shaped and homoscedastic residuals, or did so after appropriate transformation, multiple linear regression analyses were performed, yielding regression coefficients and standard errors (SE). Multiple logistic regression analysis were performed when outcome variables had to be transformed into binary variables due to their extreme skewness, yielding odds ratios with 95 % confidence intervals (OR; 95%-CI). The effect of prenatal, lactational and current exposure variables were each studied in separate regression analyses. Results are considered significant at p-values ≤ 0.05.

The following skewed outcome variables were transformed to achieve symmetric and homoscedastic residuals. Play-time was transformed by -ln(900-play-time), with 900 seconds (=15 min) as maximum. The highest value for play-time recorded was 899. Nonplay-time and nonplay-episodes were both transformed by natural logarithm e.g. ln(nonplay-time) and ln(nonplay-episodes). Due to extreme skewness of low level play, visual scanning , doing nothing and interaction, these variables were transformed into binary variables, 0 = no period vs 1 = one or more periods of the behavior in question and analyzed in logistic regression analyses. For the vigilance task, errors of commission was extremely skewed to the right and therefore transformed into a binary variable, 0 = no commission errors vs 1 = one or more commission errors. The majority of the children scored 0 for the abovementioned binary variables.

Three outcome variables of the vigilance task, the slope, the mean reaction time and the mean sequence number of omission errors were analyzed with a weighted least squares method of multiple linear regression analysis. All variables in the regression model, independent and dependent including the constant variable, were multiplied with the square root of number of observations within a child used to calculate the child's outcome variable. This takes into account of the between-subject heterogeneity in precision of the calculated outcome variable, and is performed to increase the efficiency of the analyses.

All regression analyses were adjusted for the same set of control variables. The control variables used in the final model were maternal age at birth, birth order, gender, feeding type during infancy (formula-fed or breast-fed), HOME score, maternal education (two

levels; low/middle vs higher educated), smoking and alcohol use during pregnancy (0=no vs 1= yes). Breast-feeding period was an additional covariable in the analyses of lactational PCB and dioxin exposure and current PCB exposure.

7.4 RESULTS

The original study population consists of 207 mother-child pairs. At 42 months of age 193 children (93%) were re-examined and 14 children (7%) were lost to follow-up, due to lack of interest (n=10) and inability to cooperate (n=4) such as illness and emigration. Subjects retained at the 42-months follow-up (n=193) are not different from those not retained in the sample (n=14) in terms of plasma PCB and breast milk PCB and dioxin concentrations. Hundred and seventy two children visited the Children's Hospital for their neurobehavioral assessment. Free play behavior was analyzed for 170 children, for 2 children video recording failed. Data for the vigilance task were available for 153 children. From 19 children data could not be collected because of equipment failure (n=11) and failure to cooperate (n=8) during the final testing. Children subjected to a free play observation (n=170) and those eligible for the vigilance task (n=153) were not different from those not tested with respect to exposure variables and covariables as listed in Table 7.1.

In Table 7.1 general characteristics and exposure variables are presented. The ΣPCB could be calculated in 206 maternal, 182 cord and 173 child's plasma samples. In the breast-fed group the difference in median ΣPCB concentration at 42 months of age is a factor 4 when compared with the formula-fed group. In human milk, representative dioxin-TEQ, PCB-TEQ as sum of planar and mono-ortho PCB-TEQs, and sum of non dioxin-like PCBs were calculated for 82, 91 and 99 milk samples, respectively. The remaining plasma and human milk samples were missing or not analyzed due to laboratory and organizational failure.

In Table 7.2, means, standard deviations and range for outcome variables of the free play behavior observation are given. The maximum registered period for play behavior was 899 seconds. The total time for play was significantly longer in the first 5 minutes, when compared with the second and third 5 minutes of play observation (272 s vs 260 s and 250 s, $p<0.05$). Consequently, there was a longer mean time for non play behavior in the third 5 minute block, when compared with the first 5 minutes (18 s vs 9 s, $p<0.05$). The same was concluded for the number of play episodes (14 vs 6, $p<0.01$) and non play episodes (10 vs 8, $p<0.01$).

Table 7.1 Characteristics and exposure levels of the Dutch cohort from birth until 42 months of age

Characteristic	N(%)/ mean ± SD/ median (range)
Subjects at birth	207
Feeding type during infancy:	
Number breast-fed	105 (51%)
Breast feeding period (weeks)	16 (6-72)
Gender,	
Male	109 (53%)
Parity, Firstborn	102 (49%)
Maternal age at birth (yr)	29 ± 4
Maternal education[a]	
low	40 (19%)
middle	82 (40%)
high	85 (41%)
Smoking during pregnancy, yes	48 (23%)
Alcohol use during pregnancy, yes	38 (17%)
Subjects at 42 months	193 (93%)
Number breast-fed in infancy	100 (52%)
Age (months)	42 (41-47)
Verbal IQ parent	122 ±16
HOME score	43 ±5
Exposure levels	
Measured in plasma	
-ΣPCB[b] maternal (µg/L), n=206	2.04 (0.59-7.35)
-ΣPCB cord (µg/L), n=182	0.40 (0.08-2.08)
-ΣPCB at 42 months (µg/L), n=173	0.35 (0.08-5.90)
Formula-fed group, n=82	0.21 (0.08-0.46)
Breast-fed group, n=91	0.75 (0.23-5.90)
Measured in breast milk (breast-fed group)	
- PCB-TEQ (pg/g fat), n=91	28 (11-77)
- Dioxin-TEQ(pg/g milk fat), n=82	36 (10-87)
- Nondioxin-like PCBs (ng/g fat), n=99	544(234-1760)

a: Low ;Primary school finished and secondary school not finished. Medium; secondary school finished High; high school finished/professional and university training b: sum of PCBs IUPAC nos 118, 138, 153 and 180 measured in maternal, cord and 42-month-old plasma samples and breast milk samples PCB-TEQ = toxic equivalents of planar PCBs; IUPAC nos 77, 126, 169 and mono-ortho PCBs; nos. 105, 118, 156 and dioxin-TEQ: TEQs of seventeen substituted 2,3,7,8 PCDDs and PCDFs measured in breast milk samples. Nondioxin-like PCBs: sum of 20 non dioxin-like PCB congeners measured in breast milk. Values are numbers (percentages), means ± standard deviation and median (range)

Table 7.2 Free play observation (n=170)

Variable	Mean ±SD	Minimum	Maximum
Play behavior			
Number of episodes			
Play-episodes	28 ±10	0	53
-Exploration	10±4	0	25
-Low level play	4±5	0	23
-High level play	14±5	0	33
Period of time (seconds)			
Play-time	783±137	0	899
-Exploration	133±67	0	435
-Low level play	55±88	0	430
-High level play	595±177	0	849
Nonplay behavior			
Number of episodes			
Nonplay episodes	25±17	1	83
-Manipulation	21±15	1	77
-Visual scanning	3±3	0	17
-Doing nothing	1±1	0	10
Period of time (seconds)			
Nonplay-time	44±44	1	241
-Manipulation	24±23	1	144
-Visual scanning	15±19	0	95
-Doing nothing	5±21	0	218
Interaction			
Number of episodes	3±5	0	21
Period of time	26±56	0	413

In Table 7.3, descriptive data are presented for outcome variables of the vigilance task. Most variables approximated a normal distribution, however reaction times (RT) were skewed due to the censored data, the highest possible RT for a stimulus was interrupted at 2,501 milliseconds. The mean RT was significantly longer in the third 4-minute block when compared with the first and second 4-minute blocks (mean RT is 1.89 s vs 1.81 s and 1.72 s, $p<0.01$). More errors of omissions were made in the second and third block when compared with the first block (mean error of omission is 3.6 and 4.3 vs 2.8, $p<0.01$). The mean number of commission errors were not different in the three 4-minute blocks.

Table 7.3 Vigilance computer Task and the Groninger Behavior Observation Scale

Variable	Mean ±SD	Minimum	Maximum
Vigilance task (n=154)			
Mean reaction time in seconds only correct responses	1.44±0.19	0.854	2.010
Slope	-0.023±2.58	-17.03	12.35
Mean sequence number of omission errors in the row of 1-30	16.9±2.9	9	26.3
Number of omission errors	10.7±5.8	0	27
Number of commission errors	6.8±9.1	0	49
Parent questionnaire, n=192			
GBO[a] parent (15-items)	30±7	17	52
-Activity scale (5-items)	11±3	5	18
-Inattention scale (6-items)	12±3	6	22

a: Adapted version of the Groninger Behavior Observation Scale (GBO).

In Table 7.4 results of multiple regression analyses are presented. Prenatal PCB exposure measured from lnΣPCBcord and lnΣPCBmaternal were both negatively associated with the number of high level play episodes and the duration of high level play (all p-values < 0.05). LnΣPCBcord was significantly associated with more nonplay-episodes and longer periods for nonplay-behavior. This association was not significant when related to lnΣPCBmaternal. All regression analyses were adjusted for the same set of covariables, maternal age, maternal education, gender, smoking and alcohol use during pregnancy, birth order, HOME score and type of feeding. Current PCB exposure was not related to play behavior outcome variables (results not presented).

Table 7.4 *Results from multiple linear regression analysis; effects of prenatal and current PCB exposure on free play behavior at 42 months of age*

Outcome	Prenatal PCB exposure			
	LnΣPCBmaternal Regression Coefficient (SE)	p-value	LnΣPCBcord Regression Coefficient (SE)	p-value
	n=169	n=147		
Play behavior				
Number of episodes				
Play-episodes	-1.69(1.93)	0.39	-0.88(1.54)	0.57
High level play	-2.50(0.95)	0.009	-1.69(0.73)	0.02
Period of time (seconds)				
-ln(900-total play-time)	-0.09(0.27)	0.74	-0.27(0.21)	0.21
High level play	-72.5(36.7)	0.05	-79.3(28.3)	0.006
Nonplay behavior				
Number of episodes				
ln(nonplay-episodes)	0.06(0.17)	0.73	0.26 (0.13)	0.05
Period of time (seconds)				
ln(nonplay-time)	0.06(0.24)	0.84	0.38(0.18)	0.04

In the final model of linear and logistic regression analyses the following covariates were included; maternal education (low/middle vs high level), maternal age, HOME score, Parity (first vs second), Sex(boys vs girls), feeding type (FF vs BF), alcohol and smoking during pregnancy (no vs yes) next to the exposure variable. ΣPCB=sum of PCBs IUPAC nos 118, 138, 153 and 180 measured in maternal and cord plasma samples.

\In Table 7.5 results from linear regression analyses of the vigilance task are presented. After adjustment was done for covariables, LnΣPCBcord is associated with a lower mean sequence number of omission errors, which can interpreted as more errors of omissions in the beginning rather than the end of the vigilance task. This effect was not found in relation to lnΣPCBmaternal. Current PCB body burden, measured from lnΣPCB42-months, is associated with a longer mean RT ($p<0.05$). In the breast-fed group, lnΣPCB42-months is also related to a longer mean RT ($p<0.05$) as well as a higher slope ($p<0.05$). A higher slope can be interpreted as less sustained attention. Results from the GBO-parent questionnaire showed that after adjustment was done for covariates, current PCB body burden was associated with a higher score on the whole scale, and the activity scale ($p \leq 0.05$, Table 7.5). Higher activity level reported by parents was also related with current PCB exposure in the breast-fed group, however the effect was not statistical significant ($p=0.06$, Table 7.5).

Table 7.5 Results from multiple linear regression analysis. Effects of prenatal and current PCB exposure on the vigilance RT task

Outcome	Prenatal PCB exposure		Current PCB body burden			
	Whole group LnΣPCBcord Regression Coefficient (SE)	p-value	Whole group LnΣPCB42months Regression Coefficient (SE)	p-value	Breast-fed group LnΣPCB42months Regression Coefficient (SE)	p-value
	n=132		n=141		n=79	
Vigilance RT task						
MeanRT	-0.02(0.03)	0.66	0.07(0.03)	0.03	0.09(0.04)	0.04
Slope	+0.20(0.36)	0.57	0.47(0.33)	0.15	0.91(0.40)	0.03
Mean sequence number of commission error	-1.11(0.45)	0.02	-0.03(0.40)	0.94	0.88(0.61)	0.17
	n=169		n=172		n=91	
Parent questionnaire						
GBO parent (whole scale)	-0.62(1.06)	0.56	2.02(1.0)	0.05	2.55(1.52)	0.09
-Activity subscale	-0.16(0.46)	0.73	0.94(0.43)	0.03	1.20(0.62)	0.06
-Inattention subscale	-0.05(0.48)	0.92	0.62(0.46)	0.18	0.99(0.76)	0.19

In the final model of all regression analyses the following covariates were included; maternal education (low/middle vs high level), maternal age, HOME score, Parity (first vs second), Sex(boys vs girls), feeding type (FF vs BF or breast-feeding period), alcohol and smoking during pregnancy (no vs yes) next to the exposure variable ΣPCB=sum of PCBs IUPAC nos 118, 138, 153 and 180 measured in maternal, cord and 42-month-old plasma samples

Prenatal exposure to planar and mono-ortho PCB-TEQs, as well as dioxin-TEQs measured in breast milk were not related to outcome measures of free play behavior, the vigilance task, nor were they associated with the GBO parent questionnaire. The same was concluded for lactational exposure estimated from PCB-TEQ and dioxin-TEQ levels in breast milk multiplied by the number of breast-feeding weeks (results not presented).

Spearman rank correlations (Rs) were calculated between 42-month overall cognitive score of the K-ABC and outcome variables measured during free play and the vigilance task. There was a negative relationship between cognitive score and low level play-time (Rs = -0.26, $p<0.05$), and a positive relationship with high level play-time (Rs = 0.19, $p<0.05$). Reaction time and number of omission errors were negatively correlated with the K-ABC (Rs = -0.21 and -0.21, respectively, $p<0.05$). The cognitive score on the K-ABC was negatively correlated with the total score on the GBO parent questionnaire, and with the attention scale (Rs = -0.21 and Rs = -0.27 respectively, $p<0.05$), but not with the activity scale of the GBO (Rs = -0.12, $p<0.10$).

7.5 DISCUSSION

In utero exposure to PCBs is related to shorter periods and less time spent for high level play, suggesting that the quality of play behavior is affected. Prenatal PCB exposure is also related to longer periods and more episodes of nonplay behavior. This can be interpreted as a longer period of inattention to the toys and more shifts in nonplay behavior, such as visual scanning, doing nothing, and manipulation other than play objects. Current PCB body burden is associated with slower mean reaction times (RT) and more signs of hyperactive behavior. Moreover, in children breast-fed during infancy current PCB exposure is related to less sustained attention.

This is the first time that attentional processes in preschool children are measured during free play observation, and related to perinatal and current exposure to PCBs and dioxins. Measurement of attention during free play has been validated in normal children, aged 2 to 6 years and appeared to be a fruitful method for investigating developmental differences in attentional processes in preschool children.[33] The ability to focus and sustain attention appropriately to objects and events, and to remain task oriented are considered to be important in many kinds of learning and performance.[37]

In order to detect small decrements in CNS function in relation to background exposure to PCBs and dioxins, particularly in terms of attention, reaction time and activity, we conducted a computer-operated vigilance task especially designed for preschool children.[33] During this experiment different types of attentional behavior were assessed: attentional errors (omission and commission errors), reaction time (the speed of button pressing) and sustained attention during the whole task. In principle, fast RTs and a low error rate requires the focusing of attention and maintaining such performance over time requires sustained attention. Although studies with RT tasks in preschoolers are scarce, a few studies have demonstrated that RT studies can be used successfully in young children[27] and deterioration of performance over trials found in preschool children is comparable with results reported for older children and adults.[38]

Jacobson et al described that prenatal PCB exposure, estimated from maternal milk PCB levels, is associated with slower reaction times on a visual discrimination task and cord PCB levels are related to more errors on a Sternberg memory task. They did not report any adverse effects of PCB exposure on sustained attention measured with a vigilance task.[22] We report that cord PCB levels are associated with more omission errors made in the beginning of the vigilance task, which can be interpreted as less focussed attention. In addition, we report a slower mean RT and less sustained attention in relation to the 42-month PCB body burden, which is predominantly predicted by lactational transfer of maternal PCBs.[5] Our results are difficult to compare with the results from the Michigan study, due to differences in methods of PCB determination. It is unknown if the PCB levels found in the Michigan study are comparable to the levels found in the Netherlands. Furthermore, preschool children in general are difficult to test when strict rules have to be held, depending on the type of tasks they are subjected to. This is especially difficult for the vigilance task, which at the

end is rather dull. Moreover, the attentional capacity in especially young children is more limited than for older children and adults that changes rapidly during childhood.[39] These rapid changes in information processing during the preschool years, might explain the differences in findings with respect to attentional processes in the Michigan and the Dutch cohort.

We report that current PCB exposure in 42-month-old children is associated with more signs of hyperactive behavior assessed from parent GBO reports, with slower mean RTs and less sustained attention on the vigilance task. In children with attention deficit and hyperactivity disorder (ADHD), performance on sustained attention tasks gives more difficulties and longer reaction times are measured, when compared with controls.[33,40,41] Moreover, poor vigilance performance is related to childhood hyperactivity.[42] In the Yu-Cheng cohort children born in the exposed group showed more hyperactive behavior than their matched controls.[43] Jacobson et al.[24] described that 4-year old children had a reduced activity reported on a composite measure of parent and examiner ratings, when related to their current PCB body burden. Both hypo- and hyperactivity were described in PCB-exposed monkeys[15] and rats.[14] Recently it was reported that hyperactive behavior was related to lactational exposure to PCB IUPAC nos. 153, and 126 in male rats.[44]

In our study current PCB exposure was associated with more signs of hyperactive behavior. Exposure to PCBs and dioxins in starts in utero, and in human studies it is difficult to separate prenatal from postnatal exposure in breast-fed infants. The current PCB levels in breast-fed children are significantly related with maternal PCB levels in plasma or breast milk[5], but the major predictor is breast-feeding period which accounts for the majority of PCB transfer postnatally. Therefore, it is difficult to conclude from our study whether the effects described with current PCB exposure are an effect of lactational PCB exposure only. Only in animal studies the separate effects of prenatal and postnatal exposure can be studied. In the study by Lilienthal and Winneke in which rat pups were cross-fostered, pre- plus postnatal exposure produced greatest degree of behavioral impairment, followed by in utero exposure only.[45]

Using free play observation as a tool for neurobehavioral assessment in young children has several advantages. Preschoolers in general are difficult in testing procedures. Due to the development of attachment and exploration which is characteristic for the toddler age, they need more time to get used to unknown situations and persons. They are not very task oriented and tend to be uncooperative. Since children are observed in a semi-natural environment with no restrictions like demands of an experimental situation, free play observation is easy to administer in toddlers. Disadvantages are that a free play observation is loosely structured with different types of behaviors displayed which are hard to define. The scoring of behavior categories is time consuming which makes this method less useful for studying a large study population, when compared with conventional standardized developmental tests. However, when the testing and scoring is performed appropriately, results provide us with information on which processes might be affected than could be assessed from standardized developmental tests only.

In conclusion, prenatal exposure to background PCB levels is associated with adverse effects on attentional processes in preschool children measured during free play. Moreover, 42-month PCB body burden is associated with slower mean reaction times, less sustained attention and more signs of hyperactive behavior. The here reported differences in attention and activity level in preschool children are noteworthy, since the children are healthy and from enriched home environments. Whether these adverse effects will attenuate or increase during school age remains to be determined in a further follow-up.

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CHAPTER 8

PROBLEM BEHAVIOR IN DUTCH PRESCHOOL CHILDREN IN RELATION TO BACKGROUND POLYCHLORINATED BIPHENYL AND DIOXIN EXPOSURE.

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8.1 ABSTRACT

Background: Polychlorinated Biphenyls (PCBs) may produce neurotoxic effects, including behavioral alterations. In animal and a few human studies, effects of perinatal PCB exposure have been reported mainly on activity level. Problem behavior in young children exposed to background levels of PCBs and dioxins was not investigated before. Therefore we investigated the effects of perinatal PCB and dioxin exposure on behavior in Dutch preschoolers.

Methods: In an ongoing neurodevelopmental follow-up study of 207 healthy term born babies, 193 were re-examined at 42 months of age. In addition to their neurodevelopmental outcome, problem behavior was assessed with the Dutch Child Behavior Checklist (CBCL). Mothers, fathers and day care teachers were asked to complete a CBCL. Prenatal PCB exposure was estimated from the sum of PCBs 118, 138, 153 and 180 (ΣPCB) in maternal plasma and cord plasma. Prenatal exposure to the sum of PCB and dioxin toxic equivalents (ΣTEQ) were estimated from breast milk concentrations. Current PCB body burden was estimated from ΣPCB plasma samples at 42 months of age.

Results: Logistic regression analyses showed that, after adjustment was done for covariates, in utero exposure plasma ΣPCB and breast milk ΣTEQ levels are both associated with a higher prevalence of withdrawn/depressed behavior reported by day care teachers. In addition, a lower prevalence of overactive behavior in association with 42-month PCB body burden is reported by teachers. Moreover, teacher report on child behavior is influenced by the child's cognitive development.

Conclusion: This is the first time that withdrawn/depressed behavior is reported in relation to prenatal PCB and dioxin exposure. It is hypothesized that withdrawn/depressed behavior might be secondary to poorer cognitive functioning. These results are in accordance with our recently reported poorer cognitive abilities in prenatal PCB exposed 42-month-old children. Although these children are all normal and healthy, early identification of cognitive deficits and consequently more deviant behavior among preschoolers may help to prevent later learning problems.

8.2 INTRODUCTION

Polychlorinated Biphenyls (PCBs) were used widely in industry between the 1930s and 1970s,[1] and have since become ubiquitous and persistent organic pollutants, because of their resistance to chemical and biological degradation as well as their lipophilic nature.[2] Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), summarized as dioxins, are also wide-spread contaminants formed as undesirable byproducts during a variety of chemical and thermal processes.[3] PCBs and dioxins frequently occur simultaneously in the environment, accumulate especially in fat tissue.[2] Since PCBs and dioxins can cross the placenta,[4,5] and much higher levels are

transferred during lactation to the nursing infant,[6,7] the developing child during this vulnerable period of life is especially at risk.

A broad spectrum of toxic and biochemical effects in different organs[8] have been reported for individual and complex mixtures of PCBs, PCDDs and PCDFs in animal studies [8-10], but little is known about their neurotoxicity.[11] Teratogenic effects of PCBs and related compounds in humans were documented first in Japan in 1968, Yusho incident[12] and later in Taiwan in 1979, Yu-Cheng incident.[13] In both instances PCBs and their heat-degraded by-products, PCDFs and polychlorinated quarterphenyls (PCQs), leaked into rice bran oil. Babies born to women who had consumed this rice oil showed a high infant mortality rate,[13] and surviving children had ectodermal defects, were growth retarded, showed developmental delay and poorer cognitive development up to 7 years of age.[14] The Japanese Yusho children were described as hypotonic, apathetic, and dull with IQs in the mental retardation range.[12] Mildly disordered behavior and increased activity levels in relation to in utero exposure of heat-degraded PCBs were reported in Taiwanese children.[15]

The effects on neurodevelopmental outcome of chronic exposure to PCBs from environmental sources has been examined by three prospective follow-up studies, one in Michigan and a second in North Carolina, USA, started in the early 1980s. In 1989 our cohort study was initiated by the Dutch government and in 1993 it became part of an EC-funded multi center cohort study. Results from our Dutch and the Michigan study showed that children in the higher range of prenatal 'background' PCB exposure have lower birth weights and lower postnatal growth in infancy,[16, 17] lower cognitive functioning during the preschool period.[18,19] Lower IQ scores at 11 years was reported in the Michigan cohort.[20] Although several behavioral effects, mainly based on hypo- or hyperactivity, have been described in animal[21] and in the Yu-Cheng poisoning study,[15] in background exposure studies behavioral effects are not yet described. In this paper we present results on child behavior, measured by parent and day care teacher ratings of the Child Behavior Checklist[22,23] in relation to perinatal PCB and dioxin exposure in 42-month-old children. This study is part of the above mentioned Dutch cohort, an ongoing prospective follow-up study of possible adverse effects of environmental PCB and dioxin exposure on growth and development.

8.3 METHODS

Subjects and study design

The study group consists of 207 mother-infant pairs recruited from 1990 to 1992 in Rotterdam and surroundings, a densely populated and highly industrialized area in the West of the Netherlands. All included mother-infant pairs were Caucasian. Pregnancy and delivery had to be completed without overt signs of serious illness or complications. Only infants 1st or 2nd, born at term and without perinatal complications were included in the study. To be able to study the effects of pre- and postnatal exposure to PCBs and dioxins, two groups of women were included: a group

of women who intended to breast-feed their child for at least 6 weeks and a group of women who intended to give formula to their newborn infant. The formula (Almiron M2, Nutricia NV, The Netherlands) given to the infants for at least 6 months contained no detectable concentrations of PCBs and dioxins. Details of the recruitment process, study design and exposure levels have been previously published.[7, 24] The study protocol was approved by the medical ethics committee of the University Hospital Rotterdam, the Netherlands and informed consent was given by participating parents.

Assessment of child behavior

At 42 months of age children were examined twice, first at home for their neurological and cognitive development and the second time at the Children's Hospital for their neurobehavioural assessment. During the first visit participating parents were asked to complete the Child Behavior Checklist (CBCL), a questionnaire especially designed to measure problem behavior in preschool children. The Dutch version of the CBCL for 2 and 3 year olds [23] contains 99 items for problem behavior, scored 0 (not true), 1 (somewhat true), or 2 (very true or often true). The CBCL was validated in three Dutch samples of children referred to mental health services, from the general population, and from a twin study. Six scales were derived from factor analyses and labeled oppositional, aggressive, and overactive, which constituted a broadband Externalizing grouping; withdrawn/depressed and anxious, which constituted a broadband Internalizing grouping[23]. In addition, sleep and somatic problems were also obtained from the CBCL. A Total Problem Score (TPS) is obtained by summing up all 99 items.

Both the father and the mother were asked to complete the CBCL separately and to give their own opinion about their child behavior and emotional problems. Permission was asked to obtain information regarding the behavior of their child from the nursery school teacher as an additional informant. Most of the children attended a day care center or nursery school from at least the age of 2.5 years, which made it possible for the teachers to get acquainted with the child for a longer period in order to complete the CBCL reliably. All parents agreed and nursery schools or day care centers were approached by mail. Teachers were instructed to mail the completed CBCL forms to the Children's Hospital. Given that the CBCL initially was developed for parents, and day care teachers may not be expected to have sufficient information available regarding Somatic and Sleep problems, these scales were excluded from the list. Internalizing and externalizing scores could be calculated but no TPS score comparable to the parent's TPS could be calculated from the teacher CBCL questionnaires.

Assessment of exposure variables

Plasma samples were collected from mothers during the last month of pregnancy and were analyzed for the 4 predominant PCB congeners, International Union for Pure and Applied Chemistry (IUPAC) nos. 118, 138, 153, and 180. Directly after birth, cord plasma samples were collected and also analyzed for these 4 PCB congeners. At 42 months of age, plasma samples were collected from the children and analyzed for the same four PCB congeners.

Two weeks after birth, breast milk samples were collected and analyzed for seventeen 2,3,7,8 substituted PCDD and PCDF congeners, three planar PCBs, IUPAC nos. 77, 126,169 and three mono-ortho PCBs, IUPAC nos. 105, 118 and 156, and 20 other nondioxin-like PCBs, including IUPAC nos. 138, 153, and 180. To express the total toxic potency of dioxins and dioxin-like PCBs, the toxic equivalent factors (TEF) according to the latest WHO meeting in 1997 was used[25] to calculate the toxic equivalents (TEQs). Methods of determination as well as levels of PCBs, dioxins and TEQ levels have been published elsewhere.[7, 24]

Prenatal PCB exposure is defined as the sum of the four PCB congeners measured in cord plasma (ΣPCBcord) directly after birth and in maternal plasma (ΣPCBmaternal) during the last month of pregnancy. Prenatal exposure to total-TEQ, the sum of dioxin-TEQs, planar PCB-TEQ and mono-ortho PCB-TEQs is assessed from breast milk concentrations. Lactational exposure to PCBs and dioxins is calculated by multiplying breast milk PCB and dioxin TEQ concentration with the number of weeks of breast-feeding. Current PCB body burden, which is mainly predicted by lactational exposure to PCBs,[7] is measured from the sum of four PCB congeners measured in child plasma at 42 months of age (ΣPCB42months).

Other variables

Variables which can influence the relation between exposure and child behavior or child development were assessed. These variables included socio-economic background, obstetrical and neonatal history, maternal age, parents' education level, birth order, gender, fetal exposure to alcohol and cigarette smoking, type of feeding and the duration of breast-feeding during infancy. Attendance to a nursery school or day care center (yes/no), life events such as divorce, family illness, or other family problems were recorded. The child's home environment and intellectual stimulation was assessed by the Home Observation for Measurement of the Environment (HOME)[26] as well as the verbal IQ of the primary care giver, usually the mother. The parental verbal IQ was measured with two subtests; Information and Vocabulary, from the Dutch version of the Wechsler Adult Intelligence Scale (WAIS).[27]

Statistical analysis

To compare groups for a single variable we used the chi-square test for categorical nominal variables, the Student's t-test for variables with an approximately normal distribution, and the Mann-Whitney test for categorical ordinal variables or variables with skewed distributions. Paired t-tests or Wilcoxon matched paired tests were used to study differences between the CBCL scores from three informants (mothers, fathers and teachers). PCB concentrations measured in maternal, cord and child's plasma samples were skewed to the right and therefore transformed by natural logarithm.

Given that most CBCL scales except for total problem scores (TPS) were skewed due to discrete type of data, and transformation did not result in an Gaussian-shaped dependent variable with homoscedastic residuals from *linear* regression analyses, it was decided to transform all scale scores in a categorical way. The cutoff value was chosen at the 75th

percentile (P75), in order to have a sufficient number of subjects in each group of observations for the analyses of exposure variables on problem behavior. Behavior categories were dichotomized to zero when scale scores were below the P75 (= 0) and to one if the scores exceeded the P75 (= 1). The prevalence of deviant behavior is presented as the percentage of children exceeding the P75 for one or more CBCL scale scores, e.g. scores above the P75 cut off point. The effect of PCB and dioxin exposure was studied in multiple *logistic* regression analyses, with CBCL scales as binary outcome variables, yielding odds ratios (OR) with 95 % confidence intervals (95%-CI). Prenatal, lactational and current exposure variables were each studied in separate logistic regression analyses.

Based on literature and clinical knowledge the following covariables were entered in the regression analyses to adjust for other environmental influences on child behavior; maternal age (years), maternal education (low/middle vs higher educated), HOME score, gender (boy vs girl), parity (first vs second born), smoking during pregnancy (no vs yes), alcohol use during pregnancy (no vs yes), feeding type during infancy and period of breast-feeding (formula-feeding vs breast-feeding period in weeks). Results from regression analyses are considered significant at p-values ≤ 0.05.

8.4 Results

At birth 207 children were included in this prospective follow-up study, of whom 105 were breast-fed (BF) and 102 were formula-fed (FF) during infancy. At the 42-month follow-up, 193 children (104 boys, 89 girls) participated and 14 children (7%) were lost to follow-up, due to lack of interest (n=10) or inability to cooperate (n=4) such as illnesses in the family and emigration. Subjects retained at the 42-months follow-up (n=193) are not different from those not retained in the sample (n=14) in terms of plasma and breast milk PCB and dioxin concentrations. The CBCL questionnaires were completed by 192 mothers and 186 fathers. For 10 children, parents were divorced, however for 5 of them, both parents agreed to complete a CBCL form. In addition, 160 teachers completed a CBCL questionnaire. The group not attending a day care center (n=33) was not different from the group attending a day care center (n=160) with respect to exposure variables and covariables. Examiner, teachers and parents were blind to the PCB and dioxin exposure concentrations in plasma and breast milk. The sum of four PCB congeners IUPAC nos. 118, 138, 153, and 180 could be calculated for 206 maternal, 182 cord and 173 child plasma samples. In human milk, representative dioxin-TEQ, planar PCB-TEQ and mono-ortho PCB-TEQ levels could be calculated for 82, 94 and 99 milk samples, respectively. The remaining samples were missing or could not be analyzed due to laboratory failure or too low sample volume. The median sum of 4 PCB congeners measured in maternal plasma during pregnancy and in cord plasma, were 2.04 and 0.40 µg/L, respectively. At 42 months of age children in the breast-fed group had current PCB levels that were nearly 4 times higher compared to sum PCB levels measured in the formula-fed group (0.75 vs. 0.21 µg/L).[7] Median dioxin-TEQ, planar PCB-TEQ and mono-ortho PCB-TEQ concentrations in breast milk were 36, 15 and 14 pg TEQ/g milk fat, respectively.

In Table 8.1, results of all means (SD) are given for CBCL scale scores from mothers, fathers and teachers. There are small but significant differences between the mean scores of mothers and fathers. For all scales, the mean scores of mothers were significantly higher compared to those from fathers. Externalizing and consequently the corresponding oppositional, aggressive and overactive scales syndrome scales, were scored significantly lower by teachers, when comparing those with maternal as well as paternal scores. The scores for internalizing and consequently syndrome scores of withdrawn/depressed and anxious behavior were not significantly different from maternal and paternal scale scores. The mean values from the maternal CBCL for the TPS as well as scale scores are in agreement with the values reported for the community sample of the Dutch CBCL validation study.[23] The teacher CBCL scores did not include sleep and somatic problems, therefore no TPS could be calculated from the teacher's information. Spearman rank correlations coefficient were calculated between maternal, paternal and teacher CBCL scales. Spearman rank correlations are high between maternal and paternal CBCL scales, median (range): 0.68 (0.43-0.74), all p-values <0.01. Spearman rank correlations between maternal and teacher CBCL scores are moderate, median (range): 0.31 (0.14-0.34), but significant, except for withdrawn/depressed behavior, rs=0.14, p=0.09. Spearman rank correlations between father and teacher scale scores are also moderate, median (range): 0.30 (0.1-0.41) and not significant for withdrawn/depressed behavior, rs=0.10, p=0.22.

Table 8.1 *Mean scores and standard deviations (SD) on CBCL/2-3 scales for the study group, completed by mother, fathers and teachers*

	Mother	Father	Teacher
	n=192	n=186	n=160
Total problem Score	34(18)	30(18)**^{M}	-
Internalizing	4.8(4.0)	4.1(3.7)**^{M}	4.6(4.0)
Externalizing	17.1(9.7)	15.3(9.9)**^{M}	7.7(7.6)**^{M} **^{F}
Oppositional	10.7(6.3)	9.7(6.2)**^{M}	5.3(5.7)**^{M} **^{F}
Withdrawn/depressed	1.3(1.4)	1.0(1.3)**^{M}	1.3(1.4)
Aggressive	3.2(2.4)	2.7(2.5)**^{M}	1.1(2.1)**^{M} **^{F}
Anxious	3.6(3.0)	3.1(2.3)**^{M}	3.2(2.6)
Overactive	3.2(2.3)	3.0(2.3)**^{M}	1.5(1.7)**^{M} **^{F}
Sleep problems	2.4(2.5)	2.0(2.3)*^{M}	-
Somatic Problems	0.3(0.7)	0.2(0.5)*^{M}	-

CBCL: Child Behavior Checklist: Dutch version. Paired t-test and Wilcoxon paired test:** p<0.01, *p<0.05. Significantly different from M=mother or F= father CBCL scores

Linear regression analyses for maternal and paternal TPS, revealed that after adjustment for relevant covariates mentioned in the statistics section, no significant associations were found between prenatal, lactational and current PCB and dioxin exposure. Internalizing, Externalizing and the six syndrome scores as well as somatic problem scores reported by mothers and fathers in relation to exposure variables, were analyzed in multiple logistic regression analyses with the 75th percentile as cut off value. Prevalence of problem behavior at 42 months assessed from CBCL reports of mothers and fathers were not associated with prenatal, lactational and current exposure variables.

Table 8.2 *Prevalence of problem behavior above the 75th Percentile as cut off value and effects of prenatal exposure to maternal and cord plasma PCBs, as well as breast milk PCB and dioxin toxic equivalents on the Child Behavior Checklist scales in Dutch Preschoolers scored by teachers*

Teacher score			LnΣPCBmaternal		LnΣPCBcord		Total TEQ	
	%	n	OR [95-CI]	P-value	OR [95-CI]	P-value	OR [95-CI]	P-value
Internalizing	24.1	33	2.24[0.73-6.86]	0.16	1.92[0.71-5.21]	0.20	Total-TEQ 1.04[1.00-1.09] Dioxin-TEQ 1.09[1.01-1.18] Planar PCB-TEQ 1.08[1.00-1.17]	0.04 0.03 0.05
Externalizing	24.0	31	1.50[0.56-3.99]	0.42	0.37[0.15-0.88]	0.72	1.02[0.98-1.06]	0.31
Syndromes								
Oppositional	24.6	35	0.71[0.25-2.03]	0.52	1.89[0.67-5.34]	0.23	1.0[0.98-1.04]	0.64
Withdrawn/ depressed	26.6	38	3.44[1.13-10.42]	0.03	5.22[1.59-17.1]	0.006	Total-TEQ 1.04[1.00-1.08] Dioxin-TEQ 1.06[0.99-1.14] Planar PCB-TEQ 1.10[1.01-1.19]	0.03 0.08 0.03
Aggressive	23.2	32	1.77[0.54-5.84]	0.35	4.48[1.24-16.2]	0.02	0.99[0.92-1.04]	0.69
Anxious	19.3	29	2.89[0.87-9.58]	0.08	1.59[0.56-4.53]	0.38	1.03[0.99-1.08]	0.09
Overactive	27.9	43	0.70[0.26-1.90]	0.48	0.60[0.23-1.56]	0.29	0.99[0.95-1.03]	0.52

PCB: Polychlorinated Biphenyls. TEQ: Toxic equivalents. OR[95-CI]; Odds ratios and 95% confidence intervals from multiple logistic regression analyses. Outcome variables were defined as binary variables either below (=0) or above (=1) the 75th percentile (P75). All regression analyses were adjusted for maternal age, maternal education, gender, parity, smoking and alcohol during pregnancy, breast-feeding period (formula-feeding=0 vs breast-feeding weeks) and HOME. Prenatal PCB was estimated from the lnΣPCB of 4 PCBs 118, 138, 153 and 180 in maternal plasma during pregnancy or cord plasma. Total-TEQ in breast milk was measured from the sum of Dioxin-TEQ, planar PCB-TEQ and mono-ortho PCB-TEQ, only available for the breast-fed group.

After adjustment for maternal age, maternal education, HOME score, gender, parity, maternal alcohol use and smoking during pregnancy and breast-feeding period, logistic regression analyses of the teacher CBCL scores resulted in significant effects of prenatal ΣPCB and ΣTEQ exposure. Results for the cut off point set at P75 are summarized in Table 8.2. Odds ratios and 95 % confidence intervals (OR 95-CI) are presented. Prenatal exposure to total-TEQ, respectively dioxin-TEQ and planar PCB-TEQ levels measured in breast milk are associated with a higher prevalence of

internalizing problems reported by teachers. Prenatal PCB exposure measured from maternal plasma ΣPCB levels measured during pregnancy and from cord plasma ΣPCB levels are associated with a higher prevalence of withdrawn/depressed behavior scored by teachers. Moreover, prenatal TEQ exposure assessed from breast milk total TEQ and planar PCB-TEQ levels are associated with a higher prevalence of withdrawn/ depressed behavior in 42-month-old children reported by teachers. In addition, elevated cord plasma ΣPCB levels are associated with more aggressive behavior reported by teacher CBCL scores. Current PCB exposure assessed from 42-month-old ΣPCB levels is associated with a lower prevalence of overactive behavior reported by the teacher at the P75 cutoff point (OR 95-CI; 0.33 [0.12-0.87], p=0.03).

8.5 DISCUSSION

In this paper, the prevalence of problem behavior in 42-month-old Dutch preschoolers in relation to prenatal, lactational and current PCB and dioxin exposure are summarized. We report that prenatal PCB and dioxin exposure are associated with a higher prevalence of withdrawn/depressed behavior reported by the teacher. In addition a lower prevalence of overactive behavior reported by teacher CBCL scores is found in association with the child's 42-month PCB body burden.

There are a few other human studies that studied problem behavior in relation to exposure, but most of the studies mainly focused on activity level. The Yu-Cheng case-control cohort study reported mildly disordered behavior from parents' report of the Rutter Child Behavior Scale and increased activity assessed from the Werry-Weiss-Peters Activity Scale[15] in relation to prenatal PCB exposure. The Rutter Scale was assessed in 9 to 12 year-old children and information about health, habits and behavior were asked, whereas the activity Scale was assessed in 3 to 11 year-old children.[15] Yu-Cheng children had persistent cognitive deficits up to 7 years of age, and the authors suggest that the persistent behavior and activity level alteration may be secondary to the cognitive delay, however they did not report significant relationships between behavioral outcome scores and developmental outcome.[15] The North Carolina cohort, where environmental exposure to PCBs was investigated in the general population, reported on the child's work habit and conduct extracted from report cards in the third grade. There were no significant associations between milk PCB levels as measure for transplacental exposure and unsatisfactory conduct or work habit. Along with the report cards, information on whether the child was considered hyperactive by either parents, teacher, counselor, or a doctor or showed excessive activity (possibly hyperactive), was gathered.[28] Neither pre- nor postnatal PCB exposure through breast-feeding showed any association with hyperactivity by either definition.[28] In the Michigan cohort, which studied children born to mothers who reported to have consumed PCB contaminated fish from Lake Michigan USA, a composite activity rating was derived from examiners and mothers.[29] They report a reduced activity level among

children with higher 4-year serum PCB levels.[29] These PCB levels were related to the children's breast-feeding history, and the effect was seen in children who had been breast-fed for 1 year and whose mothers had above average PCB levels.[29]

In our study, teacher reported a lower prevalence for overactive behavior in association with current PCB exposure and a higher prevalence of withdrawn/depressed behavior in association with in utero PCB and dioxin exposure. Lower prevalence of overactive behavior is not the same as hypoactive active behavior, therefore this finding is difficult to interpret. However, withdrawn/depressed behavior can be interpreted as hypoactive behavior, but this is not in agreement with the Michigan study which found a postnatal PCB exposure effect on reduced activity. In the Yu-Cheng study hyperactivity was found in association with prenatal exposure, they report no association between breast-feeding and hyperactivity, moreover the exposed mothers were advised not to breast-feed their children, and only 30 of the 118 did.[15] In another recently submitted paper we reported that more signs of hyperactive behavior reported by parents, assessed with the Groninger Behavior Observation Scale [30] and slower mean reaction times as well as less sustained attention during a vigilance task are associated with 42-month PCB plasma levels [31]. Behavioral alterations appear in animals perinatally exposed to PCBs [21] and both hyperactive [32] as well as hypoactive behavior [33] are reported among exposed animals. More studies are needed to elucidate this more thoroughly, with respect to time of exposure (prenatal, postnatal or combined exposure), persistence of these effects, and what mechanism of action may be involved for these behavioral alterations.

We report a higher prevalence of withdrawn/depressed behavior reported by teachers in association with prenatal PCB and PCB/dioxin TEQ exposure. We recently reported a 4-point deficit on the overall cognitive scale as well as the sequential and simultaneous processing scales assessed with the Dutch version of the Kaufman Assessment Battery for Children (K-ABC) in association with prenatal PCB exposure in 42-month old children[19]. When the child's cognitive outcome was entered as covariate in the logistic regression analysis, it appeared to be significantly associated with a lower prevalence of withdrawn/depressed behavior. We suggest that the higher prevalence of withdrawn/depressed behavior might be due the lower cognitive development in these children as also suggested in the Yu-Cheng study.[15]

Cognitive development could be a predictor for internalizing and withdrawn/ depressed behavior. Withdrawn/depressed behavior reported by teachers was negatively influenced by prenatal PCB exposure, however this was not found with parent reports. A possible explanation could be that the information of parents CBCL scores are different than the teacher CBCL scores. In observational studies it is important to have different informants in order to have a more detailed behavior observation. The correlations between parent CBCL scores were high, however the correlations between parent and teacher CBCL scores were moderately correlated. Moreover, no significant correlation was found between the score for withdrawn/ depressed behavior from parent and teacher CBCL reports. Information derived from the teacher CBCL scores is different and probably predicted by the cognitive

development of the child, whereas the information derived from the parent CBCL scores is predicted by other factors, for instance the HOME score. Weisglas et al[34] studied parent and clinician reports of behavior problems among very low birth weight (VLBW) children at 3.5 years of age in relation to indicators of neonatal cerebral damage, cognition and social factors. VLBW children had more depressed behavior and more internalizing problems by parent report. Depressed behavior of preschool VLBW children might be associated with parental reactions to the birth of a VLBW child, and attention problems might be linked indirectly to brain damage via cognitive impairments. According to the clinician report, cerebral damage was related to cognitive development. This study emphasizes the importance of having different informants for behavior evaluation. In another recent study scores from the CBCL 4-18 years, for 67 children and adolescents with mental retardation were examined to evaluate the factorial validity of the instrument. No child characteristics were significantly related to the CBCL Externalizing dimension, but child age and level of mental retardation were significant predictors of the CBCL Internalizing dimension.[35] Lower McCarthy general, verbal, and perceptual-performance IQ scores are associated with various types of psychopathology and it was concluded that early identification of intellectual deficits among preschoolers ages 3 to 5 may help to prevent later school difficulties and severe psychopathology. [36]

Although the CBCL is primarily validated as a parent questionnaire, several studies have indicated that more informants on child behavior can reveal several aspects of deviant behavior and about factors associated with deviant behavior. In a study of children originally aged 4 to 11 years from the general population, examined across a 6-year period, Parents' and Teachers' ratings were obtained via the CBCL and Teacher's Report Form (TRF) and tested as predictors of disturbed behavior. They concluded that it was important to include teacher information in the diagnostic assessment of children. Teachers' reports predicted poor outcomes equally well or even somewhat better than parents' reports. [37]

In conclusion, we report a higher prevalence of withdrawn/depressed behavior from day care Teacher CBCL reports in relation to prenatal PCB and PCB/dioxin TEQ exposure. These findings are reported for the first time in relation to environmental PCB and dioxin exposure. As previously reported by us and other authors prenatal PCB exposure relates to poorer cognitive functioning. Moreover, teacher report on child behavior is influenced by cognitive functioning of the child. It is hypothesized that withdrawn/depressed behavior might be secondary to poorer cognitive functioning. Although these children are all normal and healthy, early identification of cognitive deficits and consequently more deviant behavior among preschoolers may help to prevent problems in later school performance.

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CHAPTER 9

IMMUNOLOGICAL EFFECTS OF BACKGROUND EXPOSURE TO POLYCHLORINATED BIPHENYLS AND DIOXINS IN DUTCH TODDLERS

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9.1 ABSTRACT

Background: Prenatal exposure to polychlorinated biphenyls (PCBs) and dioxins is associated with changes in the T-cell lymphocyte population in healthy Dutch infants. We investigated whether these changes persist into later childhood and whether background exposure to PCBs and dioxins is associated with the prevalence of infectious or allergic diseases and humoral immunity at 42 months of age.

Methods: The study group consisted of 207 healthy mother-infant pairs. Prenatal exposure to PCBs and dioxins was estimated by the sum of PCBs 118, 138, 153 and 180 (ΣPCB) in maternal and cord plasma and in breast-fed infants by the total toxic equivalent (TEQ) level in human milk. Current exposure was estimated by the ΣPCB in plasma at 42 months of age. The prevalence of infectious and allergic diseases was assessed by parent questionnaire. Humoral immunity was measured by detecting antibody levels to mumps, measles and rubella after primary vaccination. Immunological marker analyses of lymphocytes were done in a subgroup of 89 children.

Results: At 42 months of age prenatal PCB exposure was associated with an increased number of T-cells as well as CD8+ (cytotoxic), TcR αß+ and CD3+HLA-DR+ (activated) T cells, lower antibody levels to measles, a higher prevalence of chickenpox, and less shortness of breath with wheeze while current PCB exposure was associated with a higher prevalence of recurrent middle ear infections and a lower prevalence of allergic reactions.

Conclusions: In Dutch toddlers the effects of perinatal background exposure to PCBs and dioxins persist into childhood and might be associated with a greater susceptibility to infectious diseases. Common infections acquired early in life may possibly prevent the development of atopy and PCB exposure was associated with a lower prevalence of allergic diseases.

9.2 INTRODUCTION

Polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs and PCDFs, summarized as dioxins) are lipophilic stable toxic compounds which occur widely in the environment. They are presently a pollution problem because they are resistant to either chemical or biologic degradation and accumulate in the food chain. Persistent neurobehavioral, immunological, reproductive and endocrine alterations were observed in experimental animals, following in utero and lactational exposure to PCBs and dioxins[1]. In nursing infants daily intake of PCBs and dioxins per kg body weight is 50 times higher than in adults[2], and in Dutch toddlers median plasma PCB levels are 4 times higher in previously breast-fed than formula-fed children[3]. Because of the vulnerability of the developing immune system, the potential immunotoxic effects of perinatal human exposure to PCBs and dioxins presents particular concern.

PCBs and dioxins can cause a broad range of immunologic effects in animals, including decreased host resistance to infections and suppressed humoral and cell-mediated immune responses. The most consistant finding in these studies is thymic atrophy[4]. Data regarding the potential toxic effects of in utero and lactational exposure to PCBs and dioxins in humans are scarce. In 1979, an episode of poisoning from ingestion of rice oil contaminated with PCBs and PCDFs occurred in central Taiwan (Yu-Cheng). In children born to these highly exposed women a higher incidence of respiratory symptoms during the first 6 months after birth[5] and of middle-ear diseases at school age[6] was found. Inuit infants, whose mothers have elevated levels of PCBs and dioxins in their breast-milk, experienced more episodes of acute otitis media at 3 to 6 months of age[7]. In a previous paper[8] we concluded that prenatal exposure to PCBs and dioxins was associated with changes in the T-cell lymphocyte population in Dutch infants. No effect on the health status nor on humoral immunity was found. However, concern has been raised that such early subtle changes could persist into later childhood and could presage difficulties, such as immune suppression or allergy. In this paper we therefore report on the immunological effects of perinatal background exposure to PCBs and dioxins in Dutch toddlers. We investigated whether exposure to PCBs and dioxins is associated with the prevalence of infectious or allergic diseases, changes in antibody responses or persistent changes in lymphocyte phenotype determinations in Dutch children at 42 months of age.

9.3 METHODS

Subjects

The total study group consisted of 207 healthy Caucasian mother-infant pairs, recruited between June 1990 and February 1992, living in the Rotterdam area, and described in detail in a previous paper[9]. Pregnancy and delivery were uncomplicated. Only first or second-born infants at term (37 to 42 weeks of gestation) without congenital anomalies, diseases or perinatal complications were included. One hundred and five infants were breast-fed for at least six weeks and 102 infants were exclusively bottle-fed with formula from a single batch (Almiron M2, Nutricia N.V., The Netherlands) until 7 months of age. In this formula concentrations of both PCBs and dioxins were not detectable. The study protocol was approved by the medical ethics committee of the University Hospital Rotterdam/Sophia Children's Hospital. Informed consent was given by the parents.

Measures of exposure to PCBs and dioxins

The four most abundant PCB congeners (PCB 118, 138, 153 and 180) were measured in maternal plasma collected during the last month of pregnancy, in cord plasma, and in plasma samples collected from 42 months-old children. Prenatal PCB exposure was defined as the sum of the 4 PCB congeners in maternal (ΣPCB maternal) and cord (ΣPCB cord) plasma, current PCB exposure as the sum of the same 4 PCB congeners in plasma from 42 months-old children (ΣPCB 42 months).

A 24-hour representative sample of breast-milk was collected in the second week after delivery and analyzed for 17 individual PCDD, PCDF and 26 PCB congener levels using previously described methods[9, 10]. To express the total toxic potency of PCDDs, PCDFs and dioxin-like PCBs, the toxic equivalent factor (TEF) approach was used according to Safe[11] and the WHO[12], respectively. A TEF value was assigned to the PCDD, PCDF and dioxin-like PCB congeners, which represents their relative toxic potency towards 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most toxic congener with a TEF value of one. The toxic equivalents (TEQ) were calculated by multiplying the concentration (pg/g milk fat) of each congener by its TEF value. The TEQ-sum of 17 dioxin and 6 dioxin-like PCBs were added as the total TEQ level in human milk.

Measures of immunological effects at 42 months of age

Infectious and allergic diseases were assessed by a health questionnaire for the parents, also covering topics regarding potential confounding variables such as tobacco smoking, family history of atopy (asthma, bronchitis, hay fever or eczema in first-degree relatives) and day care or nursery school attendance of the child. Parents were asked whether a doctor had ever given the child a diagnosis of otitis media, pneumonia, scarlatina, chickenpox, rubella, measles, mumps, pertussis, hepatitis, meningitis or other infectious diseases. Additional questions included: Had your child ever had eczema or an allergic reaction? Has a doctor ever said to you that your child had asthma or bronchitis? Had your child periods of coughing, chest congestion, or phlegm lasting for 10 days or attacks of shortness of breath with wheeze in the previous 12 months?

Vaccinations against mumps, measles and rubella were given to 205 of the 207 children at approximately 14 months of age as part of the National Immunization Program. The vaccines were manufactured by the National Institute of Public Health and the Environment and given at the local municipal health service. Humoral antibody levels (IgG) in plasma to mumps, measles and rubella were determined with an Enzyme-Linked-Immunosorbent-Assay (ELISA), as previously described[13].

Since fresh blood is needed, in a subgroup of 89 children monocyte, granulocyte and lymphocyte counts were determined by whole blood fluorescence-activated cell sorter analysis combined with the determination of the white blood cell count by a cell counter[14]. Using several monoclonal antibodies, absolute numbers of the following lymphocyte (sub)populations were determined: CD3+CD4+ (helper), CD3+CD8+ (cytotoxic), CD3+HLA-DR (activated), Tcαß+ and TcRγδ+, CD4+CD45RA+(naive) and CD4+CD45RO+(memory) T-lymphocytes, B-lymphocytes (CD19+ and/or CD20+) and NK cells (CD16+and/or CD56+/ CD3-).

Data analysis

Data analysis was performed using the statistical software package SPSS win 7.5. The effect of exposure to PCBs and dioxins on immunological variables were first analyzed univariately. Because of the observational nature of this study, this effect was

also estimated with adjustment made for possibly confounding variables, using multivariate analyses. The type of statistical analysis depended on the scale of the immunological variables considered.

For dichotomous (or dichotomized) immunological variables, logistic regression analysis was used, with the effects of PCB and dioxin levels on the prevalence of infectious or allergic disease expressed as odds ratio (OR) per unit increase of levels of PCBs and dioxins, with 95% confidence interval and with adjustment made for the confounding variables in a multiple logistic regression analysis.

For continuous immunological variables the Spearman rank correlation coefficient between the PCB and dioxin levels and the immunological variables was estimated and tested. Then, adjustment was made for confounding variables, by estimating and testing the partial correlation coefficient. In this latter analysis variables were logarithmically transformed when appropriate (because of positive skewness). The above analyses were performed for prenatal (ΣPCB maternal, ΣPCB cord and total TEQ) as well as current (ΣPCB 42 months) exposure to PCBs and dioxins.

Bases on literature and clinical knowledge, the confounding variables included in all analyses were gender, feeding type during infancy and duration of breast-feeding, tobacco smoking by one or both parents, family history of atopy and day care or nursery school attendance for the child. Results were considered statistically significant at the $p \leq 0.05$ level.

9.4 RESULTS

At 42 months of age 193 children (93%) were re-examined and 14 children (7%) were lost to follow-up, due to lack of interest or inability to cooperate. Subjects retained at the 42-month follow-up (n=193) were not different from those not retained in the sample in terms of cord- and maternal plasma PCB levels, and milk PCB and dioxin levels. The 4 PCBs were measured in 206 maternal-, 182 cord- and 173 42-month-old plasma samples. In human milk, representative dioxin-TEQ and PCB-TEQ levels could be calculated for 83 and 95 milk samples, respectively. The remaining human milk samples were missing or not analyzed. The median sum of the 4 PCB congeners measured in maternal plasma during pregnancy, and in cord plasma, were 2.04 and 0.40 µg/L, respectively. At 42 months of age children in the breast-fed group had current PCB levels that were nearly 4 times higher compared to sum PCB levels measured in the formula-fed group (0.75 vs. 0.21 µg/L).[3] Median dioxin-TEQ and dioxin-like TEQ concentrations in breast milk were 30.6 and 28.1 pg TEQ/g fat, respectively.

Table 9.1. Prevalence of infectious and allergic diseases and effects of prenatal and current pcb exposure

	Prevalence	Prenatal PCB Exposure		Current PCB Exposure	
		ΣPCB maternal		ΣPCB 42 months	
	n=175	n=173		n=158	
Infectious diseases	n (%)	Odds ratio (95% CI)[c]	p-value	Odds ratio (95% CI)[c]	p-value
Middle ear infections					
-1 or more episodes	103 (58.9%)	0.91 (0.66-1.24)	0.549	1.36 (0.62-3.01)	0.442
- 6 or more episodes	21 (12.0%)	1.27 (0.82-1.97)	0.276	3.58 (1.04-12.28)	0.042*
Pneumonia	5 (2.9%)	0.53 (0.15-1.89)	0.329	0.01 (0.00-24.57)	0.190
Scarlatina	13 (7.4%)	1.01 (0.57-1.80)	0.970	0.38 (0.27-5.29)	0.469
Chickenpox	130 (74.3%)	1.54 (1.01-2,35)	0.049*	6.97 (0.92-52.76)	0.060
Other infectious diseases	14 (8.0%)	1.70 (0.45-6.38)	0.431	2.55 (0.72-9.02)	0.145
Allergic diseases					
Eczema	42 (24.0%)	1.26 (0.87-1.82)	0.222	0.76 (0.28-2.05)	0.584
Allergic reaction	14 (8.0%)	0.61 (0.29-1.29)	0.200	0.01 (0.00-0.55)	0.026*
Asthma or bronchitis	30 (17.1%)	0.86 (0.54-1.37)	0.536	0.60 (0.09-3.77)	0.585
Coughing, chest congestion, or phlegm lasting for 10 days or more[1]	48 (27.4%)	1.06 (0.75-1.52)	0.729	1.09 (0.53-2.26)	0.813
Attacks of shortness of breath with wheeze[1]	17 (9.7%)	0.43 (0.19-0.95)	0.038*	0.53 (0.04-8.26)	0.649

[1] in the previous 12 months

* significant at the $\leq$.05 level, [c] corrected for gender, early feeding type (formula-fed or breast-fed), duration of breastfeeding during infancy , tobacco smoking by one or both parents, family history of atopy and day care or nursery school attendance for the child .

Of the 193 (90%) questionnaires 175 were returned by the parents. The prevalence of infectious and allergic diseases is presented in Table 9.1. According to parental report of doctors' diagnoses, the median number of episodes of middle ear infections was 1 in 103 (50th percentile) and 6 in 21 (90th percentile). Other infectious diseases were reported for 14 children (1 meningitis, 1 cystitis, 1 gastroenteritis and 11 common viral diseases such as exanthema subitum or erythema infectiosum). Of the 14 children with allergic reactions, 7 children had shown an allergic reaction to food, 1 to pollen, 5 to dust and 5 to household pets. In simple logistic regression analysis the ΣPCB 42-month levels had a significant effect on recurrent middle ear infections (6 or more episodes, 90th percentile: OR 1.79, 95%CI 1.04-3.10, p=0.032) and ΣPCB maternal had a significant effect on chickenpox (OR 1.53, 95%CI 1.01-2.31, p=0.045) and on attacks of shortness of breath with wheeze in the previous 12 months (OR 0.39, 95%CI 0.18-0.86, p=0.02). These results were confirmed in multiple logistic regression analysis. In addition, in multiple logistic regression analysis the

ΣPCB 42-month levels had a significant effect on allergic reactions (OR 0.01, 95%CI 0.00-0.55, p=0.026) (Table 9.1). For the ΣPCB cord and the total TEQ the results were not significant (data not shown).

Antibody levels at 42 months of age, after vaccination at 14 months of age, were available in 150 children. Six children showed no seroconversion at 18 months of age (2 for mumps, 2 for measles and 2 for measles and rubella). For these 6 children the respective antibody concentrations were excluded from further analysis. Antibody concentrations (mean, SD) to mumps, measles and rubella and Spearman rank and partial correlation coefficients with exposure variables are presented in Table 9.2. Antibody levels to measles were negatively correlated to ΣPCB maternal levels and antibody levels to rubella were negatively correlated to ΣPCB cord levels (Table 9.2). There were no significant correlations between antibody levels, the total TEQ or the ΣPCB at 42 months of age (data not shown).

Table 9.2 Antibody concentrations to mumps, measles and rubella in relation to prenatal pcb exposure

Antibody concentrations		Prenatal PCB exposure							
	Extinction	ΣPCB maternal n=148				ΣPCB cord n=131			
	Mean (SD)	Spearman's rho	p-value	Partial correlations[c]	p-value	Spearman's rho	p-value	Partial correlations[c]	p-value
Mumps (n=146)	0.921 (.393)	-0.14	0.085	-0.10	0.239	-0.11	0.236	-0.11	0.210
Measles (n=146)	1.000 (.271)	-0.10	0.254	-0.08	0.343	-0.20	0.026*	-0.19	0.034*
Rubella (n=148)	0.957 (.302)	-0.21	0.012*	-0.15	0.082	-0.09	0.289	-0.05	0.576

* significant at the <.05 level, [c] corrected for gender, early feeding type (formula-fed or breast-fed), duration of breastfeeding during infancy, tobacco smoking by one or both parents, family history of atopy and day care or nursery school attendance for the child

The results of the white blood cell counts and the immunological marker analyses of the lymphocytes at 42 months of age in relation to exposure to PCBs and dioxins are presented in Table 3. The results were all within the normal ranges for age-matched children15. Significant positive correlations between the ΣPCB maternal plasma levels and the number of T-cells, and of CD3+CD8+ (cytotoxic), CD4+ CD45RO+ (memory), TcR αß+ and CD3+HLA-DR+ (activated) T cells were found. The ΣPCB cord plasma levels were positively correlated with a higher number of CD3+HLA-DR+ (activated) T cells. Except for the CD4+ CD45RO+ (memory) T-cells, these results were confirmed in multivariate analysis (Table 9.3). The number of CD3+CD8+ (cytotoxic) T-cells was also correlated with the ΣPCB 42-month plasma levels (Spearman's correlation coefficient =.23, p=0.031). However, in multivariate analysis, results were not significant (partial correlation adjusted for confounders 0.10, p=0.364). There were also no significant correlations of white blood cell counts and immunological marker analyses at 42 months of age with the total TEQ levels in breast-milk (data not shown).

Table 9.3. Results of the white blood cell counts and the immunological marker analysis

white blood	absolute percentiles, 109/l			PCB exposure ΣPCB maternal				ΣPCB cord			
	n=89			n=85				n=78			
	p5	p50	p95	Spearman's rho	p-value	Partial corr.[c]	p-value	Spearman's rho	p-value	Partial corr.c	p-value
Monocytes	0.3	0.5	0.9	0.07	0.542	0.04	0.754	0.096	0.408	0.9	0.437
Granulocytes	2.2	4.2	7.4	0.10	0.357	0.16	0.180	0.14	0.228	0.17	0.162
Lymphocytes	2.2	4.1	6.7	0.21	0.060	0.26	0.025	0.18	0.108	0.21	0.07
T-CELL MARKERS											
CD3+	1.3	2.7	4.5	0.25	0.023*	0.25	0.047*	0.22	0.053*	0.19	0.102
CD3+CD4+	0.8	1.7	2.7	0.18	0.104	0.19	0.099	0.17	0.125	0.14	0.22
CD3+CD8+	0.4	0.9	1.7	0.23	0.033*	0.25	0.029*	0.22	0.058	0.21	0.08
CD4+CD45RA+	1.2	3.1	5.1	0.15	0.186	-0.03	0.764	0.15	0.186	-0.05	0.665
CD4+CD45RO+	0.2	0.6	1.1	0,23	0.035*	0.04	0.748	0.20	0.084	0.05	0.675
TcR αß+	1.1	2.5	4.2	0.23	0.035*	0.24	0.036*	0.19	0.097	0.18	0.124
TcR γδ+	0.0	0.2	0.4	0.11	0.299	0.19	0.093	0.14	0.232	0.15	0.207
CD3 +HLA-DR+	0.1	0.2	0.5	0.22	0.047*	0.26	0.023*	0.30	0.009*	0.33	0.004*
B-CELL MARKERS											
CD 19/20+	0.4	0.9	1.7	0.09	0.404	0.14	0.237	0.15	0.186	0.15	0.225
NK-CELL											
CD16+ and/or	0.1	0.3	1.1	0.15	0.166	0.13	0.251	0.11	0.357	0,10	0.412

* significant at the ≤.05 level, c corrected for gender, early feeding type (formula-fed or breast-fed), duration of breastfeeding during infancy , tobacco smoking by one or both parents, family history of atopy and day care or nursery school attendance for the child .

9.5 DISCUSSION

In our study we demonstrated that in Dutch toddlers the effects of perinatal background exposure to PCBs and dioxins persist into toddler age and influence the health status, the humoral response as well as the T-cell lymphocyte population. In agreement with the changes previously described8, prenatal PCB exposure was positively associated with the total number of T-cells as well as with a higher number of CD8+ (cytotoxic) and TcR αß+ T-cells at both 18 and 42 months of age. These results are in agreement with in vitro studies, where exposure to PCBs and dioxins may change the kinetics of thymocyte maturation and skew the thymocyte differentiation towards CD8+ phenotypically more mature TcR αß+ T-cells that concomitantly may lead to thymus atrophy[16]. Prenatal PCB exposure was also significantly correlated with more CD3+HLA-DR+ (activated) T cells, a higher prevalence of a common viral disease like chickenpox, and subtle changes in antibody levels to measles

and rubella at 42 months of age. All these findings may be associated with a greater susceptibility to infectious diseases in early childhood. In seals fed by contaminated Baltic herring an impairment of natural killer (NK) cell activity, in vitro T-lymphocyte function, antigen-specific in vitro lymphocyte proliferative responses, and in vivo delayed-type hypersensitivity and antibody responses to ovalbumin was observed.[17] In our human study T-lymphocyte functions and direct hypersensitivity responses were not tested and should be subject to further study.

Current PCB exposure was associated with a higher prevalence of recurrent middle ear infections. These results are in agreement with the observations made in the Yu-Cheng incident and in Inuit children. Although current PCB exposure in children breast-fed during infancy is mainly influenced by lactational transfer,[3] there was no relation between recurrent middle ear infections and the type of feeding during infancy, nor with the duration of breast-feeding. In other studies breast-feeding has been associated with lower rates of otitis media and other infectious diseases during infancy.[18,19] However, in the latter study, the first middle ear infection occurred significantly earlier in children who were weaned before 6 months of age, whereas in our study at that age 70% of the breast-fed children were already weaned and the positive effect of breastfeeding might have been counteracted by the relative short period of breast-feeding as wel as by the effect of PCB exposure.

Prenatal PCB exposure was also associated with a lower prevalence of attacks of shortness of breath with wheeze and current PCB exposure with a lower prevalence of allergic reactions to food, pollen, dust and/or household pets. Respiratory symptoms are frequent in very young children and the relation of these symptoms to later asthma is unknown. However, attacks of shortness of breath with wheeze through ages 3 to 4 years by parental report are associated with the subsequent diagnosis of asthma.[20] Among Japanese school children, a positive tuberculin response predicted a lower incidence of asthma,[21] in African children measles infection possibly prevented the development of atopy,[22] and in Italy the prevalence of atopy was low among Hepatitis A seropositive subjects.[23] Common infections acquired early in life might paradoxically prevent the development of atopy[24] and PCB exposure may therefore be associated with a greater susceptibility to infectious as well as a lower prevalence of allergic diseases.

In the Yu-Cheng study current dioxin levels were, and current PCB levels were not significantly different between Yu-Cheng children with and without middle-ear disease,[6] whereas in our study current PCB levels in plasma were, and dioxin TEQ levels in breast milk were not related to recurrent middle-ear diseases. These differences in findings might be explained by differences in levels of exposure and differences in assessment. In Yu-Cheng, the mothers of exposed children had consumed contaminated oils before their children were born and children's current pnCDF, hxCDF and PCBs, measured in a subgroup of 30 children, were still high and directly measured in the serum,[25] while in our study of background exposure the TEQ-sum of 17 dioxins, 6 dioxin-like PCBs were expressed as the PCB and dioxin TEQ levels in human milk and could only be measured in the breast-fed group.

In our study at 18 months of age the number of CD8+ (cytotoxic) and TcR αß+ T-cells correlated best with the dioxin-TEQ levels, while at 42 months of age with the prenatal PCB sum only. The PCB sum consist of the four most abundant congeners, constituting 46% of the total PCBs,[26] and representing a complex mixtures of environmental xenobiotics. To get an indication about other possible immunotoxicants besides PCBs and dioxins, lead and cadmium were measured at 18 months of age in a subgroup of children (n=150). Mean levels were low (Cd mean 0.5 µg/dL , Pb mean 4.8 µg/dL) and not related to the outcome variables. These measurements were therefore not repeated at 42 months of age. Immunotoxicological studies are usually carried out in laboratory animals, exposing them to a range of concentrations of a potentially immunotoxic compound. However in the environment PCBs and dioxins are present as complex mixtures of various congeners. There are 209 different PCB, 135 different PCDF and 75 different PCDD congeners, that may vary in metabolism and toxicity. Moreover PCBs form persistent and abundant metabolites that accumulate in biota.[27] Limited information is available on the immunotoxic effects of chronic background exposure to such complex mixtures of xenobiotics in the human food chain.

In conclusion the effects of perinatal background exposure to PCBs and dioxins persist into childhood and might be associated with a greater susceptibility to infectious diseases. Current PCB exposure was associated with a higher prevalence of recurrent middle ear infections. Although lactational exposure to PCBs and dioxins is much higher compared to transplacental exposure, neither the type of feeding during infancy nor the duration of breast-feeding were associated with the prevalence of recurrent middle ear infections. Moreover, as previously described in our study, breast-fed children did better in neurological[28] and cognitive outcome[29] than their formula-fed counterparts. Perinatal exposure to PCBs, dioxins and related compounds should therefore be lowered by reducing the intake through the food chain at all ages, rather than by discouraging breast-feeding.[2] Common infections acquired early in life may possibly prevent the development of atopy and PCB exposure might be associated with a lower prevalence of allergic diseases. Although most of the above mentioned immune changes seen in human toddlers may be subtle, these data indicate that, due to present levels of PCBs and dioxins in the food chain, human children are at risk to immunotoxic pollutants. Long-term follow-up studies of perinatally exposed cohorts should be conducted into later childhood, through puberty and into adulthood to investigate the long-term implications of our findings.

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CHAPTER 10
GENERAL DISCUSSION

10.1 Introduction

10.2 PCB and Dioxin related effects described in the Dutch cohort

10.3 Methodological remarks

10.4 Comparison with other studies and possible mechanisms of action

10.5 Prenatal and postnatal PCB/dioxin exposure compared with early feeding type on developmental outcome measures

10.6 References

10.1 INTRODUCTION

In this thesis adverse effects of environmental exposure to PCBs and dioxins on health, growth and neurodevelopmental outcome measures in young children living in the Netherlands are described. Levels of PCBs and dioxins in breast milk, especially in industrialized countries, are high and led to concern for possible health hazard in the nursing infant. Therefore, a large prospective follow-up study, 'the Dutch PCB/dioxin study' was initiated in 1989. In 1993, this study became part of a multicenter cohort study with Germany and Denmark, financed by the European Community.

From several animal and human 'poisoning' studies it became clear that PCBs and dioxins (PCDDs and PCDFs) can elicit a broad spectrum of toxic and biochemical responses, in particular when exposure occurred during gestation and/or lactation.[1,2] PCBs and dioxins can cross the placenta[3] during a vulnerable period of organ growth and development, and large quantities are also transferred during breast-feeding to the nursing infant.[4] Therefore, the primary goal of this longitudinal study was to gain insight into the possible toxic effects which can occur during perinatal exposure, and what consequences it may have for later development.

In this chapter relevant findings will be summed according to type of effect, age and time of exposure (prenatal or postnatal/current). In addition a few methodological remarks will be made and results discussed for possible mechanisms of action in connection with animal and human literature. The contribution of prenatal versus postnatal exposure and the role of early feeding type within the spectrum of neurobehavioral and cognitive outcome measures will be discussed.

10.2 PCB AND DIOXIN RELATED EFFECTS DESCRIBED IN THE DUTCH COHORT

In Table 10.1 and 10.2 adverse effects of prenatal respectively postnatal and current PCB/dioxin exposure for different outcome measures are summarized from birth until 42 months of age. Findings are presented according to the type of toxic effects. Prenatal PCB exposure was estimated from ΣPCB concentrations in maternal plasma and cord plasma (PCB IUPAC nos. 118, 138, 153, and 180). In the breast-fed group prenatal exposure to the sum of dioxin-TEQs and dioxin-like PCB-TEQs (ΣPCB/dioxin TEQ) were assessed from breast milk concentrations and the corresponding TEF values. Lactational exposure to PCBs and dioxins were calculated by multiplying breast milk PCB and dioxin TEQ concentrations with the number of breast-feeding weeks. Current PCB body burden was estimated from ΣPCB measured in 42-months plasma samples.

Table 10.1 Associations between prenatal exposure to PCBs and dioxins, and several outcome measures from birth until 42 months of age

Parameter	Age	Outcome
Endocrine effects Chapter 1 and reference 5		
Thyroid hormone	pregnancy	↓TT3 and ↓TT4 in mothers with ΣPCB/dioxin TEQ
	10 days	↑TSH in child with ΣPCB/dioxin TEQ
		↓TT4 and ↓FT4 with dioxin-TEQ
	3m	↑TSH in child with ΣPCB/dioxin TEQ
Growth effects Chapter 4		
Fetal and postnatal growth	birth	↓Birth weight with ΣPCBcord and ΣPCBmaternal
	0-3 m	↓Growth rate for weight, length and head circumference, with ΣPCBcord and ΣPCBmaternal
Neurodevelopmental and behavior effects Chapters 5, 6, 7 and 8 and references 6, 7, 8		
Bayley Scales	3 m	↓ Psychomotor score with ΣPCBmaternal
Neurological examination	10 days	Hypotonicity with planar PCB-TEQ
		↓NOS with ΣPCB/dioxin TEQ
	18 m	↓NOS with ΣPCBcord and ΣPCBmaternal
Cognitive abilities	42 m	↓Cognitive scale with ΣPCBmaternal
K-ABC		↓Sequential processing with ΣPCBmaternal
		↓Simultaneous processing with ΣPCBmaternal and ΣPCBcord
Free play observation	42 m	↓ High level play with ΣPCBcord and ΣPCBmaternal
		↑Non-play behavior with ΣPCBcord
Vigilance task	42 m	↑Errors of omission in the beginning of the task with ΣPCBcord
Teacher CBCL	42m	↑Prevalence Withdrawn/Depressed with ΣPCBcord ΣPCBmaternal and ΣPCB/dioxin TEQ
Immunological effects Chapter 9 and reference 9		
T-cell markers	Birth	↑ TcRγδ+ T cells with ΣPCB/dioxin TEQ
	3 m	↓Monocyte, ↓ Granulocyte with ΣPCB/dioxin TEQ
	18 m	↑ CD8+, ↑ TcRαβ+ and ↑TcRγδ+ T cells with ΣPCB/dioxin TEQ
	42 m	↑ Total T-cells, ↑ CD8+, ↑TcRαβ+ and ↑CD3+HLA-DR+ T cells with ΣPCBmaternal
Health status	42 m	↑ Prevalence of chickenpox with ΣPCBmaternal
Humoral immunity	42m	↓ antibody levels to measles with ΣPCBcord

NOS: neurological optimality score at birth according Prechtl [10] and at 18 months according to Hempel/ Touwen [11]. K-ABC: Dutch Kaufman Assessment Battery for Children. CBCL: Child behavior Checklist [12]

Table 10.2 Associations between postnatal exposure to PCBs and dioxins and several outcome measures from birth until 42 months of age

Parameter	Age	Outcome
Neurodevelopmental and behavior effects		
Chapter 7 and reference 8		
Bayley Scales	7 m	↓ Psychomotor development with lactational ΣPCB/dioxin exposure
Vigilance task	42 m	↑Mean Reaction Time with ΣPCB42-month
		↓ Sustained Attention with ΣPCB42-month
GBO	42 m	↑ Activity with ΣPCB42-month
Immunological effects Chapter 9 and reference 9		
T-cells	3 m	↓Monocytes, ↓ Granulocytes with lactational PCB/dioxin TEQ exposure
Health status	42 m	↑ Prevalence of recurrent middle ear infections and ↓ prevalence of allergic reactions to food, pollen, dust and pets with ΣPCB 42-month

GBO: Groninger Behavior Observation Scale adapted for 42 month olds. Lactational exposure was defined as ΣPCB/dioxin TEQ in breast milk multiplied by the number of breast-feeding weeks. Current PCB body burden assessed from ΣPCB 42-months represents mainly lactational transfer of maternal PCBs (the breast-fed group) and in part during gestation (the formula-fed group).[4]

10.3 METHODOLOGICAL REMARKS

Good analytical methods are indispensable for reliable exposure assessment. In our Dutch cohort the 4 most abundant PCB congeners IUPAC nos. 118, 138, 153, and 180 were measured in plasma. In addition, 26 PCB and 17 dioxin congeners were measured in breast milk. PCBs and dioxins were measured with congener specific and reliable analytical methods[13] according to today's state-of-the-art standards.[14] High correlation's between different matrices, e.g. maternal and cord plasma, and breast milk PCB concentrations (0.70-0.79) were found, and excellent correlation's between individual specific congeners within one matrix (0.71-0.99) were found.[13] Another issue for exposure assessment is that other toxic compounds could also be responsible for the observed effects. At the time of the study dioxins and dioxin-like PCBs could not be measured in plasma, due to the large volume of blood (200 ml) needed for these analyses. Maternal plasma PCB concentrations correlate very good (0.66-0.71) with maternal milk dioxin and dioxin-like PCBs.[13] Therefore, effects found with maternal or cord plasma PCBs could be due to the 4 PCB congeners measured in plasma, but these effects could also be due to other contaminants that accumulate in fat tissue, such as dioxins, dioxin-like PCBs, metabolites of PCBs (OH PCB) or other substances. Possible other neurotoxicants such as heavy metals are not a matter of concern in the Dutch group. In a

subgroup, we measured lead (Pb) and cadmium (Cd) in whole blood at 18 months of age. The influence of heavy metals as other possible neurotoxicants was studied in this group. The concentrations of Pb and Cd were very low and not related to the outcome variables.

Another issue for exposure assessment is the matrix which is the best for prenatal exposure. Although PCB levels in cord plasma are a direct measure of fetal exposure to PCBs compared to maternal plasma PCB levels measured during pregnancy, the analytical precision of PCB determination in cord plasma is lower than that for maternal plasma, because of the lower lipid content in cord plasma. Cord blood is lean and contains about 20% of the lipid content of maternal blood. Therefore, from a statistical point of view, this could increase the risk of type II error making it more difficult to detect effects in the data with cord plasma PCB values. In our study, for all cord blood samples with sufficient ml of plasma, PCBs were detected. Cord blood could not be obtained for 36 subjects and in 9 cord samples PCB 118 could not be analyzed due to low plasma volume. In all maternal plasma samples PCBs could be measured with sufficient accuracy. Therefore, both maternal as well as cord plasma PCB values were used in the prenatal exposure assessment. Maternal milk PCB and dioxin values measured 2 weeks after birth, also served as a measure for prenatal PCB exposure. But then, only the breast-fed group could be analyzed in relation to TEQ exposure, which reduced the population size with 50%. To study subtle effects, the population size is another important factor that can raise the type II error.

In this observational based study, control for several potential confounding variables is necessary. Selection of control variables which are most relevant to the outcome is based on literature or clinical knowledge. Inclusion of all possible variables could artificially inflate the error term, making it more difficult to detect the effects of an exposure, especially when dealing with the lower range of exposure (Type II error). The possibility of confounding due to unknown or unmeasured risk factors can never be definitely ruled out. Conclusions concerning causality between exposure and effect therefore can not be drawn from this type of studies. To rule out several risk factors on growth and developmental outcome, we included only healthy pregnant women and newborns according to inclusion criteria previously mentioned (Chapter 1). Additional covariables were assessed from obstetrical and neonatal history, maternal age, parents' education level, parity, gender, fetal exposure to alcohol and cigarette smoking, type of feeding during infancy and the breast-feeding period. As the study progresses, quality of intellectual stimulation by parents (HOME), parental verbal IQ, socio-economic changes, divorce, illnesses, nursery school attendance and number of siblings are also obtained as well as the reasons for families who were lost to follow-up. Fortunately, most parents agreed to the additional follow-up and those not retained in sample were not different with respect to exposure variables. In our study a control group in terms of no exposure was not possible to obtain. Unlike animal studies, human subjects cannot be randomly assigned to predetermined levels of exposure. Therefore, the abovementioned control variables were assessed and controlled for in multivariate analyses and effects of high versus low exposure on different endpoints of interest could be studied within the population. Although our sample was not randomly selected but based on volunteer mother-infant pairs, exposure measures are representative for the Dutch general population.[13,15]

10.4 COMPARISON WITH OTHER STUDIES AND POSSIBLE MECHANISMS OF ACTION

Comparison with animal and human studies

In the Dutch cohort study several adverse effects are described in association with perinatal background PCB and dioxin exposure as well as current PCB exposure. In animal studies, fetotoxic, endocrine, neurotoxic and immunotoxic effects are described.[1, 2] The distribution of PCBs and dioxins in humans differs in the body considerably from that of most laboratory species. Elimination in humans is much slower than in any other known mammal species, resulting in much higher tissue concentrations at a given exposure. When animal data are extrapolated to human risk assessment, these toxicokinetic differences between species must be taken into consideration.[16]

Most adverse effects reported in our study were also found in the two human poisoning studies, the Yusho and Yu-Cheng study.[17-21] The toxic effects in both the Yusho and Yu-Cheng exposures, are believed primarily to be attributable to PCDF congeners, which were present at unusually high levels in the contaminated oil and are structurally and toxicologically similar to the planar PCBs.[22] More in the context of our study the effects of perinatal PCB exposure from environmental sources were examined in the two prospective longitudinal studies in the USA, the Michigan fish exposure [23,24] and the North Carolina (NC) background exposure cohort.[25] The neurobehavioral effects found at preschool age were also reported in the Michigan cohort, [23,26] however these effects were not described in the NC cohort.[27] In both US studies [28,29] as well as the Dutch study adverse effects on the neonatal neurological and behavioral examination were found, with respect to the reflexes and hypotonicity.[6] Effects on physical growth (lower birth weight and postnatal growth) was also found in the Michigan cohort [30] and recently an increase in risk for low birth weight was also reported in a Swedish case-control study of fish consumers versus controls.[31]

When comparing our results with those from the two US studies, differences with respect to exposure assessment must be mentioned. In our study 4 PCB congeners were measured in maternal and cord plasma, 17 PCDD and PCDF congeners, and 22 other PCB congeners were measured in breast milk. The method of determination in our study used was gas chromatography with electron capture detection (GC-ECD) for PCBs, and for dioxins and dioxin-like PCBs gas chromatography-high-resolution mass spectrometry (GC-HRMS). In the Michigan study, exposure was defined from total PCB levels determined in cord and maternal blood as well as breast milk, by summing 10 Webb-McCall peaks. In NC two Webb McCall peaks were quantified, using packed column gas chromatography. Although the Webb-McCall method was state-of-the-art at that time, it is crude by today's standards and provides no information of individual PCB congeners.[24] In the Michigan and NC study intercorrelations between cord, maternal and milk values were low to moderate (0.16-0.42) and in 70 % of cord and in 22 % of maternal serum samples PCBs fell below detection limit.[28,33] In the NC study,

intercorrelations between milk and maternal blood samples were higher (0.56-0.77), but 88% of cord and 13-26% of the maternal blood and milk samples could not be measured for PCBs because they fell below the detection limit.[32]

The persistent effects on cognitive functioning seen Michigan[33] and Yu-Cheng cohort up to school years[21] and during preschool years in the Dutch cohort,[34] are consistent with learning deficits found in PCB-exposed laboratory animals. Combined pre- and postnatal exposure to industrial PCB mixtures has been found to impair discrimination reversal learning in monkeys,[35-37] active avoidance learning in mice[38] and rats.[39] In studies of specific congeners, prenatal exposure to planar PCB 77, led to poorer active avoidance learning in mice,[40] and perinatal exposure to either planar PCB 126 and mono-ortho PCB 118 led to impaired visual discrimination learning in rats.[41] When tested in a delayed alteration paradigm, monkeys exposed to Aroclor 1254, a PCB mixture, showed a pattern of perseverative attentional errors characteristic of monkeys with lesions in the dorsolateral prefrontal cortex.[42] In adult female rats exposed to ortho-substituted PCBs 28, 118, and 153 can result in long-lasting deficits in learning or attentional deficit rather than mnemonic deficit in female rats and suggest that the effects may be sex specific.[43] The sole animal study to use cross-fostering to attempt to discriminate the effects of pre- versus postnatal exposure[44] found poorer active avoidance learning and visual discrimination retention in rats exposed to PCBs prenatally but not in those lacking prenatal exposure who were nursed by PCB-exposed dams.

Possible mechanisms of action

As stated above developmental neurotoxicity of PCBs has been reported in humans and confirmed in several laboratory species, including non-human primates. The mechanisms of action responsible for the effects of PCB and dioxin exposure on early central nervous system (CNS) development are not well understood. During the last 20 years there has been an attempt to understand the cellular basis of PCB-induced behavioral and neurological effects in animal models. The Ah receptor has been identified in vitro as a mediator in the production of toxic compounds following exposure to TCDD and the structurally similar non-ortho planar PCBs,[48,49] but the role of the Ah receptor in PCB neurotoxicity is not clear.[36]

Exposure of adult animals to a single relatively high dose of PCBs decreases the content of several brain neurotransmitters, while repeated exposure to lower PCB doses appears to affect brain dopamine (DA) metabolism.[45] The mechanism by which PCB affects DA metabolism remains unclear.[45] Sub-chronic exposure to PCBs in the non-human primate can result in long-lasting changes in brain DA concentrations.[46] Interactive effects of several PCB congeners (three or more) found in environmental samples such as human milk and blood, contaminated fish, and brain samples from PCB-treated animals can result in biological effects. These mixtures of PCB congeners fit a dose-additive model, indicating that there is a specific site of action for these PCB congeners other than the Ah receptor. Environmental mixtures contain mostly non-planar PCB congeners, and because they appear to be biologically active, the potential human health risk by this

group of chemicals should be considered in the risk assessment of PCBs.[47]

Considerable attention has been focused on the potential of PCBs to disrupt endocrine function. On the long list of endocrine disrupting agents,[50] PCBs and dioxins are also mentioned. Several studies have provided evidence of endocrine disruption by PCBs and organochlorine pesticide contaminants in wildlife including reproductive abnormalities and decreased fertility in birds, reptiles, fish and mammals.[51,52] Much research has focused on the estrogenic properties of these compounds, although androgens and antiandrogens or other hormones can be affected as well. PCBs and dioxins have been argued to interfere with thyroid hormone action and thus may affect the developing and mature brain.[53] Such problems have not only been recorded in species inhabiting polluted areas, but were also evident in wildlife in many other regions of the world.

Maternal thyroid hormone transported to the fetus is important for the first trimester brain development prior to fetal synthesis, and contributes to 18% of fetal thyroid hormone after autonomous synthesis has begun.[53] Thyroid hormone is necessary to stimulate neuronal and glial proliferation and differentiation during late gestational and early postnatal periods.[54] Severe thyroid hormone deficiency during this period causes spasticity and mental retardation.[53,54] PCBs may mimic or block the actions of thyroid hormones and have been shown to decrease both total TT4 and free FT4 in pregnant wistar rats and in very high doses, in their fetuses.[55] In our study it was reported by Koopman-Esseboom et al,[5] that in utero exposure to total PCB/dioxin TEQs reduced infants' thyroid hormone level at 2 weeks and 3 months. These reductions are small and all values are in the normal range. Given the vulnerability of the developing brain there are numerous alternative mechanisms through which PCBs and related compounds may disrupt fetal CNS development as well.

10.5 PRENATAL AND POSTNATAL PCB/DIOXIN EXPOSURE COMPARED WITH EARLY FEEDING TYPE ON DEVELOPMENTAL OUTCOME MEASURES

Contribution of prenatal and postnatal exposure

Despite the fact that an infant receives a much greater amount of PCBs and dioxins through breast milk, than across the placenta,[56,57] most adverse associations are found with prenatal exposure (Table 10.1), rather than with postnatal and current exposure (Table 10.2). Cord and maternal plasma PCB levels are used as measures for prenatal PCB exposure. In our study we used breast milk dioxin, planar and mono-ortho PCB concentrations as a measure for prenatal exposure, because these congeners could not be measured in maternal and cord blood and they are a good reflection of the maternal body burden.

In the Michigan[23,26] and North Carolina cohort[58] no associations between neurological and behavioral outcome and postnatal exposure to PCBs through breast milk were found, when offspring was tested between infancy and early childhood. At 4 years of age, reduced activity was reported with higher contemporary PCB levels.[59] The effect was seen in children who had been breast-fed for 1 year and whose mothers had above average PCB levels.[59] These levels were mainly predicted by lactational exposure. In our cohort postnatal exposure contributed to PCB-related neurotoxicity at 7 months with lower Bayley psychomotor scores in the highest lactational exposure group.[8] Lactational exposure was defined as the milk PCB/dioxin TEQ concentration multiplied with the number of weeks of breast feeding. At 42 month of age we report neurobehavioral as well as health effects in association with 42 month PCB body burden. Since PCB levels at 42 months are predicted mainly by duration of breast-feeding and maternal PCB levels, these associations are possibly related to postnatal exposure. However as we reported in Chapter 2.2, PCB levels at 42 months in the formula-fed group are predicted by in utero transfer of PCBs, suggesting that the child's body burden at preschool age is predicted by prenatal and postnatal PCB exposure, with lactational exposure as major determinant.[4] As our study is the first background exposure study to report postnatal exposure associated effects on neurobehavior and health status, other studies, human as well as animal, must investigate this association in order to support or reject these findings.

In most animal studies exposure began in utero making it impossible to differentiate prenatal from postnatal effects. In the study by Lilienthal and Winneke[44] in which rat pups were cross-fostered, pre- plus postnatal exposure produced greatest degree of behavioral impairment, followed by in utero exposure only. However, the results from the study in monkeys exposed postnatally, in which robust deficits were observed, suggests that postnatal exposure may be sufficient to produce long-term behavioral effects.[37,60]

The issue of the potential contribution of postnatal exposure to neurobehavioral and health effects is of critical concern since it has implications for recommendations regarding breast feeding, particularly in highly exposed populations. The majority of findings in human studies suggests that in utero exposure is the most relevant, studies in animals suggest that postnatal exposure can produce postnatal cognitive deficits. In addition the most predictive biomarker may vary between several endpoints and may change as the children become older. This is an issue that requires further research.[37] In our study we report that most effects on growth, health and neurobehavior are associated with prenatal PCB/dioxin exposure.

The influence of early feeding type on developmental outcome

Numerous studies on infant nutrition show that breast-feeding has a beneficial effect on growth, morbidity and neurological and cognitive functioning later in life.[61-63] Evidence from longitudinal studies has shown repeatedly that children who are breast-fed show small increases over formula-fed children in mean test scores on measures of intelligence and academic achievement, with these differences persisting

after controlling for confounding variables.[64] Moreover, data from an experimental study of feeding practices among preterm infants showed that children who were fed with breast milk had higher developmental scores at 18 months and higher intelligence quotients assessed at 7.5 years compared to those who were not.[65] Neurodevelopmental research has suggested that factors in breast milk that may be responsible for the improved cognitive abilities of breast-fed children may involve long chain polyunsatured fatty acids (PUFA) and, particularly, docosahexaenoic acid (DHA). Recently it was reported that breast-feeding is associated with small but detectable increases in child cognitive ability and educational achievement.[66] These effects were described to be pervasive, being reflected in a range of measures including standardized tests teacher ratings and academic outcomes in high school and relatively long-lived extending throughout childhood into young adulthood.

Our study was designed to include infants who were breast-fed for *at least* six weeks to guarantee a substantial level of lactational PCB/dioxin exposure next to a formula-fed group, which represents children predominantly exposed to transplacental PCBs. In our cohort there are significant differences between the breast-fed and formula-fed group. The breast-fed group is more advantaged when compared with the formula-fed group. Educational and professional level of both parents are higher, quality of intellectual stimulation by parents (HOME score) and parental verbal IQ level are higher in the breast-fed group. On the other hand PCB/dioxin exposure is higher in children breast-fed during infancy due to their additional lactational exposure. Given the disadvantage that breast milk is polluted with persistent organic compounds, we suggest that the higher socio-economic environment in which these children are raised, or other positive influences through breast-feeding, might have counterbalanced the negative effect of lactational PCB/dioxin exposure. In addition we also investigated the influences of breast-feeding and associated factors on neurobehavioral outcome.

Results from the Dutch cohort show that breast-feeding is positively associated with psychomotor and mental development at 7 month of age.[8] At 18 months of age, an advantageous effect of breast-feeding on the quality of movements in terms of fluency was found.[7] At 42 months of age, a beneficial effect of breast-feeding on the fluency of movements was found (odds ratio for non-optimal fluency 0.56 [95% confidence interval 0.37-0.85], after adjustments for study center and social, obstetrical, perinatal, and neonatal neurological differences.[67]

Table 10.3 represents results from multivariate linear or logistic regression analyses performed in the Rotterdam cohort (n=207) with feeding type as binary variable, breast-feeding (=1) versus formula-feeding (=0), and breast-feeding period as continuos variable in number of weeks, as variable of interest. Developmental outcome measures assessed during the study from birth until preschool age in the Rotterdam cohort were entered in the regression as dependent variables. In the final model, maternal age, maternal education, parity, gender, smoking and alcohol use during pregnancy as well as maternal ΣPCB levels were entered in the regression. At 42 month

of age additional covariables were HOME score at 42 months and parental verbal IQ. After adjustment for covariables, breast-feeding was positively associated with a higher score on verbal comprehension assessed with the RDLS at 42 months of age. Moreover, assessment of attentional processes during free play, showed that breast-feeding was associated with longer period of play behavior, but was negatively associated with the period of low-level play. Breast-feeding was associated with less number episodes of play as well as nonplay behavior suggesting less shifts of attention during free play.

Table 10.3 Developmental outcome in relation to feeding type, at 3, 7, 18 and 42 months of age, after adjustment for confounding variables

Outcome	n	Breast-feeding period (wk.) [a]	regression coefficient (SE)	P-value	
42 months					
RDLS	192	6-16	0.91 (1.64)	0.58	
		>16	5.05 (1.67)	0.003	
		FF=0 vs BF=1		Breast-feeding period	
		Regr coefficient (SE)	P-value	Regr coef (SE)	P-value
Play behavior	170				
Play time					
-ln(900-playtime)		0.51(0.21)	0.02	0.016(0.007)	0.02
Low level play[b]					
OR [95 CI]		0.21[0.09-0.48]	0.0002	0.96 [0.93-0.98]	0.003
Play episodes		-5.73(1.46)	0.001	-0.16(0.05)	0.003
Non-play behavior					
Number nonplay-episodes					
-ln(nonplay-episodes)		-0.39(0.13)	0.004	-0.015(0.004)	0.001

Regressions adjustment for HOME, maternal verbal IQ, maternal age, parental education, maternal smoking and alcohol use during pregnancy, birth order and gestational age.

a: Breast-feeding period was divided into two categories, 6 to 16 weeks and more than 16 weeks. "0" = formula-feeding, "1"=breast-feeding 6-16 weeks or >16 weeks.

In the final model for free play the following covariates were included; maternal education (low/middle vs high level), maternal age, HOME score, Parity (first vs second), Sex(boys vs girls), alcohol and smoking during pregnancy (no vs yes) and maternal plasma PCB levels, next to feeding type and breast-feeding period. Logistic regression analysis was performed, due to a skewed distribution.

b: Low level play time/ episodes were dichotomized into 0= no periods/episodes and 1= at least one period/ episode of low level play.

In conclusion, breast-fed children score slightly higher on several neurodevelopmental tests and after adjustment for several confounding variables such as socio-economic background and intellectual stimulation, these differences, although small still appear to be significant. Substances in breast milk, such as long

chain PUFA's, certain hormones, e.g. thyroid hormone, prolactin, gonadotrophin hormone, adrenal gland hormones, nucleotides, epidermal growth factors (EGF).[68] which are not available in formula-feeding or factors associated with breast-feeding, other than HOME and parental education level, may have contributed to this positive influence on development. Although large quantaties of PCBs and dioxins and other persistent and lipophilic contaminants are transferred to the developing infant, we do not discourage breast-feeding based on our results that most effects are related to prenatal rather than postnatal PCB/dioxin exposure.

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CHAPTER 11

11.1 SUMMARY

In the preceding chapters results of the Dutch PCB/dioxin study, a prospective follow-up study from 50% breast-fed and 50% formula-fed infants, were presented. The primary goal of this study was to gain insight into the possible toxic effects associated with perinatal background PCB and dioxin exposure and consequences for later development.

Chapter 1 introduces the subject and relevant literature is reviewed. Results from the study until 18 months of age are summarized, and objectives of the present study up to 42 months are given. Polychlorinated biphenyls (PCBs) as well as dioxins (polychlorinated dibenzo-p-dioxins (PCDDs) and -dibenzo-furans (PCDFs), are persistent organic pollutants. These contaminants have been identified in almost every component of the global ecosystem including air, water, sediments, fish, wildlife and human tissues, in particular human milk. Dairy products, meat and meat products as well as fish consumption are the major source for PCB and dioxin accumulation in human fat tissue. In pregnancy the fetus is exposed to these toxic and teratogenic compounds during a critical period of organ growth and development. After birth, breast milk is the major source of PCB and dioxin exposure in the nursing infant. Lipids in formula milk are mainly of vegetable origin, therefore the postnatal exposure in formula-fed infants is negligible. The Dutch PCB/dioxin study started in 1989 as a collaborative study with human and animal research institutes together with laboratories for PCB and dioxin analysis in human plasma and breast milk. The follow-up of the Dutch cohort was expanded until 42 months of age in an EC-funded multicenter cohort study together with Germany and Denmark, Contract-No EV5V-CT92-0207.

From June 1990 until June 1992, 418 term born babies were included in the Dutch study, of whom 209 were breast-fed (BF) for at least six weeks and 209 were formula-fed (FF). Subjects were recruited from Rotterdam and surroundings (n=207), a highly industrialized and densely populated area in the Western part, and from the Groningen area (n=211), a semi-urban region in the North of the Netherlands. Children were examined at 10 days, 3, 7, 18 and 42 months of age. Results presented in this thesis are focused on the follow-up period at 42 months. For major developmental endpoints, the cognitive and neurological development at 42 months, combined results are presented for the Rotterdam and Groningen cohort. Outcome variables concerning physical growth, immunological and neurobehavioral endpoints were collected and presented for the Rotterdam cohort only.

In Chapter 2.1 all levels of PCBs and dioxins are summarized and the toxic equivalents (TEQs) according to the WHO meeting in 1997 are incorporated. Intrauterine exposure was estimated from PCB concentrations measured in maternal plasma in the

last month of pregnancy and from PCB concentrations measured in cord blood collected directly after birth. Prenatal exposure to PCBs and dioxins was defined from breast milk PCB and dioxin concentrations collected from the breast feeding mothers 2 weeks after birth. Lactational exposure was estimated from breast PCB and dioxin concentrations multiplied by the number of weeks of breast-feeding.

The child's current PCB body burden assessed from plasma PCB concentrations at 42 months of age, and the most important predictors for this current exposure are summarized for the breast-fed and the formula-fed group in **Chapter 2.2.** Median plasma PCB concentrations at 42 months of age are nearly 4 times higher in the BF group when compared with their FF counterparts. In children BF during infancy, PCB levels are correlated with the duration of breast-feeding and with PCB concentration in breast milk. In children FF during infancy, PCB levels are strongly related to their maternal and cord plasma PCB concentration. Dietary intake of PCBs during preschool years predicts only marginally to plasma PCB levels in preschoolers.

Dietary habits as well as daily PCB and dioxin intake in 42-month-olds was measured with a validated food questionnaire (FQ), especially designed for 1 to 4 years olds. In 42-month-old children, daily dietary intake of planar PCB-TEQ and dioxin-TEQ is measured with the FQ. After weaning, dairy products, meat and meat products and processed foods are the major contributors of PCB and dioxin intake. Fish plays only a marginal role with respect to PCB/dioxin exposure in this population. Since food is the major source for PCB and dioxin accumulation in the human body, food intake from early ages until reproduction age, e.g. 25 years is important to assess exposure risk for the next generation. Therefore, the contribution of lactational exposure to PCBs and dioxins during breast-feeding is compared with long-term dietary exposure. In **Chapter 3** a model is presented to calculate the cumulative PCB- and dioxin-TEQ intake during breast-feeding, 0-1 year. Cumulative PCB- and dioxin-TEQ intake from 1-5 years was estimated using the TEQ intake measured with the FQ. Cumulative PCB and dioxin-TEQ intake from 6-25 years was estimated using national food consumption and contamination data of PCB- and dioxin-TEQ intake. The daily TEQ intake per kg body weight (BW) in breast-fed infants is 50 times higher (118 pg TEQ/kg BW), and toddlers (6.5 pg TEQ/kg BW) is 3 times higher than in adults (2.3 pg TEQ/kg BW). These values exceed the recently proposed tolerable daily intake (TDI) of 1-4 pg TEQ/kg BW by the WHO. Breast-feeding for 6 months, contributes 12% in boys and 14 % in girls, to the cumulative PCB/dioxin TEQ intake until 25 years of age. Dairy products, processed foods and meat are major contributors of PCB and dioxin accumulation until reproduction age. Lowering maternal PCB body burden can be achieved by reducing PCB and dioxin accumulation in the food chain. Maternal transfer of PCBs and dioxins to the next generation must be avoided by enforcement of strict regulations of PCB and dioxin discharge, and by reducing consumption of animal products and processed foods, starting at all ages, rather than by discouraging breast-feeding.

In **Chapter 4**, results on fetal and postnatal growth are presented in relation to background perinatal PCB and dioxin exposure. Birth weight, length and head circumference were measured at 10 days, 3, 7, 18 and 42 months of age. After

adjustment for covariates, cord and maternal plasma PCB concentrations are both negatively associated with birth weight. Infants with high cord plasma PCB levels weighed 165 grammes less compared to infants with low cord plasma PCB levels. This effect of prenatal PCB exposure is comparable with the negative effect of maternal smoking and alcohol use during pregnancy and birth weight. Cord and maternal plasma PCB levels where both significantly associated with lower growth rate, defined as change in standard deviation score (SDS) of weight, length and head circumference from birth to 3 months. No negative effects of prenatal nor postnatal PCB and dioxin exposure on growth rate were found from 3 to 42 months of age. Although the reported effects are small, intra-uterine and postnatal growth retardation are potentially harmful to the developing human and may have consequences for later life.

In **Chapter 5**, effects of environmental exposure to PCBs and dioxins on cognitive abilities in 42-month-olds is described. Cognitive abilities were assessed with the Dutch version of the Kaufman Assessment Battery for Children (K-ABC) in 395 children living in Rotterdam (n=193) and Groningen (n=202). In the Rotterdam cohort verbal comprehension was additionally assessed with the Dutch version of the Reynell Language Developmental Scales (RDLS). After adjustment for covariables, maternal plasma PCB concentrations are significantly associated with lower scores on the overall cognitive as well as sequential and simultaneous processing scales of the K-ABC. The highest exposed group scored 4 points lower on all three scales of the K-ABC when compared with the lowest exposed group. In the FF group, prenatal PCB exposure is associated with lower scores on the three scales of the K-ABC as well as with verbal comprehension of the RDLS. In the BF group however, the effect did not show statistical significance. Both lactational and current exposure to PCBs and dioxins are not related to 42-month cognitive performance. It is concluded that in utero exposure to background PCB concentrations is associated with poorer cognitive functioning in preschool children. Children of mothers at the upper end of exposure are especially at risk.

In **Chapter 6** the neurological condition in 42-month-old children in relation to pre- and postnatal exposure to PCBs and dioxins is presented. Adverse neurological effects of exposure to PCBs have been found up to 18 months of age. The persistence of these effects found earlier were studied at 42 months of age. For this purpose 394 children living in Rotterdam and Groningen were evaluated for their neurological condition using the Touwen/Hempel method. After adjustment for covariates, no adverse effects of prenatal PCB exposure on the neurological condition were found at 42 months of age. In addition, neither the lactational exposure nor the child's current body burden was related to the neurological optimality at 42 months of age. However, as maturation of the brain is an ongoing process from conception to many years after birth, during which many morphological changes occur, it can not be concluded that there will be no effect of PCB and dioxin exposure on the child's neurological condition at later ages.

In **Chapter 7**, attentional processes and activity behaviour, which are important for later cognitive performance, are described in 42-month-old Dutch children with environmental exposure to PCBs and Dioxins. Attentional processes are measured by a structured

behavioral observation of a free play session. During free play behavior observation the periods and episodes of exploration, high level play, low level play and nonplay behavior (e.g. doing nothing, visual scanning, manipulation of other objects) were assessed. Reaction Time and sustained attention is measured by a computer game, especially designed for preschool children. Inattention and hyperactive behaviour was assessed with the Groninger Behavior Observation Scale (GBO), parent version. After adjustment for covariates, cord and maternal plasma PCB concentrations are both significantly associated with less time spent for high level play. Cord plasma PCB concentrations are also associated with longer periods and more swifts of attention during non play behaviour. Current PCB body burden is significantly related to slower mean Reaction Times and more signs of hyperactive behavior reported by GBO parent. Current PCB body burden is associated with less sustained attention. In conclusion, attentional processes might be impaired by pre- as well as postnatal PCB exposure. Whether these small but significant adverse effects will attenuate or increase in later life e.g. school age remains to be determined in a further follow-up.

In **Chapter 8**, problem behavior in Dutch preschool children is described in relation to environmental PCB and dioxin exposure. Problem behavior was assessed with the Dutch Child Behavior Checklist (CBCL). Mothers, fathers and day care teachers were asked to complete a CBCL. Logistic regression analyses showed that, after adjustment for covariates in utero exposure to maternal and cord plasma ΣPCB levels, and breast milk ΣTEQs, are associated with a higher prevalence of withdrawn/depressed behavior reported by day care teachers. Moreover, teacher report on child behavior is influenced by the child's cognitive development. This is the first time that withdrawn/depressed behavior is reported in relation to prenatal PCB and dioxin exposure. It is hypothesized that withdrawn/depressed behavior might be secondary to poorer cognitive functioning. These results are in accordance with our recently reported poorer cognitive abilities in prenatal PCB exposed 42-month-old children. Although these children are all normal and healthy, early identification of cognitive deficits and consequently more deviant behavior among preschoolers may help to prevent later learning problems.

In **Chapter 9** possible immunotoxic effects in Dutch toddlers are presented in relation to their perinatal and current PCB and dioxin exposure. The prevalence of infectious and allergic diseases was assessed by parent questionnaire. Humoral immunity was measured by detecting antibody levels to mumps, measles and rubella after primary vaccination. Immunological marker analyses of lymphocytes were done in a subgroup of 89 children. At toddler age prenatal PCB exposure was associated with an increased number of T-cells as well as CD8+ (cytotoxic), TcR αβ+ and CD3+HLA-DR+ (activated) T cells, lower antibody levels to measles, a higher prevalence of chickenpox, and less shortness of breath with wheeze while current PCB exposure was associated with a higher prevalence of recurrent middle ear infections and a lower prevalence of allergic reactions. In Dutch toddlers the effects of perinatal background exposure to PCBs and dioxins persist into childhood and might be associated with a greater susceptibility to infectious diseases. Common infections acquired early in life may possibly prevent the development of atopy and PCB exposure was associated with a lower prevalence of allergic diseases.

In Chapter 10 all adverse exposure effects are summarized until 42 months of age, according to the type of effect and time of exposure (prenatal, postnatal and current exposure). In addition our results are compared to other animal and human literature in this field. The role of early feeding type within the spectrum of neurobehavioral and cognitive outcome measures are discussed.

11.2 CONCLUSIONS

We conclude that significant associations between perinatal background PCB/dioxin exposure and adverse effects on growth, immunologic parameters, and neurodevelopmental and behavioral effects are found in a healthy population. Some effects were found during infancy (neonatal and 18 month neurological condition, birth weight and growth rate). Some of effects are still present at preschool age (immunological effects). Other effects are not revealed until preschool age (poorer cognitive functioning, less focused attention, withdrawn/depressed behavior). Based on our results the question remains to be answered whether adverse effects are transient or persistent up to older ages. Therefore this study must be followed up to school age or puberty period to study persistent neurobehavioral endpoints and reproductive outcome variables as well.

Preschool children breast-fed during infancy, have a four times higher PCB body burden than their formula-fed counterparts. Six months of breast-feeding contributes about 14% of total PCB/Dioxin TEQ intake until adulthood (25 y), e.g. reproductive age in women. The daily PCB/Dioxin TEQ intake exceeds the recently revised Tolerable Daily Intake of 1-4 pg TEQ/kg body weight at all ages, beginning at preschool years. Given that most adverse affects are associated with in utero exposure, strict regulations and enforcement of these regulations could reduce the maternal PCB/dioxin body burden, and thereby pre- and postnatal exposure. Strategies should therefore be directed towards reducing PCB and dioxin intake through the food chain at all ages, by lowering the consumption of animal products and processed foods and not at discouraging breast-feeding.

The congruence of findings between studies in humans and animal models, as well as the similarity of body burden at which neurotoxic effects are observed, support the hypothesis that PCB expo\sure produces neurotoxicity in humans at environmentally relevant levels. The mechanism of action is not elucidated. Additional research from laboratory studies on neurochemical effects and crucial developmental processes is required.

The PCB-related effects on development could provide as an example of the effects of "endocrine disrupting"environmental chemicals on human development. Endocrine disrupters could alter the hormonal and growth factor milieu of the developing fetus during a critical period of development. Future follow-up studies must look into the spectrum of hormonal-related effects of PCBs, there metabolites and other related compounds.

CHAPTER 12

12.1 SAMENVATTING

In de voorgaande hoofdstukken zijn de resultaten gepresenteerd van de Nederlandse PCB/dioxine studie, ook wel genoemd "Het Nederlandse moedermelk project", een prospectief follow-up onderzoek bij borst en fles gevoede kinderen. Het doel was inzicht te verkrijgen in de mogelijke schadelijke effecten van intra-uteriene en postnatale blootstelling aan PCBs en dioxinen, en de consequenties hiervan voor de verdere ontwikkeling van het jonge kind.

In **Hoofdstuk 1** wordt een kort overzicht gegeven van de relevante literatuur op het gebied van perinatale blootstelling aan PCBs en dioxinen. De resultaten van het onderzoek tot en met de leeftijd van 18 maanden zijn samengevat en de doelstellingen van het vervolgonderzoek tot en met de leeftijd van 42 maanden worden beschreven. Polychloorbiphenylen (PCBs) en dioxinen, polychloor-dibenzo-p-dioxinen (PCDDs) en polychloor-dibenzo-furanen (PCDFs), zijn lipofiele en zeer stabiele toxische stoffen die in ons milieu terecht zijn gekomen. Deze contaminanten zijn inmiddels in bijna alle componenten van het ecosysteem aangetoond, zoals water, lucht, vis, wild, vogels en ook de mens. De mens staat aan het einde van de voedselketen en de belangrijkste bron van PCB/dioxine blootstelling is de consumptie van melk en melkprodukten, vleesprodukten en vis. Deze stoffen accumuleren zich in het vetweefsel en tijdens de borstvoeding worden deze aan vetgebonden stoffen ook uitgescheiden in moedermelk. Gedurende de zwangerschap, in een kritische fase van groei en ontwikkeling, wordt het ongeboren kind reeds blootgesteld aan PCBs en dioxinen. Na de geboorte is borstvoeding (BV) de grootste bron van PCBs/dioxinen voor het zogende kind. In flesvoeding (FV) zijn de dierlijke vetten vervangen door plantaardige vetten, waardoor de PCB/dioxine belasting in flesgevoedde zuigelingen verwaarloosbaar klein is. De Nederlandse PCB/dioxine studie startte in 1989 in samenwerkingsverband met klinische (Sophia Kinderziekenhuis/Erasmus Universiteit te Rotterdam en Rijksuniversiteit te Groningen) en dierexperimentele (Landbouw Universiteit Wageningen en TNO te Rijswijk) onderzoeksinstituten, en laboratoria (TNO in Zeist en RIKILT in Wageningen) waar de PCBs en dioxinen in plasma en moedermelk zijn geanalyseerd. Het vervolgonderzoek van het Nederlandse cohort op de leeftijd van 42 maanden werd gefinancierd door de Europese Gemeenschap "contract-no EV5V-CT92-0207" waarin ook Duitsland en Denemarken participeren.

Vanaf juni 1990 tot en met juni 1992 werden 418 à term geboren zuigelingen in de studie geïncludeerd. Tweehonderd en negen zuigelingen kregen borstvoeding gedurende minstens 6 weken en 209 zuigelingen kregen flesvoeding. De onderzoekspopulatie is verzameld in Rotterdam en omgeving (n = 207), een sterk geïndustrialiseerde en dichtbevolkte omgeving in het westen, en in Groningen en

omgeving (n = 211), een minder verstedelijkt gebied in het noorden van Nederland. De kinderen werden onderzocht op de leeftijd van 10 dagen, 3, 7, 18 en 42 maanden. In dit proefschrift worden met name de gegevens verkregen op de leeftijd van 42 maanden beschreven. De resultaten van de cognitieve en neurologische ontwikkeling zijn gepresenteerd voor het Rotterdamse en Groningse cohort. De gegevens betreffende groei, immunologie en gedrag zijn alleen in het Rotterdamse cohort onderzocht en beschreven.

In **Hoofdstuk 2.1** worden de PCB en dioxine concentraties en toxische equivalenten (TEQs) volgens de WHO bijeenkomst in 1997 beschreven. Als maat voor de intra-uteriene blootstelling aan PCBs werd genomen de som van de 4 PCB congeneren IUPAC nummers 118, 138, 153 en 180, gemeten in maternaal bloed gedurende de laatste maand van de zwangerschap, en in navelstreng bloed direct afgenomen direct na de geboorte. De PCB/dioxine concentratie gemeten in moedermelk 2 weken na de geboorte werd ook gebruikt als maat voor de prenatale blootstelling aan PCBs en dioxinen. De postnatale blootstelling aan PCBs en dioxinen werd berekend uit het produkt van de PCB/dioxine concentratie in moedermelk en het aantal weken borstvoeding.

Op de leeftijd van 42 maanden werd de PCB lichaamsbelasting berekend uit de som van de 4 PCBs gemeten in plasma van 42 maanden oude kinderen. De belangrijkste voorspellende parameters voor deze huidige lichaamsbelasting werden beschreven voor de BV en FV groep in **Hoofdstuk 2.2**. De mediane plasma PCB concentratie op 42 maanden in de BV groep is bijna 4 keer hoger dan die in de FV groep. In de BV groep zijn plasma PCB concentraties sterk gerelateerd aan de borstvoedingsduur en de PCB concentraties gemeten in moedermelk en/of moederlijk bloed. In de FV groep is er een sterke relatie aanwezig tussen plasma PCB concentraties op 42 maanden en de PCB concentraties in moederlijk bloed of navelstrengbloed. De inname van PCBs en dioxinen via de voeding op de peuterleeftijd voorspelt slechts marginaal de plasma PCB concentratie op deze leeftijd.

Omdat de voeding de grootste bron is voor PCB/dioxine accumulatie in het menselijk lichaam zijn voedingsgewoontes vanaf de geboorte tot en met de reproduktieve leeftijd (25 jaar) een belangrijke factor voor de mate van blootstelling aan de volgende generatie. De dagelijkse inname van planaire PCBs en dioxinen via de voeding en ook de voedingsgewoontes op de leeftijd van 42 maanden werden gemeten met behulp van een gevalideerde voedingsvragenlijst ontwikkeld voor kinderen van 1 tot 4 jaar oud. Na de zuigelingen periode zijn melk- en vleesprodukten, en voedingsstoffen bewerkt met industriële oliën en vetten, de belangrijkste bronnen voor de PCB en dioxine inname. Visconsumptie draagt op de peuterleeftijd weinig bij aan de PCB/dioxine inname. De bijdrage van borstvoeding gerelateerde blootstelling aan PCBs/dioxinen werd vergeleken met de blootstelling via de voeding op de lange termijn (na de BV).

In **Hoofdstuk 3** wordt een model gepresenteerd waarmee de cumulatieve PCB en dioxine TEQ consumptie kan worden berekend tijdens de borstvoeding in het eerste levensjaar. De cumulatieve PCB en dioxine TEQ consumptie van 1 – 5 jaar werd

geschat met behulp van de gevalideerde voedingsvragenlijst. Cumulatieve PCB en dioxine TEQ consumptie van 6 – 25 jaar werd geschat met behulp van gegevens van de Nederlandse Voedsel Consumptiepeiling gegevens 1992 en gegevens van het RIVM over PCB/dioxine gehaltes in voedingsstoffen. De dagelijkse TEQ consumptie per kg lichaamsgewicht (LG) in borst gevoede zuigelingen is 50 keer hoger (118 pg TEQ/kg LG), en in peuters (1-5 jaar) 3 keer hoger (6.5 pg TEQ/kg LG) dan in volwassenen (2.3 pg TEQ/kg LG). Deze waarden overschrijden de recent geadviseerde (WHO) "tolerable daily intake" (TDI) welke 1 – 4 pg TEQ/kg LG bedraagt. Zes maanden borstvoeding draagt in jongens 12 %, en in meisjes 14 % bij aan de cumulatieve PCB- en dioxine TEQ consumptie tot 25 jarige leeftijd. Verlaging van moederlijke PCB belasting dus de overdracht van PCBs en dioxinen aan het kind, kan bereikt worden door het verlagen van PCB/dioxine gehaltes in voeding. Overdracht van moederlijke PCBs en dioxinen aan de volgende generatie moet voorkomen worden door strikte regelgeving met betrekking tot de uitstoot en verwerking van PCBs en dioxinen voordat zij in ons milieu terecht komen. De consumptie van dierlijke producten moeten worden verlaagd op alle leeftijden. Het geven van borstvoeding wordt niet ontraden om de volgende redenen. De bijdrage van BV aan de totale PCB/dioxine inname op lange termijn is relatief laag is vergeleken met de intake via de voeding. Er zijn ook positieve invloeden van BV beschreven zijn op de groei en ontwikkeling.

In **Hoofdstuk 4** werden effecten van achtergrondsblootstelling aan PCBs en dioxinen op de foetale en postnatale groei gepresenteerd. Geboortegewicht werd gemeten direct na de geboorte en gewicht, lengte en hoofdomtrek werden gemeten op de leeftijd van 10 dagen, 3, 7, 18 en 42 maanden. Na correctie voor covariabelen die van belang zijn voor de groei, bleken PCB gehalten gemeten in zowel navelstreng bloed als moederlijk bloed, negatief geassocieerd te zijn met het geboortegewicht. Kinderen met een hoge PCB concentratie in navelstrengbloed (p90) wogen 165 gram minder dan kinderen met een lage PCB concentratie (p10) in navelstreng bloed. PCBs in navelstreng en maternaal bloed zijn geassocieerd met een lagere groeisnelheid van 0 tot 3 maanden (gedefinieerd als het verschil in standaard deviatie score van gewicht, lengte en schedelomtrek tussen 0 en 3 maanden). Van 3 tot en met 42 maanden werden geen negatieve effecten gevonden van de pre- of postnatale PCB/dioxine blootstelling op de groei. De beschreven effecten zijn gering. Intra-uteriene en postnatale groeivertraging zijn echter potentieel schadelijk voor het ontwikkelende kind en kunnen consequenties hebben voor de gezondheid en ontwikkeling op latere leeftijd.

In **Hoofdstuk 5** werden de effecten beschreven van blootstelling aan PCBs/dioxinen op de cognitieve ontwikkeling van 42 maanden oude kinderen. Cognitieve vaardigheden werden gemeten met behulp van de Nederlandse versie van de Kaufman Assessment Battery for Children (K-ABC), ook wel bekend als de Groningse Ontwikkelings Schalen (GOS), bij 395 kinderen in Rotterdam (n = 193) en Groningen (n = 202). In de Rotterdamse groep werd ook het taalbegrip gemeten met de Nederlandse versie van de Reynell Language Developmental Scales (RDLS). Na correctie voor covariabelen was de maternale PCB plasma concentratie negatief geassocieerd te zijn met de scores voor de cognitieve, de simultane en sequentiële verwerkingsschaal van de K-ABC. Kinderen

met de hoogste blootstelling scoorden gemiddeld 4 punten lager op alle drie schalen van de K-ABC, dan kinderen met een lagere blootstelling. Bij het apart analyseren van de 2 voedingsgroepen blijkt dat in de FV groep de prenatale PCB blootstelling is geassocieerd met lagere scores op alle schalen van de K-ABC, en ook op de taalbegripscore van de RDLS. Echter, in de BV groep was dit effect statistisch niet significant. Postnatale blootstelling aan PCBs/dioxinen en de huidige PCB lichaamsbelasting op 42 maanden zijn beide niet gerelateerd met de uitkomsten op de K-ABC en de RDLS op de leeftijd van 42 maanden. Intra-uteriene blootstelling aan PCBs is geassocieerd is met een lagere ontwikkelingsscore in peuters. Kinderen van moeders met een hoge PCB lichaamsbelasting lopen het hoogste risico.

Hoofstuk 6 beschrijft de relatie tussen de neurologische ontwikkeling van het 42 maanden oude kind en de pre- en postnatale blootstelling aan PCBs/dioxinen. Nadelige neurologische effecten van deze blootstelling zijn reeds eerder beschreven tot de leeftijd van 18 maanden. Nu werd onderzocht in hoeverre deze effecten persisteren op de leeftijd van 42 maanden. Bij 394 Rotterdamse en Groningse kinderen werd de neurologische ontwikkeling onderzocht met behulp van de Touwen/Hempel methode. Na correctie voor covariabelen werden op de leeftijd van 42 maanden geen nadelige effecten gevonden van prenatale blootstelling aan PCBs. Er werd ook geen relatie gevonden tussen enerzijds de neurologische conditie en anderzijds totale lichaamsbelasting van PCBs en de borstvoeding gerelateerde blootstelling aan PCBs/ dioxinen. Omdat de rijping van het centraal zenuwstelsel een continu proces is beginnend bij de bevruchting en pas eindigend jaren na de geboorte, kan een nadelig effect van blootstelling aan PCBs/dioxinen op de neurologische ontwikkeling op latere leeftijd niet uitgesloten worden.

In Hoofdstuk 7 worden aandachtsprocessen bij 42 maanden oude kinderen onderzocht in relatie tot achtergrondblootstelling aan PCBs en dioxinen. Aandachtsprocessen werden gemeten tijdens de observatie van het spelgedrag. Tijdens het spelgedrag werd gekeken naar het aantal episoden en de duur van; 1) exploreren van speelgoed 2) hoog niveau spelgedrag 3) laag niveau spelgedrag en 4) gedrag dat niet tot spelen behoorde, zoals niets doen, om zich heen kijken en manipuleren van andere objecten dan speelgoed. Aandacht en concentratie van de kinderen werd ook gemeten met behulp van een computertaak, waarin de reactie tijden en de volgehouden aandacht werden gemeten tijdens een speciaal daarvoor ontworpen computerspel. Verminderde aandacht en overbeweeglijk gedrag werden gescoord middels een gedragsvragenlijst voor ouders. Na correctie voor covariabelen, blijken kinderen met een hoog navelstreng plasma en maternaal plasma PCB gehalte, een kortere periode hoog niveau spelgedrag te vertonen, vergeleken met kinderen met een laag navelstreng en maternaal PCB gehalte. Navelstreng bloed PCB concentraties waren ook geassocieerd met langere periodes van-, en vaker "switchen" van niet-spel gericht gedrag. De huidige PCB lichaamsbelasting van de kinderen op 42 maanden is gerelateerd aan langere reactie tijden en meer overbeweeglijk gedrag (gescoord door de ouders). In de BV groep is de huidige PCB lichaamsbelasting ook gerelateerd aan minder volgehouden aandacht. Aandachtsprocessen kunnen nadelig worden

beïnvloed door zowel pre- als postnatale blootstelling aan PCBs. Of deze nadelige invloed ook blijft tot op oudere leeftijd (o.a. op school) moet nog worden onderzocht.

In **Hoofdstuk 8** wordt de relatie tussen blootstelling aan PCBs/dioxinen en probleemgedrag (gerapporteerd door ouders en peuterspeelzaal of crèche leidsters) van peuters onderzocht. Probleemgedrag werd gemeten met behulp van de Dutch Child Behavior Checklist (CBCL). Moeders, vaders en peuterleidsters werden gevraagd een CBCL in te vullen. Op de CBCL ingevuld door de ouders werd geen relatie gevonden tussen PCB/dioxine blootstelling en probleemgedrag. Intra-uteriene blootstelling aan PCBs/dioxinen resulteerde in een hogere prevalentie van teruggetrokken/depressief gedrag gescoord door de peuterleidsters. Mogelijk is dit gedrag secundair aan het minder goed cognitief functioneren van deze kinderen. Deze resultaten zijn in overeenstemming met de resultaten beschreven in hoofdstuk 5, betreffende een lagere cognitieve ontwikkeling in 42 maanden oude kinderen in relatie tot prenatale PCB blootstelling.

In **Hoofdstuk 9** wordt de relatie tussen perinatale blootstelling aan PCBs/dioxinen en immunologische parameters van peuters onderzocht. De prevalentie van infecties en allergieën werd gemeten met behulp van een ziektevragenlijst voor ouders. Humorale immuniteit werd gemeten door het bepalen van antilichaam concentraties tegen de bof, mazelen en rode hond na de eerste vaccinatie. In een subgroep van 89 kinderen werd een immunologische markeranalyse verricht van lymfocyten, verkregen uit bloed afgenomen op de leeftijd van 42 maanden. Op de peuterleeftijd werd een associatie gevonden tussen prenatale PCB/dioxine blootstelling en een toegenomen aantal T-cellen, CD8+ (cytotoxische), TcR _ß+ and CD3+HLA-DR+ (geactiveerde) T-cellen, lagere antilichaam concentraties tegen mazelen, een hogere prevalentie van waterpokken, en minder aanvallen van benauwdheid. Huidige PCB belasting was geassocieerd met een hogere prevalentie van recidiverende middenoorontstekingen en een lagere prevalentie van allergische reacties. Infecties welke worden opgelopen op jonge leeftijd kunnen mogelijk de ontwikkeling van atopie tegengaan; PCB/dioxine blootstelling was gerelateerd aan een lagere prevalentie van allergieën. Perinatale blootstelling aan PCBs/dioxinen tot in de kinderleeftijd is wellicht geassocieerd met een grotere vatbaarheid voor infectieziekten.

In **hoofdstuk 10** werden alle aan PCB en dioxine blootstelling gerelateerde effecten, vanaf de geboorte tot en met de leeftijd van 42 maanden, nog eens samengevat. De effecten zijn ingedeeld naar type blootstelling (prenataal, postnataal en huidige blootstelling). Tevens werden onze resultaten vergeleken met resultaten van andere klinische en dierexperimentele studies. De rol van het type voeden (flesvoeding of borstvoeding) op de zuigelingenleeftijd in relatie tot de groei en ontwikkeling werd besproken.

12.2 CONCLUSIES

De resultaten van het Nederlandse PCB/dioxine onderzoek laten zien dat er significante negatieve associaties gevonden zijn tussen perinatale achtergrondblootstelling aan PCBs en dioxinen en de verschillende groei en ontwikkelingsparameters onderzocht in jonge kinderen tot en met de leeftijd van 42 maanden. Sommige effecten zijn alleen gevonden in de baby-periode (neonatale en 18 maanden neurologische ontwikkeling, het geboortegewicht en postnatale groei tot 3 maanden, schildklierfuncties tot 3 maanden, en de psychomotore ontwikkeling tot 7 maanden). Sommige effecten worden alleen gevonden op de peuterleeftijd (cognitieve ontwikkeling, aandachtsprocessen en het gedrag op 42 maanden) en sommige effecten persisteren vanaf de geboorte tot op de peuterleeftijd (immunologische effecten). Of deze effecten voorbijgaand zijn of persisteren tot op latere leeftijd moet nog worden onderzocht. Daarom worden deze kinderen inmiddels op de leeftijd van 7 jaar onderzocht. Uiteindelijk zouden deze kinderen tot in de puberteit en de jongvolwassen leeftijd onderzocht moeten worden om de effecten van achtergrondblootselling aan PCBs en dioxinen te onderzoeken op de verschillende ontwikkelingparameters. De effecten op de puberteitsontwikkeling en andere hormonale veranderingen kunnen dan ook worden onderzocht.

Peuters die borstvoeding kregen, hebben een PCB lichaamsbelasting die gemiddeld 4 keer hoger is dan peuters die flesvoeding hebben gehad. Zes maanden borstvoeding draagt 12% in jongens en 14% in meisjes bij aan de totale PCB/dioxine TEQ inname via de voeding tot op de volwassen leeftijd (25 jaar). De dagelijkse inname van PCBs en dioxinen overschrijdt de recent geadviseerde (WHO) Tolerable Daily Intake (TDI), welke 1-4 pg TEQ/kg lichaamgewicht bedraagt. De meeste negatieve effecten die zijn beschreven in dit onderzoek, zijn geassocieerd met de intra-uteriene PCB/dioxine blootstelling. Daarom moet de overdracht van moederlijke PCBs en dioxinen aan de volgende generatie worden voorkomen door strikte regelgeving met betrekking tot de uitstoot en verwerking van PCBs en dioxinen, voordat zij in ons milieu terecht komen. De consumptie van dierlijke producten zou moeten worden verlaagd op alle leeftijden. Het geven van borstvoeding hoeft niet te worden ontraden, omdat de bijdrage van borstvoeding aan de totale PCB/dioxine inname is relatief laag in vergelijking met de jarenlange inname via de voeding. Verder blijkt ook in onze onderzoekspopulatie, dat het geven van borstvoeding en daarmee samenhangende factoren, een positief invloed heeft op de ontwikkeling van het jonge kind.

De meeste uitkomsten van het Nederlandse PCB/dioxine onderzoek komen overeen met effecten die zijn beschreven in verschillende dierexperimentele en anderen humane studies. Dit bevestigt dat PCBs en, daaraan gerelateerde stoffen zoals dioxinen, neurotoxisch effecten kunnen veroorzaken bij gehalten zoals we die vinden bij een achtergrondblootstelling. De manier waarop deze stoffen op moleculair niveau in de verschillende weefsels hun negatieve effect veroorzaken is nog onduidelijk. Verder onderzoek is hiervoor noodzakelijk. Toekomstige studies moeten zich ook richten op de hormoon gerelateerde effecten van PCBs en aanverwante stoffen, en de effecten daarvan op de groei en ontwikkeling van de mens.

ABBREVIATIONS

Ah receptor	Aryl hydrocarbon receptor
AHH	Aryl Hydrocarbon Hydroxylase
BF	Breast-fed
BW	Body Weight
CBCL	Child Behavior Checklist from 2 to 3 year
CNS	Central Nervous System
EC	European Community
ELISA	Enzyme-Linked-Immunosorbent-Assay
EROD	Ethoxyresorufin-O-deethylase
FCS	Dutch Food Consumption Survey
FF	Formula-fed
FQ	Food Questionnaire
GC-ECD	Gas chromatography with electron capture detection
GC-HRMS	Gas chromatography-high-resolution mass spectrometry
hcSDS	Head circumference standard deviation score
HOME	Home Observation for Measurement of the Environment
hSDS	Height standard deviation score
IUPAC	International Union of Pure and Applied Chemistry
K-ABC	Kaufman Assessment Battery for Children (Dutch version)
ml	milliliter
µg	10^{-6} gram
ng	10^{-9} gram
NOS	Neurological Optimality Score
OR	Odds Ratio
PAH	Polyhalogenated Aromatic Hydocarbon
PCB	Polychlorinated Biphenyl
PCDD	Polychlorinated dibenzo-p-dioxin
PCDF	Polychlorinated dibenzo-p-furan
pg	10^{-12} gram
RDLS	Reynell Developmental Language Scales (Dutch Version)
RIVM	Rijks Instituut voor Volksgezondheid en Milieu National Institute of Public Health and the Environment
RT	Reaction Time
s	second
TCDD	2,3,7,8-Tetrachlorodibenzo-p-dioxin
TDI	Tolerable Daily Intake
TEF	Toxic equivalent factor
TEQ	Toxic equivalent
TH	Target Height
WAIS	Wechsler Adult Intelligence Scale (Dutch version)
WHO	World Health Organization
wSDS	Weight standard deviation score
ΣPCB	sum of PCB IUPAC nos. 118, 138, 153, and 180
ΔSDS	Change in standard deviation score

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Svati

CURRICULUM VITAE

Svati Patandin was born on 3 december 1967 in Paramaribo, Surinam, South America. She finished her High School (VWO) in 1985 at the Vrije Atheneum te Paramaribo. In 1985 she studied for one year at the Agricultural University Wageningen in Environmental Sciences and Human Nutrition. In 1986 she started her Medical training at the Erasmus University Rotterdam. As a medical student she performed a population based study from September 1990 until February 1991, in a rural part of South India, to study the prevalence of type II Diabetes Mellitus. In Oktober 1991 she started her internship and obtained her Medical Degree in November 1993. From January 1994 until June 1996 she worked as a fellow researcher to obtain her PhD degree at the Department of Pediatrics, Division of Neonatology (Promotor, Prof. Dr. P.J.J Sauer, copromotor Dr. N. Weisglas-Kuperus) from the Sophia Children's Hospital and Erasmus University Rotterdam. The research project, entitled "neonatal PCB exposure and neurodevelomental deficits" was financed by the European Community as part of a multi center cohort study (Coordinator: Prof. Dr. G. Winneke, Dusseldorf Germany). In oktober 1995 she passed her exam after the course on Classical Statistical Methods for Data-analysis. In june 1996 she went to the Faroer Islands, Denmark to work on a final report for combined data-analysis from the Dutch, German and Danish cohort.

From August 1995 until December 1995 she worked as a medical doctor at the outpatient clinic from the Department of Neonatology. From July 1996 until March 1997 she worked as a resident at the Department of Pediatrics, mainly on the Neonatal Intensive and High Care Unit of the Sophia Children's Hospital Rotterdam. From April 1997 until Oktober 1998 she worked at home to finish her thesis. From November 1998 she started her residency at the Department of Anesthesiology, Dijkzigt University Hospital Rotterdam (Head Prof Dr. W Erdmann).

Since 1991, she is married to Jesse Ashruf, surgeon in training, and they have a daughter, Talin, born on the 20th of April 1998.