EFFECTS OF PERINATAL EXPOSURE TO PCBs AND DIOXINS ON EARLY HUMAN DEVELOPMENT

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Koopman-Esseboom, Corine

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EFFECTS OF PERINATAL EXPOSURE TO PCBs AND DIOXINS ON EARLY HUMAN DEVELOPMENT

Effecten van perinatale blootstelling aan PCBs en dioxinen op de vroege ontwikkeling van de mens

PROEFSCHRIFT

ter verkrijging van de graad van doctor

aan de Erasmus Universiteit Rotterdam

op gezag van de rector magnificus

Prof. Dr. P.W.C. Akkermans M.A.

en volgens besluit van het college voor promoties.

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Promotor : Prof. Dr. P.J.J. Sauer

Overige leden : Prof. Dr. H.K.A. Visser

Prof. Dr. F.C. Verhulst

Prof. Dr. J.H. Koeman

The studies described in this thesis were performed at the Department of Pediatrics, Division of Neonatology, Sophia Children's Hospital, Erasmus University Rotterdam.

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Voor alle zuigelingen en hun ouders

EFFECTS OF PERINATAL EXPOSURE TO PCBs AND DIOXINS ON EARLY HUMAN DEVELOPMENT

CONTENTS

LIST OF ABBREVIATIONS

PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo-p-dioxins
PCDFs	Polychlorinated dibenzofurans
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
Ah-receptor	Aryl-hydrocarbon receptor
TEQ	Toxic equivalent
TEF	Toxic equivalence factor
TT3	Total triiodothyronine
TT4	Total thyroxine
TSH	Thyroid stimulating hormone
FT4	Free thyroxine
TBG	Thyroxine binding globuline
TTR	Transthyretine
NOS	Neurological optimality score
MDI	Mental developmental index
PDI	Psychomotor developmental index
LCPUFAs	Long-chain polyunsaturated fatty acids
TcR	T cell receptor
NK	Natural killer

CHAPTER 1

GENERAL INTRODUCTION

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1.1 Background of the study

Polychlorinated biphenyls (PCBs) and dioxins are hazardous compounds, which are widespread in the environment. Several experimental animal studies and a few human studies have shown deleterious effects on different organ systems.[1-4]

Before this study was started it was well known that the PCB and dioxin levels in animal products and human milk samples from the Netherlands belong to the highest background levels in the world.[5] This was the reason that the Program Committee Toxicology (PCT) in co-operation with the Dutch Health Research Promotion Program (SGO) developed a large project in which the possible harmful effects of perinatal exposure of humans to PCBs and dioxins were investigated.

The clinical part of the study involving human volunteers (mother-infant pairs) was done by the Department of Pediatrics of the Sophia Children's Hospital and Erasmus University Rotterdam, and by the Department of Obstetrics and Gynaecology and Developmental Neurology of the University Hospital Groningen. The animal experimental part of the study was done by the Department of Toxicology of the Agricultural University Wageningen, the Department of Biological Toxicology of TNO in Zeist, and the Department of Neurotoxicology of MBL-TNO in Rijswijk. PCB measurements in human plasma were done by the Institute for Toxicology and Food of TNO in Zeist, the PCB and dioxin levels in human milk were analysed by the State Institute for Quality Control of Agricultural Products (RIKILT) in Wageningen.

1.2 Polychlorinated biphenyls and dioxins

Polychlorinated biphenyls (PCBs), formulations prepared by the chlorination of biphenyls, were commercially produced on a large scale and worldwide between 1930 and 1970. They had diverse industrial application possibilities because of their wide range of physical properties, chemical stability and miscibility with organic compounds. These characteristics have resulted in the use of PCBs as hydraulic fluids, plasticizers, adhesives, heat transfer fluids, wax extenders, dedusting agents, organic dilutents and extenders, lubricants and flame retardants. These PCB sources are called "open systems", because humans can be exposed directly to them. Besides, PCBs are also used in "closed systems", as dielectric fluids in capacitors and transformers, meaning an indirect exposure for human beings. The detection of large amounts of these stable compounds in the environment resulted in a worldwide ban in the early 1980s. The total amount of PCBs produced since 1929 is approximately 1.5 million metric tons.[6] However, still a few countries in the world produce PCBs, signifi-

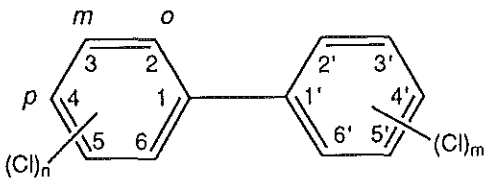
cant quantities of PCBs are till the present day employed as dielectric fluids in older transformers and capacitors, and large amounts of PCBs were dumped or leaked from older apparatus, so that land, water and especially sludge can still be highly polluted. Recently it was shown, that de novo synthesis of PCBs possibly occurs during combustion processes.[7,8]

Polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), summarized as dioxins, are mainly formed as by-products during the process of combustion and manufacturing of organochlorine chemicals. Recently, one of the main sources of pollution in the Netherlands: the output of dioxins during the combustion of municipal and hazardous waste, declined because better absorbing filters were installed into the incinerators.[9] However, the output was not reduced until zero, and there are still a lot of incinerators especially in the densely populated parts of Western Europe which produce a considerable amount of dioxins. Besides, during all kind of combustion processes, like the production of iron and steel, (accidental) fires, wood combustion, traffic, metal industry, use of wood preservatives, bleaching of pulp with chlorine, but also during smoking, dioxins are still emitted in the environment.[10] Also, during many chemical reactions PCDDs and PCDFs are formed as unwanted by-products. As a result, many pesticides and technical products, including chlorophenols, chlorophenoxy herbicides and PCBs, have been contaminated with these compounds. Although the production is currently banned or strictly regulated in most countries, these products still form a major source of contamination in the environment. Like PCBs, dioxins are also very stable compounds, with half-lives ranging from 2 to 7 years, depending on the congener.[11] They have also been detected in a wide range of specimens in the environment. Mass balance calculations indicate a gap between the observed deposition of PCDDs and PCDFs and known sources. This can be explained by long-range transport from unidentified sources.[12]

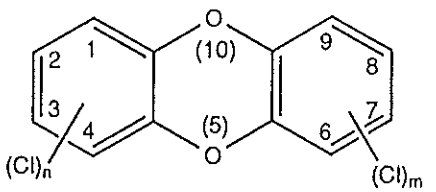
Both PCBs and dioxins are aromatic structures with different possibilities of chlorine atom substitution (Figure 1.1). There are 209 different PCB congeners and 210 different dioxin congeners (75 PCDDs and 135 PCDFs). Because of the double bond, the dioxins are almost planar tricyclic aromatic compounds which are much more toxic compared to the PCBs which only have one bond between the two phenyl rings and are less planar. Within the group of PCBs, different subgroups can be defined such as the planar PCBs which have no chlorine atom on the ortho position of the biphenyl ring structure, they resemble closely the dioxins because of their planar structure. Secondly, the mono-ortho PCBs which have one chlorine atom on the ortho position and the di-ortho PCBs which have two chlorine atoms on the ortho position. Having more chlorine atoms on the ortho position means that the coplanarity between the two phenyl rings is reduced and thus the toxicity of the congener.

Of the 210 possible isomers of the dioxins, usually only the 2,3,7,8-substituted congeners are present in biotic samples. In general, higher chlorinated congeners of 2,3,7,8-PCDD/Fs and PCBs are more abundant than lower chlorinated compounds.[13] The absence of the non-2,3,7,8-substituted congeners in environmental biotic samples can be explained by rapid metabolism and excretion of these congeners.

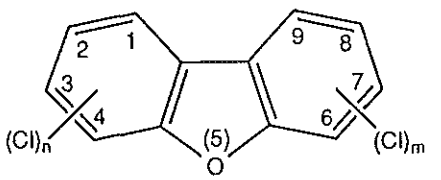
Figure 1.1 Molecular structures of PCBs, PCDDs and PCDFs.



Polychlorinated biphenyls (PCBs)



Polychlorinated dibenzo-*p*-dioxins (PCDDs)



Polychlorinated dibenzofurans (PCDFs)

O = Ortho position

M = Meta position

P = Para position

Working mechanism: Strong evidence exists that many toxic and biochemical responses observed in animals that are experimentally exposed to these compounds, are mediated by an aryl-hydrocarbon (Ah) receptor mechanism of action.[14] Especially, the 2,3,7,8-substituted PCDD/F congeners are potent in inducing Ah receptor mediated toxic responses. In addition, good correlations have been found between their Ah receptor-mediated cytochrome P450 gene expression (CYP1A1 and CYP1A2) and associated enzyme activities (aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-O-deethylase (EROD)), and their toxic potency including body weight reduction, hepatotoxicity and thymic atrophy.[15] However, it should be emphasized that, for non-planar PCBs in particular, a number of important toxic and biochemical responses induced in experimental animals, e.g. neurobehavioural, neurochemical, carcinogenic and endocrine effects may not, or only partly be mediated by the Ah receptor.[16,17]

The TEF concept for risk management: Based on the Ah-receptor mechanism, a toxic equivalence factor (TEF) concept has been developed to express the toxic potency of a mixture of different dioxin and PCB congeners, like human milk, by a single sum parameter, the toxic equivalent (TEQ). A TEF is assigned to the different congeners which represents their relative toxic potency towards 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which is the most toxic dioxin congener with a TEF value of one. By multiplying the concentration (pg/g fat) and TEF value, the toxic equivalent (TEQ) of each congener can be calculated (pg TEQ/g fat). By adding up the TEQs of all congeners the total-TEQ value is obtained.[18,19] A limitation of the present TEF approach is that it only takes into account additive but no synergistic or antagonistic effects of PCDDs, PCDFs and PCBs.

1.3 Animal data

Many studies have been done on the toxic and biochemical responses observed in laboratory animals exposed to PCBs, PCDDs and PCDFs.[1,2,20,21] These responses include dermal, immuno- and hepatotoxicity, carcinogenic, teratogenic and neurobehavioural effects, reproductive toxicity, endocrine responses, as well as numerous biochemical responses, such as the induction of several drug metabolizing enzymes. The responses are sex-, age-, strain- and species-specific. The monkey, guinea pig and mink appear to be more sensitive to PCB and dioxin toxicity than rats and mice are. The reasons for the variation in species sensitivity have not been elucidated, but variation cannot be fully explained by different rates of metabolism.

The toxic effects commonly observed after acute, subchronic and chronic administration of PCB or dioxin mixtures and/or individual congeners to animals include a *wasting syndrome*: a progressive weight loss which is not simply related to decreased food consumption. The *skin* may show signs of chloracne, hyperkeratosis, hyperpigmentation, facial oedema, finger nail loss, alopecia, erosions, and ulcerations. *Immunotoxic* effects which have been reported are: increased sensitivity to infections, a decrease in thymic and splenic weight, a decrease in the number of germinal centres in spleen and lymphnodes, the number of bone marrow cells, leukocytes, lymphocytes, splenic plaque forming cells, T-helper/T-suppressor cell ratio, serum IgA and IgG levels, and antibody production. The *gastrointestinal tract* can show signs of gastritis with bleeding. *Liver* damage is characterised by increased liver size, fatty degeneration and focal necrosis. The liver enlargement is associated with hepatocyte enlargement and an increase in smooth endoplasmatic reticulum and/or increased enzyme activity. Induction of metabolic enzymes may result in increased detoxication or enhanced toxicity, due to an increased formation of reactive metabolites. *Carcinogenicity*: PCBs and dioxins are found to be tumor promotors more than tumor initiators. Hepatocellular carcinomas are most frequently detected. Besides, tumors of the lung, nasopharynx and skin are described. Effects on the *lung* are peribronchial cell infiltration, formation of cellular lipid vacuoles and lamellar bodies. In the *nervous system* the cholinergic receptors, the dopamin level and the uptake of neurotransmitters may be decreased. Behavioral effects

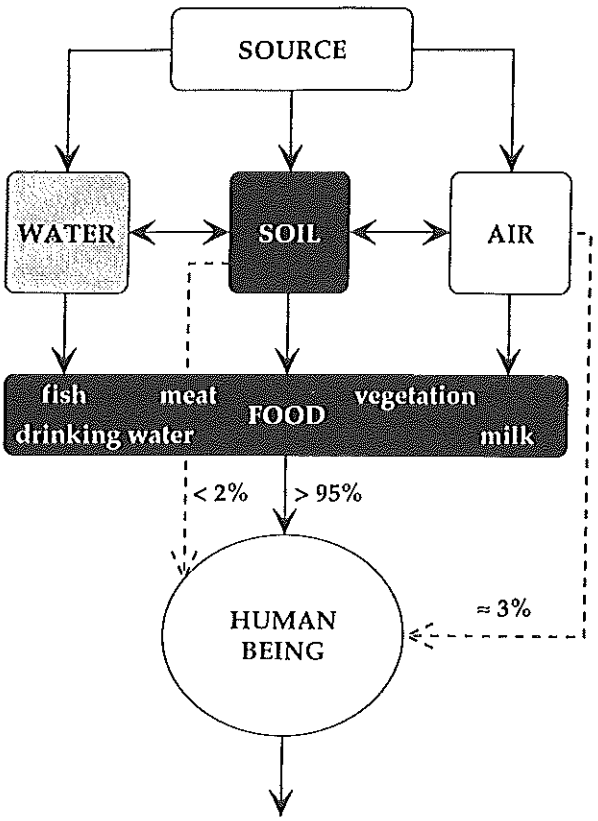
in the offspring such as spinning syndrome, hyperactivity and impaired learning have been reported. Effects on *reproduction* constitute of decreased fertility, matings, pregnancies, testicular spermatozoan concentrations, ventral prostate weight, and seminal vesicle weight in the male. In the female there is anovulation, effects on the estrogen cycle, decreased weight of the uterus, ovaries, and accessory glands. The number of implantations, litter size, fetal development and perinatal survival have been decreased. In mice an increase in teratogenic effects such as cleft palate and hydronephrosis has been reported. The *lipid metabolism* can be influenced by an increase in the hepatic and serum lipids and a decrease in the output of bile acids. The excretion of hepatic *porphyrins* may be enhanced. The *vitamin A and thyroid hormone* levels can be decreased by occupation of the transport proteins by dioxins or PCB metabolites, or by inducing hepatic enzymes like uridine diphosphate-glucuronyltransferase by which the biliary excretion is enhanced.

1.4 Human data

There is considerable uncertainty in the extrapolation of toxicological information on PCDD/Fs and PCBs from experimental animals to man, because of the observed large species differences in toxicokinetics, sensitivity and pattern of toxic responses.[22,23]

Primarily, humans can be exposed to PCBs, PCDDs and/or PCDFs accidentally which means a sudden exposure to high levels of the contaminants during a short period. Secondly, they can be exposed for a longer period of time during their occupation such as the production of PCBs, production of chemical products where PCBs, PCDDs or PCDFs are formed as unwanted by-products, the production or maintenance of apparatus that contain PCBs, like older transformers and capacitors. The average population is exposed to relatively low background levels of PCBs and dioxins. Worldwide these contaminants have been detected in all kinds of human specimen like blood, adipose tissue, human milk, different organs like the brain and liver. Accidentally and occupationally exposed populations have the highest detectable levels. People living in densely populated areas like Western Europe, have higher PCB, PCDD and PCDF background levels compared to people living in sparsely populated areas.[5] Human background levels of these contaminants are mainly caused by consuming meat, fish and dairy products. Since PCBs, PCDDs and PCDFs are dumped or formed during all kinds of combustion processes, they are widely spread in the air, water and ground by long-range atmospheric transport. At the end of the food chain these lipophilic contaminants are concentrated in human adipose tissue. The higher chlorinated congeners of 2,3,7,8-PCDD/Fs and PCBs, which are the most toxic ones, are most abundant and very stable with half-lives between 2 and 7 years. The less chlorinated congeners are excreted by the human body within a shorter time, mainly in the feces. Besides the dietary intake, humans can be exposed by inhaling air, dermal contact or drinking water. However, these exposure routes would only account for 5% of the total amount.[24] (Figure 1.2)

Figure 1.2 Exposure routes of PCBs and dioxins for human beings.



This figure has been borrowed from Dr. A.K.D. Liem, with kind permission.

Babies form a special group of exposed humans. PCBs, PCDDs and PCDFs have been detected in umbilical cord blood, in placenta tissues and in stillborn babies.[25,26] Although the levels in these specimen were much lower compared to the adult levels, the fetus and embryo are exposed during a critical period of organ development and growth. Besides, many studies have detected relatively high levels of PCBs and dioxins in human milk. PCBs in breast-feeding were firstly detected by Jenssen in 1966, PCDDs and PCDFs could only be measured accurately since 1984. After the prenatal exposure to which all infants are exposed, breast-fed infants are additionally exposed postnatally to relatively high levels of both contaminants during a period of further organ growth and development. Children being breast-fed for a longer time had the highest levels of PCBs and dioxins in their blood just after the breast-feeding ceased.[27,28] Because of tissue growth, the levels declined relatively fast, but even at an older age, the children breast-fed the longest time with the highest levels of PCBs and dioxins, had the highest levels of these contaminants in their blood.[29,30,31]

1.4.1 Accidental exposure:

Data on the human toxicology of PCBs, PCDDs and PCDFs are primarily derived from the results of accidental and occupational exposure.

In the western part of Japan, a mass food poisoning was caused in 1968, by ingestion of a commercial brand of rice oil contaminated with PCBs, PCDFs, PCDDs and polychlorinated quaterphenyl ethers (PCQEs). It was called 'Yusho', which literally means 'oil disease' in Japanese, and involved more than 1850 people.

A very similar poisoning caused by consuming contaminated rice oil occurred in Central Taiwan in 1979, yielding more than 2000 victims. The poisoning was called 'Yu-Cheng': oil disease in Chinese. It is generally believed that Kanochlor, a mixture of different PCB congeners, which was used for heating the rice oil over 200 degrees Celsius in order to remove odorous matter from it, must have contaminated the rice oil through a few pinholes in a heating pipe. The victims showed severe dermal lesions like chloracne (severe acne-like eruptions), pigmentation of the skin, mucous membranes and nails, distinctive hair follicles, increased sweating at the palms, itching, increased eye discharge, hyperemia of the conjunctiva, a delay in eruption of the permanent teeth, and jaundice. Besides, they complained of swelling, numbness and spasm of the limbs, transient visual disturbances, feeling of weakness, fever, hearing difficulties, headache, bronchitis, vomiting, and diarrhea. Additional clinical examinations for the neurological situation showed sensory neuropathy but no weakness, with decreased vibration sense, hyperalgesia, and decreased ankle jerks. Electrophysiologic testing showed reduced sensory and/or motor nerve conduction in some cases. Immunological testing in a subpopulation of Yu-Cheng patients showed an increase in the total leukocyte count, a reduction in T-helper cells and an increase in T-suppressor cells, with a decrease in the T4/T8 ratio. However, the T4/T8 ratio in Yusho patients was increased.[30,32]

Babies born to exposed mothers had a dark-brown colored skin, which gave them the name 'coca-cola babies', they showed deformed pigmented nails, natal teeth, gum hypertrophia, hypersecretion of the Meibomian glands, acne, decreased birth weight and growth, and abnormal lung auscultation. Between 1985 and 1990 a follow-up study of 117 exposed Taiwanese children and 108 control children was performed.[33,34,35] The exposed children appeared to be smaller than the controls regarding weight and height. Gum hypertrophy, hyperpigmentation and nail deformities were still apparent, neither acne nor conjunctival cysts were more common in the exposed. There were also marked differences in eyebrow flare, hypertelorism and clinodactyly. The diagnosis bronchitis was made in several of the exposed children. There were no abnormal reflexes or any localizing findings during the neurological examination. However, the exposed children were delayed compared to controls in performing tasks such as formulating phrases and sentences, turning pages, carrying out requests, pointing to body parts, holding pencils, imitating drawn circles, or catching a ball. There was an overall clinical impression of developmental or psychomotor delay in 10% of the exposed children compared with 3% of the control children, and of a speech problem in 7% versus 3%. Age-appropriate testing of cognitive development and behavioral assessment was also done. The

Bayley Scales of Infant Development were used until 30 months (mental and motor scale), the Stanford-Binet yields an intelligence quotient and was used from 30 to 72 months. The Wechsler Intelligence Scales for Children (WISC) were used after 72 months. Except for verbal IQ on the WISC, the exposed children always scored lower on the three developmental and cognitive tests compared to the controls. On the Rutter scales the exposed children showed lower scores on all three scales concerning health problems, habits, and behavior. Children born up to 6 years after their mothers' exposure were as affected as children born within a year or two after exposure when examined at 6 and 7 years of age. However, at 8 years of age the congnitive development of the exposed Taiwanese children had a tendency to catch up, and did not differ significantly anymore from their controls.[36] Older siblings who were not exposed prenatally resembled the control children.

Although a general tendency to spontaneous recovery could be seen in most of the patients, a considerable number of them showed clinical signs, mainly of the skin, even 20 years after the outbreak of the poisoning. An excess mortality was seen for malignant neoplasms of the liver, lung, trachea and bronchus.

In Seveso, Italy, a 2,4,5-trichlorophenol reactor exploded in 1976, which caused the release of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), the most toxic dioxin congener. More than 17,000 individuals were contaminated, and chloracne, especially in children, was the most remarkable clinical sign. Laboratory tests showed very slight modifications in some liver enzymes and a mild increase in lymphocyte count. There was no significant increase in reproductive pathology, birth defects, neurological abnormalities or cancer mortality up until now. It was remarkable that domestic animals and some wildlife, particularly rabbits, domestic poultry and other birds, started dying spontaneously within 3 days after the accident.[37]

There have been several accidental exposures to PCBs from transformer accidents. The most extensive investigation was undertaken after the Binghamton accident in 1986. Like in several other studies, there was a direct relationship between blood triglyceride concentrations and PCB concentrations. It has been suggested that this relationship is an artifact resulting from the tendency of lipophilic compounds such as PCBs to partition between adipose tissue and serum lipids in direct proportion to the lipid content of the serum.[38] There was a slight impairment in liver function tests, without hepatomegaly. The most prevalent symptoms for several accidents were: headache, skin disorders, irritation of the eyes and respiratory tract, abdominal pain and nausea, numbness, and dizziness. Two months after the accident there was a slight impairment in the sensory conduction velocities of peripheral nerves. Six months after the accident, the nerve conduction velocities were close to normal values.

1.4.2 Occupational exposure:

Studies in occupationally exposed persons, like heavily exposed chemical workers[39] and less heavily exposed US Air Force Ranch Hand personnel, during the use of Agent Orange in the Vietnam War, showed no chronic effects on the peripheral nervous system in studies conducted more than 15 years after

removal from exposure. In these studies, there was no increase in liver or gastrointestinal diseases, porphyria or pulmonary diseases.[40-42] In the Ranch Hand study, in which serum TCDD measurements were used to estimate exposure, glucose, cholesterol and HDL levels increased significantly with increasing serum levels of TCDD.[43]

Some evidence that TCDD is a carcinogen in heavily exposed humans was provided by three studies of chemical production workers, where serum TCDD levels were used as measures of exposure.[44-46] Statistically significant excesses of total cancer were found in the high exposure groups of all three studies, and a significant excess of respiratory cancer and soft tissue sarcoma was associated with high exposure in one study. Exposure to chlorophenols or phenoxy acids without simultaneous exposure to TCDD also correlates with soft tissue sarcoma or non-Hodgkins lymphoma in other studies; this complicates the interpretation of the data. There have been few studies on carcinogenicity of PCBs in humans and these provide inconclusive results.[47]

1.4.3 Background environmental exposure:

Little is known about possible adverse health effects from the chronic, low background levels of PCBs, PCDDs and PCDFs present in human populations. The two most well known studies in which effects of perinatal exposure to PCBs were examined, were done in the USA. Rogan and Gladen et al. followed a cohort of 930 children in North Carolina. The families were volunteers and, with very few exceptions, had no unusual chemical exposures. The second study was done by Jacobson et al. in Michigan in a cohort of 313 children; 242 of the mothers had consumed Lake Michigan sport fish presumed to be contaminated by PCBs.

In both studies the infants were examined in the first week with the Brazelton Neonatal Behavioral Assessment Scales (BNBAS). Rogan et al. described a significant relationship between the in utero exposure to background levels of PCBs and a negative outcome on the muscle tone and reflex cluster score of the BNBAS.[48] Jacobson et al. described a negative effect on the autonomic maturity, reflex and range of state cluster score.[49]

At 7 months of age, Jacobson et al. reported a dose-dependent decrease in the infant's visual recognition memory with the Fagan Test of Infant Intelligence, due to intrauterine PCB exposure.[50]

Both cohorts were examined at different ages for their mental and psychomotor developmental index scores with the Bayley Scales of Infant Development. In North Carolina they described an association between the prenatal PCB exposure and a poorer performance on the psychomotor developmental index (PDI) at 6, 12, 18, and 24 months of age.[51,52] The pattern was similar at all ages, with only the top 5-10% of children affected, and the size of the effect about 5-8 Bayley points. They could not find a negative influence of the prenatal PCB exposure on the mental developmental index (MDI) at these ages. Jacobson et al. reported that the cord serum PCB level was associated with poorer performance on a cluster of items focusing primarily on fine motor coordination, but this effect fell short of the conventional level of

statistical significance.[53]

In North Carolina as well as in Michigan the children were studied with the McCarthy Scales of Children's Abilities (Kaufman and Kaufman, 1977. Clinical Evaluation of Young Children with the McCarthy Scales. New York, Grune and Stratton). Rogan and Gladen et al. could not find a negative influence of the prenatal or postnatal PCB exposure on any of the McCarthy Scales examined at 3, 4, and 5 years of age.[54] The Jacobsons studied their cohort at 4 years of age: children with the highest 5% cord serum PCB levels scored 6 points lower on the Memory scale of the McCarthy Scales, and there was a trend toward poorer performance on the Verbal scale.[55]

A few adverse effects on the infants' development due to lactational exposure have been described: Jacobson et al.[56] reported a relationship between postnatal PCB exposure and a decrease in activity level at 4 years of age. The effect on activity was only seen in children whose mothers had above average PCB levels and who breast-fed for more than a year.

The North Carolina cohort submitted report cards, and performance at third grade and higher was analysed. Grades in English and mathematics showed no statistically significant relationship with transplacental or breast milk exposure. There was neither an association with hyperactivity.[54]

In the Netherlands another cohort of 34 babies was examined neurologically according to Prechtl in the first week and 26 weeks after birth, in relation to PCDD and PCDF exposure. The study did not indicate that perinatal exposure to background levels of dioxins influences the neurological development of the infant during the first six months of life.[57]

1.5 Aims of the study

"The Dutch PCB and dioxin study", is a collaborative study between animal experimental research workers and human clinical research workers. The main question was:

"Does perinatal exposure to PCBs, PCDDs or PCDFs affect the early development of animals and/or human beings ?".

In this thesis a part of the human study has been described. The work was done in cooperation with the Department of Gynecology and Obstetrics and the Department of Developmental Neurology of the University of Groningen.

The main questions of the human study were:

1. What are the levels of PCBs, PCDDs and PCDFs in maternal plasma, cord plasma, and human milk in the Netherlands?
2. Is there a correlation between the different contaminant levels in maternal plasma, cord plasma, and human milk?
3. Can we predict the levels in human milk from the levels in maternal plasma during pregnancy?

3. Is there a difference in the contaminant levels between the Rotterdam region, a highly industrialized area in the western part of the Netherlands, and Groningen which is a more semi-urban region in the northern part of the Netherlands.
4. Does the prenatal in utero and/or postnatal exposure to PCBs, PCDDs and/or PCDFs influence the neurological development in the second week or 18th month after birth?

Additional questions of the Department of Pediatrics of the Sophia Children's Hospital/Erasmus University in Rotterdam were:

5. Does perinatal exposure to PCBs, PCDDs and PCDFs affect the thyroid hormone levels of the infants?
   (In cooperation with the Department of Toxicology of the Agricultural University Wageningen).
6. Is there a relationship between the perinatal exposure to PCBS, PCDDs and PCDFs and the infant's visual recognition memory?
7. Is there a relationship between the perinatal exposure to these contaminants and the mental or psychomotor development?
8. Is the immunological status of infants influenced by perinatal exposure to PCBs, PCDDs or PCDFs?

1.6 Subjects and methods

The study group consisted of 418 mother-infant pairs. They were enrolled in the study between June 1990 and July 1992. Two hundred and seven pairs lived in the Rotterdam area and 211 in the Groningen area. The following inclusion criteria were used: first or second born term infants (37 to 42 weeks of gestation), no congenital anomalies or diseases. Pregnancy and delivery had to be passed without signs of serious illness, or complications like a caesarian section, forceps or vacuum extraction. All infants were of the Caucasian race. From other studies it is well known that differences in these inclusion criteria may influence the neurological outcome.[58,59]

In order to study the effects of postnatal exposure to PCBs, PCDDs and PCDFs by breast-feeding, 50% of the women enrolled intended to give breast-feeding for at least 6 weeks, the other 50% volunteered to give formula-feeding from one batch as a reference (Almiron M2, Nutricia N.V., the Netherlands) during 7 months, both groups were equally divided over the Rotterdam and Groningen area.

An overview of the biochemical parameters and the neurodevelopmental parameters collected in the study is presented in Table 1.1 and Table 1.2.

Table 1.1 Biochemical parameters.

	Human Milk	Maternal Plasma	Cord Plasma	Child Plasma
PCB 118,138,153,180 [R+G]	+	+	+	-
PCBs (22 congeners) [R+G]	+	-	-	-
Dioxins (17 congeners) [R+G]	+	-	-	-
Immunological parameters [R]	-	-	+	+
Thyroid functions (TT3, TT4, TSH, FT4) [R]	-	+	+	+

[R] = Rotterdam, [G] = Groningen.

Table 1.2 Neurodevelopmental parameters.

	10 days	3 months	7 months	18 months
Neurological Examination Prechtl/Touwen [R+G]	+	-	-	+
Obstetrical Optimality Score [R+G]	+	-	-	-
Mental and Psychomotor Development Bayley Scales of Infant Development [R]	-	+	+	+
HOME score [R]	-	-	-	+
Visual Recognition Memory Fagan Infantest [R]	-	+	+	-

[R] = Rotterdam, [G] = Groningen

1.7 Structure of the thesis

In the following chapters of this thesis the different assessments at the different ages will be described in detail and the consequences of these findings will be discussed:

Chapter 2 describes the levels of the different PCB and dioxin congeners measured in maternal plasma, cord plasma or human milk. It also describes the relations between the levels in maternal plasma, cord plasma and human milk.

Chapter 3 describes the differences in PCB, PCDD and PCDF levels in plasma and human milk between the Rotterdam and the Groningen area.

Chapter 4 describes the influences of PCBs, PCDDs and PCDFs on the thyroid hormone status of mother and infant.

Chapter 5 describes the clinical neurological diagnosis of the infants in the second week after birth in relation to the perinatal exposure to PCBs, PCDDs and PCDFs.

Chapter 6 describes the neurological optimality score of the infants in the second week after birth in relation to the perinatal exposure to PCBs, PCDDs and PCDFs.

Chapter 7 describes the relationship between perinatal exposure to PCBs, PCDDs and PCDFs, and the feeding type on the visual recognition memory of the infants at 3 and 7 months of age.

Chapter 8 describes the relationship between perinatal exposure to PCBs, PCDDs and PCDFs, and the feeding type on the mental and psychomotor development of the infants at 3, 7, and 18 months of age.

Chapter 9 describes the effects of perinatal exposure to PCBs, PCDDs and PCDFs on the neurological development at 18 months of age.

Chapter 10 describes the effects of perinatal exposure to PCBs, PCDDs and PCDFs on the immunologic status of the infants.

Chapter 11 gives a summary of all findings. Final conclusions and recommendations for the future are made.

1.8 References

1. Safe S. Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology, and mechanism of action. CRC Crit Rev Toxicol 1984;13:319-395.
2. Ahlborg UG, Hanberg A, Kenne K. Risk assessment of polychlorinated biphenyls (PCBs). Nord 1992: 26. ISBN 9291200751.
3. Ahlborg UG, Brouwer A, Fingerhut MA et al. Impact of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls on human and environmental health, with special emphasis on application of the toxic equivalency factor concept. Eur J of Pharmacol - Envir Toxicol and Pharmacol Section 1992;228:179-199.
4. Skene SA, Dewhurst IC, Greenberg M. Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans: The risks to human health. A review. Human Toxicol 1989; 8: 173-203.
5. World Health Organisation. Levels of PCBs, PCDDs, and PCDFs in breast milk: results of WHO-coordinated interlaboratory quality control studies and analytical field studies. FADL, Copenhagen. Environmental Health series 1989;34.
6. De Voogt P, Brinkman UATh, 1989. Production, properties and usage of polychlorinated biphenyls. In: Kimbrough RD, Jensen AA (eds). Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Elsevier, Amsterdam, pp 3-45.

7. Rappe C, Buser HR, 1989. Chemical and physical properties, analytical methods, sources and environmental levels of halogenated dibenzodioxins and dibenzofurans. In: Kimbrough RD, Jensen AA (eds). Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Elsevier, Amsterdam, pp 71-102.
8. Van Bavel B, Fängmark I, Marklund S et al. 1992. Quantitative analysis of organic trace compounds by isotope dilution : formation of the non-ortho PCBs 77, 126 and 169 during incineration. In: Organohalogen Compounds Vol. 8:225-228. Finish Institute of Occupational Health, Helsinki.
9. Bremmer HJ, Troost LM, Kuipers G, De Koning J, Sein AA. Emissions of dioxins in the Netherlands. RIVM/TNO Report nr. 770501003 (summary in English) 1993.
10. Rappe C, Andersson R, Bergqvist PA, Brohede C, Hansson M, Kjeller LO et al. Overview of environmental fate of chlorinated dioxins and dibenzofurans, sources, levels and isomeric pattern in various matrices. Chemosphere 1987;16:1603-1618.
11. Poiger H, Schlatter C. Pharmacokinetics of 2,3,7,8-TCDD in man. Chemosphere 1986;15:1489.
12. Rappe C, 1991. Sources of, and human exposure to PCDDs and PCDFs. Banbury Report 35:121-131. Biological Basis for Risk Assessment of Dioxins and Related Compounds. Cold Spring Harbor Laboratory Press, New York.
13. Van den Berg M, Blank F, Heeremans C, Veenhoven E, Olie K. The presence of polychlorinated dibenzodioxins and dibenzofurans in fish-eating birds and fish from the Netherlands. Arch Environ Contam Toxicol 1987;16:149.
14. Poland A, Knutson JC. 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. Ann Rev Pharmacol Toxicol;1982:22:517.
15. Goldstein JA, Safe SH. Mechanisms of action and structure-activity relationship for the chlorinated dibenzo-p-dioxins and related compounds. In: Kimbrough RD, Jensen AA (eds). Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Elsevier, Amsterdam, 1989:239-293.
16. Seegal RF, Bush B, Shain W. Lightly chlorinated ortho-substituted PCB congener decrease dopamine in nonhuman primate brain and in tissue culture. Toxicol Appl Pharmacol 1990;106:136.
17. Brouwer A. Role of biotransformation in PCB-induced alterations in vitamin A and thyroid hormone metabolism in laboratory and wildlife species. Biochem Soc Transact 1991;19:731.
18. NATO/CCMS. Pilot study on International Information Exchange on Dioxins and Related Compounds. International Toxicity Equivalency Factor (I-TEF). Method of Risk Assessment for Complex Mixtures of Dioxins and Related Compounds. Report number 176, 1988.
19. Ahlborg UG, Becking GC, Birnbaum LS, Brouwer A, Derks HJGM, Feeley M, Golor G, Hanberg A, Larsen JC, Liem AKD, Safe SH, Schlatter C, Waern F, Younes M, Yrjänheikki E. Toxic Equivalency Factors for dioxin-like PCBs. Report on a WHO-ECEH and IPCS consultation, December 1993. Chemosphere 1994;28:6.
20. McConnell EE. Acute and chronic toxicity and carcinogenesis in animals. In: Kimbrough RD, Jensen AA (eds). Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Elsevier, Amsterdam, 1989:-161-193.

21. Brouwer A, Ahlborg UG, Van den Berg M, Birnbaum LS, Boersma ER, Bosveld B, Denison MS, Earl Gray L, Hagmar L, Holene E, Huisman M, Jacobson SW, Jacobson JL, Koopman-Esseboom C, Koppe JG, Kulig BM, Morse DC, Muckle G, Peterson RE, Sauer PJJ, Seegal RF, Smits-Van Prooije, Touwen BCL, Weisglas-Kuperus N, Winneke G. Functional aspects of developmental toxicity of polyhalogenated aromatic hydrocarbons in experimental animals and human infants. Eur J of Pharmacol - Envir Toxicol and Pharmacol Section 1995;293:1-40.
22. Kimbrough RD. Human health effects of polychlorinated biphenyls and polybrominated biphenyls 9PBBs). Ann Rev Pharmacol Toxicol 1987;27:87.
23. Gallo MA, Scheuplein RJ, Van der Heijden CA (eds.). Biological Basis for Risk Assessment of Dioxins and Related Compounds. Banbury Report 1991;35. New York, Cold Spring Harbor Laboratory Press.
24. Theelen RMC. Modeling of human exposure to TCDD and I-TEQ in the Netherlands: Background and occupational. In: Gallo MA, Scheuplein RJ, Van der Heijden KA (eds). Biological basis for risk assessment of dioxins and related compounds. Banbury report 1991;35:277-290. Cold Spring Harbor Laboratory Press.
25. Schecter A, Päpke O, Ball M. Evidence for transplacental transfer of dioxins from mother to fetus: chlorinated dioxins and dibenzofuran levels in livers of stillborn infants. Chemosphere 1990;21:1017.
26. Beck H, Droß A, Kleeman WJ, Mathar W. PCDD and PCDF concentrations in different organs from infants. Chemosphere 1990;20:903-910.
27. Gezondheidsraad: Commissie Moedermelk. Verontreiniging van moedermelk. Den Haag: Gezondheidsraad 1991; publicatie nr. 1991;13:66-67.
28. Niessen KH, Ramolla J, Binder M, Brügmann G, Hofmann U. Chlorinated hydrocarbons in adipose tissue of infants and toddlers: inventory and studies on their association with intake of mothers' milk. Eur J Pediatr 1984;142:238-243.
29. Kuratsune M. Yusho, with reference to Yu-Cheng. In: Halogenated biphenyls, terphenyls, naphtalenes, dibenzodioxins and related products. Kimbrough and Jensen (eds.) Elsevier Science Publishers B.V. Amsterdam, 1989:381-396.
30. Rogan WJ. Yu-Cheng. In: Halogenated biphenyls, terphenyls, naphtalenes, dibenzodioxins and related products. Kimbrough and Jensen (eds.) Elsevier Science Publishers B.V. Amsterdam, 1989:401-413.
31. Jacobson JL, Humphrey HEB, Jacobson SW, Schantz SL, Mullin MD, Welch R. Determinants of polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), and dichlorodiphenyl trichloroethane (DDT) levels in the sera of young children. Am J Public Health 1989;79:1401-1404.
32. Harada M. Intrauterine poisoning: clinical and epidemiological studies and significance of the problem. Bull. Inst. Constit. Med. (Kumamoto Univ), 1976:25(suppl);1-69.
33. Rogan WJ, Gladen BC, Hung KL, Koong SL, Shih LY, Taylor JS, Wu YC, Yang D, Ragan NB, Hsu CC. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 1988;241:334-336.
34. Chen YCJ, Guo YL, Hsu CC, Rogan WJ. Cognitive development of Yu-Cheng ('Oil Disease') children prenatally exposed to heat-degraded PCBs. JAMA 1992;268:3213-3218.
35. Chen YCJ, Hsu CC. Effects of prenatal exposure to PCBs on the neurological function of children: a neuropsychological and neurophysiological study. Dev Med Child Neurol. 1994;36:312-320.

36. Lai TJ, Guo YL, Chen SJ, Yu ML, Hsu CC. Cognitive development in Yucheng children. In: Fiedler H, Hutzinger O, Birnbaum L, Lambert G, Needham L, Safe S, eds. Organohalogen Compounds. Kyoto, Japan: Dept of Environmental and Sanitary Engineering 1994;21:513-516.
37. Reggiani GM. The Seveso accident: medical survey of a TCDD exposure. In: Halogenated biphenyls, terphenyls, naphtalenes, dibenzodioxins and related products. Kimbrough and Jensen (eds.) Elsevier Science Publishers B.V. Amsterdam, 1989:445-471.
38. Brown JF, Lawton RW. Bull Environ Contam Toxicol 1984;33:277-280.
39. Sweeney MH, 1990. Evaluation of the peripheral nervous system among workers employed in the production of chemicals contaminated with 2,3,7,8-tetrachloro-dibenzo-p-dioxin (dissertation) Ann Arbor MI: University of Michigan.
40. Calvert GM, Hornung RW, Sweeney MA, Fingerhut MA, Halperin WE. Hepatic and gastrointestinal effects in an occupational cohort exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Am Med Assoc 1992;267:2209.
41. Calvert GM, Sweeney JA, Morris JA, Fingerhut MA, Hornung RW, Halperin WE. Evaluation of chronic bronchitis, chronic obstructive pulmonary disease, and ventilatory function among workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Am Rev Respir Dis 1992;144:1302.
42. Wolfe WH, Michalek JE, Miner JC, 1991. An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides. In: Air Force Health Study Vol I-IX, analysis of 1987 Examination Results, Contract No. F41689-85-D-0010, SAIC Project No. 1-813-X4-195/254/4-37/011/942/943).
43. Wolfe W, Michalek J, Miner J, Needham L, Patterson D: Diabetes versus dioxin body burden in veterans of operation ranch hand. In: Organohalogen Compounds, 1991;10:279-282. Finnish Institute of Occupational Health. Helsinki, 1992.
44. Fingerhut MA, Halperin WE, Marlow LA, Piacitelli LA, Honchar PA, Sweeney MH et al. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. New Engl J Med 1991;324:212.
45. Manz A, Berger J, Dwyer JH et al. Cancer mortality among workers in chemical plant contaminated with dioxin. Lancet 1991;338 (8773):959.
46. Zober A, Messerer P, Huber P. Thirty-four-year mortality follow-up of BAsf employees exposed to 2,3,7,8-TCDD after the 1953 accident. Int Arch Occup Environ Health 1990;62:139.
47. Silberhorn EM, Glauert HP, Robertson LW. Carcinogenicity of polyhalogenated bephenyls: PCBs and PBBs. Crit Rev Toxicol 1990;20:439.
48. Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, Tinglestad J, Tully M. Neonatal effects of transplacental exposure to PCBs and DDE. J Pediatr 1986;109: 335-341.
49. Jacobson JL, Jacobson SW, Schwartz PM, Fein GG, Dowler JK. Prenatal exposure to an environmental toxin: a test of the multiple effects model. Dev Psychol 1984;20:523-532.
50. Jacobson SW, Fein GG, Jacobson JL, Schwartz PM, Dowler JK. The effect of intrauterine PCB exposure on visual recognition memory. Child Dev 1985;56:853-860.
51. Gladen BC, Rogan WJ, Hardy P, Thullen J, Tingelstad J, Tully M. Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. J Pediatr 1988;113:991-995.

52. Rogan WJ, Gladen BC. PCBs, DDE, and child development at 18 and 24 months. Ann Epidemiol 1991;1:407-13.
53. Tilson HA, Jacobson JL, Rogan WJ. Polychlorinated biphenyls and the developing nervous system: Cross-species comparisons. Neurotoxicol Teratol 1990;12:239-248.
54. Gladen BC, Rogan WJ. Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. J Pediatr 1991;119:58-63.
55. Jacobson JL, Jacobson SW, Humphrey HEB. Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J Pediatr. 1990;116:38-45.
56. Jacobson JL, Jacobson SW. Effects of exposure to PCBs and related compounds on growth and activity in children. Neurotoxicol Teratol 1990;12:319-326.
57. Pluim HJ. Dioxins - pre- and postnatal exposure in the human newborn [dissertation]. Amsterdam (NL): Univ of Amsterdam, 1993.
58. Freedman DG. Genetic influences on development of behaviour. In: Stoelinga GBA, Van der Werff ten Bosch JJ (eds). Normal and abnormal development of brain and behaviour. Boerhaave series for postgraduate medical education. Leiden University Press, 1971:208-229.
59. Touwen BCL. The preterm infant in the extrauterine environment. Implications for neurology. Early Hum Dev 1980;4/3:287-300.

CHAPTER 2

PCB AND DIOXIN LEVELS IN PLASMA AND HUMAN MILK OF 418 DUTCH WOMEN AND THEIR INFANTS. PREDICTIVE VALUE OF PCB CONGENER LEVELS IN MATERNAL PLASMA FOR FETAL AND INFANT'S EXPOSURE TO PCBs AND DIOXINS.

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PCB AND DIOXIN LEVELS IN PLASMA AND HUMAN MILK OF 418 DUTCH WOMEN AND THEIR INFANTS. PREDICTIVE VALUE OF PCB CONGENER LEVELS IN MATERNAL PLASMA FOR FETAL AND INFANT'S EXPOSURE TO PCBs AND DIOXINS.

Corine Koopman-Esseboom[1], Marcel Huisman[2], Nynke Weisglas-Kuperus[1], Cornelis G. Van der Paauw[3], Louis G.M.Th. Tuinstra[4], E. Rudy Boersma[2], Pieter J.J. Sauer[1].

1. Department of Paediatrics, Division of Neonatology, Erasmus University and University Hospital/Sophia Children's Hospital Rotterdam.
2. Department of Obstetrics and Gynaecology, University of Groningen.
3. TNO Nutrition and Food Research, Zeist.
4. DLO State Institute for Quality Control of Agricultural Products, Wageningen, The Netherlands.

2.1 Abstract

Polychlorinated biphenyls (PCBs) as well as dioxins (polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs)) are potentially hazardous compounds in the environment for human beings.

In order to investigate PCB and dioxin exposure of Dutch women and their neonates, levels were examined in 418 mother-infant pairs. Four non-planar PCB congener levels (PCB 118, 138, 153 and 180) were measured in maternal plasma and in umbilical cord plasma. The 208 mothers who breast-fed their infants collected human milk samples for the analysis of seventeen 2,3,7,8-substituted PCDD and PCDF congener levels, three planar PCB and twenty-three non-planar PCB congener levels.

The dioxin and planar PCB levels we measured in human milk (mean 30 respectively 16 pgTEQ/g fat), belong to the highest background levels analysed all over the world but they are in the normal range for highly industrialised, densely populated countries in Western Europe.

Correlation coefficients between PCB 118, 138, 153 and 180 congener levels in maternal plasma and PCB levels in cord plasma or PCB and dioxin levels in human milk are highly significant. However, the 95% predictive interval is too wide to predict accurately the PCB and dioxin levels to which an individual infant is exposed in-utero or postnatally by breast-feeding, from the PCB levels in maternal plasma.

2.2 Introduction

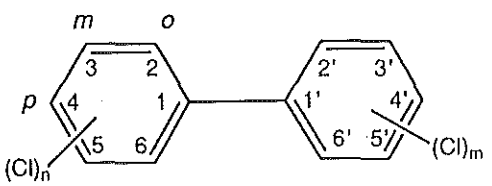
Polychlorinated biphenyls (PCBs) are a compilation of 209 possible congeners with different chlorine substitutions. Within the group of PCB congeners different subgroups can be defined (Figure 2.1). The planar PCBs have no chlorine atom on the ortho position of the biphenyl ring structure and they resemble closely the dioxins because of their planar structure. The mono-ortho PCBs have one chlorine atom on the ortho position and the di-ortho PCBs have two chlorine atoms on the ortho position. All three kinds of PCB structures

are also chlorinated in both para and at least two meta positions. Having more chlorine atoms on the ortho positions the coplanarity between the two phenyl rings is reduced and thus the toxicity of the congener. The degree of biphenyl chlorination also alters the properties and applications of commercial mixtures.[1] After the detection of ubiquitous levels of PCBs in the environment, the production and use of these compounds were banned in the late 1970s. However, large amounts of PCBs were dumped so that land, water and especially sludge can still be highly polluted and thus form a source of contamination for animals and humans.

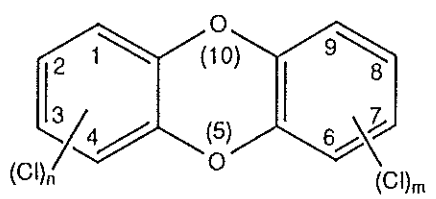
Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), summarized as dioxins, are tricyclic aromatic compounds. Since the number of chlorine atoms can vary between 1 and 8, there are potentially 75 different PCDD and 135 PCDF congeners. They are mainly formed as by-products during the combustion of municipal and hazardous waste and during the manufacturing of organochlorine chemicals.[2]

Higher chlorinated PCB and dioxin congeners are more abundant in most biotic samples because they are highly lipophilic, chemically stable and accumulate in the food chain. For humans the main sources of exposure to these compounds are dairy products, meat and fish.[3] PCBs, PCDDs and PCDFs may produce a wide spectrum of toxic effects in animals and humans.[4-10]

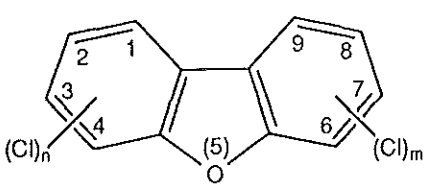
Figure 2.1 Molecular structures of PCBs, PCDDs and PCDFs.



Polychlorinated biphenyls (PCBs)



Polychlorinated dibenzo-*p*-dioxins (PCDDs)



Polychlorinated dibenzofurans (PCDFs)

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

In this paper we wish to answer the following questions: what are the PCB levels in maternal and cord plasma, and what are the PCB, PCDD and PCDF levels in human milk of Dutch women? Is there a decline in PCB or dioxin levels in human milk samples of the second and sixth week after delivery? As dioxin measurements are time-consuming, expensive and require large volumes of blood (100-200 ml), we investigated if one of the more easily measurable PCB congener levels in maternal plasma could predict the PCB and dioxin exposure of the developing fetus and breast-fed infant.

This study is part of the Dutch PCB/Dioxin study, a prospective longitudinal study on possible adverse health effects of these pollutants.

2.3 Methods

The study group consisted 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 industrialised 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.

In order to establish an optimal study population, only infants born at term (37 to 42 weeks of gestation) without congenital anomalies or diseases were included. Pregnancy and delivery had to be passed without overt signs of serious illness or complications. All mothers and infants were of the Caucasian race. In order to study the effects of postnatal dioxin and PCB exposure a group of pregnant women was selected of whom 50% had the intention to breast-feed their infants and the other 50% to give formula feeding. Formula countains negligible levels of dioxins and PCBs. The mothers who breast-fed their infants (n = 208) had to do so for at least 6 weeks. The bottle-fed infants (n = 210) received formula milk from one batch during 7 months.

A blood sample was taken from the mothers in the last month of their pregnancy (36th to 40th week) for the measurement of PCB congener levels 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 2,2'3,4,4',5'-hexachlorobiphenyl (PCB 138), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) and 2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB 180).[11] A blood sample of the umbilical cord was taken to measure the same PCB congener levels as an indication of prenatal exposure. Blood was collected in a vacuum system EDTA-tube, transported in a cooling-pail, and centrifuged within 24 hours during 15 minutes at 3000 rpm; plasma was stored at -20 °C until analysis.

In the second and sixth week after delivery, the mothers collected a 24-hour representative sample of breastmilk by collecting before each feeding as much milk as possible from both breasts with a vacuum pump. An aliquot of ten percent of each sample was pooled and stored in the refrigerator, the remainder was given to the infants in a bottle. After 24 hours the milk was stored at -20 °C until analysis.

PCB congeners in plasma were measured by gas chromatography with electron capture detection (GC-ECD).[12] 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 ng/g 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 analysed to assure the quality.

The human milk samples were analysed 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).[13] Twenty-three non-planar PCB congeners (IUPAC no. 28, 52, 66, 70, 99, 101, 105, 118, 128, 137, 138, 141, 151, 153, 156, 170, 177, 180, 183, 187, 194, 195 and 202)[11] were measured by gas chromatography with electron capture detection (GC-ECD).[14]

To express the toxic potency of the mixture of dioxins and PCBs in breastmilk samples, the toxic equivalence factor (TEF) approach was used according to Safe[15] for the PCDDs and PCDFs and according to the WHO 1993 for the PCBs.[16] 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 milkfat) and TEF value, the toxic equivalent (TEQ) of each congener was calculated (pg TEQ/g milkfat). By adding up the TEQs of all congeners the total-TEQ value was obtained. The TEQ-sum of the 17 dioxin congeners yielded a dioxin-TEQ, the three planar PCB (77, 126, 169), three mono-ortho PCB (105, 118, 156) and two di-ortho PCB congeners (170, 180) revealed a planar, mono-ortho and di-ortho PCB-TEQ respectively. The other PCB congeners did not receive a TEF value because they have negligible dioxin-like activity. The study protocol had been approved by the medical ethical committee of the University Hospital Rotterdam/Sophia Children's Hospital and the University of Groningen. Informed consent had been given by the parents.

Data analysis was performed by means of the statistical soft-ware package SPSS/PC. Means, medians, ranges and standard deviations are reported in terms of original distributions. Spearman rank correlation coefficients were measured between the different congener levels in plasma and human milk. A 95% interval for predicting the total-TEQ in human milk from the PCB 153 level in maternal plasma for an individual subject, was calculated after log transformation of the variables. The paired Student's t test was used to determine differences in mean PCB and dioxin levels in paired human milk samples of the second and sixth week, after log transformation.

2.4 Results

The mean, median and range of PCB 118, 138, 153 and 180 congener levels in maternal and cord plasma are summarized in Table 2.1. Three maternal plasma samples missed, 36 cord plasma samples missed for the PCB 138, 153 and 180 analysis and 45 for the PCB 118 analysis, due to organization failure.

The mean, median, range and available TEQ values of all PCDD, PCDF and PCB congener levels in human milk samples of the second week after birth are summarized in Table 2.2 and 2.3. Representative PCDD, PCDF and planar PCB congener levels are presented of 176 respectively 194 human milk samples and non-planar PCB congener levels of 195 samples.

Spearman rank correlation coefficients between levels of the four different non-planar PCB congeners (118, 138, 153, 180) within one biological sample such as maternal plasma, cord plasma or human milk are all highly significant and vary from 0.71 to 0.98. However, the correlation coefficients in human milk between all other PCB and dioxin congener levels vary considerably from -0.16 to 0.85.

Relations between PCB and dioxin congener levels in different biological samples were also analysed. The correlation coefficients between levels of the four PCB congeners 118, 138, 153 and 180 in maternal plasma and cord plasma vary from 0.52 to 0.74. In Table 2.4 Spearman rank correlation coefficients between these four PCB congener levels in maternal plasma and human milk are presented. The correlation coefficients are all significant, the corresponding congeners having the highest correlations. In Table 2.5 Spearman rank correlation coefficients between these four PCB congener levels in human milk and dioxin- and PCB-TEQ levels in human milk have been summarized. The correlation coefficients are highly significant. In Table 2.6 Spearman rank correlation coefficients between these PCB congener levels in maternal plasma and dioxin-TEQ and PCB-TEQ levels in human milk are summarized. The correlation coefficients are less compared to the coefficients in Table 2.5. In Figure 2.2 the 95% confidence interval for the regression line between the PCB 153 level in maternal plasma and the total-TEQ level in human milk is shown as well as the 95% predictive interval for the total-TEQ from the PCB 153 level for an individual subject. For instance, if the PCB 153 level in maternal plasma would be equal to the mean, 0.91 ng/g plasma, the upper limit of the 95% predictive interval of the total-TEQ level in her breastmilk would be 100.0 pg TEQ/g fat, the lower limit 39.8 pg TEQ/g fat. Since the predictive interval is wide, it is impossible in this way to predict accurately the PCB and dioxin levels to which an individual fetus or breast-fed infant is exposed.

Paired human milk samples collected in the second and sixth week after birth were analyzed for dioxin- and PCB-TEQ levels. Results are presented in Table 2.7. The Student's t test for paired samples showed no significant decrease in mean dioxin-, planar PCB- or total-TEQ level over a four weeks period. However the mean mono-ortho and di-ortho PCB-TEQ level was significantly decreased in this period.

2.5 Discussion

We measured 17 dioxin and 26 PCB congener levels in human milk and 4 PCB congener levels in maternal and cord plasma. The mean dioxin-TEQ level (30 pg TEQ/g fat) is elevated compared to dioxin background levels in the Scandinavian countries (20 pg TEQ/g fat), Spain (13 pg TEQ/g fat) and the United States (17 pg TEQ/g fat). However the dioxin and PCB levels found in this study are comparable to the levels in other highly industrialised, densely populated countries in Western Europe such as Belgium, The United Kingdom and the Federal Republic of Germany.[17] In the Netherlands the National Institute of Public Health and Environmental Protection (RIVM), measured PCDD, PCDF and PCB congener levels in human milk, in a WHO coordinated international study.[18] In 1983 only PCB congener levels (IUPAC no. 28, 52, 101, 118, 153, 138, 180 and 194) were measured. In 1988 both PCBs and dioxins were measured. The PCB levels tended to decrease over the 5-year-period, as is the tendency all over the world. The median PCB congener levels we measured from 1990 to 1992 are somewhat higher (PCB 138 and 153) or similar (PCB 118, 180 and 194) to the results the RIVM presented over the year 1983. This may be due to differences in analytical methods.

PCB congener levels in maternal plasma are about five times higher compared to the levels in umbilical cord plasma. When expressed on a lipid base the levels are comparable[19], because PCBs are highly bound to lipids which contents in cord blood are low compared to maternal blood.[20,21]

The total-TEQ value and the contribution of individual dioxin and PCB congener levels to the TEQ value, depend on the TEF values used. According to the model we used, the dioxins contribute for 46%, the planar PCBs for 24%, the mono-ortho PCBs for 23% and the di-ortho PCBs for 7% to the total-TEQ value (65.2 pgTEQ/g fat). Although the mono-ortho and di-ortho PCBs have low TEFs, they can contribute considerably to the total-TEQ because of their high concentrations in human milk.

Correlation coefficients between the non-planar PCB congener levels (PCB 118, 138, 153 and 180) in maternal plasma, cord plasma and human milk are quite high within one biological sample as plasma or human milk (0.71 to 0.98), as well as between different biological samples. However, correlation coefficients between other PCB and dioxin congener levels in human milk differ considerably. One explanation for this wide range in correlation coefficients could be that different congeners have different half-lives. Secondly, due to limitations of determination, levels of some congeners in plasma as well as in human milk might be less accurate. Finally, correlation coefficients between non-planar PCB levels in maternal plasma and dioxin- or PCB-TEQ levels in human milk are lower compared to the correlation coefficients between the same non-planar PCB congener levels in human milk and the TEQ levels in human milk. Levels in plasma are detected in ng/g plasma while levels in human milk are expressed as ng/g fat. However, when we correlated the PCB plasma levels with the TEQ levels in human milk on product base (ng/g milk), the correlation coefficients were lower. Since new fat is produced in the breast, plasma and milk might be different pools for PCB and dioxin congeners.

We found a decline in mono-ortho and di-ortho PCB-TEQ levels in human milk over a 4-week-period. However, the dioxin-, planar PCB- and total-TEQ levels did not change in this period. Fürst[22] and Norén[23] did report a decline in dioxin levels over a longer period of breast-feeding.

Non-planar PCB levels in maternal plasma are relatively easy and cheap to analyse instead of dioxin and PCB levels in human milk, which are time-consuming, expensive measurements. Moreover it is easier to collect a blood sample than a 24-hour representative human milk sample which is an onerous task for the mothers who collect the milk. Our results demonstrate that non-planar PCB analysis in maternal plasma during pregnancy or even before conception can give an indication about in-utero and postnatal breast-feeding exposure to PCDDs, PCDFs and PCBs, but the 95% predictive interval for an individual infant is wide.

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Table 2.1 Mean, median and range of PCB 118, 138, 153 and 180 levels (ng/g 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

Table 2.2 Mean, median and range (pg/g fat) and mean TEQ (pg TEQ/g fat) of PCDD, PCDF (N = 176) and planar PCB (N = 194) levels in human milk samples of the second week after birth.

PCDD'S	IUPAC	TEF	mean	median	range	TEQ
2,3,7,8-TCDD	48	1	4.0	3.6	0.03-15.8	4.0
1,2,3,7,8-PECDD	54	0.5	10.6	10.2	0.1-30.1	5.3
1,2,3,4,7,8-HXCDD	66	0.1	8.7	8.7	0.04-28.4	0.9
1,2,3,6,7,8-HXCDD	67	0.1	47.4	45.8	18.7-131.8	4.7
1,2,3,7,8,9-HXCDD	70	0.1	6.7	6.7	0.04-21.0	0.7
1,2,3,4,6,7,8-HPCDD	73	0.01	63.2	58.1	0.1-178.4	0.6
1,2,3,4,6,7,8,9-OCDD	75	0.001	799.6	670.4	224.2-3113.6	0.8
PCDF'S	IUPAC	TEF	mean	median	range	TEQ
2,3,7,8-TCDF	83	0.1	0.8	0.7	0.02-3.2	0.08
1,2,3,7,8-PECDF	94	0.05	0.3	0.1	0.02-4.7	0.01
2,3,4,7,8-PECDF	114	0.5	22.7	21.6	8.4-55.0	11.3
1,2,3,4,7,8-HXCDF	118	0.1	6.6	6.4	0.1-15.3	0.7
1,2,3,6,7,8-HXCDF	121	0.1	5.7	5.5	0.1-12.5	0.6
2,3,4,6,7,8-HXCDF	130	0.1	3.6	3.0	0.04-16.9	0.4
1,2,3,7,8,9-HXCDF	124	0.1	0.3	0.1	0.02-21.5	0.03
1,2,3,4,6,7,8-HPCDF	131	0.01	7.9	6.3	0.1-72.3	0.08
1,2,3,4,7,8,9-HPCDF	134	0.01	0.2	0.2	0.05-1.8	0.0
1,2,3,4,6,7,8,9-OCDF	135	0.001	2.2	0.4	0.1-215.5	0.0
DIOXIN-TEQ						30.2
PLANAR PCB'S	IUPAC	TEF	mean	median	range	TEQ
3,3',4,4'-PCB	77	0.0005	19.3	14.8	3.7-143.2	0.01
3,3',4,4',5-PCB	126	0.1	152.0	137.5	39.4-443.9	15.2
3,3',4,4',5,5'-PCB	169	0.01	84.3	79.5	33.2-282.9	0.8
PLANAR PCB-TEQ						16.0

IUPAC = International Union of Pure and Applied Chemistry
TEF = Toxic Equivalence Factor
TEQ = Toxic Equivalent

Table 2.3 Mean, median and range (ng/g fat) and mean TEQ (pg TEQ/g fat) of non-planar PCB levels in human milk samples of the second week after birth. N = 195.

	IUPAC	TEF	mean	median	range	TEQ
2,4,4'	28		12.1	5.8	0.2-188.6	
2,2',5,5'	52		2.6	1.5	0.2-32.7	
2,3',4,4'	66		11.6	9.4	0.4-79.6	
2,3,'4',5	70		18.5	17.4	0.5-54.2	
2,2',4,4',5	99		19.7	18.1	1.4-54.1	
2,2',4,5,5'	101		1.5	1.1	0.2-10.0	
2,3,3',4,4'	105*	0.0001	9.4	8.7	0.4-22.9	0.9
2,3',4,4',5	118*	0.0001	35.5	32.7	9.7-94.0	3.6
2,2',3,3',4,4'	128		4.0	3.7	0.4-16.1	
2,2',3,4,4',5	137		16.8	13.4	1.4-174.6	
2,2',3,4,4',5'	138		129.9	124.2	43.8-314.3	
2,3,4,5,2'5'	141		1.1	0.8	0.3-7.1	
2,2',3,5,5',6	151		0.9	0.8	0.2-7.5	
2,2',4,4',5,5'	153		186.3	174.7	59.9-475.7	
2,3,3',4,4',5	156*	0.0005	21.0	20.1	2.9-78.0	10.5
2,2',3,3',4,4',5	170**	0.0001	37.1	35.4	11.4-219.9	3.7
2,2',3,3',4',5,6	177		6.3	5.9	0.4-22.4	
2,2',3,4,4',5,5'	180**	0.00001	76.8	71.3	2.5-418.8	0.8
2,2',3,4,4',5',6	183		12.2	11.5	2.9-34.6	
2,2',3,4',5,5',6	187		20.0	17.9	2.9-103.8	
2,2',3,3',4,4',5,5'	194		8.6	7.8	0.6-74.2	
2,2',3,3',4,4',5,6	195		2.9	2.7	0.5-15.5	
2,2',3,3',5,5',6,6'	202		0.9	0.7	0.2-8.6	
Mono-ortho-PCB-TEQ						15.0
Di-ortho-PCB-TEQ						4.5

IUPAC = International Union of Pure and Applied Chemistry
TEF = Toxic Equivalence Factor
TEQ = Toxic Equivalent
* = mono-ortho PCB
** = di-ortho PCB

Table 2.4 Spearman rank correlation coefficients between PCB congener levels in human milk (ng/g fat) and PCB congener levels in maternal plasma (ng/g). All correlations have a significance of $p \leq 0.0001$. n = 193.

Human milk	Maternal plasma			
	PCB 118	PCB 138	PCB 153	PCB 180
PCB 118	0.77	0.69	0.64	0.51
PCB 138	0.63	0.79	0.76	0.67
PCB 153	0.62	0.78	0.77	0.72
PCB 180	0.48	0.63	0.66	0.70

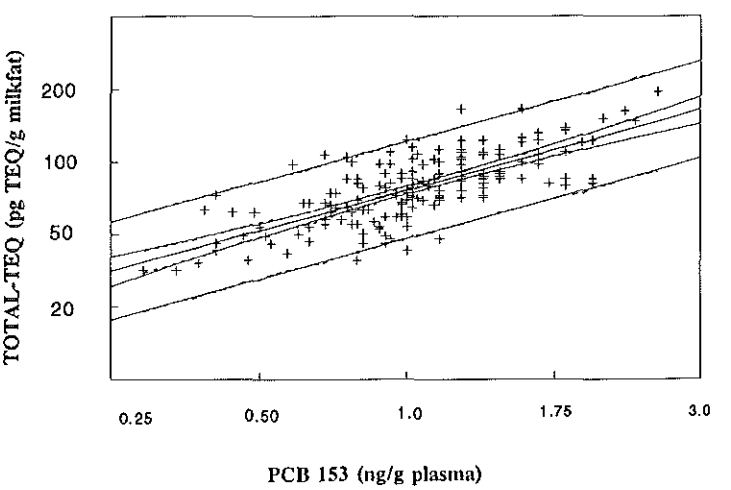
Table 2.5 Spearman rank correlation coefficients between PCB congener levels in human milk (ng/g fat) and dioxin and PCB levels in human milk (pg TEQ/g fat). All correlations have a significance of $p \leq 0.0001$.

Human milk	Human milk				
	dioxins n = 168	planar PCBs n = 186	mono-ortho PCBs n = 195	di-ortho PCBs n = 195	Total TEQ n = 168
PCB 118	0.69	0.81	0.85	0.64	0.84
PCB 138	0.77	0.61	0.93	0.86	0.85
PCB 153	0.78	0.59	0.94	0.90	0.86
PCB 180	0.70	0.47	0.80	0.91	0.73

Table 2.6 Spearman rank correlation coefficients between PCB congener levels in maternal plasma (ng/g) and dioxin and PCB levels in human milk (pg TEQ/g fat). All correlations have a significance of $p \leq 0.0001$.

Maternal plasma	Human milk				
	dioxins	planar PCBs	mono-ortho PCBs	di-ortho PCBs	Total TEQ
	n=174	n = 192	n = 193	n = 193	n = 166
PCB 118	0.64	0.70	0.64	0.51	0.71
PCB 138	0.65	0.51	0.75	0.69	0.71
PCB 153	0.67	0.50	0.75	0.72	0.71
PCB 180	0.63	0.42	0.70	0.76	0.66

Figure 2.2 95% Predictive interval of the total-TEQ level in human milk from the PCB 153 level in maternal plasma.



Log Y = 1.82 + 0.62 Log X

r = 0.73

Table 2.7 Mean dioxin, planar PCB, mono-ortho PCB, di-ortho PCB and total TEQ levels (pg TEQ/g fat) in human milk samples of the second and sixth week after delivery.

	Sample I mean s.d.	Sample II mean s.d.	P*	corr. coeff.**	N
dioxins	34.4 ± 12.4	31.8 ± 10.3	0.07	0.88	27
planar PCBs	16.7 ± 8.0	16.7 ± 7.7	0.91	0.67	44
mono-ortho PCBs	15.2 ± 5.8	14.3 ± 5.4	0.002	0.85	180
di-ortho PCBs	4.5 ± 2.4	4.3 ± 2.3	0.001	0.92	180
total TEQ	72.3 ± 31.3	66.0 ± 23.9	0.10	0.94	19

* = Paired Student's t test, for log normalized values

** = Correlation coefficient between the paired milk samples of one woman

s.d. = standard deviation

2.6 References

1. De Voogt P, Brinkman UATh. Production, properties and usage of polychlorinated biphenyls. In: Kimbrough RD, Jensen AA (eds). Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Elsevier 1989, pp 3-45.
2. Rappe C, Andersson R, Bergquist PA et al. Overview of environmental fate of chlorinated dioxins and dibenzofurans, sources, levels and isomeric pattern in various matrices. Chemosphere 1987; 16: 1603-1618.
3. Theelen RMC. Modeling of human exposure to TCDD and I-TEQ in the Netherlands: Background and occupational. In: Gallo MA, Scheuplein RJ, Van der Heijden KA (eds). Biological basis for risk assessment of dioxins and related compounds. Cold Spring Harbor Laboratory Press; Banbury report 1991; 35: 277-290.
4. Safe S. Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology, and mechanism of action. CRC Crit Rev Toxicol 1984; 13 (4): 319-395.
5. Skene SA, Dewhurst IC, Greenberg M. Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans: The risks to human health. A review. Human Toxicol 1989; 8: 173-203.
6. Murai K, Okamura K, Tsuji H, Kajiwara E, Watanabe H, Akagi K, Fujishina.

Thyroid function in "Yusho" patients exposed to polychlorinated biphenyls. Environ Res 1987; 44: 179-187.
7. Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lutke-Schipholt IJ, Van der Paauw CG, Tuinstra LGMTh, et al.. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatr Res 1994;36:468-473.
8. Rogan WJ, Gladen BC, Hung K-L, Koong S-L, Shih L-Y, Taylor JS, Wu Y-C, Yang D, Ragan NB, Hsu C-C. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 1988; 241: 334-336.
9. Jacobson SW, Fein GG, Jacobson JL, Schwartz PM, Dowler JK. The effect of intrauterine PCB exposure on visual recognition memory. Child Development 1985; 56: 853-860.
10. Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, Tinglestad J, Tully M. Neonatal effects of transplacental exposure to PCBs and DDE. J Pediatr 1986; 109: 335-341.
11. Ballschmiter K, Bacher R, Mennel A, Fischer R, Riehle U, Swerev M. The determination of chlorinated biphenyls, chlorinated dibenzodioxins and chlorinated dibenzofurans by GC-MS. J High Resolut Chromatogr 1992; 15: 260-270.
12. Burse VW, Korver MP, Needham LL, Lapeza CR, Boozer EL, Head SL, et al.. Gas chromatographic determination of polychlorinated biphenyls (as Aroclor 1254) in serum: Collaborative study. J Assoc Off Anal Chem 1989; 72: 649-659.
13. Van Rhijn JA, Traag WA, Van de Spreng PG, Tuinstra LGMTh. Simultaneous determination of planar chlorobiphenyls and polychlorinated dibenzo-p-dioxins and -furans in Dutch milk using isotope dilution and gas chromatography-high-resolution mass spectrometry. J Chromatogr 1993; 630: 297-306.
14. Tuinstra LGMTh, Van Rhijn JA, Traag WA, Van de Spreng P, Zuidema T, Horstman HJ. Method for the determination of dioxins, planar and other PCBs in human milk. In preparation. In: Fiedler H, Frank H et al (eds). Organohalogen Compounds 11, Analytical Methods, Formation and Sources. Federal Environmental Agency, Austria 1993; pp 181-183.
15. Safe S. Polychlorinated biphenyls (PCBs): Environmental impact, biochemical and toxic responses, and implications for risk assessment. CRC Crit Rev Toxicol 1994; 24: 1-63.
16. Ahlborg UG, Becking GC, Birnbaum LS et al. Toxic equivalency factors for dioxin-like PCBs. Report on a WHO-ECEH and IPCS consultation, December 1993. Chemosphere 1994;28:6.
17. World Health Organisation. Levels of PCBs, PCDDs, and PCDFs in breast milk: results of WHO-coordinated interlaboratory quality control studies and analytical field studies. FADL, Copenhagen. Environmental Health series 1989; 34.
18. Albers JMC, Kreis IA, Van Zoonen P, Liem AKD, Theelen RMC. Contamination of breastmilk with chlorinated organic compounds in the Netherlands, 1988. RIVM Rapport nr 638815002 (in Dutch, summary in English) 1993.
19. Huisman M, Koopman-Esseboom C, Touwen BCL, Van der Paauw CG, Sauer

PJJ, Huisjes HJ, Muskiet FAJ, Boersma ER. Risk assessment of fetal exposure to polychlorinated biphenyls (PCBs). 13th Europian congress of perinatal medicine; Amsterdam (abstracts).
J Perinat Med 1992;20 suppl 1:125.
20. Jensen AA. Transfer of chemical contaminants into human milk. In: Jensen AA, Slorach SA (eds). Chemical contaminants in human milk. CRC Press 1991:9-19.
21. Van den Berg M, De Jongh J, Poiger H, Olson JR. The toxicokinetics and metabolism of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), and their relevance for toxicity. CRC Critical Reviews in Toxicology 1994;24:1-74.
22. Fürst P et al. PCDD and PCDF levels in human milk. Dependence on the period of lactation. Chemosphere 1989;18:439.
23. Norén K. Changes in the levels of organochlorine pesticides, polychlorinated biphenyls, dibenzo-p-dioxins and dibenzofurans in human milk from Stockholm, 1972-1985. Chemosphere 1988;17:39-49.

CHAPTER 3

DIOXIN AND PCB LEVELS IN BLOOD AND HUMAN MILK IN RELATION TO LIVING AREAS IN THE NETHERLANDS.

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DIOXIN AND PCB LEVELS IN BLOOD AND HUMAN MILK IN RELATION TO LIVING AREAS IN THE NETHERLANDS.

Corine Koopman-Esseboom[1], Marcel Huisman[2], Nynke Weisglas-Kuperus[1], E. Rudy Boersma[2], Maria A.J. de Ridder[3], Cees G. Van der Paauw[4], Louis G.M.Th. Tuinstra[5], Pieter J.J. Sauer[1].

[1]Department of Paediatrics, Division of Neonatology, Erasmus University and University Hospital/Sophia Children's Hospital, Rotterdam.
[2]Department of Obstetrics and Gynaecology, University of Groningen.
[3]Institute of Epidemiology and Biostatistics, Erasmus University Rotterdam.
[4]TNO Nutrition and Food Research, Zeist.
[5]DLO State Institute for Quality Control of Agricultural Products, RIKILT-DLO, Wageningen, The Netherlands.

3.1 Abstract

Dioxins and polychlorinated biphenyls (PCBs) are ubiquitous toxic compounds in the environment. Negative influences of these compounds on the health status of human beings have been described. Especially susceptible might be the fetus, which is exposed in utero, and the newborn breast-fed infant, since both are exposed to relatively high levels of dioxins and PCBs during a critical period of organ growth and development.

We investigated PCB levels in 406 maternal plasma samples as well as PCB and dioxin levels in 172 human milk samples with relation to living area of women living for at least five years in the western industrialized part of the Netherlands or the northern more rural part. The western part was further subdivided into one urban and two highly industrialized areas.

After correction for covariates, we found significantly higher levels of PCB 118 in maternal plasma as well as significantly higher levels of the dioxin-TEQ and of ten individual dioxin and PCB congener levels in human milk in the western more industrialized areas of the Netherlands compared to the northern more rural part. We did not find significant differences in planar, mono-ortho or di-ortho PCB-TEQ levels in human milk between all different areas.

We conclude that significantly higher levels of a number of dioxin and PCB congeners are found in women living in industrialized areas compared to women living in rural areas in the Netherlands.

3.2 Introduction

Dioxins (polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs)), are formed as unwanted by-products mainly during the production of chlorinated chemicals and during the combustion of municipal and hazardous waste.[1,2] After an explosion in a herbicide factory in Seveso, Italy, a large amount of 2,3,7,8-TCDD, the most toxic dioxin congener, was released, and

high levels (up to 56,000 ppt) could be measured afterwards in the blood of people living close to that factory.[3] Elevated levels have also been detected in Vietnam veterans, who sprayed Agent Orange, a defoliant contaminated with dioxins during the production process.[4] In the Netherlands elevated dioxin levels (>6 pg TEQ/g fat) have been detected in the milk of cows, grazing close to waste incinerators.[5]

Polychlorinated biphenyls (PCBs) are commercial chemical mixtures produced on a large scale since 1930 because of their wide range of application in industry,[6] such as coolant fluids in transformers and dielectric fluids in capacitors. Elevated levels could be measured in blood of transformer repair workers[7] and in blood of humans exposed accidentally to high levels of PCBs and PCDFs in rice bran oil for consumption.[8] When their widespread occurrence in the environment and subsequent adverse health effects became clear, PCB production and use were banned in the late 1970s. However, humans are still exposed through PCB leakage of old capacitors and transformers and disposal of contaminated materials such as old paints and fire retardants. Otherwise large amounts of PCBs were dumped on land and in water and became widespread in the environment by long-range atmospheric transport.

The higher chlorinated congeners are chemically stable, highly lipophilic substances which accumulate in the food chain. Food, such as dairy products, meat and fish, is regarded to be the main source of background exposure for humans.[9] Inhaling and dermal absorption could be additional sources of exposure to dioxins and PCBs.

It is well known that differences in human dioxin and PCB levels exist between countries.[10] In this paper we wish to answer the question if differences exist, within a small country as the Netherlands, between heavily industrialized areas and more rural areas, assuming that food habits are not different.

3.3 Methods

The study group consisted of 418 healthy women recruited between June 1990 and June 1992. 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 obstetric optimality scale[11] was used to evaluate pregnancy and delivery. As far as smoking is concerned the women were categorized into 3 subgroups: non-smokers, 1 to 10 cigarettes a day, or more than 10 cigarettes a day. Additional questions were asked about the living area from birth until the time of the study and about the duration of breast-feeding periods of older children. Pregnancy and delivery had to be passed without overt signs of serious illness or complications. All infants were born between the 37th and 42th weeks of pregnancy. All women were of the Caucasian race. Nobody was likely to be exposed to elevated levels of dioxins or PCBs during occupation.

One hundred and ninety-eight women who have been living for at least five years in Rotterdam city and the surrounding industrialized region in the western part of the Netherlands were selected for this study. Nine mothers in this region had been living in different living areas in the Netherlands or abroad during the

last five years and were therefore excluded from this part of the study. The other 211 selected women were living in Groningen and the surrounding area, which is a semi-urban and rural region in the northern part of the Netherlands (see map, Figure 3.1).

Figure 3.1 Map of the Netherlands

1 = Rotterdam
2 = Vlaardingen/Schiedam
3 = Spijkenisse
4 = Groningen

The group of 198 women living in the western part of the Netherlands was subdivided into three subgroups. One hundred and nine women were living in Rotterdam (map nr 1), which is an urban area; 49 women lived in Vlaardingen or Schiedam (map nr 2) which is located on the northern edge of a highly industrialized zone containing a large waste incinerator and a number of large oil refineries and chemical industries; 40 women lived in Spijkenisse (map nr 3) which is located on the southern edge of the same industrialized zone. The distance between the industrialized area and the living area is less than 6 kilometers and for some women it was less than 2 kilometers. Of the group of 409 women, 202 breast-fed their infants. Fifty-two were living in Rotterdam, 30 in Vlaardingen or Schiedam, 17 in Spijkenisse and 103 in Groningen (map nr 4).

A blood sample was taken from the women in the last month of their pregnancy (36th to 40th week) for the measurement of the PCB congeners 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) and 2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB 180).[12] Blood was collected in a vacuum system EDTA-tube, transported in a cooling-pail, and within 24 hours centrifuged for 15 minutes at 3000 rates per minute; plasma was stored at -20 °C until analysis.

In the second week after delivery, the women who breast-fed their infants collected a 24-hour representative sample of breastmilk by collecting before each feeding as much milk as possible from both breasts with a vacuum pump. An aliquot of ten percent of each sample was pooled and stored in the refrigerator, the remainder was given to the infants in a bottle. After 24 hours the milk was stored at -20 °C until analysis.

PCB congeners in plasma were measured by gas chromatography with electron capture detection (GC-ECD).[13] The detection limit was 0.01 ng/g plasma.

The human milk samples were 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).[14] Twenty-three non-planar PCB congeners (IUPAC no. 28, 52, 66, 70, 99, 101, 105, 118, 128, 137, 138, 141, 151, 153, 156, 170, 177, 180, 183, 187, 194, 195 and 202)[12] were measured by gas chromatography with GC-ECD.[15]

To express the toxic potency of the mixture of dioxins and PCBs in breastmilk samples, the international toxic equivalence factor (I-TEF) approach[16] was used for the PCDDs and PCDFs and the WHO 1993 approach for the PCBs.[17] A TEF value was assigned to the dioxin and dioxin-like PCB congeners which represents their relative toxic potency compared to 2,3,7,8-TCDD, the most toxic dioxin congener which is assigned a TEF value of one. By multiplying the concentration (pg/g milkfat) and TEF value, the toxic equivalent (TEQ) of each congener was calculated (pg TEQ/g milkfat). By adding up the TEQs for all congeners the total-TEQ value was obtained. The TEQ-sum of the 17 dioxin congeners yielded a dioxin-TEQ, the three planar PCB (77, 126, 169), three mono-ortho PCB (105, 118, 156) and two di-ortho PCB congeners (170, 180) gave a planar, mono-ortho and di-ortho PCB-TEQ respectively. The other PCB congeners did not receive a TEF value because they have negligible dioxin-like activity.

The study protocol had been approved by the medical ethical committee of the University Hospital Rotterdam/Sophia Children's Hospital and the University of Groningen. Informed consent had been given by the parents.

Data analysis was performed by means of the statistical soft-ware package SPSS/PC. Means, 95% confidence intervals, ranges and standard deviations are reported in terms of original distributions. Multiple regression analysis was used to test the influences of different covariates, which are well-known from the literature, on the dioxin and PCB levels. The four living areas as well as the three categories of smoking were entered in the regression analysis as dummy variables. Age in years, Quetelet index (weight/length^2) of the women before

pregnancy, number of children delivered, weeks of breast-feeding to older children, fat percentage of the human milk sample and birthweight of the delivered infant were entered as continuous covariables. To correct for non-linear relationships, age and weeks of breast-feeding were also raised to a square and entered in the analyses. The PCB and dioxin levels were transformed by natural logarithmics if necessary.

3.4 Results

In Table 3.1 the mean, standard deviation, 95% confidence interval and range of the four PCB congener levels measured in maternal plasma are demonstrated for the four subgroups. One plasma sample in the Rotterdam group and two in the Groningen group were not determined. Oneway analysis of variance showed a significant difference in the PCB 118 level of women living in the different areas.

In Table 3.2 the results of the dioxin, planar-, mono-ortho- and di-ortho-PCB TEQ levels, measured in human milk, are presented for the four subgroups. Due to analytical problems not all TEQ values could be calculated in the human milk samples, therefore the number of samples analysed in the four subgroups differ. There were no significant differences for these levels in the four living areas with the oneway analysis of variance test.

In Table 3.3 demographic data of the four subgroups are presented, which are used as covariates in the multiple regression analysis. Oneway analysis of variance showed a significant difference in the covariates birthweight of the infants ($p = 0.04$) and age of the women ($p = 0.002$) within the breast-feeding groups of the different areas. For the other covariates there was no significant difference.

Multiple regression analysis was performed for all individual PCB and dioxin congener levels in plasma or human milk and for the TEQ levels in human milk. In Table 3.4 results are given for the PCB 118 level in plasma and the dioxin-TEQ level in milk. Both levels are significantly higher in Rotterdam and Spijkenisse compared to Groningen. Analysis of the individual congener levels in human milk showed that 1,2,3,4,7,8-HxCDD (D66), 1,2,3,6,7,8-HxCDD (D67), 2,3,7,8-TCDF (F83), 1,2,3,4,7,8-HxCDF (F118), 1,2,3,6,7,8-HxCDF (F121), 2,3,4,6,7,8-HxCDF (F130) and 2,3',4,4'-tetrachlorobiphenyl (PCB 66), 2,2',3,4,4',5-hexachlorobiphenyl (PCB 137) and 2,2',3,4',5,5',6-heptachlorobiphenyl (PCB 187) were also significantly higher in the western areas compared to the northern area. The PCB 118 level in maternal plasma was also higher in Spijkenisse compared to Vlaardingen/Schiedam. There was no statistical significant difference in the planar, mono-ortho, di-ortho PCB-TEQ levels or other individual congener levels for the different areas.

3.5 Discussion

We investigated PCB levels in plasma and PCB and dioxin levels in human milk of Dutch women living in highly industrialized, urban or more rural areas.

After correction for covariates we found significantly higher levels of PCB 118 in maternal plasma, and of dioxin-TEQ and 10 individual dioxin and PCB congener levels in human milk from the western industrialized areas compared to the more rural areas in the northern part of the Netherlands.

It is well-known that dioxin and PCB levels in human milk can vary considerably in different countries. The WHO published data of a coordinated study in 1988[10]. The highest dioxin levels (30-40 pg TEQ/g fat) were found in Belgium, the Federal Republic of Germany, the Netherlands and the United Kingdom and these data are in agreement with our results; the lowest levels (10 pg TEQ/g fat) were measured in human milk samples from Hungaria and former Yugoslavia. Middle-range values were detected in samples from the USA, Canada, Japan, Austria, Poland and Scandinavia (15-24 pg TEQ/g fat). Some results from the WHO study indicate also that there are differences between the dioxin-TEQ level and specific dioxin congener levels in different geographic areas within one country. Such as is the case in the Hanoi area in Vietnam (low levels) compared to areas in southern Vietnam where the levels are elevated and this may be associated with the spraying of large amounts of Agent Orange, a herbicide contaminated with dioxins, during the war in the 1970s. In some countries (e.g. Austria, Belgium, Canada, Norway and Sweden) the levels are slightly but not significantly elevated in industrial and urban areas compared to rural areas. However, no such differences have been observed in former Yugoslavia or in human milk sampled in the Netherlands in 1988.

PCB results from the WHO study are not comparable for all participating countries because they used different analytical techniques. The mean sum of the measured PCB levels (28, 52, 101, 138, 153 and 180) in Western Europe was equally high (392-762 ug/kg) compared to the mean PCB sum of these same congeners in our study (409 ug/kg). The levels measured in Finland, the USA, Vietnam and Thailand were lower. Comparisons with the Scandinavian countries and former Yugoslavia were impossible because they used packed column rather than capillary column gas chromatography analysis. Results from different areas within one country, like Belgium, do not show significant differences between areas with different geographical, population or pollution profiles. In contrast to our study, PCB levels in the Netherlands reported in the WHO study, were slightly but not significantly elevated in rural compared to urban areas. In this study, significant differences in levels of several PCB and dioxin congeners were observed for women living in different areas in the Netherlands. It is possible that these congeners are emitted by some industries in close proximity to the living areas. In 1989 the milk of cows grazing close to a municipal solid waste incinerator, located in between Vlaardingen and Spijkenisse (a sampling area in this study), was excluded for human consumption because the dioxin level of 6 pg I-TEQ/g milk fat was exceeded.[5] Cows' milk in this area contained mainly higher chlorinated PCDDs and PCDFs. Near other incinerators lower as well as higher chlorinated PCDDs were found.

Waste incineration accounted in 1991 for approximately 85% of the total annual dioxin emission into the air in the Netherlands.[2] Municipal waste incinerators are mainly located in the western part of the Netherlands. Besides incinerators there are other industries in the western area that produce dioxins as unwanted by-products. The consumption of dairy products, meat or fish

(estimated as being 95% of the total human exposure[9]) from this area may be a source of elevated dioxin levels in human plasma and milk in this part of the Netherlands. However, in the Netherlands milk is transported throughout the whole country and consumption of milk products solely from the western industrialized area is unlikely.

Inhaling or dermal absorption of dioxins and PCBs is another route of exposure. However, it is estimated that less than 5% of the daily intake occurs via these routes. Since the combustion of leaded-petrol is also a source of dioxins[18], living in densely populated areas with high vehicular traffic might be an additional source of exposure for humans.

We conclude that the dioxin-TEQ level as well as some individual dioxin and PCB congener levels in women are significantly higher in the western industrialized area of the Netherlands compared to the northern more rural area. Further studies have to be carried out to determine the sources that account for these differences.

3.6 References

1. Rappe C, Andersson R, Bergqvist PA, Brohede C, Hansson M, Kjeller LO, et al. Overview of environmental fate of chlorinated dioxins and dibenzofurans, sources, levels and isomeric pattern in various matrices. Chemosphere 1987; 16: 1603-1618
2. Bremmer HJ, Troost LM, Kuipers G, De Koning J, Sein AA. Emissions of dioxins in the Netherlands. RIVM/TNO Report nr. 770501003 (summary in English) 1993.
3. Mocarelli P, Needham L, Marocchi A, Patterson GD, Brambilla P, Gerthoux PM, et al. Serum correlation of 2,3,7,8 tetrachlorodibenzo-p-dioxin and test results from selected residents of Seveso, Italy. J Toxicol Environ Health 1991; 32: 357
4. Wolfe WH, Michalek JE, Miner JC, Rahe AJ, Silva J, Thomas WF, et al.. Health status of Air Force veterans occupationally exposed to herbicides in Vietnam. 1. Physical health. J Am Med Assoc 1990; 264: 1824-1831
5. Liem AKD, Hoogerbrugge R, Kootstra PR, Van der Velde EG, De Jong APJM. Occurrence of dioxins in cow's milk in the vicinity of municipal waste incinerators and a metal reclamation plant in the Netherlands. Chemosphere 1990; 23: 1675-1684
6. De Voogt P, Brinkman UATh. Production, properties and usage of polychlorinated biphenyls. In: Kimbrough RD, Jensen AA (eds). Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Elsevier 1989; pp 3-45
7. Hara I. Health status and PCBs in blood of workers exposed to PCBs and of their children. Environ Health Perspect 1985; 59: 85-90
8. Kuratsune M, Yoshimura T, Matsuzaka J, Yamaguchi A. Epidemiological study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. Environ Health Perspect 1972; 1: 119-128

9. Theelen RMC. Modeling of human exposure to TCDD and I-TEQ in the Netherlands: Background and occupational. In: Gallo MA, Scheuplein RJ, Van der Heijden KA (eds). Biological basis for risk assessment of dioxins and related compounds. Cold Spring Harbor Laboratory Press. Banbury report 1991; 35: 277-290
10. World Health Organisation. Levels of PCBs, PCDDs, and PCDFs in breast milk: results of WHO-coordinated interlaboratory quality control studies and analytical field studies. FADL, Copenhagen. Environmental Health Series, 1989; Vol 34
11. Touwen BCL, Huisjes HJ, Jurgens-v.d. Zee, Bierman-van Eendenburg MEC, Smrkovsky M, Olinga AA Obstetrical condition and neonatal neurological morbidity. An analysis with the help of the optimality concept. Early Hum Dev 1980; 4/3:207-228
12. Ballschmiter K, Bacher R, Mennel A, Fischer R, Riehle U, Swerev M. The determination of chlorinated biphenyls, chlorinated dibenzodioxins and chlorinated dibenzofurans by GC-MS. J High Resolut Chromatogr 1992; 15: 260-270
13. Burse VW, Korver MP, Needham LL, Lapeza CR, Boozer EL, Head SL, et al.. Gas chromatographic determination of polychlorinated biphenyls (as Aroclor 1254) in serum: Collaborative study. J Assoc Off Anal Chem 1989; 72: 649-659
14. Van Rhijn JA, Traag WA, Van de Spreng PG, Tuinstra LGMTh. Simultaneous determination of planar chlorobiphenyls and polychlorinated dibenzo-p-dioxins and -furans in Dutch milk using isotope dilution and gas chromatography-high-resolution mass spectrometry. J Chromatogr 1993; 630: 297-306
15. Tuinstra LGMTh, Van Rhijn JA, Traag WA, Van de Spreng P, Zuidema T, Horstman HJ. Method for the determination of dioxins, planar and other PCBs in human milk. In: Fiedler H, Frank H, Hutzinger O, Parzefall W, Riss A, Safe S (eds). Organohalogen Compounds 11, Analytical Methods, Formation and Sources. Federal Environmental Agency, Austria 1993; pp 181-183
16. NATO/CCMS. Pilot study on International Information Exchange on Dioxins and Related Compounds. International Toxicity Equivalency Factor (I-TEF). Method of Risk Assessment for Complex Mixtures of Dioxins and Related Compounds. Report number 176.
17. Ahlborg UG, Becking GC, Birnbaum LS, Brouwer A, Derks HJGM, Feeley M et al. Toxic Equivalency Factors for dioxin-like PCBs. Report on a WHO-ECEH and IPCS consultation, December 1993. Chemosphere 1994; 28:6.
18. Ballschmiter K, Buchert H, Niemczyk R, Munder A, Swerev M. Automobile exhausts versus municipal-waste incineration as sources of the polychloro-dibenzodioxins (PCDD) and -furans (PCDF) found in the environment. Chemosphere 1986; 15: 901-915

Table 3.1 Mean, standard deviation, 95% confidence interval and ranges of PCB levels in maternal plasma (ng/g plasma).

	Rotterdam n = 108	Vlaardingen/ Schiedam n = 49	Spijkenisse n = 40	Groningen n = 209	P
PCB 118					
mean	0.17 ± 0.07	0.16 ± 0.08	0.20 ± 0.12	0.15 ± 0.08	
95% c.i.	0.16-0.18	0.14-0.18	0.16-0.24	0.14-0.17	
range	0.06-0.38	0.02-0.39	0.06-0.60	0.04-0.58	.03
PCB 138					
mean	0.58 ± 0.24	0.58 ± 0.25	0.66 ± 0.31	0.60 ± 0.25	
95% c.i.	0.54-0.63	0.51-0.65	0.57-0.76	0.56-0.63	
range	0.16-1.40	0.19-1.40	0.30-1.50	0.13-1.60	.43
PCB 153					
mean	0.90 ± 0.38	0.88 ± 0.36	0.99 ± 0.44	0.89 ± 0.36	
95% c.i.	0.83-0.98	0.77-0.98	0.85-1.13	0.84-0.94	
range	0.24-2.20	0.29-1.90	0.44-2.00	0.18-2.30	.55
PCB 180					
mean	0.53 ± 0.24	0.50 ± 0.19	0.58 ± 0.25	0.53 ± 0.22	
95% c.i.	0.48-0.57	0.45-0.56	0.50-0.66	0.50-0.56	
range	0.13-1.30	0.18-1.10	0.25-1.20	0.08-1.30	.50

95% c.i. = 95% confidence interval
P' = overall p-value, oneway analysis of variance on logarithmic scale.

Table 3.2 Mean, standard deviation, 95% confidence interval and ranges of dioxin and PCB levels in human milk (pg TEQ/g fat).

	Rotterdam	Vlaardingen/ Schiedam	Spijkenisse	Groningen	P
Dioxin-TEQ	n = 38	n = 26	n = 15	n = 93	
mean	30.8 ± 9.3	30.3 ± 13.2	34.3 ± 12.1	28.9 ± 9.7	
95% c.i.	27.7-33.9	25.0-35.6	27.6-41.0	26.9-30.9	
range	11.1-56.5	13.8-76.4	15.8-56.7	12.2-58.6	.34
Planar PCB TEQ	n = 44	n = 30	n = 16	n = 99	
mean	16.4 ± 5.9	15.2 ± 8.3	18.0 ± 9.7	15.8 ± 7.6	
95% c.i.	14.6-18.2	12.1-18.3	12.9-23.2	14.3-17.3	
range	7.5-37.6	4.4-35.9	7.9-45.7	4.6-39.8	.38
Mono-ortho PCB TEQ	n = 49	n = 30	n = 14	n = 95	
mean	13.7 ± 4.4	14.1 ± 5.9	15.0 ± 5.5	15.5 ± 5.5	
95% c.i.	12.5-15.0	11.9-16.3	11.8-18.2	14.4-16.6	
range	3.2-25.4	6.4-24.0	7.0-25.8	5.9-30.4	.23
Di-ortho PCB TEQ	n = 49	n = 30	n = 14	n = 95	
mean	4.0 ± 1.4	4.0 ± 1.6	4.3 ± 1.8	4.6 ± 1.7	
95% c.i.	3.6-4.4	3.4-4.6	3.2-5.3	4.3-5.0	
range	1.6-9.7	1.8-7.0	2.2-8.3	1.5-10.4	.15

95% c.i. = 95% confidence interval

P* = p-value, oneway analysis of variance on logarithmic scale.

Table 3.3 Means and standard deviation of the covariates in the four living areas.

	Rotterdam		Vlaardingen/Schiedam		Spijkenisse		Groningen	
	blood	milk	blood	milk	blood	milk	blood	milk
	n=108	n=38	n=49	n=26	n=40	n=15	n=209	n=93
age	28.8±4.0	28.2±3.2	28.6±3.6	28.8±3.0	28.9±3.7	28.9±2.7	29.1±3.8	30.4±3.5
smoking								
1	82	30	39	21	29	13	149	80
2	19	7	5	4	4	1	46	12
3	7	1	5	1	7	1	14	1
quetele index	22.7±4.0	22.6±3.9	22.1±3.3	22.5±3.7	22.6±2.5	22.6±2.3	22.4±3.2	22.0±2.8
child number	1.5±0.6	1.5±0.6	1.5±0.5	1.5±0.5	1.7±0.6	1.7±0.5	1.6±0.6	1.5±0.6
breast-feeding	5.4±13.3	8.7±14.8	7.1±14.0	12.0±16.6	5.4±9.7	11.91±2.9	5.5±11.9	11.5±16.8
fatperc		3.0±0.6		3.2±0.7		2.8±0.8		3.0±0.8
birth-weight	3450±418	3460±418	3453±451	3392±400	3429±494	3429±494	3564±442	3627±460

Smoking 1 = non-smoking
Smoking 2 = 1-10 cigarettes a day
Smoking 3 = > 10 cigarettes a day.

Breast-feeding = weeks of given breast-feeding to older children.
Fatperc = fat percentage in human milk.
Blood = maternal plasma samples.
Milk = human milk samples.

Table 3.4 Results of multiple regression analysis.

	R^2		C	Age	Age^2	Fat perc.	Breast-fed.	Rotter-dam	Vlaard Schie-dam	Spijke-nisse	Area overall p	S1	S2	Smoking overall p
Ln PCB118 maternal plasma	22	b	-5.27	0.18	-0.002		-0.005	0.12	0.009	0.23		-0.16	-0.26	
		se	0.94	0.07	0.001		0.002	0.05	0.07	0.08		0.06	0.08	
DF = 405		p	0.00	0.005	0.04		0.015	0.02	0.90	0.003	0.007	0.007	0.002	0.006
Ln DIOXIN-TEQ human milk	26	b	-0.87	0.27	-0.004	-0.11	-0.005	0.15	0.10	0.19				
		se	1.51	0.10	0.002	0.03	0.002	0.06	0.07	0.09				
DF = 171		p	0.57	0.009	0.03	0.0006	0.003	0.01	0.13	0.03	0.02			

DF = degrees of freedom
C = constant
b = regression coefficient
SE = standard error
Fatperc = fat percentage in human milk
Breast-fed = weeks of given breast-feeding to older children
S1 = 1-10 cigarettes a day
S2 = > 10 cigarettes a day
Baseline area = Groningen
Baseline smoking = non-smoking

CHAPTER 4

EFFECTS OF DIOXINS AND POLYCHLORINATED BIPHENYLS ON THYROID HORMONE STATUS OF PREGNANT WOMEN AND THEIR INFANTS

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EFFECTS OF DIOXINS AND POLYCHLORINATED BIPHENYLS ON THYROID HORMONE STATUS OF PREGNANT WOMEN AND THEIR INFANTS

Corine Koopman-Esseboom[1], Dennis C. Morse[2], Nynke Weisglas-Kuperus[1], Ineke J. LutkeSchipholt[2], Cornelis G. Van der Paauw[3], Louis G.M.Th. Tuinstra[4], Abraham Brouwer[2], Pieter J.J. Sauer[1].

[1]Department of Pediatrics, Division of Neonatology, Erasmus University and University Hospital/Sophia Children's Hospital, Rotterdam.
[2]Department of Toxicology, Agricultural University, Wageningen.
[3]TNO Nutrition and Food Research, Zeist.
[4]DLO State Institute for Quality Control of Agricultural Products, Wageningen, The Netherlands.

4.1 Abstract

Dioxins (polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs)) and polychlorinated biphenyls (PCBs) are potentially hazardous compounds. Animal studies have demonstrated that PCDDs, PCDFs and PCBs can alter thyroid hormone homeostasis.

We investigated thyroid hormone levels in 105 mother-infant pairs. To estimate maternal and infant exposure, four non-planar PCB congeners were measured in maternal plasma during the last month of pregnancy and in umbilical cord plasma. Seventeen PCDD and PCDF congeners, three planar, and 23 non-planar PCB congeners were measured in human milk.

Higher PCDD, PCDF and PCB levels in human milk, expressed as toxic equivalents (TEQs), correlated significantly with lower plasma levels of maternal total triiodothyronine (TT_3) and total thyroxine (TT_4), and with higher plasma levels of thyroid stimulating hormone (TSH) in the infants in the 2nd week and 3rd month after birth. Infants exposed to higher TEQ levels had also lower plasma free thyroxine (FT_4) and TT_4 levels in the 2nd week after birth.

We conclude that elevated levels of dioxins and PCBs can alter the human thyroid hormone status.

4.2 Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), summarized as dioxins, are tricyclic aromatic compounds. Because the number of chlorine atoms can vary between one and eight, there are potentially 75 different PCDD and 135 PCDF congeners. They are mainly formed as by-products during the synthesis of organochlorine chemicals and during the combustion of municipal and hazardous waste.[1] Polychlorinated biphenyls (PCBs) are a compilation of 209 possible congeners with different chlorine

substitutions. Because of their unique physical properties and chemical stability, mixtures were used for diverse industrial applications, such as coolant fluids in transformers and dielectric fluids in capacitors, since 1930.[2] In the late 1970s, the production and the use of these compounds were banned because adverse health effects had become evident.[3,4] However, there are still significant quantities of PCBs used in older transformers and capacitors.

Both dioxins and PCBs are highly lipophilic and chemically stable compounds that accumulate in the food chain. The higher chlorinated congeners are more abundant in most biotic samples. For human beings the main sources of environmental exposure to these toxic compounds are dairy products, meat, and fish. They are stored mainly in the human adipose tissue, with elimination half-lives of 6 to 10 years.[5] Small amounts of dioxins and PCBs can reach the fetus by means of transplacental transport[6], whereas much higher levels reach the breast-fed infant. Dioxin levels can be especially high (10-100 pg TEQ/g milkfat) in human milk samples from highly industrialised, densely populated countries such as the Netherlands, Belgium, the United Kingdom, and Germany.[7]

PCDDs, PCDFs and PCBs produce a wide spectrum of toxic effects in animals including body weight loss, immunotoxicity, thymic atrophy, hepatotoxicity, teratogenicity, carcinogenicity, and reproductive toxicity.[8,9] Furthermore, they can alter thyroid hormone status in laboratory animals. In rats and monkeys, exposure to PCDDs, PCDFs, or PCBs generally results in decreased plasma thyroxine (T_4) levels, accompanied by increased concentrations of thyroid stimulating hormone (TSH).[10-13] There is evidence that human exposure to these compounds may result in altered thyroid hormone status as well. Reduced serum T_4 levels have been observed in transformer repair workers exposed to PCBs.[14] A recent study in a small group of breast-fed infants suggests that elevated levels of PCDDs and PCDFs in human milk might be associated with increases in plasma TT_4 and TSH levels.[15]

The aim of this study was to evaluate the effects of PCDDs, PCDFs and PCBs on the thyroid hormone status of pregnant women and their infants. This study is part of the Dutch PCB/Dioxin Study, a larger prospective longitudinal study on possible adverse health effects of these pollutants on human beings.

4.3 Methods

The study group consisted of 105 healthy mother-infant pairs recruited between June 1990 and February 1992, living in Rotterdam and the surrounding area, which is a highly industrialised region in The Netherlands. 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 obstetric, delivery, and neonatal conditions were assessed by means of the obstetrical optimality scale.[16] To establish an optimal study population, only infants born at term (37 to 42 weeks of gestation) without congenital anomalies or diseases were included. All mother-infant pairs were of the Caucasian race. Pregnancy and delivery had to be completed without overt signs of serious illness or complications, and the infants had to be breast-fed for at least 6 weeks.

A blood sample was taken from the mothers in the last month of their pregnancy (36th to 40th week) for the measurement of the PCB congeners 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) and 2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB 180).[17] A blood sample of the umbilical cord was taken to measure the same PCB congeners as an indication of prenatal exposure. Blood was collected in a vacuum system EDTA-tube, transported in a cooling-pail, and centrifuged within 24 hours during 15 minutes at 3000 rpm; plasma was stored at -20 °C until analysis.

The detection of PCDDs and PCDFs in human tissues and milk became possible only in 1984. Because large amounts of blood (100-200 mL) are still needed to measure PCDD and PCDF levels, we analysed them in human milk samples. Because these levels in human milk correlate well with the dioxin levels in adipose tissue[18], they are good indicators of maternal load and of prenatal and postnatal exposure of the breast-fed infant.

In the 2nd week after delivery, the mothers collected a 24-hour representative sample of breast milk by collecting before each feeding as much milk as possible from both breasts with a vacuum pump. An aliquot of 10% of each sample was pooled and stored in the refrigerator, the remainder was given to the infants in a bottle. After 24 hours the milk was stored at -20 °C until analysis.

PCB congeners in plasma were measured by gas chromatography with electron capture detection (GC-ECD).[19] The human milk samples were 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).[20] Twenty-three non-planar PCB congeners (PCB 28, 52, 66, 70, 99, 101, 105, 118, 128, 137, 138, 141, 151, 153, 156, 170, 177, 180, 183, 187, 194, 195, 202)[17] were measured by GC-ECD.[21]

To express the toxic potency of the mixture of dioxins and PCBs in breast milk samples, the toxic equivalence factor (TEF) approach was used.[22,23] A TEF value was assigned to the dioxin and dioxin-like PCB congeners to represent their relative toxic potency toward 2,3,7,8-TCDD, the most toxic dioxin congener, which has a TEF value of one. By multiplying the concentration (pg/g milkfat) and TEF value, the toxic equivalent (TEQ) of each congener was calculated (pg TEQ/g milkfat). By adding up the TEQs for all congeners, the total PCB-dioxin TEQ value was obtained. The TEQ sum of the dioxin and PCB congeners yielded a dioxin-TEQ, planar-PCB-TEQ (PCB 77, 126, and 169), and non-planar-PCB-TEQ (PCB 105, 118, 156, 170, and 180). The other PCB congeners did not receive a TEF value because they have negligible dioxin-like activity.

Total thyroxine (TT_4), total triiodothyronine (TT_3), free thyroxine (FT_4), and thyroid stimulating hormone (TSH) were measured in maternal plasma during the last month of pregnancy (maternal pregnancy), 9 to 14 days after delivery (maternal post delivery), in plasma of the umbilical cord (umbilical cord plasma), and in infants' plasma 9 to 14 days (infant 2 weeks) as well as three months (infant 3 months) after birth. TT_3, TT_4, FT_4, and TSH were determined by

chemiluminescence immunoassay, using standard Amerlite assay kits (Amersham, England).

The study protocol was approved by the medical ethical committee of the University Hospital Rotterdam/Sophia Children's Hospital. Informed consent was given by the parents.

Data analysis was performed by means of the statistical software package SPSS/PC. Means, medians, ranges, and standard deviations are reported in terms of original distributions. Spearman rank correlation coefficients were measured between TT_4, TT_3, FT_4, TSH levels and individual PCB and dioxin congener levels as well as dioxin-TEQ, PCB-TEQ and total PCB-dioxin TEQ levels. Because of the large number of analyses, a p-value <0.01 was estimated as being statistically significant. The Mann-Whitney test was used to analyze the significance of differences in thyroid hormone status between a low and high exposed breast-fed group; a p-value <0.05 was estimated as being statistically significant.

4.4 Results

Of the initial 105 mother-infant pairs, 78 fulfilled all criteria and were included in the analysis. The median and range of TT_3, TT_4, FT_4, and TSH levels in maternal and infant plasma are summarized in Table 4.1. One mother appeared to have an autoimmune hypothyroidism with high TSH levels. The levels of all the other mother-infant pairs were in the normal range for age-appropriate controls.

The mean level of the total PCB-dioxin TEQ in human milk was 74.86 pg TEQ/g fat (SD 26.19, range 30.85-154.21), the dioxin-TEQ 32.06 pg TEQ/g fat (SD 11.26, range 12.44-76.43), the planar-PCB-TEQ 19.95 pg TEQ/g fat (SD 8.54, range 6.39-51.11) and the non-planar-PCB-TEQ 22.75 pg TEQ/g fat (SD 8.96, range 8.52-58.19).

The significant correlation coefficients between TEQ levels and thyroid hormone plasma levels are summarized in Table 4.2. Higher levels of total PCB-dioxin TEQ, dioxin-TEQ and both planar- and non-planar-PCB-TEQ in human milk were significantly correlated with lower maternal plasma TT_3 levels in the last month of pregnancy and with lower maternal plasma TT_3 and TT_4 levels in the 2nd week after delivery. There was a trend toward a decline in the maternal plasma TT_4 level in the last month of pregnancy with higher planar-PCB-TEQ levels ($r=-0.27$, $p<0.05$). There were no significant correlations with maternal plasma FT_4 or TSH levels. The total PCB-dioxin TEQ, dioxin-TEQ and PCB-TEQ levels demonstrated no significant correlations with TT_3, TT_4, FT_4 or TSH levels in umbilical cord plasma. However, higher TEQ levels were significantly correlated with higher plasma TSH levels in the infants in the 2nd week and 3rd month after birth.

Analyses of all individually measured PCDD, PCDF, and PCB congeners in human milk demonstrated that five PCDD congeners (2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD), three PCDF congeners (2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF), 2 planar PCB congeners (PCB 126 and 169) and twelve non-planar PCB congeners

(PCB 105, 118, 128, 138, 153, 156, 170, 177, 180, 183, 187, and 194) gave correlation coefficients with maternal or infants' thyroid hormone levels of the same order of magnitude as did the total PCB-dioxin TEQ, dioxin-TEQ, and PCB-TEQ levels. Higher levels of three non-planar PCB congeners (PCB 137, 138, and 153) in human milk also correlated significantly with higher TSH levels in umbilical cord plasma ($r = 0.31$, $p < 0.01$). Higher levels of PCB 118, 138, 153, and 180 in maternal and cord plasma correlated significantly with higher plasma TSH levels in the infants in the 2nd week after birth.

In Table 4.3, the infants have been divided into a low (less than or equal to the median (30.75 pg TEQ/g fat)) and a high (greater than the median) dioxin-exposed breast-fed group. The mean plasma TT_4 level was significantly lower, and the mean plasma TSH level was significantly higher in the high exposed group in the 2nd week after birth. In umbilical cord plasma and at 3 months, only the mean plasma TSH level was significantly higher in the high dioxin-exposed breast-fed group (8.5 ± 6.0 *versus* 11.6 ± 8.0 μIU/mL, $p < 0.05$, and 1.6 ± 0.6 *versus* 2.3 ± 1.0 μIU/mL, $p < 0.0004$). When the infants were divided into low and high total PCB-dioxin TEQ exposed breast-fed groups (median = 72.43 pg TEQ/g fat), the mean plasma FT_4 level was also significantly lower in the high exposed group in the 2nd week after birth (24.6 ± 3.5 *versus* 23.0 ± 3.3 pmol/L, $p < 0.05$).

4.5 Discussion

In this study, we found mean dioxin levels in human milk (32 pg TEQ/g fat) that are elevated compared to the mean dioxin levels of countries in Northern and Southern Europe such as Sweden and Spain, which have levels of 20 and 13 pg TEQ/g fat, respectively. The dioxin and PCB levels are comparable to the levels in other highly industrialised countries in Western Europe, such as Belgium, the United Kingdom, and Germany.[7] Except for one mother with high TSH levels, plasma TT_3, TT_4, FT_4, and TSH levels of all mother-infant pairs were in the normal range. We demonstrated that higher dioxin and PCB-TEQ levels in human milk were significantly correlated with lower maternal plasma TT_3 and TT_4 levels and with higher infant plasma TSH levels in the 2nd week and 3rd month after birth. Twenty-two individual PCDD, PCDF and PCB congener levels measured in human milk gave correlation coefficients with maternal and infant thyroid hormone levels of the same order of magnitude as did the TEQ levels. It is impossible from this study to define the dioxin or PCB congeners that really have an effect on thyroid hormone levels because there are associations between the congener patterns in humans[18], who are exposed mainly to one source, food.

Reduced serum T_4 levels have also been described in transformer repair workers exposed to PCBs.[14] Murai et al.[24] described an increase in serum T_3 and T_4 levels in adults 16 years after exposure to high levels of PCBs and PCDFs via food compared to non-exposed controls. Serum FT_4, TSH and thyroxine binding globulin (TBG) levels were not altered. However, they did not find significant correlations between PCB levels and thyroid hormone levels in serum. The results might be different from our data because PCBs were measured as total

PCB level, which is less accurate compared to our congener specific analysis. Furthermore, a short exposure to high PCB levels might have different effects on thyroid hormone parameters compared to a long-term background exposure. Pluim et al.[15] divided a group of 38 healthy newborns into a high- and low-dioxin exposed breast-fed group compared to the median dioxin-TEQ (29.0 pg TEQ/g fat) in human milk. In agreement with our study, they found a significantly higher mean plasma TSH level 11 weeks after birth in the high exposed group. In contrast to our study, they found significantly higher mean TT_4 levels in the infants' plasma at 1 week and 11 weeks after birth.

In general, the effects of dioxins and PCBs in our human study resemble the effects on plasma TT_3, TT_4, FT_4, and TSH levels in rats and monkeys.[10-13, 25] In these animals, dioxins and PCBs cause a reduction of plasma TT_4 and FT_4 levels and a concomitant increase in TSH levels. Plasma TT_3 levels are usually much less affected by dioxins and PCBs. In rats, hydroxy-metabolites of PCBs cause a decline in plasma TT_4 levels. The metabolites have a marked structural resemblance to T_4 and occupy the T_4 binding pocket on transthyretin (TTR), the most important plasma binding protein for T_4 in rats.[26] In *in vitro* studies, hydroxy-metabolites of PCBs and dioxins also compete with T_4 for human TTR.[27] The binding capacity of TBG, which is the most important binding protein for T_4 in human plasma, does not seem to be influenced by PCBs or dioxins.[28] Although the competition for binding to TTR is unlikely to be the cause for a decreased plasma TT_4 and TT_3 level in humans, it might still be of importance for T_4 passage through the blood-brain barrier. Herbert et al. postulated that, also in humans, T_4 transport from the circulation into the cerebrospinal fluid is based on the binding of T_4 to TTR produced in the choroid plexus.[29] Hydroxy-metabolites of PCBs, which can also be measured in human tissues[30], could cause a decline in the local brain T_4 level by interrupting the T_4 transport on TTR.

Besides affecting plasma thyroid hormone transport, dioxins and PCBs can also alter peripheral T_4 metabolism. In rats, dioxins and PCBs decrease plasma TT_4 levels by inducing hepatic uridine diphosphate-glucuronyltransferase. As a consequence, the biliary excretion of T_4-glucuronide is enhanced.[11,31] Morse et al.[32] demonstrated that decreases in plasma TT_4 and FT_4 levels, induced by PCBs, were accompanied by increases of type II thyroxine 5'-deiodinase, the enzyme responsible for the deiodination of T_4 to biologically active T_3 in the brain of adult, fetal, and neonatal rats.

Small amounts of maternal thyroid hormone pass the placental barrier[33] and could be important for the early development of the fetus, which is able to produce its own thyroid hormones from the 12th week on. Further research is necessary to elucidate the mechanism by which dioxins and PCBs alter the thyroid hormone status in humans.

We did not find significant correlations between PCB levels in cord plasma or dioxin levels in human milk and TT_3, TT_4, FT_4, or TSH levels in umbilical cord plasma. However, when we related all individual PCB congener levels measured in human milk with the thyroid hormone levels in umbilical cord plasma, higher PCB 137, 138, and 153 congener levels correlated significantly with higher TSH levels in cord plasma. There are a few explanations for these differences in results. The PCB levels we measured in umbilical cord plasma were about 5

times lower compared to the levels in maternal plasma, about 20 times lower compared to PCB levels in human milk on volume base, and close to the detection limit. Secondly, the PCB levels in plasma were expressed on volume base and the dioxin and PCB levels in human milk on lipid base. Because dioxins and PCBs are bound mainly to lipids, which contents can vary, PCB levels in plasma expressed on lipid base might be more representative.

The higher plasma TSH levels in the 2nd week and 3rd month after birth may be a sustained reaction to prenatal exposure to dioxins and PCBs to remain euthyroid. An alternative explanation for these increases in TSH levels is additional postnatal exposure to dioxins and PCBs through breast-feeding. When neonatal plasma TT_3, TT_4, FT_4, and TSH levels were divided into low- and high-dioxin exposed breast-fed groups, plasma TT_4 was significantly lower and TSH significantly higher in the high exposed group in the 2nd week after birth. When the thyroid hormone levels were divided into a low and high total PCB-dioxin TEQ exposed breast-fed group, plasma FT_4 was also significantly lower in the high exposed group. At the age of 3 months, we found no significant differences in the infants' plasma TT_3, TT_4, or FT_4 levels, although TSH was still significantly higher. The infants' thyroid hormone production could have come into a new balance by TSH stimulation.

We demonstrated that Dutch levels of dioxins and PCBs correlate with alterations of the human thyroid hormone status. Sufficient levels of T_4, which is deiodinated into active T_3 on the tissue level, are necessary for normal brain development.[34] Although the thyroid hormone levels were found to be in the normal range, the small changes observed in this study might be of influence on the development of the fetus and infant; this will be the subject of further study.

4.6 References

1. Rappe C, Andersson R, Bergqvist PA, Brohede C, Hansson M, Kjeller LO, Lindström G, Marklund S, Nygren M, Swanson SE, Tysklind M, Wiberg K 1987 Overview of environmental fate of chlorinated dioxins and dibenzofurans, sources, levels and isomeric pattern in various matrices. Chemosphere 16:1603-1618.
2. De Voogt P, Brinkman UATh 1989 Production, properties and usage of polychlorinated biphenyls. In: Kimbrough RD, Jensen AA (eds) Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Elsevier, Amsterdam, pp 3-45.
3. Kuratsune M, Yoshimura T, Matsuzaka J, Yamaguchi A 1972 Epidemiological study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. Environ Health Perspect 1: 119-128.
4. Rogan WJ, Gladen BC, Hung K-L, Koong S-L, Shih L-Y, Taylor JS, Wu Y-C, Yang D, Ragan NB, Hsu C-C 1988 Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 241: 334-336.
5. Michalek JE, Tripathi RC, Caudill SP, Pirkle JL 1992 Investigation of TCDD half-life heterogeneits in veterans of operation ranch hand. J Toxicol Environ Health 35: 29-38.

6. Schecter A, Päpke O, Ball M 1990 Evidence for transplacental transfer of dioxins from mother to fetus: chlorinated dioxins and dibenzofuran levels in livers of stillborn infants. Chemosphere 21:1017-1022.
7. World Health Organisation 1989 Levels of PCBs, PCDDs, and PCDFs in breast milk: results of WHO-coordinated interlaboratory quality control studies and analytical field studies. FADL, Copenhagen. Environmental Health series 34.
8. Safe S 1984 Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology, and mechanism of action. CRC Crit Rev Toxicol 13:319-395.
9. Skene SA, Dewhurst IC, Greenberg M 1989 Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans: The risks to human health. A review. Human Toxicol 8:173-203.
10. Ness DK, Schantz SL, Moshtaghian J, Hansen LG 1993 Effects of perinatal exposure to specific PCB congeners on thyroid hormone concentrations and thyroid histology in the rat. Toxicol Letters 68:311-323.
11. Bastomsky CH 1977 Enhanced thyroxine metabolism and high uptake goiters in rats after a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxine. Endocrinology 101:292-296.
12. Van den Berg KJ, Zurcher C, Brouwer A 1988 Effects of 3,4,3′,4′-tetrachlorobiphenyl on thyroid function and histology in marmoset monkeys. Toxicol Appl Pharmacol 41:77-86.
13. Brewster DW, Elwell MR, Birnbaum LS 1988 Toxicity and disposition of 2,3,4,7,8-pentachlorodibenzofuran (4PeCDF) in the rhesus monkey (Macaca mulatta). Toxicol Appl Pharmacol 93:231-246.
14. Emmett EA, Maroni M, Jefferys J, Schmith J, Levin BK, Alvares A 1988 Studies of transformer repair workers exposed to PCBs: II: Results of clinical laboratory investigations. Am J Ind Med 14:47-62.
15. Pluim HJ, Koppe JG, Olie K, Van der Slikke JW, Vulsma T, Kok JH, Vulsma T, van Tijn D, de Vijlder JJM 1992 Effects of dioxins on thyroid function in newborn babies. The Lancet 339:1303.
16. Touwen BCL, Huisjes HJ, Jurgens-v.d. Zee, Bierman-van Eendenburg MEC, Smrkovsky M, Olinga AA 1980 Obstetrical condition and neonatal neurological morbidity. An analysis with the help of the optimality concept. Early Hum Dev 4/3:207-228.
17. Ballschmiter K, Bacher R, Mennel A, Fischer R, Riehle U, Swerev M 1992 The determination of chlorinated biphenyls, chlorinated dibenzodioxins and chlorinated dibenzofurans by GC-MS. J High Resolut Chromatogr 15:260-270.
18. Jensen AA 1991 Transfer of chemical contaminants into human milk. In: Jensen AA, Slorach SA (eds) Chemical contaminants in human milk. CRC Press, pp 9-19.
19. Burse VW, Korver MP, Needham LL, Lapeza CR, Boozer EL, Head SL, et al. 1989 Gas chromatographic determination of polychlorinated biphenyls (as Aroclor 1254) in serum: Collaborative study. J Assoc Off Anal Chem 72:649-659.

20. Van Rhijn JA, Traag WA, Van de Spreng PG, Tuinstra LGMTh 1993 Simultaneous determination of planar chlorobiphenyls and polychlorinated dibenzo-p-dioxins and -furans in Dutch milk using isotope dilution and gas chromatography-high-resolution mass spectrometry. J Chromatogr 630:297-306.
21. Tuinstra LGMTh, Van Rhijn JA, Traag WA, Van de Spreng P, Zuidema T, Horstman HJ 1993 Method for the determination of dioxins, planar and other PCBs in human milk. In: Fiedler H, Frank H, Hutzinger O, Parzefall W, Riss A, Safe S (eds) Organohalogen Compounds 11, Analytical Methods Formation and Sources. Federal Environmental Agency, Austria, pp 181-183.
22. NATO/CCMS. Pilot study on International Information Exchange on Dioxins and Related Compounds. International Toxicity Equivalency Factor (I-TEF). Method of Risk Assessment for Complex Mixtures of Dioxins and Related Compounds. Report number 176.
23. Ahlborg UG, Becking GC, Birnbaum LS, Brouwer A, Derks HJGM, Feeley M, Golor G, Hanberg A, Larsen JC, Liem AKD, Safe SH, Schlatter C, Waern F, Younes M, Yrjänheikki E 1994 Toxic Equivalency Factors for dioxin-like PCBs. Report on a WHO-ECEH and IPCS consultation, December 1993. Chemosphere 28:6.
24. Murai K, Okamura K, Tsuji H, Kajiwara E, Watanabe H, Akagi K, Fujishina 1987 Thyroid function in "Yusho" patients exposed to polychlorinated biphenyls. Environ Res 44:179-187.
25. Van Birgelen A, Van der Kolk J, Fase K, Poiger H, Brouwer A, Van den Berg M 1992 Toxicity and biochemical potencies of polychlorinated biphenyl congeners relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin in three months feeding studies in the rat. In: Organohalogen Compounds 10, Toxicology, Epidemiology, Risk Assesment and Management. Painotalo Miktor, Helsinki, pp 373-376.
26. Brouwer A, Van den Berg KJ 1986 Binding of a metabolite of 3,4,3',4'-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxin. Toxicol Appl Pharmacol 85:301-312.
27. Lans MC, Klasson-Wehler E, Willemsen M, Meussen E, Safe S, Brouwer A 1993 Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, dibenzo-p-dioxins and dibenzofurans with human transthyretin. Chem Biol Interact 88:7-21.
28. Lans MC, Spiertz C, Brouwer A, Koeman JH. Differential, competitive interaction of hydroxy-PCBs, PCDDs and PCDFs with transthyretin(TTR) and thyroxine-binding globulin (TBG). Eur J Pharmacol, Toxicology Section. In press.
29. Herbert J, Wilcox JN, Pham K-TC, Fremeau RT, Zeviani M, Dwork A, Soprano DR, Makover A, Goodman, DeWitt S, Zimmerman EA, Roberts JL, Schon EA 1986 Transthyretin: a choroid plexus-specific transport protein in human brain. Neurology 36:900-911.

30. Klasson-Wehler E, Kuroki H, Athanasiadou M, Bergman Å 1992 Selective retention of hydroxylated PCBs in blood. In: Organohalogen compounds 10, Toxicology, Epidemiology, Risk Assesment and Management. Painotalo Miktor, Helsinki, pp 121-122.
31. Beetstra JB, Van Engelen JGM, Karels P, Van der Hoek HJ, De Jong M, Docter R, Krenning EP, Henneman G, Brouwer A, Visser TJ 1991 Thyroxine and 3,3',5-triiodothyronine are glucuronidated in rat liver by different uridine diphosphate-glucuronyltransferases. Endocrinology 128:741-746.
32. Morse DC, Groen D, Veerman M, Van Amerongen CJ, Koëter HBWM, Smits van Prooije AE, Visser TJ, Koeman JH, Brouwer A 1993 Interference of polychlorinated biphenyls in hepatic and brain thyroid hormone metabolism in fetal and neonatal rats. Toxicol Appl Pharmacol 122:27-33.
33. Vulsma T, Gons MH, De Vijlder JJM 1989 Maternal-fetal transfer of thyroxine in congenital hypothyroidism due to a total organification defect or thyroid agenesis. N Engl J Med 321:13-16.
34. Porterfield SP, Hendrich CE 1993 The role of thyroid hormones in prenatal and neonatal neurological development - current perspectives. Endocr Rev 14:94-106.

Table 4.1 Median and range of plasma TT_4, TT_3, FT_4, and TSH levels of women in the last month of pregnancy and in the second week after delivery, of the umbilical cord and of infants in the 2nd week and 3rd month after birth*

Thyroid hormone parameter	Maternal pregnancy (n = 78)	Maternal after delivery (n = 77)	Umbilical cord (n = 75)	Infant second week (n = 78)	Infant third month (n = 78)
TT_4 nmol/l	152.2 (89.3-210.3)	134.7 (77.2-190.1)	127.1 (78.3-187.3)	168.7 (83.9-245.7)	139.3 (72.3-214.5)
TT_3 nmol/l	2.5 (1.9-3.9)	2.1 (1.4-2.5)	1.0 (0.6-1.9)	2.9 (2.0-4.3)	3.0 (2.0-3.8)
FT_4 pmol/l	11.7 (8.0-16.0)	15.3 (11.6-21.5)	16.0 (12.0-23.7)	23.7 (17.2-34.1)	17.2 (12.4-24.5)
TSH μIU/ml**	1.4 (0.02-18.0)	1.0 (0.03-6.6)	10.0 (2.8-33.7)	2.3 (0.7-9.8)	2.0 (0.8-6.0)

* Values are median with range in parentheses.
** One mother with high TSH levels due to an autoimmune hypothyroidism included.

Table 4.2 Spearman rank correlation coefficients between TT_3 and TT_4 levels in maternal plasma and TSH levels in infants' plasma and toxic equivalents (TEQs) of dioxins, planar, and non-planar PCBs in human milk.

	Maternal pregnancy TT_3 (n = 78)	Maternal after delivery TT_3 (n = 77)	Maternal after delivery TT_4 (n = 77)	Infant 2nd week TSH (n = 78)	Infant 3rd month TSH (n = 78)
DIOXIN-TEQ	-.47***	-.35**	-.34**	.38***	.41***
PLANAR-PCB-TEQ	-.39***	-.38***	-.33**	.37***	.31**
NON-PLANAR-PCB-TEQ	-.36***	-.33**	N.S.	.38***	N.S.
TOTAL PCB-DIOXIN TEQ	-.46***	-.37***	-.35**	.40***	.39***

** = $p \leq 0.01$
*** = $p \leq 0.001$
N.S. = not significant

Table 4.3 Plasma TT_3, TT_4, FT_4, and TSH levels in infants in the 2nd week of life in a low- and high-dioxin exposed breast-fed group.

	Low-dioxin exposed (≤30.75 pg TEQ/g fat)		High-dioxin exposed (>30.75 pg TEQ/g fat)		
	Mean ± S.D.	n	Mean ± S.D.	n	P*
TT_3 (nmol/l)	2.9 ± 0.5	39	2.9 ± 0.5	39	0.66
TT_4 (nmol/l)	177.5 ± 39.2	39	159.9 ± 31.6	39	0.04
FT_4 (pmol/l)	24.3 ± 3.4	39	23.1 ± 3.4	39	0.16
TSH (μIU/ml)	1.9 ± 0.8	39	2.6 ± 1.5	39	0.004

* Mann-Whitney test

CHAPTER 5

NEWBORNS DIAGNOSED AS NEUROLIGALLY ABNORMAL WITH RELATION TO PCB/DIOXIN EXPOSURE AND THEIR THYROID HORMONE STATUS

Submitted

NEWBORNS DIAGNOSED AS NEUROLIGALLY ABNORMAL WITH RELATION TO PCB/DIOXIN EXPOSURE AND THEIR THYROID HORMONE STATUS

Corine Koopman-Esseboom[1], Marcel Huisman[2], Bert C.L. Touwen[3], E. Rudy Boersma[2], Abraham Brouwer[4],
Pieter J.J. Sauer[1], Nynke Weisglas-Kuperus[1].

[1]Department of Pediatrics, Division of Neonatology, Erasmus University and University Hospital/Sophia Children's Hospital, Rotterdam.
[2]Department of Obstetrics and Gynaecology, University of Groningen.
[3]Department of Medical Physiology, Developmental Neurology, University Hospital, Groningen.
[4]Department of Toxicology, Agricultural University, Wageningen, The Netherlands.

5.1 Abstract

Objective: Polychlorinated biphenyls (PCBs) and dioxins are both widespread environmental pollutants, which can alter thyroid hormone metabolism. We examined if in utero or lactational exposure to these contaminants is related to neonatal neurological abnormalities in human infants. In addition, we studied the infants' thyroid hormone status in relation to their neurological outcome.

Study Design: PCB levels were measured in maternal and umbilical cord plasma as an estimation of prenatal exposure. In human milk, PCB as well as dioxin levels were examined as a measure of the pre- and postnatal exposure to these contaminants. The neonatal neurological examination technique according to Prechtl, was applied to 418 newborns. In a subgroup of 207 mother-infant pairs, thyroxine (TT4), total triiodothyronine (TT3), free thyroxine (FT4) and thyroid stimulating hormone (TSH) plasma levels were measured.

Results: Four neonates were diagnosed as neurologically definitively abnormal and twenty as mildly abnormal. We did not find a significant relationship between either the perinatal PCB and dioxin exposure, or the infants' thyroid hormone status, and their neonatal neurological status.

Conclusion: In utero or early lactational exposure to PCBs and dioxins is not related to clinically relevant neonatal neurological abnormalities.

5.2 Introduction

During the last decades it became evident that polychlorinated biphenyls (PCBs) and dioxins (polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzo-furans (PCDFs)), which are both widespread and stable toxins in the environment, may cause central nervous system dysfunctioning in animals (Safe 1984). Mice displayed a neurobehavioural syndrome, called "spinning syndrome", after exposure to PCBs in utero (Chou et al. 1979). Rhesus monkeys exposed perinatally to PCBs, exhibited locomotor hyperactivity

during their infant-stage but once they reached the age of 44 months, they were hypoactive (Bowman et al. 1981).

In human adults a decrease in nerve conduction time, and motor neuropathy have been found after accidental exposure to PCBs, PCDFs and 2,3,7,8-tetrachlorodibenzo-p-dioxin (Filippini et al. 1981). After two outbreaks of PCB, PCDF and polychlorinated quaterphenyl poisoning in Japan and Taiwan, infants exposed in utero showed mainly dermal lesions as chloracne and hyperpigmentation (Kuratsune et al. 1972; Rogan et al. 1988). In follow up studies these children showed signs of apathy, hypotonia, clumsiness and sluggishness as well as a delay in psychomotor development (Harada 1976; Chen et al. 1994[A]; Chen et al. 1994[B]). Two studies conducted in the USA describe a relationship between in utero exposure to background levels of PCBs and a negative outcome on the muscle tone and reflex cluster score on the one hand (Rogan et al. 1986), and on the autonomic maturity, reflex and range of state cluster score (Jacobson et al. 1984) of the Brazelton Neonatal Behavioural Assessment Scales (BNBAS) on the other hand. In a previous paper we described that higher levels of PCBs and dioxins in human milk, as an estimation of the *in utero* and early lactational exposure to these compounds, were significantly related to a reduced neonatal neurological optimality and to a higher incidence of hypotonia (Huisman et al. 1995). The clinical significance of these subtle effects is not clear.

PCBs and dioxins can also alter the thyroid hormone status in animals by decreasing the circulating thyroxine (T4) and increasing the thyroid stimulating hormone (TSH) level (Bastomsky 1977; Ness et al. 1993). In a previous paper we described a decrease in maternal total triiodothyronine (TT3) and total thyroxine (TT4) plasma level and an increase in the infants' thyroid stimulating hormone (TSH) level with an increase in PCB and dioxin load (Koopman-Esseboom et al. 1994[A]). Adequate levels of thyroid hormones are essential for a normal development of the central nervous system (Porterfield et al. 1993). Alterations in thyroid hormone levels might be the direct cause of central nervous system dysfunctioning in infants exposed to PCBs and dioxins.

In this article the clinical neonatal neurological findings, in relation to perinatal PCB and dioxin exposure, as well as thyroid hormone status, are described. This study is part of the Dutch PCB/dioxin study, a prospective longitudinal study on possible adverse health effects of these pollutants in human beings.

5.3 Methods

Study group: Women were recruited for the study by obstetricians and midwives in the last trimester of pregnancy, between June 1990 and June 1992. An explanation of the study protocol was given at home by one of the examiners (CKE and MH). In order to study the effects of postnatal exposure to PCBs and dioxins, we enrolled women who intended to give breast-feeding, which contains relatively high levels of both toxins, as well as women who would voluntarily give formula-feeding from one batch as a reference (Almiron M2, Nutricia N.V. the Netherlands), with negligible levels of PCBs and dioxins

during a period of 7 months. The women who breast-fed their infants had to do so for at least 6 weeks.

Half of the population was recruited in the Rotterdam area, an industrialized zone, located in the western part of the Netherlands. The other half of the study population was enrolled in the Groningen area, which is a semi-urban region in the northern part of the Netherlands. In order to study a homogeneous population, the mother-infant pairs had to meet the following criteria: absence of serious illness and complications during pregnancy and delivery, Caucasian race, first or second born term infant (37-42 weeks of gestation), no Caesarian section, forceps or vacuum extraction. The obstetrical condition of the mother and her infant was described by means of the obstetrical optimality score (Touwen et al. 1980), consisting of 72 items which were classified according to 7 categories. These categories comprise: social background, nonobstetric conditions during pregnancy, obstetric history, obstetric aspects of present pregnancy, diagnostic and therapeutic measures, parturition, and neonatal condition immediately after birth.

The study protocol had been approved by the medical ethical committee of the University Hospital Rotterdam/Sophia Children's Hospital and the University of Groningen. Informed consent had been given by the parents.

Neonatal neurological examination: At fourteen days after birth (range 6-30 days) the infants were examined at home using the neonatal neurological examination technique described by Prechtl (Prechtl 1977). Posture, motility, muscle tone, stability of states, tendon, and neonatal reflexes with their thresholds were examined in the appropriate behavioural state. Each infant was clinically diagnosed as neurologically normal, mildly abnormal, or definitively abnormal. Mildly abnormal implies inconclusive indications of a syndrome, e.g. mild hypotonia or a slight, coarse tremor. Definitively abnormal implies the presence of overtly syndromes such as hyperexcitability, hypotonia or hypertonia, or a hemisyndrome.

The examination can be summarized according to the neurological optimality score by means of 60 items (Touwen et al. 1980). A separate postural tone cluster score, consisting of 10 items, and a reflex cluster score, consisting of 11 items, were also constructed (Huisman et al. 1995).

Measures of exposure: A blood sample was taken from the mothers in the last month of their pregnancy (36th to 40th week) and from the umbilical cord, for the measurement of the PCB congener levels 118, 138, 153 and 180 as a measure of prenatal exposure. The technique used for these analyses was gas chromatography with electron capture detection (GC-ECD) (Burse et al 1989). The sum of these four congeners in maternal as well as in cord plasma (PCB-plasma-sum), was used in the statistical analyses.

In the second and sixth week after delivery, the mothers who breast-fed their infants collected a 24-hour representative sample of breastmilk with an electric pump (Babyluxus 2, KAWECO, Stuttgart, Germany), for the analysis of seventeen 2,3,7,8-substituted PCDD and PCDF congeners and three planar PCB congeners (International Union of Pure and Applied Chemistry (IUPAC) no. 77, 126 and 169) by gas chromatography-high-resolution mass spectrometry (GC-HRMS) (Van Rhijn et al. 1993). Twenty-three non-planar PCB congeners (IUPAC no. 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 (Tuinstra et al. 1994). The same measurements were done in samples of the formula-feeding batch.

To express the toxic equivalent (TEQ) of dioxins and the dioxin-like PCBs (planar PCBs 77, 126 and 169; mono-ortho PCBs 105, 118 and 156; di-ortho PCBs 170 and 180) in breastmilk samples, the toxic equivalence factor (TEF) approach was used as described in detail in a previous paper (Koopman-Esseboom et al. 1994[B]).

For twenty-four infants, dioxin and PCB levels were not available in human milk, sampled in the second week after birth. Since there is no significant decline in levels over a 4-week period (Koopman-Esseboom et al. 1994[B]), we used their levels of the sixth week sample. Both examiners were unaware of the results of the chemical analyses.

Assessment of the thyroid hormone metabolism: In the Rotterdam subgroup TT3, TT4, FT4 and TSH levels were measured in maternal and cord plasma samples as well as in infants' plasma collected after the neurological examination, by chemiluminescence immunoassay, using standard Amerlite assay kits (Amersham, England).

Statistical analyses: Data analysis was performed by means of the statistical software package SPSS/PC. Means and standard deviations (s.d.) are reported in terms of original distributions. The Mann-Whitney Test was used to compare group means of the neurologically normal versus abnormal group for the obstetric optimality, the neurological optimality and cluster scores, and thyroid hormone levels. The Spearman rank test was used for univariate correlation analyses.

5.4 Results

At birth 489 mother-infant pairs fulfilled the inclusion criteria of the study protocol. At six weeks 71 pairs were excluded, mainly because they ceased giving breast-feeding before the sixth week after birth. Of the remaining 418 women, 209 breast-fed and 209 formula-fed their infants. Two hundred and seven pairs lived in the Rotterdam area (105 breast-fed and 102 formula-fed infants), and 211 pairs lived in the Groningen area (104 breast-fed and 107 formula-fed infants).

Through the neonatal neurological examination, four infants were clinically diagnosed as neurologically definitively abnormal (2 in Rotterdam, and 2 in Groningen). Twenty infants were found to be neurological mildly abnormal (11 in Groningen, 9 in Rotterdam). In Table 5.1 the clinical diagnoses of these infants are summarized. One infant diagnosed as neurologically definitively abnormal, had an Erb's palsy. This diagnosis is related with birth trauma and therefore, the baby was excluded from further analysis. Since the number of infants diagnosed as mildly abnormal or definitively abnormal is small, for further analysis infants were divided into 2 groups: neurologically normal (n = 394) and neurologically abnormal (n = 23).

In Table 5.2 the obstetrical optimality score with its 7 cluster scores, as well as the neurological optimality score with its 2 cluster scores are presented for

both groups. There are no statistically significant differences in any of the obstetric scores of both groups. As was expected, the neurologically normal infants had significantly higher neurological optimality scores compared to the abnormal infants.

In Table 5.3 the mean PCB-sum in maternal and cord plasma as well as the mean PCB- and dioxin-TEQ levels in human milk are presented for both groups. In formula-feeding the PCB and dioxin levels were below the limits of determination. The mean planar-PCB-TEQ, and the mean total-PCB-dioxin-TEQ level of the neurologically normal infants were significantly higher ($p = 0.04$ for both) compared to the levels of the neurologically abnormal infants. We also examined the PCB and dioxin congener levels, to which the abnormal infants were exposed individually. For neither one of the abnormal infants the prenatal or postnatal exposure levels were significantly higher compared to the normal neonates.

In Table 5.4 the mean thyroid hormone levels in infants' plasma of the second week after birth are presented for both groups in the Rotterdam cohort ($n = 207$). When corrected for chance finding, neither the overall neonatal neurological optimality scores nor the postural tone cluster or the reflex cluster scores of the infants in the Rotterdam cohort were significantly correlated with the TT3, TT4, FT4 or TSH levels in maternal, cord or second week plasma (Spearman rank correlation coefficients).

5.5 Discussion

In this study of 418 Caucasian full-term Dutch infants, 24 neonates (6%) were diagnosed as neurologically abnormal. We did not find a significant relationship between the PCB and dioxin levels in plasma or human milk, and abnormal neonatal neurological examinations. There was also no significant difference in the obstetrical optimality score of the neurologically normal compared to the neurologically abnormal infants.

In humans subtle effects of these compounds have been mainly reported in neonates who were exposed in utero. Rogan described Taiwanese children who were exposed in utero to accidentally high levels of PCBs, PCDFs and polychlorinated quaterphenyls (Rogan et al 1988). As neonates they were not examined neurologically. During follow-up these children did not show abnormalities at the neurological examination. However, Japanese children exposed to comparably high levels of these contaminants in utero did show hypotonia and jerky movements in the follow-up evaluations (Harada 1976). Jacobson reported that infants whose mothers consumed high amounts of PCB contaminated fish from Lake Michigan, were more likely to have a motor immaturity, poorer lability of states, and weak reflexes than controls assessed with the BNBAS (Jacobson et al. 1984).

In North Carolina, Rogan studied 912 neonates with the BNBAS, who were exposed perinatally to background levels of PCBs. Only the highest exposed infants (6%) were found to be significantly more hypotonic and to have more abnormally weak reflexes (Rogan et al. 1986).

We made a clinical diagnostic classification of the study group in

neurologically normal, mildly abnormal or abnormal, as well as a neonatal neurological optimality score (NOS). Previously, we described a significant relationship between higher PCB and dioxin levels in human milk on the one hand, and a reduced NOS on the other hand. PCB levels in cord and maternal plasma were not related to the NOS (Huisman et al. 1995). It must be emphasized that optimal is not identical with normal, and non-optimal does not always mean abnormal (Prechtl 1980).

The mechanisms through which PCBs and dioxins could negatively influence the development of the nervous system remain unclear. Some studies reported effects on neurotransmitter levels (Seegal et al. 1992). Another hypothesis is that the site of action is in the developing muscle (Huisman et al. 1995). Other studies described PCB metabolites acting as thyroid hormone antagonists (Brouwer et al. 1986). Although we previously reported effects of PCB and dioxin exposure on the thyroid hormone status of mother-infant pairs in our cohort (Koopman-Esseboom et al. 1994[A]), we could not find a significant relationship between the neonatal thyroid hormone levels and the neonatal neurological outcome. This suggests that there is no direct relationship between PCB and dioxin exposure, thyroid function and neonatal neurological optimality or abnormality.

In conclusion: although subtle signs of neurological dysfunctioning due to PCB and dioxin exposure have been described, we found no significant relationship between perinatal exposure to background levels of these contaminants and clinically relevant neonatal neurological abnormalities. Since the neonatal brain is still immature, follow-up studies of the neurological and psychomotor development of the children at school age are necessary to evaluate whether there are also no clinical consequences of background exposure to PCBs and dioxins at a later age.

5.6 References

1. Bastomsky CH, 1977. Enhanced thyroxine metabolism and high uptake goiters in rats after a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxine. Endocrinology;101;292-296.
2. Bowman RE, Heironimus MP, Barsotti DA, 1981. Locomotor hyperactivity in PCB-exposed rhesus monkeys. Neurotoxicity;2;251-268.
3. Brouwer A, Van den Berg KJ, 1986. Binding of a metabolite of 3,4,3',4'-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxin. Toxicol Appl Pharmacol;85;301-312.
4. Burse VW, Korver MP, Needham LL et al. 1989. Gas chromatographic determination of polychlorinated biphenyls (as Aroclor 1254) in serum: Collaborative study. J Assoc Off Anal Chem;72;649-659.
5. Chen YJ, Hsu CC, 1994. Effects of prenatal exposure to PCBs on the neurological function of children: a neuropsychological and neurophysiological study. Dev Med Child Neurol;36;312-320.
6. Chen YCJ, GUO YL, Hsu CC, Rogan WJ, 1992. Cognitive development of Yu-Cheng ('Oil Disease') children prenatally exposed to heat-degraded PCBs.

JAMA;268;3213-3218.
7. Chou SM, Miike T, Payne WM, Davis GJ, 1979. Neuropathology of "spinning syndrome" induced by prenatal intoxication with a PCB in mice. Ann NY Acad Sci;320:373-395.
8. Filippini G, Bordo B, Crenna P, Massetto N, Musicco M, Boeri M, 1981. Relationship between clinical and electrophysiological findings and indicators of heavy exposure to 2,3,7,8-tetrachlorodibenzo-dioxin. Scan J Work Envir Health;7;257-262.
9. Harada M, 1976. Intrauterine poisoning: clinical and epidemiological studies and significance of the problem. Bull Inst Constit Med Kumamoto Univ;25(Suppl);1-60.
10. Huisman M, Koopman-Esseboom C, Touwen BCL et al. 1995. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. Early Hum Dev 1995;41:111-127.
11. Jacobson JL, Jacobson SW, Fein GG, Schwartz PM, Dowler JK, 1984. Prenatal exposure to an environmental toxin: a test of the multiple effects model. Dev Psychol;20;523-532.
12. Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N et al. 1994. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatr Res;36;4;468-473.
13. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N et al. 1994. PCB and dioxin level in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere;28:1721-1732.
14. Kuratsune M, Yoshimura T, Matsuzaka J, Yamaguchi A, 1972. Epidemiological study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. Environ Health Perspect;1;119-128.
15. Ness DK, Schantz SL, Moshtaghian J, Hansen LG, 1993. Effects of perinatal exposure to specific PCB congeners on thyroid hormone concentrations and thyroid histology in the rat. Toxicol Letters;68;311-323.
16. Porterfield SP, Hendrich CE, 1993. The role of thyroid hormones in prenatal and neonatal neurological development - current perspectives. Endocr Rev;14;94-106.
17. Prechtl HFR, 1977. The neurological examination of the full-term newborn infant, 2nd edn. Clin Dev Med. SIMP. London, Heinemann Medical Books, vol 63.
18. Prechtl HFR, 1980. The optimality concept. Early Hum Dev;4;201-205.
19. Rogan WJ, Gladen BC, McKinney JD et al. 1986. Neonatal effects of transplacental exposure to PCBs and DDE. J Pediatr;109;335-341.
20. Rogan WJ, Gladen BC, Hung K-L et al. 1988. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science;241;334-336.
21. Safe S, 1984. Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology, and mechanism of action. CRC Crit Rev Toxicol;13;319-395.
22. Seegal RF, Shain W, 1992. Neurotoxicity of polychlorinated biphenyls. In: The Vulnerable Brain and Environmental Risks. RL Isaacson, KF Jensen, eds.

New York: Plenum Press, vol 2: 169-195.
23. Touwen BCL, Huisjes HJ, Jurgens-v.d. Zee AD, Bierman-van Eendenburg MEC, Smrkovsky M, Olinga AA, 1980. Obstetrical condition and neonatal neurological morbidity. An analysis with the help of the optimality concept. Early Hum Dev;4;207-228.
24. Tuinstra LGMTh, Traag WA, Van Rhijn JA, Van de Spreng PF, 1994. The Dutch PCB/dioxin study. Development of a method for the determination of dioxins, planar and other PCBs in human milk. Chemosphere;29;1859-1875.
25. Van Rhijn JA, Traag WA, Van de Spreng PG, Tuinstra LGMTh, 1993. Simultaneous determination of planar chlorobiphenyls and polychlorinated dibenzo-p-dioxins and -furans in Dutch milk using isotope dilution and gas chromatography-high-resolution mass spectrometry. J Chromatogr; 630;297-306.

Table 5.1 Clinical neurological diagnosis of newborns classified as mildly or definitively abnormal.

Diagnosis	Total	Neonatal neurological classification
Hypotonia	4	Mildly abnormal
Hypotonia and hypokinesia	5	Mildly abnormal
Hypokinesia	1	Mildly Abnormal
Hypertonia and hypotonia	1	Mildly Abnormal
Hypertonia and hyperkinesia	2	Mildly abnormal
Hyperexcitability	6	Mildly abnormal
Clonical motility	1	Mildly abnormal
Erb's palsy*	1	Definitively Abnormal
Hypotonia with hypertonic bursts	1	Definitively Abnormal
Hypertonic syndrome	2	Definitively Abnormal

Normal infants:	394	(94%)
Mildly abnormal infants:	20	(5%)
Definitively abnormal infants:	4	(1%)

*Excluded from further analysis

Table 5.2 **Means and standard deviations of the obstetrical and neonatal optimality score of infants clinically diagnosed as neurologically normal (n = 394) versus abnormal (n = 23).**

Optimality score	Items	Neonatal neurological diagnosis				P-value*
		Normal		Abnormal		
		mean	SD	mean	SD	
Obstetrical optimality	72	64	3	63	4	NS
social background	12	9	1	9	2	NS
nonobstetric conditions during pregnancy	9	8	1	8	0.4	NS
obstetric history	14	13	1	13	1	NS
obstetric aspects of present pregnancy	11	10	1	10	1	NS
diagnosis and therapeutic measures	8	7	1	7	1	NS
parturition	11	10	1	10	1	NS
neonatal condition	7	7	1	6	1	NS
Neurological optimality	60	57	3	52	3	.0000
postural tone cluster	10	9	1	7	2	.0000
reflex cluster	11	10	1	9	2	.0001

*Mann-Whitney Test
NS = not significant

Table 5.3 Mean PCB and dioxin levels in plasma and human milk of neurologically normal compared to neurologically abnormal infants.

Parameter	Neurologically Normal			Neurologically Abnormal			P*
	Mean	s.d.	N	Mean	s.d.	N	
PCB-sum cord plasma	0.5	0.3	352	0.4	0.1	20	.75
PCB-sum maternal plasma	2.2	0.9	393	2.0	0.6	21	.70
Dioxin-TEQ	30.0	10.2	189	25.4	7.9	12	.08
Planar-PCB-TEQ*	16.3	7.4	194	11.7	3.8	12	.04#
Mono-ortho-PCB-TEQ**	14.8	5.3	189	12.6	4.6	12	.21
Di-ortho-PCB-TEQ***	4.4	2.2	189	3.7	1.5	12	.18
Total PCB-dioxin-TEQ	65.3	21.6	182	52.5	15.6	12	.04#

PCB plasma sum ng/g plasma
TEQ level pg TEQ/g fat
s.d. Standard deviation
P* Mann-Whitney Test
Not significant if corrected for the number of analyses using Bonferroni correction
Planar PCBs* PCB congeners 77, 126 and 169
Mono-ortho PCBs** PCB congeners 105, 118 and 156
Di-ortho PCBs*** PCB congeners 170 and 180

Table 5.4 Thyroid hormone levels of neurologically normal versus abnormal infants in the second week after birth.

Parameter	Neurologically Normal			Neurologically Abnormal			P*
	Mean	s.d.	N	Mean	s.d.	N	
TT3	2.8	0.5	195	2.9	0.5	11	.90
TT4	173.5	32.8	195	174.7	30.4	11	.96
FT4	24.4	3.5	195	22.3	2.9	11	.05#
TSH	2.1	1.1	195	2.2	1.3	11	.56

TT3 nmol/l
TT4 nmol/l
FT4 pmol/l
TSH mIU/ml

s.d. Standard deviation
P* Mann-Whitney Test
Not significant if corrected for the number of analyses using Bonferroni correction

CHAPTER 6

PERINATAL EXPOSURE TO POLYCHLORINATED BIPHENYLS AND DIOXINS AND ITS EFFECT ON NEONATAL NEUROLOGICAL DEVELOPMENT

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PERINATAL EXPOSURE TO POLYCHLORINATED BIPHENYLS AND DIOXINS AND ITS EFFECT ON NEONATAL NEUROLOGICAL DEVELOPMENT

Marcel Huisman[1], Corine Koopman-Esseboom[2], Václav Fidler[3], Mijna Hadders-Algra[4], Cornelis G. van der Paauw[5], Louis G.M.Th. Tuinstra[6], Nynke Weisglas-Kuperus[2], Pieter J.J. Sauer[2], Bert C.L. Touwen[7], E. Rudy Boersma[1].

[1]Dept. of Obstetrics & Gynaecology, Nutrition and Development Unit, University Hospital, Groningen.
[2]Dept. of Pediatrics, Erasmus University & University Hospital/Sophia Children's Hospital, Rotterdam.
[3]Dept. of Health Sciences, Epidemiology & Statistics unit, University of Groningen.
[4]Dept. of Medical Physiology, Developmental Neurosciences, University Hospital, Groningen.
[5]TNO Nutrition and Food Research Institute, Zeist.
[6]DLO State Institute for Quality Control of Agriculture Products, Wageningen.
[7]Dept. of Medical Physiology, Developmental Neurology, University Hospital, Groningen, The Netherlands.

6.1 Abstract

Polychlorinated biphenyls (PCBs) and dioxins (polychlorinated dibenzo-p-dioxins (PCDDs), and dibenzofurans (PCDFs)) are widespread environmental contaminants, which are neurotoxic in animals. Perinatal exposure to PCBs, PCDDs, and PCDFs occurs prenatally via the placenta and postnatally via breast milk.

To investigate whether such an exposure affects the neonatal neurological condition, the neurological optimality of 418 Dutch newborns was evaluated with the Prechtl neurological examination. Half of the infants were breast-fed, the other half were formula-fed, representing a relatively high against a relatively low postnatally exposed group, respectively. As an index of prenatal exposure, four non-planar PCBs in cord and maternal plasma were used. These PCB levels were not related to neurological function. As measures of combined pre- and early neonatal exposure, seventeen dioxin congeners, three planar, and twenty-three non-planar PCB congeners were determined in human milk in the second week after delivery.

Higher levels of PCBs, PCDDs, and PCDFs in breast milk were related to reduced neonatal neurological optimality. Higher levels of planar PCBs in breast milk were associated to a higher incidence of hypotonia. This study confirms previous reports about neurotoxic effects of these compounds on the developing brain of newborn infants.

6.2 Introduction

Polychlorinated biphenyls (PCBs) and dioxins (polychlorinated dibenzo-*p*-dioxins (PCDDs), and dibenzofurans (PCDFs)) are polycyclic aromatic compounds that are widespread environmental contaminants.[1] They are toxic for animals and human beings. PCBs have a total number of 209 possible congeners that differ in degree of chlorination and chlorine position. PCBs have electrical insulating properties. They are resistant to high temperatures and can easily conduct heat. Consequently, they have been widely used as plasticizers, fire retardants, in carbonless copy paper, in hydraulic fluids and as dielectric fluids in capacitors and transformers. In the late 1970s, they were banned world-wide because of their environmental persistence. However, production continued until the mid 1980s in e.g. the Czech Republic.[2] Dioxins are a mixture of possibly 75 (PCDD) and 135 (PCDF) congeners. They are unwanted byproducts of industrial and thermal processes. In the Netherlands, the incineration of municipal and industrial waste is the most important source for atmospheric deposition.[3] Since PCBs, PCDDs, and PCDFs are highly lipophilic and chemically stable[4], they tend to accumulate in the food chain. Considerable concentrations have been found in dairy products, fish, meat, and breast milk.[5] Food is the major source (>90%) of human exposure to PCBs, PCDDs, and PCDFs.[6]

Numerous animal studies have indicated that PCBs, PCDDs, and PCDFs are toxic to the nervous system.[7-11] Perinatal exposure of rats has been shown to induce effects on brain dopamine concentrations[12] and alterations in steroidal and thyroid hormone status.[13]

In Japan in 1968 (Yusho incident) as well as in Taiwan in 1979 (Yu-cheng incident), about 2,000 people were accidentally exposed to PCBs, PCDFs, and polychlorinated quaterphenyls through the consumption of contaminated rice oil. The most evident clinical sequelae were dermal lesions such as chloracne and comedones. PCBs and dioxins cross the placenta and can affect the developing fetus. The offspring of exposed women were small-for-gestational age and showed hyperpigmentation of the skin and nails. In follow-up studies, these children demonstrated signs of apathy, hypotonia, and a low performance on intelligence tests.[14,15]

Two studies in the USA indicated toxic effects of prenatal exposure to background PCB levels. Jacobson et al.[16] followed 242 infants whose mothers consumed PCB-contaminated fish from Lake Michigan and 71 control infants whose mothers did not eat fish. In a study of 912 infants, Rogan et al.[17] evaluated the neonatal effects of transplacental exposure to PCBs. Both studies found a significant negative effect on neonatal behavioural performance, assessed with the Brazelton Neonatal Behavioral Assessment Scale (BNBAS).[18] Until now effects have been evaluated behaviourally, and not yet with help of an age-adequate neurological examination. The present paper aims to fill this gap.

As in other industrialized countries in Western Europe, contamination of Dutch breast milk with PCBs, PCDDs, and PCDFs is high.[19] In 1989, this contamination of breast milk with high levels of possibly neurotoxic substances developed into a great public concern. As a result, the Dutch government initiated a prospective longitudinal study on possible adverse effects, starting in the neonatal period. In order to examine health risks associated with postnatal

PCB and dioxin exposure, half of the study population was selected on the basis of realized maternal intention to breast-feed her infant; the other half intended to formula-feed her infant. In formula-feeding, milk lipids are replaced by lipids of vegetable origin with a negligible content of PCBs and dioxins. An evaluation of the postnatal effects of PCB and dioxin exposure includes necessarily eventual effects caused by transplacental exposure during the prenatal period. PCB levels in cord and maternal plasma were used as a direct index of prenatal PCB exposure. PCB and dioxin levels in human milk are closely correlated with levels in maternal adipose tissue.[6] Therefore, PCB and dioxin levels in breast milk are good indicators of maternal load and subsequently to prenatal and postnatal exposure. In the present study, we report on the relationship between perinatal background exposure to PCBs, PCDDs, and PCDFs and the neurological condition of 418 Dutch newborns.

6.3 Subjects and methods

Study design: The study was carried out in two widely apart and different areas of the Netherlands: Groningen, a semi-urban area in the Northeast, and Rotterdam, a highly industrialized region in the Southwest of the Netherlands. Approval was given by the ethics committees of the Groningen and the Rotterdam university hospitals. Intake took place from June 1990 till June 1992. Two hundred healthy pregnant women were planned to be included in each area: 50% who intended to breast-feed their infants for at least 6 weeks and 50% who preferred formula-feeding. The latter agreed to use Almiron M2 (Nutricia N.V., The Netherlands) from one batch as a reference. The women were asked to cooperate by their midwife or obstetrician and were visited at home for an explanation of the study protocol. The women and their children had to meet the following criteria: absence of serious illness and complications during pregnancy and delivery; Caucasian race; first or second born term infants (37-42 weeks of gestation); no caesarian section; no forceps- nor vacuum extraction; availability of a maternal blood sample in the last month of gestation and of a cord blood sample.

Obstetrical and neurological optimality scores: Obstetrical data were evaluated according to the obstetrical optimality list, as described by Touwen et al.[20] The list used in this study consisted of 72 items that measure socioeconomic situation and pre-, intra-, and immediate post-partum conditions. By counting the number of items that fulfilled preset criteria for optimality[21], the optimality score was calculated.

The neonatal neurological examination was scheduled between the 10th and the 21th day after delivery. For the assessment of the neonatal neurological condition, the comprehensive age-adequate neurological examination, as described by Prechtl[22], was used. This technique, in contrast to the BNBAS[23], has proven to be predictive[24-27] for later major and minor neurological dysfunctions. The examination leads to a clinical diagnostic classification: normal, suspect, or abnormal. Furthermore, two clusters of items were formed: one describing postural tone, the other reflexes and responses. The latter cluster consisted of 11 items, the former consisted of 10 items (Table 6.1). For each

item score 0 represents a low value, score 1 an intermediate value and score 2 a high value; as may occur in healthy vigorous infants. By summation of scores a postural tone and a reflex cluster score was calculated, which could range from 0-17 and 0-22 respectively. A postural tone cluster score of ≤ 9 was considered to reflect a low muscle tone, and a reflex cluster score of ≤ 10 a low responsiveness. The cut-off points were arbitrarily chosen close to the median score.

Finally, the neurological findings were also interpreted in terms of optimality.[21] A neurological optimality score was calculated, consisting of 60 items for each of which an optimal range was defined.[20] After giving a point for each item meeting these criteria, the neurological optimality score (NOS) was calculated by the summation of optimal items. It must be emphasized that optimal is not identical with normal, and non-optimal does not always mean abnormal.[21] The neurological examinations were carried out by M.H. in Groningen and by C.K.E. in Rotterdam. Both observers were unaware of the results of the chemical analyses of the plasma and milk samples but were not blinded to the feeding status.

Samples: Maternal blood was collected in the last month of gestation and cord blood immediately after birth. Blood was collected in a vacuum system EDTA-tube and centrifuged for 10 minutes at 3000 rpm; plasma was stored at -20° Celsius until analysis. Human milk was collected as a 24 hour sample in the second and 6th week, and if possible 3 months after delivery. This was achieved by emptying both breasts with an electric pump (Babyluxus 2, KAWECO, Stuttgart, Germany). Volumes were recorded and 10% aliquots were pooled and stored at -20° Celsius until analysis. The remaining milk was administered to the infants by bottle.

Analytical methods: Plasma samples were analyzed for the four non-planar PCB congeners 118, 138, 153, and 180 only. Plasma has a relatively low fat content compared to human milk and a too large volume of blood would be needed to measure all the PCDD, PCDF, and PCB congeners, such as were analyzed in human milk. Plasma was denaturated with methanol and 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. A part of the cleaned extract was analyzed by gas-liquid chromatography using electron capture detection. The analysis was carried out on two capillary columns of different polarity. The limits of determination for PCB 118, 138, 153, and 180 were 0.01 μg/L. The recovery of chlorinated biphenyl congeners added to the plasma before extraction, and determined as described above, was >95%. Control samples were analyzed to estimate the reliability.

The milk samples were analyzed for the seventeen 2,3,7,8-substituted PCDDs and PCDFs, which are usually found in biotic samples, three planar PCBs, and 23 non-planar PCB congeners. Human milk was fortified with sixteen ^{13}C labelled PCDDs and PCDFs, and three ^{13}C labelled PCB standards. The procedure for quantitative fat extraction has been described in detail, as well as the gas chromatography-high-resolution mass spectrometry determination.[28,29] The non-planar PCB congeners were measured by gas chromatography using electron capture detection.[30]

Data processing: In the present study, the possible toxicity of the individual congeners for the neonatal nervous system was studied in plasma and breast milk. In addition, the toxicity of a mixture of PCBs and dioxins in human milk was investigated. Finally, a comparison was made between breast-fed and formula-fed infants, in order to analyze a possible postnatal effect.

In order to express the toxic potency of the mixture of dioxins[31] and dioxin-like PCBs[32], we used the toxic equivalence factor approach. For each congener, these factors express in breast milk the relative toxicity towards 2,3,7,8 TCDD, the most toxic congener of which toxic equivalence factor (TEF) is one. In order to calculate the toxic equivalent (TEQ) of each congener, concentrations of all 2,3,7,8 compounds and planar PCBs (PCB 77, 126, and 169) were multiplied by their TEF value. By adding up the individual TEQs, a dioxin TEQ and planar PCB TEQ score could be acquired. Mono-ortho PCB TEQ (PCB 105, 118, and 156) and di-ortho PCB TEQ (PCB 170 and 180) were calculated by multiplying the concentrations and their proposed TEF value.[32] The total PCB/dioxin TEQ is a summation of the dioxin TEQ, the planar, mono-ortho, and di-ortho PCB TEQs.

To evaluate a postnatal effect, the sum (ΣPCB) of the four PCB congeners (PCB 118, 138, 153, and 180) was calculated as a measure of PCB exposure separately for cord plasma and breast milk. The ΣPCB_{cord} in cord plasma was used as a continuous independent variable, whereas the ΣPCB_{milk} in formula milk and breast milk were used as a categorial independent variable (no-; low-; and high-postnatal exposure) in the statistical analysis.

Statistical analysis: The 5th, 50th, and 95th percentiles were used to describe the distribution of the concentrations. For a univariate comparison of the results in Groningen and Rotterdam, we used the Chi square test and the Wilcoxon rank sum test performed at the 5% level. Both univariate analysis and logistic regression analysis[33] were used to examine the relation between the NOS, the postural tone cluster, and the reflex cluster on one hand and levels of PCB and dioxin exposure, and obstetrical variables on the other hand. We dichotomized the NOS at the median of the pooled population. The postural tone cluster and the reflex cluster scores were dichotomized as (≤ 9, >9) and (≤ 10, >10), respectively. The PCB and dioxin values were logarithmically transformed. After adjusting for obstetrical variables, the effect of each chemical compound and total TEQ scores was examined. The results are reported as odds ratios (ORs) associated with doubling of concentrations, together with 95% confidence intervals (CI), without any correction for multiple hypothesis testing.

6.4 Results

Description of the study group: Initially, 489 mother-infant pairs fulfilled the criteria. Seventy-one mother-infant pairs were lost mainly because of difficulties in sustaining breast-feeding, leaving a study population of 418 pairs. This number consisted of 104 breast-fed and 107 formula-fed infants in Groningen, and 105 breast-fed and 102 formula-fed infants in Rotterdam. Obstetrical characteristics of the total groups are presented in Table 6.2. No differences were found between the study centers Groningen and Rotterdam for maternal age, weight, the percentage of smoking during pregnancy for both women and

their partners, gender, one-minute Apgar scores and obstetrical optimality scores. In Groningen the education of both mothers and their partners was higher as compared to Rotterdam, as was the maternal alcohol consumption during pregnancy. Duration of gestation based on reported last menstrual period was also significantly different (40.6 ± 1.1 for Groningen versus 40.1 ± 1.2 weeks for Rotterdam), but the difference was considered to be too small to carry biological significance. In Groningen, mean birth weight was slightly higher (3.56 ± 0.44 versus 3.47 ± 0.44 kg).

Neurological findings: Sixty-three percent of the newborns were examined in the second postnatal week, 31% in the third week, and 6% in the fourth week of life. From the 418 children, 394 were neurologically classified as normal, 20 newborns as suspect, and 4 newborns as abnormal. There were no differences in the clinical diagnoses between the two study centers; these data will be reported elsewhere. The percentages of infants with a postural tone cluster score of ≤ 9 and a reflex cluster score of ≤ 10 were 43% and 22%, respectively. The distribution of the NOS in Groningen and Rotterdam is shown in Figure 6.1. In Rotterdam, the optimality score was shifted to the left compared to Groningen. There appeared to be a systematic difference between the two observers in the assessment of 9 items: stability of states; posture in supine position; abdominal skin reflex; active power; knee jerk; posture of head during traction test; Moro reaction, amplitude of abduction and extension; Bauer response; and Galant response. In Groningen, these items (except for active power) were more often considered optimal. Therefore in the logistic regression model, we adjusted for the study center. The median NOS of the pooled population used as the cut-off point was 57; the score 57 or higher was considered optimal. The NOS of the 24 neonates who were clinically diagnosed as suspect or as abnormal were all but one below the median.

PCBs, PCDDs, and PCDFs: The 5th, 50th, and 95th percentiles of PCB 118, 138, 153, and 180 congener concentration in cord and plasma are shown in Table 6.3. The percentiles of the PCB, PCDD, and PCDF content in breast milk are presented in Tables 6.4 and 6.5.[34] In Rotterdam, some congener levels were higher than in Groningen.[35] Three maternal plasma samples were missing. In 382 cord plasma samples, concentrations of PCB 138, 153, and 180 congeners were analyzed. For the analysis of PCB 118 in cord plasma, 9 samples were missing. In human milk, representative dioxin, planar and non-planar PCB congeners were available in 176, 194 and 195 milk samples, respectively. The Spearman correlation between ΣPCB_{cord} levels in cord plasma and ΣPCB_{milk} in human milk was 0.68 ($p<0.001$). In the formula milk, we found PCB and dioxin levels below limits of determination.

NOS and clusters versus PCBs in maternal and cord plasma: The results of logistic regression analysis with the dichotomized NOS as the dependent variable in relation to PCB 118, 138, 153, and 180 in maternal as well as in cord plasma are shown in Table 6.3. After adjusting for the age of the mother, the study center, alcohol consumption, and an interaction between age and alcohol consumption, no significant relation was found between the NOS and PCB congeners in maternal or cord plasma. Plasma PCB levels affected neither the cluster scores for reflexes and responses nor for postural tone.

NOS and clusters versus PCBs, PCDDs, and PCDFs in human milk: In breast

milk, logistic regression analysis with covariate adjustment showed significant effects on the NOS of some PCB and dioxin congeners: five PCDD, two PCDF, one planar PCB, two mono-ortho PCB, one di-ortho PCB, and seven non-planar PCB congeners as well as the dioxin, mono-ortho PCB, di-ortho PCB, and total PCB/dioxin TEQ values (Tables 6.4 and 6.5). After adjusting for the study center, logistic regression analysis with the postural tone cluster score as the dependent variable demonstrated a significant higher percentage of hypotonia with an increase in planar PCB TEQ (OR: 1.64, 95% CI: 1.03-2.63). No effect on the reflex cluster score was found.

After adjusting for the study center and for the ΣPCB_{cord} in cord plasma, a reduced neonatal neurological optimality and a higher prevalence of hypotonia was found in the highest exposed group at a turning point at 540 ng ΣPCB_{milk}/g milk fat. The percentage of breast-fed infants with a ΣPCB_{milk} content of $\geq$ 540 ng/g milk fat was 23%. In this group, the odds ratio for the NOS was 3.4 (95% CI: 1.6-7.1). Such an odds ratio corresponds, for example, to an increase in the prevalence of non-optimality from 50% to 75%.

6.5 Discussion

The neurological optimality of 418 Dutch newborns was evaluated with the use of a comprehensive age-adequate neurological examination.[22] PCB levels in cord and maternal plasma were assumed to be a direct index of prenatal PCB exposure, whereas PCB and dioxin levels in breast milk in the second week after delivery were expected to reflect the extent of intrauterine and neonatal exposure during the first 2 weeks after birth.[5] PCB levels in cord and maternal plasma were not related to (mild) nervous system dysfunction. Higher levels of PCB, PCDD, and PCDF congeners in breast milk were found to be significantly related to reduced neonatal neurological optimality. Higher levels of planar PCBs in breast milk were associated with a higher incidence of hypotonia.

The study cohort consisted of infants born in Groningen, a semi-urban area, and infants born in Rotterdam, a highly industrialized region. The two populations were obstetrically comparable. On the basis of the inclusion criteria, the study group can be considered as a 'low-risk' population for neurological abnormality. In order to specify subtle dysfunctions of the neonate's nervous system, Prechtl's optimality concept was used.[21] The neurological optimality score in Groningen was higher than in Rotterdam. It is likely that this difference is mainly due to a systematic difference in the assessment of some items by the two observers. The logistic regression analysis attempts to correct for this systematic dissimilarity (Tables 6.3, 6.4, and 6.5).

Our neurological findings are consistent with the two studies in the United States. Rogan et al.[17] followed 912 infants with whom prenatal exposure to background levels of PCBs was estimated on the basis of the concentration of PCBs in fat of their mother's breast milk. On the BNBAS, the highly exposed newborns were found to be more hypotonic and to have a higher incidence of abnormally weak reflexes. We could confirm these results as far as the hypotonia is concerned. In a study in Michigan of 312 neonates, Jacobson et al.[16] reported a significant effect of maternal consumption of PCB-contaminated

fish on neonatal neurobehavioural performance, described as motor immaturity, more limited lability of states, and weak reflexes. It is essential to note that in that study the patients were examined on the third day after delivery. Beintema[36] demonstrated that neurological examinations on the first two or three days after birth have less predictive value, than those on later days of the neonatal period, because of the infant's adaptation to extrauterine life. In Michigan, the behavioural deficits were correlated to maternal fish consumption, but could not be predicted by PCB levels in cord blood. In the present study, only in breast milk fat did PCB levels show significant negative effects on the NOS. Although our congener-specific analysis differs from the total PCB determinations performed in the USA studies, the estimated levels of exposure on the basis of maternal milk samples are reasonably comparable.

Jacobson et al.[37] suggested that prenatally the central nervous system would be more vulnerable to teratogenic agents than postnatally. In the present study, the correlation between ΣPCB_{cord} levels in cord plasma and ΣPCB_{milk} in human milk was fairly strong. We calculated that the total amount of PCB 118, 138, 153, and 180 (ΣPCB) reaching the fetus via the transplacental route is similar to that ingested by the infant during two-weeks of breast-feeding. We found a significant negative effect of a high postnatal exposure ($\Sigma PCB_{milk} > 540$ ng/g fat) even when prenatal exposure was adjusted for. This finding suggests an adverse effect from breast-feeding during the first two weeks in the presence of a high PCB level in breast milk.

Our study does not confirm prenatal effects of PCBs which have been found in other studies. It is feasible, however, that prenatal exposure in our groups remained sufficiently low, so that we did not find neurological effects in the neonatal period. Considering the high correlation between plasma and breast milk contents of PCBs, a high plasma PCB level is frequently concurrent with a high PCB level in breast milk. The combination of a high intrauterine and a high postnatal exposure might then result in neurological non-optimality as reflected by the decreased optimality score. Such a negative effect is not found in formula-fed infants with merely high plasma PCB levels (cf. Figure 6.2, which shows a hypothetical model).

So in our study of a low-risk population, only breast-fed children who were perinatally exposed to higher dioxin, mono-ortho PCB, di-ortho PCB, and total PCB/dioxin TEQ values showed a reduced neonatal neurological optimality. Breast-fed children exposed to higher planar PCB TEQ appeared to have a higher incidence of hypotonia. Since reflexes and responses were normal and the minor dysfunction mainly consisted of hypotonia, it is possible that the site of action is in the developing muscle. This is a subject for further study.

It should be stressed that severe neurological deviancies were absent. Considering the multitude of beneficial effects of human milk and the very minor character of the deviations, it would seem premature to advice against breast-feeding during the first weeks of life. The neonatal brain is, however, different from the brain of the older child, so that it will be necessary to investigate later neurological, but also cognitive and behavioural development, e.g. at school age.

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6.6 References

1. Kimbrough, R.D. (1974): The toxicity of polychlorinated polycyclic compounds and related chemicals. CRC Crit. Rev. Toxicol., 2, 445-498.
2. Nondek, L., Frolikova, N. (1991): Polychlorinated biphenyls in the hydrosphere of Czechoslovakia. Chemosphere, 23, 269-280.
3. Bremmer, H.J. (1991): Sources of Dioxins in the Netherlands. RIVM report no. 730501014, February 1991 (in Dutch, summary in English).
4. Kimbrough, R.D. and Jensen, A.A., Editors. (1989): Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. New York; Elsevier/North-Holland.
5. Jensen, A.A. (1987): Polychlorobiphenyls (PCBs), Polychlorodibenzo-p-dioxins (PCDDs) and Polychlorodibenzofurans (PCDFs) in human milk, blood and adipose tissue. Sci. Total Eviron, 64, 259-293.
6. Theelen, R.M.C., Knaap, A.G.A.C., Sangster, B. (1991): Dioxine: een norm aanbevolen door de Wereldgezondheidsorganisatie. Ned. Tijdschr. Geneeskd., 135, 875-877.
7. Bowman, R.E., Heironimus, M.P., Barsotti, D.A. (1981): Locomotor hyperactivity in PCB-exposed rhesus monkeys. Neurotoxicology, 2, 251-268.
8. Chou, S.M., Miike, T., Payne, W.M., Davis, G.J. (1979): Neuropathology of "spinning syndrome" induced by prenatal intoxication with a PCB in mice. Ann. NY Acad. Sci., 320, 373-395.
9. Schantz, S.L., Bowman, R.E. (1989): Learning in monkeys exposed perinatally to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Neurotoxicol. Teratol., 11, 13-19.
10. Shain, W., Bush, B., Seegal, R.F. (1991): Neurotoxicity of polychlorinated biphenyls: structure-activity relationship of individual congeners. Toxicol. Appl. Pharmacol., 111, 33-42.
11. Tilson, H.A., Davis, G.J., McLachlan, J.A., Lucier, G.W. (1979): The effects of polychlorinated biphenyls given perinatally on the neurobehavioral development of mice. Environ Res., 18, 466-474.
12. Seegal, R.F. (1992): Perinatal exposure to Aroclor 1016 elevates brain dopamine concentrations in the rat. Toxicologist, 12, 320.
13. Morse, D.C., Groen, D., Veerman, M., et al. (1993): Interference of polychlorinated biphenyls in hepatic and brain thyroid hormone metabolism in fetal and neonatal rats. Toxicol. Appl. Pharmacol. 122, 27-33.
14. Harada, M. (1976): Intrauterine poisoning: clinical and epidemiological studies and significance of the problem. Bull. Inst. Constit. Med. (Kumamoto Univ), 25(suppl), 1-69.

15. Rogan, W.J., Gladen, B.C., Hung, K.-L., et al. (1988): Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science, 241, 334-336.
16. Jacobson, J.L., Jacobson, S.W., Schwartz, P.M., Fein, G.G., Dowler, J.K. (1984): Prenatal exposure to an environmental toxin: a test of the multiple effects model. Dev. Psychol., 20, 523-532.
17. Rogan, W.J., Gladen, B.C., McKinney, J.D., et al. (1986): Neonatal effects of transplacental exposure to PCBs and DDE. J. Pediatr., 109, 335-41.
18. Brazelton, T.B. (1973): Neonatal Behavioral Assessment Scale. Philadelphia: Lippincott.
19. World Health Organization - Regional Office for Europe. (1989): Levels of PCBs PCDDs, and PCDFs in Breast Milk. Environmental Health Series no. 34, Editor: Yrjänheikki, E.J. (WHO, Geneva).
20. Touwen, B.C.L., Huisjes, H.J., Jurgens-van der Zee, A.D., et al. (1980): Obstetrical condition and neonatal neurological morbidity. An analysis with the help of the optimality concept. Early Hum. Dev., 4, 207-228.
21. Prechtl, H.F.R. (1980): The optimality concept. Early Hum. Dev., 4, 201-205.
22. Prechtl, H.F.R. (1977): The neurological examination of the full-term newborn infant, 2nd edn. Clinics in Developmental Medicine, No. 63. SIMP. London: Heinemann Medical Books.
23. Sameroff, A.J. (1978): Summary and conclusions: the future of newborn assessment. In: Monographs of the Society for Research in Child Development: Organization and Stability of Newborn Behaviour Assessment Scale, Serial No. 177, vol. 43, pp. 102-103. Editor: A.J. Sameroff. Chicago: University Press.
24. Bierman-van Eendenburg, M.E.C., Jurgens-van der Zee, A.D., Olinga, A.A., Huisjes, H.J., Touwen, B.C.L. (1981): Predictive value of neonatal neurological examination: A follow-up study at 18 months. Dev. Med. Child Neurol., 23, 296-305.
25. Hadders-Algra, M., Touwen, B.C.L., Huisjes, H.J. (1986): Neurologically deviant newborns: neurological and behavioural development at the age of six years. Dev. Med. Child Neurol., 28, 569-578.
26. Hadders-Algra, M., Huisjes, H.J., Touwen, B.C.L. (1988): Perinatal correlates of major and minor neurological dysfunction at school age: a multivariate analysis. Dev. Med. Child Neurol., 30, 472-481.
27. Touwen, B.C.L., Lok-Meijer, T.Y., Huisjes, H.J., Olinga, A.A. (1982): The recovery rate of neurologically deviant newborns. Early Hum. Dev., 7, 131-148.
28. Van Rhijn, J.A., Traag, W.A., Kulik, W., Tuinstra, L.G.M.Th. (1992): Automated clean-up procedure for the gas chromatographic-high-resolution mass spectrometric determination of polychlorinated dibenzo-p-dioxins and dibenzofurans in milk. J. Chromatogr., 595, 289-299.

29. Van Rhijn, J.A., Traag, W.A., van de Spreng, P.F., Tuinstra, L.G.M.Th. (1993): Simultaneous determination of planar chlorobiphenyls and polychlorinated dibenzo-p-dioxins and -furans in Dutch milk using isotope dilution and gas chromatography-high-resolution mass spectrometry. J. Chromatogr., 630, 297-306.
30. Tuinstra, L.G.M.Th., Van Rhijn, J.A., Traag, W.A., et al. (1993): Method for the determination of dioxins, planar and other PCBs in human milk. Halogenated Compounds 11, 181-183.
31. North Atlantic Treaty Organisation (NATO), Committee on the Challenges of Modern Society. Pilot study on International Information Exchange on Dioxins and Related Compounds. International Toxicity Equivalence Factor (I-TEF). Method of Risk Assessment for Complex Mixtures of Dioxins and Related Compounds. Brussels: NATO 1988. (Report number 176).
32. Ahlborg, U.G., Becking, G.C., Birnbaum, L.S., et al. (1994): Toxic equivalency factors for dioxin-like PCBs. Chemosphere, 28, 1049-1067.
33. Hosmer, D.W. and Lemeshow, S. (1989): Applied Logistic Regression. Editors: D.W. Hosmer, S. lemeshow. Toronto: John Wiley & Sons, Inc.
34. Koopman-Esseboom, C., Huisman, M., Weisglas-Kuperus, N., et al. (1994): PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere, 28, 1721-1732.
35. Koopman-Esseboom, C., Huisman, M., Weisglas-Kuperus, N., et al. (1994): Dioxin and PCB levels in blood and human milk in relation to living areas in the Netherlands. Chemosphere, 29, 2327-2338.
36. Beintema, D.J. (1968): A Neurological Study of Newborn Infants. In: Clinics in Developmental Medicine, No. 28. London: Heinemann Medical.
37. Jacobson, J.L., Fein, G.G., Jacobson, S.W., Schwartz, P.M., Dowler, J.K. (1984): The transfer of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) across the human placenta and into maternal milk. J. Public Health, 74, 378-379.

Table 6.1 The reflex, and postural tone cluster score

	score#				score#		
Reflex cluster	0	1	2	Postural tone cluster	0	1	2
Lip reflex	+	+ +	+ + +	Overshooting movements	+	-	
Glabella reflex	+	+ +	+ + +	Posture of arms in supine suspension (extended)	+	-	
Abdominal skin reflex	+	+ +	+ + +	Posture of legs in supine suspension (extended)	+	-	
Biceps reflex	+	+ +	+ + +	Resistance against passive movements	+	+ +	+ + +
Knee reflex	+	+ +	+ + +	Recoil of arms	+	+ +	+ + +
Threshold tendon reflexes	high	medium	low	Posture of arms during traction test	+	+ +	+ + +
Palmar grasp	+	+ +	+ + +	Posture of head during traction test	+	+ +	+ + +
Plantar grasp	+	+ +	+ + +	Head balance during sitting	+	+ +	+ + +
Moro reaction, intensity	+	+ +	+ + +	Spontaneous head movements in prone position	+	+ +	+ + +
Ankle jerk	+	+ +	+ + +	Posture in prone position	+	+ +	+ + +
Galant response	+	+ +	+ + +				

Technique described by Prechtl.[22]

Table 6.2 Baseline characteristics of the study group

Variables	Outcome (n = 418)
Education*	
lower secondary school / higher secondary school / university training	20% / 37% / 43%
Parity of child	
first born / second or third born	48% / 52%
Smoking during pregnancy	
yes / no	26% / 74%
Alcohol consumption during pregnancy*	
no / sporadic / regular (at least 1/wk)	72% / 25% / 3%
Sex of child	
male / female	54% / 46%
Neonatal jaundice	
no / mild / severe	43% / 57% / 0%
Maternal age (yrs)◊	
mean (SD)	29 (4)
Maternal weight (kg)	
mean (SD)	65 (10)
Maternal height* (cm)	
mean (SD)	170 (6)
Quetelet index	
mean (SD)	22 (3)
Birth weight* (kg)	
mean (SD)	3.52 (0.44)
Gestational age* (wks)	
mean (SD)	40.3 (1.2)
Age of examination* (days)	
mean (SD)	14 (4)
Apgar 1 min	
median (range)	9 (3-10)
Obstetrical Optimality Score	
median (range)	64 (50-70)

◊ In the logistic regression analysis categorised as 18-26, 27-29, 30-32, 33-39
*Significant difference between Groningen and Rotterdam ($p < 0.05$)

Table 6.3 Results of logistic regression analysis[◇]: neonatal neurological optimality[†] in relation to maternal and cord plasma levels of PCB 118, 138, 153, and 180 (IUPAC nomenclature).

Congener	IUPAC	n	Concentration Percentiles			Relation with NOS Odds	95% confidence
			P5	P50	P95	Ratio	interval OR
maternal plasma (µg/l)							
2,4,5,3',4'-PECB	118	415	0.07	0.15	0.31	1.05	0.76-1.45
2,3,4,2',4',5'-HXCB	138	415	0.27	0.56	1.10	1.19	0.81-1.73
2,4,5,2',4',5'-HXCB	153	415	0.40	0.84	1.70	1.08	0.73-1.61
2,3,4,5,2',4',5',-HPCB	180	415	0.25	0.50	0.94	1.07	0.72-1.58
$\Sigma PCB_{maternal}$[#]		415	1.00	2.04	3.81	1.11	0.74-1.65
cord plasma (µg/l)							
2,4,5,3',4'-PECB	118	373	0.02	0.04	0.09	1.06	0.76-1.47
2,3,4,2',4',5'-HXCB	138	382	0.04	0.11	0.25	1.09	0.81-1.48
2,4,5,2',4',5'-HXCB	153	382	0.06	0.15	0.34	1.05	0.77-1.45
2,3,4,5,2',4',5',-HPCB	180	382	0.04	0.08	0.19	1.02	0.75-1.39
ΣPCB_{cord}[#]		373	0.18	0.38	0.86	0.96	0.68-1.36

[◇] After adjusting for obstetrical factors
[†] Dichotomized NOS: optimal = 0, non-optimal = 1
IUPAC = International Union of Pure and Applied Chemistry
[#] ΣPCB = PCB 118 + 138 + 153 + 180

Table 6.4 Results of logistic regression analysis◇: neonatal neurological optimality† in relation to PCDD, PCDF, and planar PCB congeners in breast milk fat.

Congener	IUPAC	TEF	Content Percentiles P5	P50	P95	TEQ	Relation with NOS Odds ratio	95% confidence interval OR
PCDDs (ng/kg fat)								
2,3,7,8-TCDD	48	1	1.55	3.61	7.68	4.04	1.54	0.94-2.55
1,2,3,7,8-PECDD	54	0.5	5.20	10.25	19.96	5.37	2.23*	1.10-4.52
1,2,3,4,7,8-HXCDD	66	0.1	1.69	8.71	14.57	0.87	1.64*	1.08-2.50
1,2,3,6,7,8-HXCDD	67	0.1	23.10	45.98	76.26	4.72	3.03**	1.34-6.81
1,2,3,7,8,9-HXCDD	70	0.1	0.09	6.72	12.47	0.68	1.23*	1.03-1.48
1,2,3,4,6,7,8-HPCDD	73	0.01	20.18	57.38	125.22	0.63	1.61*	1.01-2.56
1,2,3,4,6,7,8,9-OCDD	75	0.001	274.60	660.64	1579.51	0.79	1.35	0.82-2.23
PCDFs (ng/kg fat)								
2,3,7,8-TCDF	83	0.1	0.04	0.73	2.17	0.08	1.31*	1.04-1.66
1,2,3,7,8-PECDF	94	0.05	0.04	0.09	0.91	0.01	0.88	0.69-1.12
2,3,4,7,8-PECDF	114	0.5	11.41	21.76	40.23	11.40	2.48*	1.16-5.32
1,2,3,4,7,8-HXCDF	118	0.1	3.11	6.48	10.84	0.67	1.43	0.90-2.27
1,2,3,6,7,8-HXCDF	121	0.1	2.03	5.59	10.01	0.57	1.26	0.92-1.72
1,2,3,7,8,9-HXCDF	124	0.1	0.04	0.08	0.40	0.02	0.92	0.71-1.20
2,3,4,6,7,8-HXCDF	130	0.1	0.08	3.00	9.11	0.35	1.15	0.96-1.37
1,2,3,4,6,7,8-HPCDF	131	0.01	0.25	6.32	18.40	0.08	1.16	0.94-1.43
1,2,3,4,7,8,9-HPCDF	134	0.01	0.09	0.19	0.49	0.00	1.22	0.77-1.93
1,2,3,4,6,7,8,9-OCDF	135	0.001	0.18	0.38	2.26	0.00	1.07	0.82-1.41
dioxin TEQ						30.19	3.12**	1.36-7.18
Planar PCBs (µg/kg fat)								
3,4,3'4'-TCB	77	0.0005	6.53	14.77	42.35	0.01	1.29	0.85-1.96
3,4,3'4'5-PECB	126	0.1	63.28	137.54	302.16	15.20	1.62	0.96-2.74
3,4,5,3'4'5'-HXCB	169	0.01	42.35	79.53	143.70	0.84	2.33*	1.13-4.80
planar PCB TEQ						16.05	1.67	0.97-2.87

◇ After adjusting for obstetrical factors
† Dichotomized NOS: optimal = 0, non-optimal = 1
TEF = Toxic Equivalency Factor
TEQ = Toxic Equivalent = mean content x TEF
PCDD/Fs (n = 176); planar PCBs (n = 194)
IUPAC = Internat Union of Pure and Appl Chem
* Significant odds ratios ($p < 0.05$, two-tailed)
** Significant odds ratios ($p < 0.01$, two-tailed)

Table 6.5 Results of logistic regression analysis◇: neonatal neurological optimality[1] in relation to non-planar PCBs, mono-ortho PCB TEQ, di-ortho PCB TEQ, and total TEQ in breast milk fat.

Congener	IUPAC	TEF	Content Percentiles P5	P50	P95	TEQ	Relation with NOS Odds ratio	95% confidence interval OR
PCB (µg/kg fat)								
2,4-4'	28		0.25	5.79	46.05		0.96	0.85-1.09
2,5-2'5'	52		0.24	1.46	8.91		1.17	0.96-1.42
2,4-3'4'	66		1.20	9.43	26.42		1.06	0.82-1.36
2,5-3'4'	70		2.09	17.37	34.67		1.88**	1.29-2.72
2,4,5-2'4'	99		8.37	18.14	35.10		2.01**	1.19-3.40
2,4,5-2'5'	101		0.28	1.08	4.18		1.06	0.82-1.37
2,3,4-3'4'	105[A]	0.0001	3.66	8.72	16.90	0.94	1.42	0.91-2.20
2,4,5-3'4'	118[A]	0.0001	15.10	32.75	61.57	3.55	2.21**	1.24-3.95
2,3,4-2'3'4'	128		1.00	3.67	7.58		1.46	0.99-2.15
2,3,4,5-2'4'	137		5.03	13.43	39.32		0.99	0.69-1.42
2,3,4-2'4'5'	138		59.70	124.22	221.60		2.73**	1.40-5.35
2,3,4,5-2'5'	141		0.37	0.79	2.65		1.26	0.84-1.89
2,3,5,6-2'5'	151		0.21	0.76	2.04		1.08	0.79-1.49
2,4,5-2'4'5'	153		90.31	174.71	317.96		2.76**	1.39-5.48
2,3,4,5-3'4'	156[A]	0.0005	9.90	20.09	36.24	10.52	2.55**	1.31-4.95
2,3,4,5-2'3'4'	170[B]	0.0001	17.28	35.37	65.05	3.71	2.33*	1.19-4.56
2,3,5,6-2'3'4'	177		2.96	5.89	10.66		1.96*	1.14-3.39
2,3,4,5-2'4'5'	180[B]	0.00001	31.00	71.33	138.20	0.77	1.55	0.98-2.46
2,3,4,6-2'4'5'	183		5.98	11.48	20.41		2.32*	1.21-4.46
2,3,5,6-2'4'5'	187		9.04	17.85	36.62		1.87*	1.07-3.28
2,3,4,5-2'3'4'5'	194		3.28	7.84	15.23		1.29	0.80-2.07
2,3,4,5,6-2'3'4'	195		0.83	2.65	5.00		1.15	0.73-1.80
2,3,5,6-2'3'5'6'	202		0.36	0.71	1.97		1.01	0.67-1.52
ΣPCB_{milk}[#]			205.11	404.77	722.67		2.93**	1.45-5.90
mono-ortho PCBs TEQ						15.02	2.78**	1.40-5.59
di-ortho PCBs TEQ						4.47	2.38*	1.21-4.71
total PCB/dioxin TEQ						65.25	3.21**	1.37-7.48

Legends to Table 6.5

◇ After adjusting for obstetrical factors
† Dichotomized NOS: optimal = 0, non-optimal = 1

[A] mono-ortho PCB; [B] di-ortho PCB
TEF = Toxic Equivalency Factor
TEQ = Toxic Equivalent = mean content x TEF (ng TEQ/kg fat)
Total PCB/dioxin TEQ = dioxin TEQ + planar PCB TEQ + mono-ortho PCB TEQ + di-ortho PCB TEQ
Non-planar PCBs (n = 194); PCB 180 (n = 192); total PCB/dioxin TEQ (n = 168)
[#] ΣPCB = PCB 118 + 138 + 153 + 180

IUPAC = International Union of Pure and Applied Chemistry
* Significant odds ratios ($p < 0.05$, two-tailed)
** Significant odds ratios ($p < 0.01$, two-tailed)

Figure 6.1 Distribution of the neonatal neurological optimality scores for Groningen and Rotterdam.

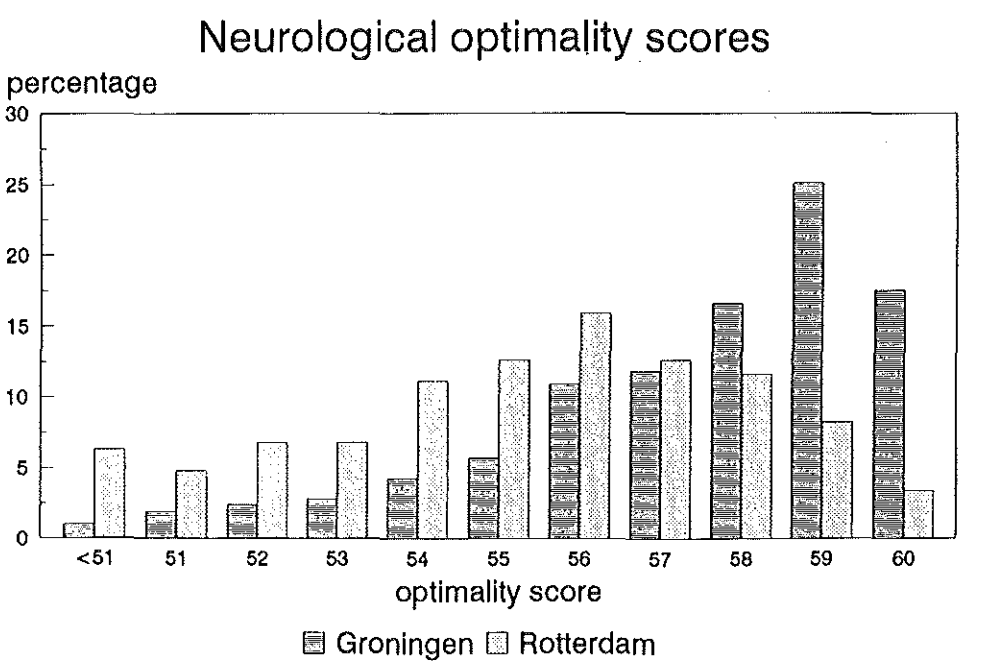
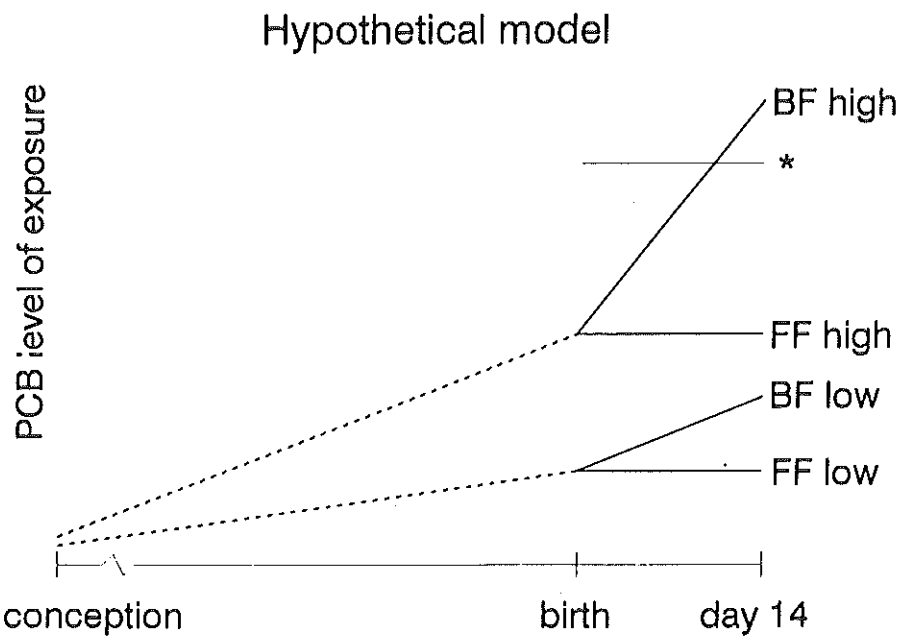


Figure 6.2 Hypothetical model of the relation between the summation of intrauterine PCB exposure (PCB levels in cord plasma), and early postnatal PCB exposure (from breast milk or formula milk) versus neurological signs in the newborn period.



BF high: high PCB content in plasma and breast milk exceeds critical level: neurological signs.
BF low: low PCB content in plasma and breast milk does not exceed critical level: no neurological signs.
FF high: high PCB content in plasma of the formula-feeding group does not exceed critical level: no neurological signs.
FF low: low PCB content in plasma of the formula-feeding group does not exceed critical level: no neurological signs.
* *critical theoretical PCB level for neurological signs.*

CHAPTER 7

EFFECTS OF PCB/DIOXIN EXPOSURE AND FEEDING TYPE ON THE INFANT'S VISUAL RECOGNITION MEMORY.

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EFFECTS OF PCB/DIOXIN EXPOSURE AND FEEDING TYPE ON THE INFANT'S VISUAL RECOGNITION MEMORY.

Corine Koopman-Esseboom[1], Nynke Weisglas-Kuperus[1], Maria A.J. de Ridder[2], Cornelis G. Van der Paauw[3], Louis G.M.Th. Tuinstra[4], Pieter J.J. Sauer[1].

[1]Department of Pediatrics, Division of Neonatology, Erasmus University and University Hospital/Sophia Children's Hospital, Rotterdam.
[2]Institute of Epidemiology and Biostatistics, Erasmus University Rotterdam.
[3]TNO Nutrition and Food Research, Zeist.
[4]DLO State Institute for Quality Control of Agricultural Products, Wageningen, The Netherlands.

7.1 Abstract

Objective: Adverse effects of perinatal exposure to polychlorinated biphenyls (PCBs) on the psychomotor development of young children have been described by different research groups. Effects of PCB exposure on the mental development are less clear: some report negative effects, others could not affirm these findings. Effects of perinatal exposure to dioxins and dioxin-like PCBs on the early mental development are unknown. In this study effects of intrauterine and lactational exposure to PCBs as well as dioxins and dioxin-like PCBs are evaluated on the short term memory function of infants.

Design: Prenatal PCB exposure was estimated from the levels in maternal plasma during the last month of pregnancy. Postnatal PCB and dioxin exposure of breast-fed infants was calculated from levels in human milk samples and the duration of breast-feeding. Infants were examined at 3 and 7 months of age for their visual recognition memory by means of the Fagan Test of Infant Intelligence. This test is one of the earliest tests to measure the infant's cognitive brain function and to predict intelligence at a later age.

Setting: General community.

Participants: Voluntary sample of 207 mother-infant pairs. One hundred and five infants were breast-fed and 102 were formula-fed.

Interventions: None.

Results: There was no significant relationship between the pre- or postnatal PCB or dioxin exposure and cognitive development as assessed by the visual recognition memory test at 3 months of age. At 7 months of age, breast-fed infants had significantly higher mean scores, compared to formula-fed infants. Moreover, the scores were positively correlated with the duration of breast-feeding.

Conclusions: Intrauterine or lactational exposure to Dutch background levels of PCBs and dioxins has no adverse effects on the infant's cognitive development assessed by means of the visual recognition memory test. On the contrary, breast-feeding has a positive, dose-dependent influence on the cognitive development.

7.2 Introduction

Polychlorinated biphenyls (PCBs) and dioxins (polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzo furans (PCDFs)) are both widespread resistant toxins in the environment with possible adverse health effects on human beings.[1,2] PCBs are industrial chemicals which have been utilized for diverse commercial applications such as dielectric fluids for capacitors and transformers, plasticizers, fire retardants, paint additives, and waxes. The production and use of these compounds started in the thirties and was mainly banned in the late seventies. Dioxins are formed as by-products during the process of combustion and manufacturing of organochlorine chemicals. Both toxins are lipophilic and accumulate in the food chain. The PCBs are a compilation of 209 possible congeners, the dioxins of 210 congeners, with different chlorine substitution.

Adults are mainly exposed through the consumption of dairy products, meat and fish.[3] Both compounds can pass the placental barrier and are present in relatively high amounts in human milk.[4] The embryo, fetus and breast-fed infant are exposed to PCBs and dioxins during a critical period of organ growth and differentiation. In formula, milk lipids are replaced by lipids of vegetable origin with a negligible content of PCBs and dioxins.

Adverse effects of perinatal exposure to PCBs on the psychomotor development of young children have been described by different research groups.[5-10] Effects of PCB exposure on the mental development are less clear: some report negative effects, others could not confirm these findings.

In the USA, two different studies were done on possible effects of perinatal exposure to background levels of PCBs on cognitive functions of children. Dioxin and dioxin-like PCB levels were not measured in these studies. Jacobson et al. reported a dose-dependent decrease in the infant's visual recognition memory with the Fagan Test of Infant Intelligence at 7 months of age, due to intrauterine PCB exposure.[11] The mothers of these infants consumed Lake Michigan sport fish which was considerably contaminated with PCBs. At 4 years of age, high prenatal PCB exposure predicted poorer short-term memory function on both verbal and quantitative scales of the McCarthy Scales of Children's Abilities.[12] Gladen et al. could not measure a negative influence of the transplacental or breast-feeding exposure to PCB background levels in North-Carolina, on any of the mental developmental indices (MDI) of the Bayley Scales of Infant Development at 6, 12, 18 or 24 months,[7,8] nor on any subscale of the McCarthy Scales at 3, 4 or 5 years of age.[13] We did not find a relationship between the perinatal PCB or dioxin exposure and MDI-scores of Dutch children at 3, 7 and 18 months of age.[9]

After accidentally high, intrauterine exposure to PCBs and PCDFs in Japan and Taiwan, mainly dermal lesions as chloracne, hyperpigmentation and skeleton deformities are reported in newborns.[14] In follow-up studies a delay in mental and psychomotor development is described as well.[15,16]

Rhesus monkeys perinatally exposed to 2,3,7,8-tetrachloro-dibenzo-dioxin (TCDD), the most toxic dioxin congener, were facilitated on discrimination reversal learning (DRL) at lower doses, and exhibited a dose-dependent decrement with an increase in TCDD dose.[17] Perinatal PCB exposure, caused

deficits in DRL as well as in delayed spatial alternation of these monkeys.[18]
A beneficial effect of breast-feeding on especially the cognitive development of children has been described in several other studies.[19-21] It was postulated that either long-chain polyunsaturated fatty acids (LCPUFAs), trophic factors or hormones, which are not present in formula-feeding, account for this difference.

In this paper we examine the effects of pre- and postnatal exposure not only to PCBs, but also to dioxins, which levels are relatively high in the Netherlands, and the effects of breast-feeding versus formula-feeding, on the early cognitive development. The Fagan Test of Infant Intelligence was chosen to measure the infant's actual visual recognition memory function and to predict the effects of perinatal exposure to PCBs and dioxins on later cognitive performance.[22] Most conventional sensorimotor tests, such as the Bayley Scales of Infant Development, give an assessment of the infant's current mental development, no prediction. Rose et al.[23] reported that visual recognition memory scores determined at 7 months of age in a group of 45 full-term infants with a low social economical status, predicted significantly the MDI/IQ from 2 to 5 years of age (r_s = 0.37 - 0.65).
This study, is the first which evaluates effects of perinatal exposure to dioxins and dioxin-like PCBs. It is part of the Dutch PCB/Dioxin study, a larger prospective longitudinal study on possible adverse health effects of these pollutants on human beings.

7.3 Methods

Subjects: During the last trimester of pregnancy women were asked to volunteer for the study by obstetricians and midwives, between June 1990 and February 1992, in the Rotterdam area. They were given written information about the study design and purposes. When they agreed to take part in the study, they were visited at their homes for further explanation of the study protocol by the examiner (CKE). There is no information on the women who refused to take part in the study. In order to establish a homogeneous study population, only first or second born term infants (37 to 42 weeks of gestation) without congenital anomalies or diseases were included. Pregnancy and delivery had to be passed without overt signs of serious illness, or complications like a caesarian section, forceps or vacuum extraction. All infants were of the Caucasian race. In order to study the effects of postnatal exposure to PCBs and dioxins, women were enrolled who intended to give breast-feeding, which contains relatively high levels of both toxins, for at least 6 weeks, as well as women who volunteered to give formula-feeding from one batch as a reference (Almiron M2, Nutricia N.V., the Netherlands), during 7 months.
The study protocol had been approved by the medical ethical committee of the University Hospital Rotterdam/Sophia Children's Hospital. Informed consent had been given by the parents.

Measures of exposure: A blood sample was taken from the mothers during the last month of their pregnancy (36th to 40th week) and from the umbilical cord for the measurement of the PCB congener levels 118, 138, 153, and 180 by gas chromatography with electron capture detection (GC-ECD).[24] These

congeners are known to have concentrations in human blood which are high enough to be measured with high accuracy. The four congener levels were added and summarized as the PCB-plasma-sum. One maternal and 30 cord blood samples were missing for this analysis due to failures in the organization. Dioxin levels could not be measured in the maternal or cord plasma samples since large amounts of blood would have been necessary to measure accurately the low dioxin levels. In the second week after delivery, the mothers who breast-fed their infants, collected a 24-hour representative human milk sample, that is 10% of each feeding, by means of a vacuum pump (Babyluxus 2, KAWECO, Stuttgart, Germany). Seventeen individual dioxin congener and 24 PCB congener levels were measured by gas chromatography-high-resolution mass spectrometry (GC-HRMS)[25,26], and GC-ECD respectively. According to the toxic equivalent (TEQ) concept, the dioxin and dioxin-like PCBs (IUPAC no. 77, 126, 169, 105, 118, 156, 170 and 180) were added and summarized as the total PCB-dioxin TEQ level, as described in a previous paper.[4] The PCB congener levels 118, 138, 153 and 180 in human milk were also added and summarized as the PCB-milk-sum in comparison with the PCB-plasma-sum. The same measurements were done in samples of the formula-feeding. Of the 105 human milk samples, 80 could be measured with sufficient accuracy for the total PCB-dioxin TEQ level, and 100 for the PCB-milk-sum. The other analytical measurements were inaccurate due to either interferences in the chromatograms or small volumes of human milk samples; these measurements were not used in the statistical analyses.

Test material and procedure: All infants were tested by means of the Fagan Test of Infant Intelligence[22] at 53 and 69 weeks of postconceptional age (± 1 week). All Fagan tests were performed at the infants' homes, in the presence of the parent(s), by one examiner (CKE) who was unaware of the infant's PCB and dioxin exposure. The test-retest reliability was 95%. The Fagan test is based on the baby's ability to recognize pictures. Recognition is tested by presenting the baby with one picture to study for a brief time, after which the studied picture is paired with a new picture, which constitutes a novelty problem. The manner in which babies distribute their visual attention between novel and previously shown pictures reveals the operation of memory and abstraction processes which are the same as those employed by older children and adults in solving intelligence tests. At 3 months of age the Fagan test consists of 6 novelty problems and at 7 months of age it consists of 10 problems. Visual recognition is measured by differential fixation to the novel over the previously-seen pictures and is expressed as mean percentage of the time looking at the novel pictures. Infants who look for at least 58% of the time at the novel pictures are classified as normal, between 54 and 57% as suspect, and less than 53% as at risk for a delay in mental development. At three months of age the Fagan test was missing for 1 infant.

Data analysis:

Exposure: Data analysis was performed by means of the statistical soft-ware package SPSS/PC. In the analysis the relationship between mean Fagan scores and the PCB/dioxin exposure was studied. As a measure of the prenatal exposure, the PCB-plasma-sum in maternal and umbilical cord plasma was examined, both after natural logarithm transformation. Dioxin levels in human

milk are known to be highly correlated with the dioxin levels in maternal plasma and in adipose tissue and are therefore a good estimation of the prenatal dioxin exposure.[27] Since in this study dioxins and dioxin-like PCBs could only be measured in human milk, the total PCB-dioxin TEQ level in human milk was separately studied as an estimate of the prenatal dioxin exposure of the breast-fed infants. Since it is assumed that breast-feeding *per se* has positive effects on the development of children, the amount of breast-feeding received was studied separately. This variable called "duration of breast-feeding in weeks", was divided into 3 categories: zero (formula-fed), short and long (Table 7.1). In the multiple regression analysis this categorial variable was entered as a continuous independent variable with the value 0, 1 or 2. We chose to use linearity since the difference between category 1 and 2 is half the difference between category 1 and 3.

The postnatal PCB exposure and the total PCB-dioxin TEQ exposure were calculated separately as a multiplication of the PCB-milk-sum respectively the total PCB-dioxin TEQ level in human milk and the duration of breast-feeding in weeks (Exposure = PCB/dioxin level x duration BF). Both variables were divided into 3 categories: low, medium and high-exposed (Table 7.2).

Confounders: The socio-economic, obstetric and neonatal conditions were assessed by means of the obstetrical optimality scale.[28] The following potential confounding variables were identified with univariate analyses: the level of education of the parents (high (1), at least secondary education completed, or low (0)), profession (divided into 3 categories: student, unemployed or unskilled worker; skilled worker or middle class employe; independent middle class man or higher profession), maternal smoking and alcohol usage (yes or no), parity (first or second, reference category is "first"), duration of gestation, birthweight, Apgar score after 1 and 5 minutes, sex of the infant, duration of breast-feeding (in weeks). The Home Observation for Measurement of the Environment, the HOME Inventory[29], was examined at the age of 18 months.

7.4 Results

Two hundred and sixty-eight women were involved in the study. After birth 231 mother-infant pairs fulfilled the inclusion criteria of the study protocol. A vacuum extraction or caesarian section formed the main cause for exclusion of these 37 women. Six weeks after delivery 24 pairs were excluded from the study mainly due to ceasing breast-feeding before this time. Of these 61 excluded pairs no PCB or dioxin analysis were done, nor were the infants tested with the Fagan Test. Of the remaining 207 infants, 105 were breast-fed and 102 formula-fed. Eighty out of 105 breastmilk samples could be measured with sufficient accuracy for the total PCB-dioxin TEQ level: the caracteristics of the mother-infant pairs whose milk samples were analysable did not differ significantly from those whose samples were not analysable.

The median value of the PCB-cord-plasma-sum was 0.4 ng/g plasma (range 0.1 - 2.1); the median of the PCB-maternal-plasma-sum was 2.0 ng/g plasma (range 0.6 - 7.4); and the median of the total-PCB-dioxin-sum in human milk was 64.8 pg TEQ/g fat (range 28.0 - 155). Levels in formula feeding were

below the limits of determination. There was a high significant relation between the PCB-sum in maternal plasma and in cord plasma: Ln PCB-maternal-plasma = 1.22 + 0.55 Ln PCB-cord-plasma-sum. ($r = 0.73$, $P < 0.001$, $N = 182$).

In Table 7.3 the mean Fagan scores (± S.D.) are summarized for the whole group, as well as for the breast- and formula-fed infants separately. There was no significant correlation between the Fagan scores of each infant measured at 3 and 7 months ($r = -0.07$). At 7 months of age, the mean Fagan score of the breast-fed infants was significantly higher compared to the formula-fed infants ($p = .002$).

The potential confounders gestational age, birthweight, parity, HOME-score and maternal education, were entered into the multiple regression analysis as independent variables, for characteristics see[9]. Since there were high correlations between the socio-economic variables, the level of education of the mother was chosen as confounding variable in the multivariate regression. There were no significant correlations between the outcomes of the Fagan test at 3 or 7 months of age and any of the covariables. There were neither significant simple correlations between the outcomes at 3 or 7 months of age and individual PCB congener levels in maternal plasma, or PCB and dioxin congener levels in human milk.

Using multiple regression analyses we could not measure a significant effect of the pre- or postnatal PCB or dioxin exposure on the Fagan outcome at 3 months of age. Table 7.4 shows that there was no significant effect of transplacental PCB exposure, estimated in maternal plasma, on the Fagan outcome at 7 months of age. There was neither a significant influence of the total PCB-dioxin TEQ level in human milk as an estimation of the prenatal dioxin and dioxin-like PCB exposure of the breast-fed infants. However, there is a significantly positive relationship between the "duration of breast-feeding", and the Fagan outcome at 7 months of age. The mean Fagan score of the group of infants being breast-fed for a period of 6 to 16 weeks, respectively 17 weeks or longer, would be 1.6 respectively 3.2 points higher compared to the mean score of the group of formula-fed infants.

In Table 7.5 the effects of the postnatal total PCB-dioxin TEQ exposure on the Fagan outcome at 7 months of age are presented. There is a significantly positive effect of the postnatal total PCB-dioxin TEQ exposure on the Fagan outcome at this age. The "duration of breast-feeding" is no longer significantly related with the Fagan test outcome. This suggests that it might be the total PCB-dioxin TEQ exposure that the infants received via breast-feeding, or another lipophilic substance which increases comparably to the total PCB-dioxin TEQ level in breast-feeding, that accounts for the increase in the Fagan score at 7 months of age. Infants who received a medium or high total PCB-dioxin TEQ exposure through breast-feeding had significantly higher Fagan scores at 7 months of age (+ 4.4, respectively + 4.6), compared to formula-fed infants and breast-fed infants who received a low total PCB-dioxin TEQ exposure (overall $p = .04$). Figure 7.1 shows that infants being breast-fed between 6 and 16 weeks (short), with a low total PCB-dioxin TEQ exposure, would score 0.5 points lower on the Fagan test at 7 months of age, and infants being breast-fed between 17 and 30 weeks (long), with a low total PCB-dioxin TEQ exposure would score 1 point lower compared to formula-fed infants, which is of no

significance. Infants being shortly breast-fed with a medium total PCB-dioxin TEQ exposure would score 3.9 points higher, and infants being breast-fed for a long period with a medium total PCB-dioxin TEQ exposure would score 3.4 points higher compared to formula-fed infants, which is a significant difference. Infants being breast-fed shortly with a high total PCB-dioxin TEQ exposure would score 4.5 points higher and infants being breast-fed for at least 17 weeks with a high exposure would score 3.6 points higher compared to formula-fed infants, which is also a significant difference.

When entering the prenatal PCB exposure in this regression analysis, the significant effect of the postnatal total PCB-dioxin TEQ exposure remained. There was no significant effect on the Fagan outcome at 7 months of age, when we analysed the effects of the postnatal exposure to the non-dioxin like PCBs in human milk.

7.5 Discussion

In this paper we evaluate both the effects of prenatal and postnatal exposure to PCBs and dioxins, and the effects of breast-feeding versus formula on the visual recognition memory of infants by using the Fagan Test of Infant Intelligence. This test measures early cognitive functions of the infant's brain, and predict later intelligence from 2 years onwards.[22,23]
At the age of 3 months we neither found an effect of the PCB or dioxin exposure nor of the feeding type (breast versus formula). At the age of 7 months there was no significant influence of the infants' prenatal PCB or dioxin exposure. However, at this age we found a significantly positive effect of the duration of breast-feeding on the visual recognition memory. Moreover, the visual recognition memory score was significantly higher with an increase in total PCB-dioxin TEQ exposure via human milk. In contrast to our findings, Jacobson et al. found a dose-dependent negative influence of the prenatal PCB exposure on the mean visual recognition memory score at 7 months of age.[14] There was no effect of the postnatal PCB exposure. They only tested the first three novelty problems instead of all ten problems used by us. When examining the same three novelty problems as Jacobson et al. did, we neither found a negative influence of the prenatal exposure. Since they measured a total PCB level in blood and human milk, without dioxin levels, it is difficult to compare their levels to our congener specific measurements which are more accurate. It remains possible that the PCB levels in their study population are higher compared to the levels in our cohort.

Rhesus monkeys exposed perinatally to TCDD, the most toxic dioxin congener, were facilitated on spatial discrimination reversal learning at a lower TCDD dose, and exhibited a dose-dependent decrement as the dose increased.[17] These results may be comparable to our measurements, that exposure to low background levels of dioxins may have a facilitating effect on the infants' visual recognition memory. We did not find a negative influence on the visual recognition memory outcome at 7 months of age in the infants who belonged to the highest exposed group. The highest dioxin and dioxin-like PCB levels in Dutch human milk might be below the level above which decrements in early

cognitive development could appear. Perinatal exposure to PCBs resulted in a deficit in the monkeys on delayed spatial alternation, a spatial learning and memory task.[18] The deficit was mostly apparent in the shorter delays, suggesting that it was not due to memory impairment but to impairments in associational or attentional processes. We neither found a negative influence of the prenatal nor of the postnatal non-dioxin like PCB exposure on the Fagan outcome, maybe because the exposure levels are below a no-effect level.

Besides a possible facilitating effect of the Dutch background levels of PCBs and dioxins in human milk on the visual recognition memory of infants, it is more reasonable to assume that lipids like LCPUFAs, or other lipophilic factors like hormones or trophic factors, that are present in breast-milk, and which increase comparably to the total PCB-dioxin TEQ level, are responsible for the better outcome in visual recognition memory. Lucas et al.[19] described a significant advantage in IQ of children at the ages of 1,5 and 8 years, who were born preterm and received breast-feeding by tube compared to preterms who received formula-feeding by tube. Birch et al.[20] found a significant positive effect of LCPUFAs on the visual development of pre- and full-terms who were breast-fed compared to infants who were formula-fed. Higher visual aquity outcomes were significantly correlated with higher LCPUFA levels in the red blood cells. These LCPUFAs, formed from essential fatty acids in breast-feeding, are crucial for retinal and other neural tissue growth and development. The formula-fed infants of our cohort received formula without these essential fatty acids.

We conclude that the Dutch perinatal PCB and dioxin exposure has no negative effect on the infant's cognitive development, measured by means of the visual recognition memory test at the ages of 3 and 7 months. Visual recognition memory scores examined at 7 months of age were positively related to the duration of breast-feeding and total PCB-dioxin TEQ exposure in breast-feeding. We assume that compounds like lipids or lipophilic factors, present in human milk, are responsible for the dose-dependent increase in visual recognition memory outcome of breast-fed infants. Follow-up studies are underway at the moment to examine possible effects of perinatal PCB and dioxin exposure on the cognitive function at school age.

7.6 References

1. Ahlborg UG, Brouwer A, Fingerhut MA et al. Impact of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls on human and environmental health, with special emphasis on application of the toxic equivalency factor concept. Eur J of Pharmacol - Envir Toxicol and Pharmacol Section 1992;228:179-199.
2. Skene SA, Dewhurst IC, Greenberg M. Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans: the risks to human health. A review. Human Toxicol 1989;8:173-203.
3. Theelen RMC. Modeling of human exposure to TCDD and I-TEQ in the Netherlands: Background and occupational. In: Gallo MA, Scheuplein RJ, Van der Heijden KA (eds). Biological basis for risk assessment of dioxins and related compounds. Cold Spring Harbor Laboratory Press; Banbury report

1991;35:277-290.
4. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N et al. PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 1994;28:1721-1732.
5. Jacobson JL, Jacobson SW, Schwartz PM, Fein GG, Dowler JK. Prenatal exposure to an environmental toxin: a test of the multiple effects model. Dev Psychol 1984;20:523-532.
6. Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, Tinglestad J, Tully M. Neonatal effects of transplacental exposure to PCBs and DDE. J Pediatr 1986;109:335-341.
7. Gladen BC, Rogan WJ, Hardy P, Thullen J, Tinglestad J, Tully M. Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. J Pediatr 1988;113:991-995.
8. Rogan WJ, Gladen BC. PCBs, DDE, and child development at 18 and 24 months. Ann Epidemiol 1991;1:407-413.
9. Koopman-Esseboom C, Weisglas-Kuperus N, De Ridder MAJ, Van der Paauw CG, Tuinstra LGMTh, Sauer PJJ. Effects of PCB/dioxin exposure and feeding type on the infant's mental and psychomotor development. Pediatr 1995, in press.
10. Huisman M, Koopman-Esseboom C, Fidler V et al. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. Early Hum Dev 1995;41:111-127.
11. Kuratsune M, Yoshimura T, Matsuzaka J, Yamaguchi A. Epidemiological study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. Environ Health Perspect 1972;1:119-128.
12. Rogan WJ, Gladen BC, Hung K-L, Koong S-L, Shih L-Y, Taylor JS, Wu Y-C, Yang D, Ragan NB, Hsu CC. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 1988;241:334-336.
13. Chen YJ, Hsu CC. Effects of prenatal exposure to PCBs on the neurological function of children: a neuropsychological and neurophysiological study. Dev Med Child Neurol 1994;36:312-320.
14. Jacobson SW, Fein GG, Jacobson JL, Schwartz PM, Dowler JK. The effect of intrauterine PCB exposure on visual recognition memory. Child Dev 1985;56:853-860.
15. Jacobson JL, Jacobson SW, Humphrey HEB. Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J Pediatr 1990;116:38-45.
16. Gladen BC, Rogan WJ. Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. J Pediatr 1991;119:58-63.
17. Bowman RE, Schantz SL, Ferguson SA. Controlled exposure of female rhesus monkeys to 2,3,7,8-TCDD; cognitive behavioral effects in their offspring. Chemosphere 1990;20:1103-1108.
18. Schantz SL, Levin ED, Bowman RE, Heironimus MP, Laughlin NK. Effects of

perinatal PCB exposure on discrimination-reversal learning in monkeys. Neurotoxicol Teratol 1989;11:243-250.
19. Lucas A, Morley R, Cole TJ, Lister G, Leeson-Payne C. Breast milk and subsequent intelligence quotient in children born preterm. Lancet 1992;339:261-264.
20. Birch E, Birch D, Hoffman D, Hale L, Everett M, Uauy R. Breast-feeding and optimal visual development. J Pediatr Ophthalmol Strabismus 1993;30:33-38.
21. Rogan WJ, Gladen BC. Breast-feeding and cognitive development. Early Hum Dev 1993;31:181-193.
22. Fagan JF, Shepherd PA. The Fagan test of infant intelligence, manual. Cleveland: Infantest corporation, 1990.
23. Rose SA, Feldman JF, Wallace IF, McCarton C. Infant visual attention: relation to birth status and developmental outcome during the first 5 years. Dev Psychol 1989;25:560-576.
24. Burse VW, Korver MP, Needham LL et al. Gas chromatographic determination of polychlorinated biphenyls (as Aroclor 1254) in serum: Collaborative study. J Assoc Off Anal Chem 1989;72:649-659.
25. Van Rhijn JA, Traag WA, Van de Spreng PG, Tuinstra LGMTh. Simultaneous determination of planar chlorobiphenyls and polychlorinated dibenzo-p-dioxins and -furans in Dutch milk using isotope dilution and gas chromatography-high-resolution mass spectrometry. J Chromatogr 1993;630:297-306.
26. Tuinstra LGMTh, Traag WA, Van Rhijn JA, Van de Spreng PF. The Dutch PCB/dioxin study. Development of a method for the determination of dioxins, planar and other PCBs in human milk. Chemosphere 1994;29:1859-1875.
27. Van den Berg M, De Jongh J, Poiger H, Olson JR. The toxicokinetics and metabolism of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), and their relevance for toxicity. CRC Critical Reviews in Toxicology 1994;24:1-74.
28. Touwen BCL, Huisjes HJ, Jurgens-v.d. Zee AD, Bierman-van Eendenburg MEC, Smrkovsky M, Olinga AA. Obstetrical condition and neonatal neurological morbidity. An analysis with the help of the optimality concept. Early Hum Dev 1980;4:207-228.
29. Caldwell BM, Bradley RH. Home observation for measurement of the environment. Administration manual. Little Rock, Arkansas, 1984.

Table 7.1 Categories of duration of breast-feeding.

	3 Months		7 Months	
	N	weeks of breast-feeding	N	weeks of breast-feeding
Category I	102	0	102	0
Category II	26	6 - 10	54	6 - 16
Category III	79	11 - 12	51	17 - 30

Table 7.2 Categories of total PCB-dioxin TEQ exposure via breast-feeding.

	Exposure by breast-feeding	N	3 Months pg PCB-dioxin TEQ/g fat times weeks	7 Months pg PCB-dioxin TEQ/g fat times weeks
Category I	low	27	168 - 617	168 - 769
Category II	medium	26	618 - 810	770 - 1289
Category III	high	27	811 - 1860	1290 - 4340

Table 7.3 Visual Recognition Memory Scores at 3 and 7 months of age.

	All	Breast-fed	Formula-fed	P
Fagan 3 months				
N	206	105	101	
Mean (SD)	61.8 (9.8)	61.5 (9.0)	62.2 (10.7)	0.62
Range	37.0 - 87.0	37.0 - 87.0	38.0 - 86.8	
Fagan 7 months				
N	207	105	102	
Mean (SD)	58.6 (6.0)	59.9 (5.9)	57.3 (5.9)	0.002
Range	42.1 - 75.4	46.0 - 75.4	42.0 - 72.7	

SD = Standard Deviation
P = Student's t-test

Table 7.4 Results of the multiple regression analysis of the visual recognition memory at 7 months of age related to the prenatal PCB exposure.

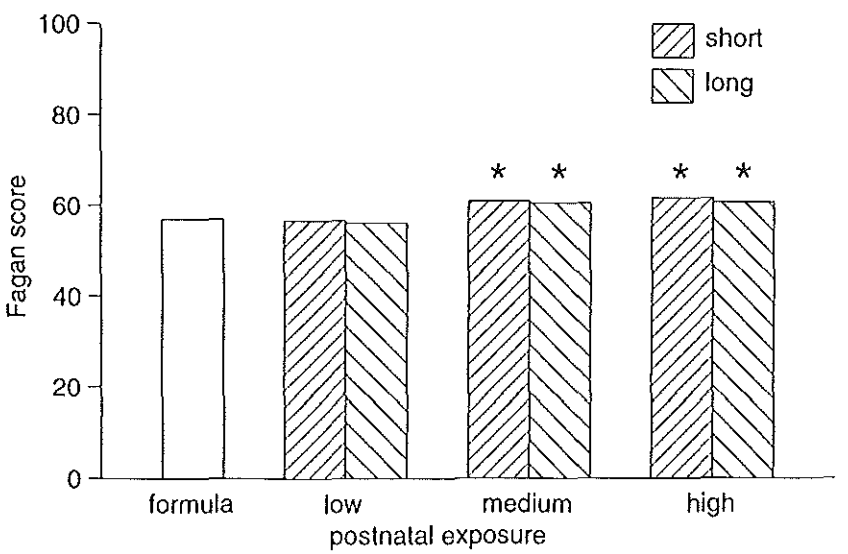
Independent Variables	Coefficient	Standard Error	P
Constant	52.3	17.0	.002
Birthweight (grams)	-.002	.001	.09
Parity (no sibs, one or more sibs)	.21	.90	.82
HOME-score	.12	.18	.52
Gestational Age (weeks)	.18	.39	.65
Education of mother (low, high)	.56	.98	.57
Ln PCB-plasma-sum	-.50	1.07	.64
Duration of breast-feeding (0, 6-16 or 17-30 weeks)	1.63	.56	.004

N = 181

Table 7.5 Results of the multiple regression analysis of the visual recognition memory at 7 months of age related to the postnatal total PCB-dioxin TEQ exposure. N = 182

Independent Variables	Coefficient	Standard Error	P
Constant	42.3	16.6	.01
Birthweight (grams)	-.002	.001	.10
Gestational Age (weeks)	.39	.39	.32
Parity (no sibs, one or more sibs)	.35	.88	.69
HOME-score	.14	.18	.44
Education of mother (low, high)	.28	.96	.77
Duration of breast-feeding (0, 6-16 or 17-30 weeks)	-.46	1.0	.66
PCB-dioxin-TEQ exposure medium	4.4	1.7	.01
PCB-dioxin-TEQ exposure high	4.6	2.2	.03
PCB-dioxin-TEQ exposure, overall-P			.04

Figure 7.1 Visual recognition memory at 7 months of age related to breast-feeding.



CHAPTER 8

EFFECTS OF PCB/DIOXIN EXPOSURE AND FEEDING TYPE ON THE INFANT'S MENTAL AND PSYCHOMOTOR DEVELOPMENT.

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EFFECTS OF PCB/DIOXIN EXPOSURE AND FEEDING TYPE ON THE INFANT'S MENTAL AND PSYCHOMOTOR DEVELOPMENT.

Corine Koopman-Esseboom[1], Nynke Weisglas-Kuperus[1], Maria A.J. de Ridder[2], Cornelis G. Van der Paauw[3], Louis G.M.Th. Tuinstra[4], Pieter J.J. Sauer[1].

[1]Department of Pediatrics, Division of Neonatology, Erasmus University and University Hospital/Sophia Children's Hospital, Rotterdam.
[2]Institute of Epidemiology and Biostatistics, Erasmus University Rotterdam.
[3]TNO Nutrition and Food Research Institute, Zeist.
[4]DLO State Institute for Quality Control of Agricultural Products, Wageningen.

8.1 Abstract

Objective: To evaluate the effects of in utero and lactational exposure to polychlorinated biphenyls (PCBs) and dioxins on the mental and psychomotor development of infants.

Design: Prenatal PCB exposure was estimated from the levels in maternal plasma during the last month of pregnancy. Postnatal PCB and dioxin exposure of breast-fed infants was calculated from levels in human milk samples and the duration of breast-feeding. Infants were examined at 3, 7 and 18 months of age with the Bayley Scales of Infant Development.

Setting: General community.

Participants: Voluntary sample of 207 mother-infant pairs. One hundred and five infants were breast-fed and 102 were bottle-fed.

Interventions: None.

Results: Higher in utero exposure to PCBs was associated with lower psychomotor scores at 3 months of age: a doubling of the PCB load resulted in a decrease of 3 points. Breast-fed infants scored significantly higher on the psychomotor score at 7 months of age, compared to formula-fed infants. However, when corrected for confounders, the psychomotor score of the 66% highest exposed breast-fed infants (>756 pg total PCB-dioxin TEQ), was negatively influenced by this postnatal exposure to PCBs and dioxins, and was comparable to the psychomotor score of the formula-fed infants. Breast-fed infants scored also higher on the mental scale at 7 months of age in a dose-dependent way. There was no significant influence of the perinatal PCB and dioxin exposure on the mental outcome at 3 and 7 months of age. At 18 months of age neither the mental nor the psychomotor score was related to perinatal PCB or dioxin exposure, nor to the duration of breast-feeding.

Conclusions: Prenatal PCB exposure has a small negative effect on the psychomotor score at 3 months of age. PCB and dioxin exposure through breast-feeding has an adverse effect on the psychomotor outcome at 7 months of age. The mental outcome at 7 months of age is positively influenced by breast-feeding *per se*, the perinatal exposure to PCBs and dioxins does not influence this outcome. At 18 months of age the development is neither affected by PCB and dioxin exposure nor by feeding type.

8.2 Introduction

Polychlorinated biphenyls (PCBs) and dioxins (PCDDs and PCDFs) are both widespread, highly resistant pollutants in the environment with possible adverse health effects on human beings.[1,2] PCBs are industrial chemicals which have been utilized for diverse commercial applications such as dielectric fluids for capacitors and transformers. The production and use of these compounds were mainly banned in the late seventies. Dioxins are formed as by-products during the process of combustion and manufacturing of organochlorine chemicals. Both toxins are lipophilic and accumulate in the food chain. Adults are mainly exposed through the consumption of dairy products, meat and fish.[3] Both compounds can pass the placental barrier and are present in relatively high amounts in human milk.[4] The embryo, fetus and breast-fed infant are exposed to PCBs and dioxins during a critical period of organ growth and differentiation. In formula, milk lipids are replaced by lipids of vegetable origin with a negligible content of PCBs and dioxins. As a consequence the postnatal exposure to these toxins of formula-fed infants is of no concern. A subpopulation of Taiwanese and Japanese women who were accidentally exposed to high levels of PCBs and PCDFs, through contaminated rice oil, gave birth to infants who were small for gestational age and who showed mainly dermal lesions as chloracne and hyperpigmentation. In follow-up studies a delay in mental and psychomotor development has been described up until 7 years of age, in these prenatally high-exposed children.[5-9] However, at 8 years of age the cognitive development of the exposed Taiwanese children had a tendency to catch up, and did not differ significantly anymore from their controls.[10]

Negative effects on both the mental and psychomotor development have also been measured after prenatal exposure to PCB background levels in the USA. Jacobson et al. described a significant dose-dependent relationship between a higher cord serum PCB level and a poorer performance on the Verbal and Memory scale of the McCarthy Scales of Children's Abilities, in children at 4 years of age, whose mothers consumed Lake Michigan sport fish, which was contaminated with PCBs.[11] Gladen and Rogan et al. described an association between the prenatal PCB exposure in North Carolina, and poorer performance on the psychomotor developmental index (PDI) of the Bayley Scales of Infant Development at 6, 12, 18 and 24 months of age.[12,13] They could not find a negative influence of the prenatal PCB exposure on the mental developmental index (MDI) at these ages, nor on any of the McCarthy Scales examined at 3, 4 and 5 years of age.[14] Few adverse effects on the infants' development due to lactational exposure have been described: Jacobson et al. reported a relationship between postnatal PCB exposure and a decrease in activity level at 4 years of age.[15]

A beneficial effect of breast-feeding, particularly on the cognitive development of children, has been described in several studies. It was postulated that hormones, trophic factors or long-chain polyunsaturated fatty acids (LCPUFAs), which are absent in formula-feeding, may account for these differences in development.[16-18]

In this paper we wish to examine the effects of pre- and postnatal exposure not only to PCBs, but also to dioxins, which levels are relatively high in human

milk samples from the Netherlands, and the effects of breast-feeding versus formula-feeding, on the mental and psychomotor development of infants at 3, 7 and 18 months of age. This study is part of the Dutch PCB/Dioxin study, a larger prospective longitudinal study on possible adverse health effects of these pollutants on human beings.

8.3 Methods

Subjects: During the last trimester of pregnancy women were asked to volunteer for the study by their obstetrician or midwife, between June 1990 and February 1992, in the Rotterdam area. They were given written information about the study design and purposes. When they agreed to take part in the study, they were visited at their homes for further explanation of the study protocol by the examiner (CKE). There is no information on the women who refused to take part in the study. In order to establish a homogeneous study population, only first or second born term infants (37 to 42 weeks of gestation) without congenital anomalies or diseases were included. Pregnancy and delivery had to be passed without overt signs of serious illness, or complications like a caesarian section, forceps or vacuum extraction. All infants were of the Caucasian race. In order to study the effects of postnatal exposure to PCBs and dioxins, women were enrolled who intended to give breast-feeding, which contains relatively high levels of both toxins, for at least 6 weeks, as well as women who volunteered to give formula-feeding from one batch as a reference (Almiron M2, Nutricia N.V., the Netherlands), during 7 months.

The study protocol had been approved by the medical ethical committee of the University Hospital Rotterdam/Sophia Children's Hospital. Informed consent had been given by the parents.

Measures of exposure: A blood sample was taken from the mothers in the last month of their pregnancy (36th to 40th week) and from the umbilical cord for the measurement of the PCB congener levels 118, 138, 153 and 180 by gas chromatography with electron capture detection (GC-ECD).[19] These congeners are known to have concentrations in human blood which are high enough to be measured with high accuracy. The four congener levels were added and summarized as the PCB-plasma-sum. One maternal and 30 cord blood samples were missing for this analysis due to failures in the organization. Dioxin levels could not be measured in the maternal or cord plasma samples since larger amounts of blood would have been necessary to measure accurately the low dioxin levels, in relatively lean blood. In the second week after delivery, the mothers who breast-fed their infants, collected a 24-hour representative human milk sample with a vacuum pump (Babyluxus 2, KAWECO, Stuttgart, Germany). Seventeen individual dioxin congener and 24 PCB congener levels were measured by gas chromatography-high-resolution mass spectrometry (GC-HRMS)[20,21] respectively GC-ECD. According to the toxic equivalent (TEQ) concept, the dioxin and dioxin-like PCBs (IUPAC no. 77, 126, 169, 105, 118, 156, 170 and 180) were added and summarized as the total PCB-dioxin TEQ level, as described in a previous paper.[4] The PCB congener levels 118, 138, 153 and 180 in human milk were also added and summarized as the PCB-milk-sum in

comparison with the PCB-plasma-sum. The same measurements were done in samples of the formula-feeding. Of the 105 human milk samples, 80 could be measured with sufficient accuracy for the total PCB-dioxin TEQ level, and 100 for the PCB-milk-sum. The other analytical measurements were inaccurate due to either interferences in the chromatograms, or to too small volumes of human milk samples; these measurements were not used in the statistical analyses.

Test material and procedure: The Mental (MDI) and Psychomotor Developmental Index (PDI) of the infants was determined with the Dutch standardized version of the Bayley Scales of Infant Development[22], the BOS 2-30[23], at 3, 7 and 18 months of age. The Bayley Scales were originally standardized for the Dutch population to a mean of 100 and a standard deviation of 15. All Bayley tests were performed at the infants' homes in the presence of the parent(s) by one examiner (CKE), who was unaware of the infants' PCB and dioxin exposures. At the age of 3 months, MDI scores were missing for 7 infants and PDI scores for 9 infants. At 18 months of age, the PDI score was missing for 1 child.

Data analysis: Data analysis was performed by means of the statistical software package SPSS/PC. In the analysis the relationship between mean Bayley scores and the perinatal PCB/dioxin exposure was studied.

Exposure: As a measure of the prenatal exposure, the PCB-plasma-sum in maternal and umbilical cord plasma was examined, both after log transformation. Dioxin levels in human milk are known to be highly correlated with the dioxin levels in maternal plasma and in adipose tissue and are therefore a good estimation of the prenatal dioxin exposure.[24] Since in this study dioxins and dioxin-like PCBs could only be measured in human milk, the total PCB-dioxin TEQ level in human milk was separately studied as an estimation of the prenatal dioxin exposure of the breast-fed infants.

Since it is assumed that breast-feeding *per se* has positive effects on the development of children, the amount of breast-feeding received was studied separately. This variable called "duration of breast-feeding in weeks", was divided into 3 categories: zero (formula-fed), short and long (Table 8.1). In the multiple regression analysis this categorial variable was entered as a continuous independent variable with the value 0, 1 or 2. We chose to use linearity since the difference between category 1 and 2 is half the difference between category 1 and 3.

The postnatal PCB exposure and the total PCB-dioxin TEQ exposure were calculated separately as a multiplication of the PCB-milk-sum respectively the total PCB-dioxin TEQ level in human milk, and the duration of breast-feeding in weeks. Both variables were divided into 3 categories: low, medium and high-exposed (Table 8.2).

With multiple regression analysis the effects of prenatal and postnatal exposure to PCBs and dioxins were studied separately as well as combined.

Confounders: The socio-economic, obstetric and neonatal conditions were assessed by means of the obstetrical optimality scale.[25] The following potential confounding variables were identified with univariate analyses: the level of education of the parents (high (1), at least secondary education completed, or low (0)) and profession (divided into 3 categories: student, unemployed or unskilled worker; skilled worker or middle class employe; independent middle

class man or higher profession), maternal smoking and alcohol usage (yes or no), parity (first or second, reference category is "first"), duration of gestation, birthweight, apgar score after 1 and 5 minutes, sex of the infant, duration of breast-feeding (in weeks). Total triiodothyronine (TT3), total thyroxine (TT4), free thyroxine (FT4) and thyroid stimulating hormone (TSH) were measured in maternal plasma during the last month of pregnancy, in cord plasma and in the infants' plasma in the second week, and in the third and eighteenth month after birth by chemiluminescence immunoessay, using Amerlite assay kits (Amersham, England). The Home Observation for Measurement of the Environment, the HOME Inventory[26], was examined at the age of 18 months.

8.4 Results

Two hundred and sixty-eight women were involved in the study. After birth 231 mother-infant pairs fulfilled the inclusion criteria of the study protocol. A vacuum extraction or caesarian section formed the main cause for exclusion of these 37 women. Six weeks after delivery 24 pairs were excluded from the study mainly due to ceasing breast-feeding before this time. Of these 61 excluded pairs no PCB or dioxin analysis were done, nor were the infants tested with the Bayley Scales. Of the remaining 207 infants, 105 were breast-fed and 102 formula-fed. Eighty out of 105 breastmilk samples could be measured with sufficient accuracy for the total PCB-dioxin TEQ level: the characteristics of the mother-infant pairs whose milk samples were analysable did not differ significantly from those whose samples were not analysable.

In Table 8.3 the mean Bayley scores (± S.D.) are summarized for the whole group, as well as for the breast- and formula-fed infants separately. Breast-fed infants scored significantly higher on the MDI-7 ($p = .03$), the MDI-18 ($p = .01$) and on the PDI-7 ($p = .05$) compared to the formula-fed infants. The PCB-cord-plasma-sum averaged 0.5 ± 0.3 ng/g; the PCB-maternal-plasma-sum 2.2 ± 1.0 ng/g; and the total-PCB-dioxin-sum in human milk 66.6 ± 24.2 pg TEQ/g fat. The two measurements of prenatal exposure: the PCB-plasma-sum in maternal and cord plasma were highly correlated ($r = 0.72$, $p < 0.001$). Levels in formula feeding were below the limits of determination.

Table 8.4 gives an overview of the potential confounders (gestational age, parity, HOME-score and maternal education), which were correlated at a level $p < 0.10$, with at least one of the dependent outcome variables, and which were entered into the multiple regression analysis as independent variables. Since there were high correlations between the socio-economic variables, we chose maternal education as potential confounding variable in the multivariate regression. The sex of the infants was equally divided in the breast- and formula-fed group, and was not significantly related to any of the outcome variables. There was a weak positive relation between the PCB and dioxin exposure levels and the education of the mothers and the HOME-score of the infants. The other possible confounders were not significantly related to the exposure levels.

In Table 8.5 the effects of the prenatal PCB exposure, measured in maternal plasma, on the PDI at 3 months of age, examined with multiple regression

analysis are presented: there is a significantly negative relation between the prenatal PCB exposure and the PDI score. A doubling of the PCB-plasma-sum (e.g. 1 ng/g compared to 0.5 ng/g, or 2 ng/g compared to 1 ng/g) would result in a decrease by 3 points of the PDI-3 (= -4.8 x Ln 2). When the PCB-sum in cord plasma was used, only 175 cases could be analysed. No effect of prenatal PCB exposure could then be found. This cannot be due to selection, because in this group the maternal PCB-plasma-sum still had a significantly negative influence on the PDI-3. When the total PCB-dioxin TEQ level in human milk was entered into the regression analysis, instead of the PCB-plasma-sum, as an estimation of the prenatal dioxin and dioxin-like PCB exposure of the breast-fed infants, there was also a negative influence of this transplacental exposure on the PDI-3 outcome (B = -7.4, S.E. = 4.0, p = .07). The duration of breast-feeding is not significantly related to the PDI-3. Of the other potential confounders, only the gestational age was of a significantly positive influence (p = .0001). Examination of the postnatal exposure revealed no significant effect of the total PCB-dioxin TEQ exposure on the PDI-3 outcome. When the prenatal PCB exposure and the postnatal total PCB-dioxin TEQ exposure were entered together in the multiple regression analysis the results remain almost the same: a decrease by 3 points of the PDI-3 when the prenatal PCB exposure would double, and no significant effect of the postnatal exposure.

At 7 months of age there was no significant effect of the prenatal exposure on the PDI outcome. Table 8.6 shows the effects of the postnatal total PCB-dioxin TEQ exposure on the PDI-7 outcome in the multiple regression. There is a significantly positive relationship with the duration of breast-feeding: infants being breast-fed between 6 and 16 weeks would score 7 points higher and infants being breast-fed between 17 and 30 weeks would score 14 points higher on the PDI-7 compared to formula-fed infants. This is a positive influence of breast-feeding itself. However, there is a negative influence of the postnatal PCB and dioxin exposure by breast-feeding on the PDI-7: infants who received a medium or high total PCB-dioxin TEQ exposure through breast-feeding had significantly lower PDI-7 scores (-10 respectively -8 points), compared to formula-fed infants and breast-fed infants who received a low total PCB-dioxin TEQ exposure (overall p = .05). Figure 8.1 shows that infants being breast-fed between 6 and 16 weeks (short), with a low total PCB-dioxin TEQ exposure, would score 7 points higher on the PDI-7, and infants being breast-fed between 17 and 30 weeks (long), with a low total PCB-dioxin TEQ exposure would score 14 points higher on the PDI-7, compared to formula-fed infants. Infants being shortly breast-fed with a medium or high total PCB-dioxin TEQ exposure would score 3 points respectively 1 point lower, and infants being breast-fed for a long period with a medium or high total PCB-dioxin TEQ exposure would score 4 respectively 6 points higher compared to formula-fed infants, which is of no significant difference. Furthermore, the PDI-7 is significantly negatively related to the number of older sibs (p < .0001). When the prenatal PCB exposure and the postnatal total PCB-dioxin TEQ exposure were entered together in the multiple regression analysis the results showed the same trends: no effect of the prenatal exposure, and a negative influence of the postnatal exposure on the PDI-7 outcome, although of no significance anymore (overall p = .09).

The PDI outcome at 18 months of age was neither significantly influenced

by the pre- or postnatal PCB and dioxin exposure, nor by the duration of breast-feeding, nor by the other confounders.

The MDI score at 3 months' of age was neither significantly related to the perinatal PCB or dioxin exposure, nor to the duration of breast-feeding. Gestational age was the only confounder with a significant relationship to the MDI-3 ($p < .0001$).

Table 8.7 shows the results of the multiple regression analysis with the MDI at 7 months of age as the dependent variable. The duration of breast-feeding is significantly positively related to the MDI-7. Infants who received breast-feeding for a short or long period, would have an advantage of 2 and respectively 4 points, compared to formula-fed infants. Neither the prenatal nor the postnatal PCB or total PCB-dioxin TEQ exposure have significant influences on the MDI-7 outcome.

The MDI-18 was neither significantly influenced by the duration of breast-feeding, nor by the PCB/dioxin exposure. However, there is a strong positive relationship between the HOME score and the MDI-18 outcome ($p < .0001$). Secondly, a higher level of education of the mother, gives an increase by 7 points on the MDI-18 score ($p = .01$).

Instead of the postnatal total PCB-dioxin TEQ exposure, all analyses were repeated with the PCB-milk-sum multiplied by the duration of breast-feeding as an estimation of the postnatal exposure. The effect of this postnatal PCB exposure on the Bayley scores, was not significant.

There was no significant relationship between the thyroid hormone levels and the mental or psychomotor outcome at any age.

8.5 Discussion

In this study we examined whether the early mental and psychomotor development of full-term, healthy infants was influenced by perinatal exposure to PCBs and dioxins or by breast-feeding versus formula. We found a significantly negative relationship between prenatal PCB exposure and the PDI-3 outcome, there was no influence of this exposure at 7 and 18 months of age. In contrast to our study, Rogan and Gladen et al. described a negative relationship between prenatal PCB exposure and PDI scores at the ages of 6, 12, 18 and 24 months examined with the Bayley Scales, although the negative influence found at 18 months was of no significance.[12,13] It remains difficult to compare the exposure levels of our study to the levels reported by Rogan and Gladen et al. because of differences in analytical methods. They measured a total PCB level in human milk as an estimation of the prenatal PCB exposure, instead of that we measured four specific PCB congener levels in maternal plasma, which is a more accurate method. The PCB exposure we measured is assumed to be as high as the levels in the USA. In addition to the negative effect of prenatal PCB exposure on the PDI-3, we found a significantly positive effect of the gestational age on the PDI-3 outcome. Although all infants were born at term, a range in gestational age of 5 weeks (37-42 weeks) is of significant influence on the PDI outcome at this age. The longer the infants were in utero, the higher the points were of the psychomotor outcome (increase by 2 points per week). In general,

developmental test outcomes of term infants are not corrected for gestational age.

At 7 months of age we found a significant, dose-dependent positive effect of breast-feeding *per se* on the PDI, the effect of which was decreased when the infants received a postnatal medium or high total PCB-dioxin TEQ exposure via breast-feeding. Rogan and Gladen et al. studied influences of breast-feeding on the psychomotor scales of the Bayley test as well. They measured a significantly higher PDI score at 24 months of age in the infants who received breast-feeding for at least 20 weeks.[18] Although they reported negative effects of the prenatal PCB exposure on the psychomotor development, they never found a negative influence of the postnatal PCB exposure by breast-feeding on the infants' development. They did not measure dioxin levels in human milk. Since the dioxin levels in human milk samples of industrialized countries in Western Europe are relatively high compared to the dioxin levels in the USA[27], it might be possible that the dioxin exposure is responsible for the negative effect that we measured on the psychomotor development. At 18 months of age, we no longer detected a negative effect of the postnatal exposure on the PDI outcome. Since most infants were no longer breast-fed after one year of age, and since the PCB and dioxin levels in childrens' tissues decline considerably due to growth, the actual exposure is much lower at this age.

We could not find an adverse effect of transplacental or breast-feeding exposure to PCBs and dioxins on the mental development of the infants. Rogan and Gladen et al. neither found an effect of perinatal PCB exposure on mental development.[12,13] However, adverse effects have been reported on children's cognitive development after accidentally high prenatal exposure to PCBs, up till 7 years of age.[5-9] Jacobson et al. also described a significant dose-dependent relationship between higher prenatal PCB exposure and a poorer performance on the Verbal and Memory scale of the McCarthy Scales of Children's Abilities in children at 4 years of age, whose mothers consumed Lake Michigan sport fish, which was contaminated with PCBs.[11]

We measured a significant positive and dose-dependent relationship between the duration of breast-feeding and the MDI score at 7 months of age. At the age of 18 months breast-fed children scored also significantly higher on the MDI outcome, compared to the formula-fed children. At this age, however, this higher score on the MDI outcome was not related to breast-feeding itself but to better environmental (HOME-score) and socio-economical circumstances: in the breast-fed group 53% of the mothers received higher education compared to 28% in the formula-fed group. Advantages of breast-feeding on the mental development have been described in different studies of preterm as well as full-term children. Morrow-Tlucak[29] described a dose-dependent positive effect of breast-feeding on the MDI score at 12 and 24 months of age with the Bayley Scales. Gladen and Rogan et al.[18] investigated also influences of the duration of breast-feeding on the MDI scores at 6, 12, 18 and 24 months of age. Children who were breast-fed the longest (>20 weeks) scored significantly higher compared to formula-fed and short breast-fed (0-4 weeks) children at 24 months of age. At later ages these children had also higher scores on the cognitive skills of the McCarthy Scales. Lucas et al.[16] described an advantage on the cognitive development of children born prematurely, who received breast-milk by tube

compared to formula-fed prematures, at the ages of 1,5 and 8 years. Birch et al.[17] described a dose-dependent positive influence of breast-feeding on the visual development of preterms as well as full-terms. The visual development was positively related to the level of long-chain polyunsaturated fatty acids (LCPUFAs) in red blood cells of the infants. These LCPUFAs, formed from essential fatty acids in breast-feeding, are crucial for retinal and other neural tissue growth and development. The formula-fed infants in our cohort received formula without these essential fatty acids. It is possible that LCPUFAs or other essential fatty acids, trophic factor or hormones which are present in breast milk and absent in formula, are responsible for the positive effects we measured on the development.

We described an influence of perinatal PCB and dioxin exposure on the infants' thyroid hormone levels[30], which are essential for normal brain development.[31] However, in this study there was no relationship between thyroid hormone levels and development.

The study group is not an at random chosen group, which might be a source of selection bias. It was ethically not possible to randomize the mothers for giving their infants breast-feeding or formula-feeding. Besides, the women were asked to give blood samples of themselves and of their infants, to collect human milk samples during 24 hours and to have examined their infants at different time points at their homes. It would not have been possible to collect all these data completely than in a group who volunteered for this study protocol. Education and profession of the parents in the breast-fed group was higher compared to the parents in the formula-fed group. Overall, the characteristics of the mother-infant pairs in this study, measured with several possible confounding variables, conforms well to data of the general Dutch population.

We expressed the postnatal dioxin and dioxin-like PCB exposure by breast-feeding as the total PCB-dioxin TEQ, which is a summation of individual congener levels multiplied by their toxic equivalent factor (TEF)[4], corresponding to the toxic equivalent (TEQ) concept[28]. This method is based on animal experiments in which effects mediated by the aryl-hydrocarbon (AH) receptor were the studied end points e.g. AHH/EROD enzyme induction, LD50, hepatotoxic effects, body weight loss and thymic atrophy, and not the neurologic or developmental outcome. Individual PCB and dioxin congeners may have a different effect on these outcome parameters and might receive other TEF values for neurotoxicity. There are studies indicating other routes for neurotoxicity by PCBs and dioxins like altering hormone and neurotransmitter levels.[32,33] The TEQ concept neither accounts for additive nor inhibiting effects, although it is known that these effects exist after exposure to a mixture of congeners. When considering the individual congener levels in human milk we did not find a clear relationship between certain congeners and developmental outcome. At this moment, the TEQ concept is the best available method for studying effects of a mixture of many different congeners such as human milk.

In conclusion: prenatal as well as postnatal exposure to Dutch levels of PCBs and dioxins has a small adverse effect on the early psychomotor development. Breast-feeding *per se* has an important positive influence on the mental and psychomotor development at 7 months of age. Although the postnatal dioxin and dioxin-like PCB exposure through breast-feeding had a

negative effect on the PDI outcome at 7 months of age, breast-fed infants never scored significantly lower compared to formula-fed infants. Therefore, mothers can be supported, also in the western industrialized part of the world, to breast-feed their infants. However, it is not clear if the small adverse effects we found on early development, and which are caused in a critical period of organ growth and differentation, might represent differences in neurobehaviour that become apparent in later life. Therefore, it remains necessary for governments all over the world to reduce the expulsion and dumping of these toxins as much as possible. Follow-up studies are performed in school-age children at the moment.

8.6 References

1. Ahlborg UG, Brouwer A, Fingerhut MA et al. Impact of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls on human and environmental health, with special emphasis on application of the toxic equivalency factor concept. Eur J of Pharmacol - Envir Toxicol and Pharmacol Section. 1992;228:179-199.
2. Skene SA, Dewhurst IC, Greenberg M. Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans: the risks to human health. A review. Human Toxicol. 1989;8:173-203.
3. Theelen RMC. Modeling of human exposure to TCDD and I-TEQ in the Netherlands: Background and occupational. In: Gallo MA, Scheuplein RJ, Van der Heijden KA, ed. Biological basis for risk assessment of dioxins and related compounds. Cold Spring Harbor Laboratory Press; Banbury report 1991;35:277-290.
4. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N et al. PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 1994;28:1721-1732.
5. Kuratsune M, Yoshimura T, Matsuzaka J, Yamaguchi A. Epidemiological study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. Environ Health Perspect. 1972;1:119-128.
6. Rogan WJ, Gladen BC, Hung K-L, Koong S-L, Shih L-Y, Taylor JS, Wu Y-C, Yang D, Ragan NB, Hsu CC. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 1988;241:334-336.
7. Harada M. Intrauterine poisoning: clinical and epidemiological studies and significance of the problem. Bull Inst Constit Med Kumamoto Univ. 1976;25(Suppl):1-60.
8. Chen YCJ, Hsu CC. Effects of prenatal exposure to PCBs on the neurological function of children: a neuropsychological and neurophysiological study. Dev Med Child Neurol. 1994;36:312-320.
9. Chen YCJ, Guo YL, Hsu CC, Rogan WJ. Cognitive development of Yu-Cheng ('Oil Disease') children prenatally exposed to heat-degraded PCBs. JAMA. 1992;268:3213-3218.
10. Lai TJ, Guo YL, Chen SJ, Yu ML, Hsu CC. Cognitive development in

Yucheng children. In: Fiedler H, Hutzinger O, Birnbaum L, Lambert G, Needham L, Safe S, eds. Organohalogen Compounds. Kyoto, Japan: Dept of Environmental and Sanitary Engineering; 1994;21:513-516.
11. Jacobson JL, Jacobson SW, Humphrey HEB. Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J Pediatr. 1990;116:38-45.
12. Gladen BC, Rogan WJ, Hardy P, Thullen J, Tingelstad, Tully M. Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. J Pediatr. 1988;113:991-995.
13. Rogan WJ, Gladen BC. PCBs, DDE, and child development at 18 and 24 months. Ann Epidemiol. 1991;1:407-413.
14. Gladen BC, Rogan WJ. Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. J Pediatr. 1991;119:58-63.
15. Jacobson JL, Jacobson SW. Effects of exposure to PCBs and related compounds on growth and activity in children. Neurotoxicol and Teratol. 1990;12:319-326.
16. Lucas A, Morley R, Cole TJ, Lister G, Leeson-Payne C. Breast milk and subsequent intelligence quotient in children born preterm. Lancet 1992;339:261-264.
17. Birch E, Birch D, Hoffman D, Hale L, Everett M, Uauy R. Breast-feeding and optimal visual development. J Pediatr Ophthal Strabismus 1993;30:33-38.
18. Rogan WJ, Gladen BC. Breast-feeding and cognitive development. Early Hum Dev. 1993;31:181-193.
19. Burse VW, Korver MP, Needham LL et al. Gas chromatographic determination of polychlorinated biphenyls (as Aroclor 1254) in serum: Collaborative study. J Assoc Off Anal Chem. 1989;72:649-659.
20. Van Rhijn JA, Traag WA, Van de Spreng PG, Tuinstra LGMTh. Simultaneous determination of planar chlorobiphenyls and polychlorinated dibenzo-p-dioxins and -furans in Dutch milk using isotope dilution and gas chromatography-high-resolution mass spectrometry. J Chromatogr. 1993;630:297-306.
21. Tuinstra LGMTh, Traag WA, Van Rhijn JA, Van de Spreng PF. The Dutch PCB/dioxin study. Development of a method for the determination of dioxins, planar and other PCBs in human milk. Chemosphere;29:1859-1875.
22. Bayley N. Manual for the Bayley scales of infant development, New York: the psychological corporation, 1969.
23. Van der Meulen BF, Smrkovsky M. Bayley ontwikkelingsschalen, handleiding Swets & Zeitlinger BV, Lisse, 1993.
24. Van den Berg M, De Jongh J, Poiger H, Olson JR. The toxicokinetics and metabolism of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), and their relevance for toxicity. CRC Critical Reviews in Toxicol. (in press).
25. Touwen BCL, Huisjes HJ, Jurgens-v.d. Zee AD, Bierman-van Eendenburg MEC, Smrkovsky M, Olinga AA. Obstetrical condition and neonatal neurological morbidity. An analysis with the help of the optimality concept. Early Hum Dev. 1980;4:207-228.

26. Caldwell BM, Bradley RH. Home observation for measurement of the environment. Administration manual. Little Rock, Arkansas, 1984.
27. World Health Organisation. Levels of PCBs, PCDDs, and PCDFs in breast milk: results of WHO-coordinated interlaboratory quality control studies and analytical field studies. In: Yrjänheikki EJ, eds. Environmental Health series. Copenhagen: FADL, 1989 vol 34.
28. NATO/CCMS. Pilot study on International Information Exchange on Dioxins and Related Compounds. International Toxicity Equivalency Factor (I-TEF). Method of Risk Assessment for Complex Mixtures of Dioxins and Related Compounds. Report number 176, 1988.
29. Morrow-Tlucak M, Haude RH, Ernhart CB. Breast-feeding and cognitive development in the first 2 years of life. Soc Sci Med. 1988;26:635-639.
30. Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N et al. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatr Res. 1994;36:468-473.
31. Porterfield SP, Hendrich CE. The role of thyroid hormones in prenatal and neonatal neurological development: current perspectives. Endocr Rev. 1993;14:94-106.
32. Brouwer A, Van den Berg KJ. Binding of a metabolite of 3,4,3′,4′-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxin. Toxicol Appl Pharmacol. 1986;85:301-312.
33. Seegal RF, Shain W. Neurotoxicity of polychlorinated biphenyls: The role of ortho-substituted congeners in altering neurochemical function. In: Isaacson RL, Jensen KF eds. The Vulnerable Brain and Environmental Risks. New York: Plenum Press; 1992;2:169-195.

Table 8.1 Categories of duration of breast-feeding.

	3 Months		7 and 18 Months	
	N	weeks of breast-feeding	N	weeks of breast-feeding
Category low	102	0	102	0
Category medium	26	6 - 10	54	6 - 16
Category high	79	11 - 12	51	>16

Table 8.2 Categories of total PCB-dioxin TEQ exposure via breast-feeding.

	Exposure by breast-feeding	N	3 Months pg PCB-dioxin TEQ/g fat times weeks	7 and 18 Months pg PCB-dioxin TEQ/g fat times weeks
Category I	low	27	168 - 617	168 - 769
Category II	medium	26	618 - 810	770 - 1289
Category III	high	27	811 - 1860	1290 - 4340

Table 8.3 Mental (MDI) and Psychomotor (PDI) scores on the Bayley Scales of Infant Development at 3, 7 and 18 months of age.

	All	Breast-fed	Formula-fed	P*
MDI-3	N = 201	N = 101	N = 100	
mean (SD)	127 (13)	128 (13)	126 (13)	
range	84 - 149	84 - 149	84 - 149	0.21
MDI-7	N = 207	N = 105	N = 102	
mean (SD)	113 (10)	115 (11)	112 (9)	
range	87 - 139	87 - 133	90 - 139	0.03
MDI-18	N = 207	N = 105	N = 102	
mean (SD)	110 (18)	113 (18)	107 (17)	
range	68 - 150	74 - 149	68 - 150	0.01
PDI-3	N = 199	N = 99	N = 100	
mean (SD)	117 (12)	118 (12)	117 (12)	
range	89 - 155	89 - 155	89 - 137	0.92
PDI-7	N = 207	N = 105	N = 102	
mean (SD)	113 (14)	115 (15)	111 (13)	
range	86 - 149	91 - 149	86 - 149	0.05
PDI-18	N = 206	N = 105	N = 101	
mean (SD)	109 (15)	110 (17)	108 (14)	
range	51 - 149	51 - 149	58 - 141	0.17

* Mann-Whitney Test

Table 8.4 Demographic and toxicologic covariables used in the multiple regression analysis.

Covariables		All N = 207	Breast-fed N = 105	Formula-fed N = 102
Gestational age in weeks	Mean (SD)	40 (1)	40 (1)	40 (1)
HOME-score	Mean (SD)	40 (3)	41 (3)	40 (3)
Older sibs	Yes (%)	105 (51)	52 (50)	53 (52)
Education of mother	High (%)	85 (41)	56 (53)	29 (28)
PCB-plasma-sum in ng/g plasma	Mean (SD)	2.2 (1.0)	2.3 (1.0)	2.2 (1.0)
PCB-milk-sum in ng/g milk fat N = 100	Mean (SD)	-	419 (173)	-
Total PCB-dioxin TEQ in pg TEQ/g milk fat. N = 80	Mean (SD)	-	66.6 (24.2)	-

SD = Standard Deviation

Table 8.5 Results of the multiple regression analysis of the PDI at 3 months of age.

	Coefficient	Standard Error	P
Constant	34.1	32.0	.29
Gestational Age (weeks)	2.6	.7	.0002
Parity (no sibs, one or more sibs)	-1.6	1.6	.33
HOME-score	-.43	.33	.19
Education of mother (low, high)	1.1	1.8	.53
Ln PCB-plasma-sum (ng/g)	-4.8	2.0	.02
Duration of breast-feeding (zero, 6-10 weeks or 11-12 weeks)	.91	.91	.32

N = 198

Table 8.6 Results of the multiple regression analysis of the PDI at 7 months of age.

	Coefficient	Standard Error	P
Constant	110.8	36.8	.003
Gestational Age (weeks)	0.12	.80	.88
Parity (no sibs, one or more sibs)	-9.0	1.9	.0001
HOME-score	-.01	.41	.98
Education of mother (low, high)	2.2	2.1	.31
Duration of breast-feeding (zero, 6-16 or 17-30 weeks)	6.9	2.3	.004
PCB-dioxin TEQ exposure medium	-9.5	3.9	.01
PCB-dioxin TEQ exposure high	-7.7	4.9	.12
PCB-dioxin TEQ exposure overall-P			.05

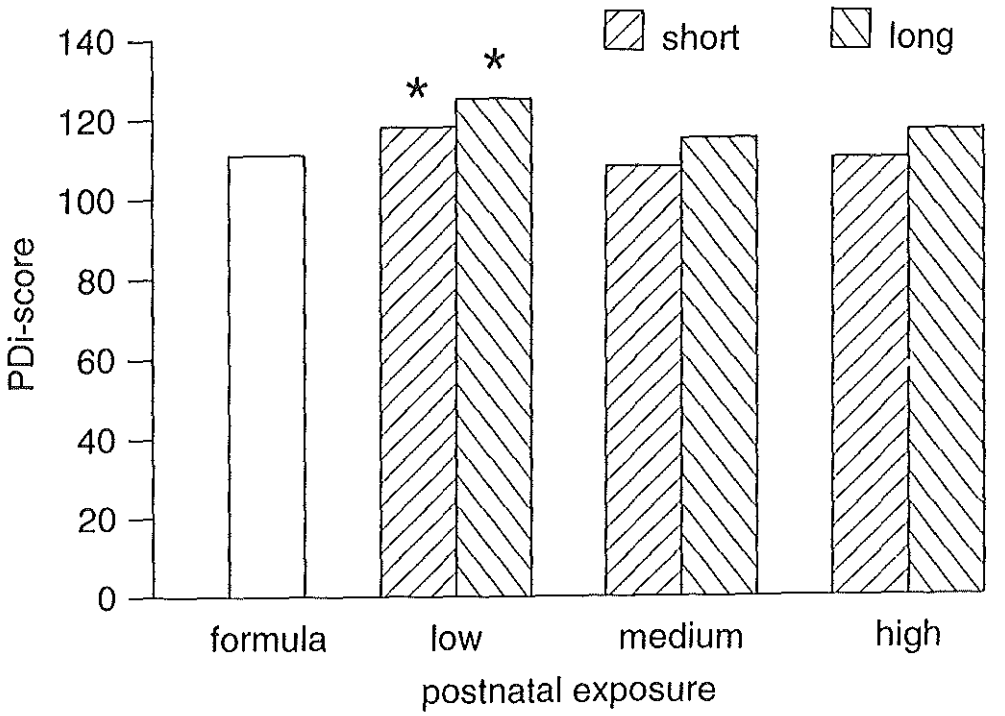
N = 182

Table 8.7 Results of the multiple regression analysis of the MDI at 7 months of age.

	Coefficient	Standard Error	P
Constant	88.1	27.1	.001
Gestational Age (weeks)	.12	.59	.84
Parity (no sibs, one or more sibs)	-1.4	1.4	.31
HOME-score	.46	.28	.10
Education of mother (low, high)	-1.8	1.5	.24
Ln PCB-plasma-sum (ng/g)	2.3	1.7	.18
Duration of breast-feeding (zero, 6-16 or 17-30 weeks)	2.0	0.9	.02

N = 206

Figure 8.1. Psychomotor development at 7 months of age related to breast-feeding.



CHAPTER 9

NEUROLOGICAL CONDITION IN 18-MONTH-OLD CHILDREN PERINATALLY EXPOSED TO POLYCHLORINATED BIPHENYLS AND DIOXINS.

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NEUROLOGICAL CONDITION IN 18-MONTH-OLD CHILDREN PERINATALLY EXPOSED TO POLYCHLORINATED BIPHENYLS AND DIOXINS.

Marcel Huisman[1], Corine Koopman-Esseboom[2], Caren I. Lanting[1], Cornelis G. Van der Paauw[3], Louis G.M.Th. Tuinstra[4], Vaclav Fidler[5], Nynke Weisglas-Kuperus[2], Pieter J.J. Sauer[2], E. Rudy Boersma[1], Bert C.L. Touwen[6].

[1]Department of Obstetrics and Gynaecology, Nutrition and Development Unit, University of Groningen.
[2]Department of Paediatrics, Division of Neonatology, Erasmus University and University Hospital/Sophia Children's Hospital, Rotterdam.
[3]TNO Nutrition and Food Research Institute, Zeist.
[4]DLO State Institute for Quality Control of Agricultural Products, RIKILT-DLO, Wageningen.
[5]Department of Health Sciences, Epidemiology and Statistics Unit, University of Groningen.
[6]Department of Medical Physiology, Developmental Neurology, University Hospital Groningen, The Netherlands.

9.1 Abstract

The neurological optimality of 418 Dutch children was evaluated at the age of 18 months, in order to determine whether prenatal and breast milk mediated exposure to polychlorinated biphenyls (PCBs) and dioxins affected neurological development. Half of the infants were breast-fed, the other half were formula-fed. PCB concentrations in cord and maternal plasma were used as a measure of prenatal exposure to PCBs. To measure postnatal exposure, PCB and dioxin congeners were determined in human milk and in formula milk.

After adjusting for covariates, transplacental PCB exposure was negatively related to the neurological condition at 18 months. Although greater amounts of PCBs and dioxins are transferred via nursing than via placental passage, an effect of lactational exposure to PCBs and dioxins could not be detected. We even found a beneficial effect of breast-feeding on the fluency of movements.

We conclude that transplacental PCB passage has a small negative effect on the neurological condition in 18-month-old toddlers.

9.2 Introduction

Polychlorinated biphenyls (PCBs) and dioxins are widespread toxic environmental pollutants. Until the late 1970s, PCBs were produced for use as fire retardants, plasticizers, dielectric fluids in capacitors and transformers, and hydraulic fluids. There is a total number of 209 possible PCB congeners, which all differ in their degree of chlorination and the position of the chlorine atom. PCBs can be divided into planar and non-planar PCBs. The planar PCBs resemble

the dioxins to the largest extent. Dioxins are unwanted by-products of thermal and industrial processes, consisting of 75 possible polychlorinated dibenzo-*p*-dioxin congeners and 135 possible polychlorinated dibenzofuran congeners. Due to a high persistency, they can be detected in food products of animal origin, human adipose tissue and blood[1]. Once entered into the food-chain, these lipophilic compounds are bioconcentrated exposing human beings who continuously absorb very small doses. Substantially greater amounts of PCBs are transferred via nursing than as a result of placental passage in both animals[2,3] and humans[4,5]. Infant formulae contain only lipids of a vegetable origin with a negligible content of PCBs and dioxins. Since breast-fed children receive considerably more of these compounds compared to those formula-fed, controversy exists over whether breast-feeding should be encouraged.

Rogan *et al.* showed that higher levels of transplacental exposure to PCBs were associated with hypotonicity and hyporeflexia in neonates[6]. We partly confirmed these results in our study concerning neonates. The combination of a high prenatal and a high lactational exposure during the first 2 weeks after birth was associated with an increase in the prevalence of neonatal neurological non-optimality and a higher incidence of hypotonia[5].

So far, the effects of PCB exposure have been evaluated as regards mental and psychomotor development[7]. We now report on the relationship between prenatal exposure to PCBs and lactational exposure to PCBs and dioxins and the neurological condition at 18 months.

9.3 Subjects and Methods

From June 1990 until June 1992 pregnant women were recruited for the study in Groningen and Rotterdam. The planned sample size was 100 breast-feeding and 100 formula-feeding mothers in each centre. The women were approached between the 32nd and 34th week of pregnancy and provisionally assigned, on the basis of their intention, to the formula-feeding or the breast-feeding group. Women suffering from serious illnesses or complications during pregnancy and delivery were excluded, as were mothers having an instrumental delivery. From the provisional breast-feeding group, only the mothers were included in the study who breast-fed their infants for at least six weeks. Formula milk from a single batch (Almiron M2; Nutricia N.V., The Netherlands) was used in the formula-feeding group. Approval was obtained from the ethics committees of the University Hospitals in both centres.

Social, obstetrical, and perinatal circumstances were recorded by means of a questionnaire with 72 representative items. The number of items that fulfilled predefined optimality criteria[8] was used as an obstetrical optimality score[9]. All newborns underwent a neurological examination according to Prechtl[10].

A maternal blood sample was taken in the last month of pregnancy and cord blood was collected immediately after birth. Plasma samples were analyzed for four non-planar PCB congeners, such as were analyzed in human milk. The sum of the concentrations of the four PCB congeners 118, 138, 153, and 180 in plasma (i.e. $\Sigma PCB_{maternal}$ and ΣPCB_{cord}, respectively) was used as a measure of prenatal exposure to PCBs. Postnatal exposure to PCBs and dioxins via breast

milk was reflected by the levels of these compounds in a 24-hour sample taken in the second week after delivery. Contents of seventeen 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins and dibenzofurans, three planar PCBs, and 23 non-planar PCB congeners were determined in breast milk fat as well as in the formula milk fat. In order to express the toxicity of the mixture of compounds in breast milk and in artificial feeding, the toxic equivalency approach was used[11]. With this approach, the relative toxicity (TEQ) of each congener towards the most toxic dioxin congener was calculated. By adding up the TEQs of all congeners, the total TEQ value was obtained (in ng TEQ/kg milk fat). The sampling, analytical, and data processing procedures have previously been described[5].

At 18 months of age, the neurological condition was assessed using an age-specific neurological examination[12]. This technique focuses on the observation of motor functions (grasping, sitting, crawling, standing, and walking) in a standardized free field situation[13,14]. On the basis of this examination each toddler was classified as normal, mildly abnormal, or abnormal. The classification 'abnormal' implies the presence of an overt circumscript neurological syndrome, which usually leads to a handicap in daily life, such as cerebral palsy. 'Mildly abnormal' signifies the presence of mild signs which do not necessarily lead to a handicapping condition, e.g. slight asymmetries, or mild hypo-, and hypertonia. The neurological findings were also evaluated in terms of optimality[8]. A list of 57 neurological items was composed, for each of which an optimal range was defined (Appendix). By giving a point for each item meeting the criteria for optimality, the neurological optimality score was calculated by counting the number of optimal items. It must be emphasized that optimality is not identical with normality, and that a reduced optimality not always mean abnormal[8]. Special attention was given to the quality of movements in terms of fluency. Fluency of motility has been shown to be an indicator for the integrity of brain function in fetuses and prematures[15,16]. The quality of movements during prehension, sitting, crawling, standing, and walking was scored separately as a fluency cluster (Appendix). The neurological examinations were carried out in Groningen by M.H. and in Rotterdam by C.K.E. after extensive training. The examiners were aware of the feeding status but not of the results of the chemical analyses of the plasma and milk samples.

Chi-square, Student's *t*, and the Mann-Whitney *U* tests were used to compare groups. The effect of PCB and dioxin exposure on the neurological condition was investigated by a multiple linear regression analysis. The dependent variables were the neurological optimality score and the fluency cluster score at 18 months. The distribution of the neurological optimality score was skew to the left. The highest possible score is 57. In order to achieve normality, the neurological optimality score was transformed into: $-log(57.5 - \textit{the neurological optimality score})$. The independent variables in the regression analysis were the logarithmically transformed PCB and dioxin levels, social, perinatal and obstetrical variables from the obstetric optimality list, and the study centre. A p-value of 0.05 or less was considered significant.

9.4 Results

The study group consisted of 418 mother-infant pairs: 209 in the breast-feeding group and 209 in the formula-feeding group. On the basis of the neurological examination, 408 toddlers were classified as neurologically 'normal'. Nine children were categorized as 'mildly abnormal': mild hpertonia was found in six toddlers, one child showed non-fluent movements in several positions, one toddler had a poor variability of the motor functional repertoire, and in one case instability for the behavioural states was found during two independent sessions. One toddler had a hypertonic syndrome which was diagnosed as 'abnormal'. In the normal group, the median neurological optimality score was 48 (range: 34-55), whereas in the group classified as mildly abnormal or abnormal the median was found to be 42 (range: 38-45). Characteristics of the study group are presented in Table 9.1. Three maternal blood samples were missing. No cord blood samples could be obtained from 36 mother-infant pairs. For the analysis of PCB 118 in cord plasma, nine samples were missing. In human milk, representative dioxin, planar and non-planar PCB congeners were available in 176, 194 and 195 milk samples, respectively.

Table 9.2 presents the results of the regression analysis for the neurological optimality score. Neither PCB nor dioxin exposure via breast milk were associated with the neurological optimality score. The final model included education of the father, parity, study centre, smoking of the father during pegnancy, and ΣPCB_{cord}. The first three variables had about the same effect (regression coefficients $\beta \approx 0.15$). The children of less educated fathers scored lower than the children of well educated fathers; the first born children had a higher score than the second or third born children. The model also included a significant ($p = 0.011$) interaction between ΣPCB_{cord} and smoking of the father. To facilitate the interpretation of regression coefficients, we worked with $\log(\Sigma PCB_{cord})$ minus its minimal value log(0.08). With this definition the regression coefficient of smoking of the father ($\beta = -0.402$, $p = 0.002$) estimates the effect of smoking at the lowest ΣPCB_{cord} exposure. Similarly, the coefficient for ΣPCB_{cord} estimates the effect of ΣPCB_{cord} in the non-smoking-father group. This effect was negative ($\beta = -0.149$, $p = 0.003$), in contrast to hardly any effect in the smoking-father group ($\beta = -0.051$, $p = 0.404$). The children of non-smoking fathers had the highest adjusted neurological optimality score in the presence of a low ΣPCB_{cord} value. In case of a high ΣPCB_{cord} value, the optimality score was similar to that of children of smoking fathers. Nearly the same results are obtained if ΣPCB_{cord} is replaced by $\Sigma PCB_{maternal}$.

The size of the estimated prenatal PCB effect on the neurological optimality score is elucidated in the following example. A first born toddler living in Groningen with a highly educated non-smoking father has an estimated neurological optimality score of 49.9 in case of a ΣPCB_{cord} value at the 5th percentile (i.e. 0.18) and a score of 47.9 in case of a ΣPCB_{cord} value at the 95th percentile (i.e. 0.86). Thus, the difference is only 2 points.

The fluency cluster score was neither related to the ΣPCB_{cord} and $\Sigma PCB_{maternal}$ concentrations nor to the PCB and dioxin levels in breast milk. Breast-fed children had a higher fluency cluster score compared to formula-fed children ($p = 0.01$; Table 9.3).

9.5 Discussion

Prenatal PCB exposure had a small negative effect on the neurological condition of 18 month-old toddlers whose fathers did not smoke. Such an effect seemed to be blurred in children of fathers who smoked. No effect of lactational exposure to PCBs and dioxins through breast milk on the neurological condition could be detected. In contrast, breast-fed children had a higher fluency cluster score compared to formula-fed children.

A negative effect of prenatal PCB exposure on psychomotor development was found by Rogan and Gladen[7,17] in North Carolina. They followed 802 children from birth through to five years of age by means of the Bayley - and McCarthy Scales. At 6, 12, 18, and 24 months of age a significant relation between prenatal PCB exposure and a lower psychomotor performance was found, but at 3, 4, and 5 years of age no effect of prenatal PCB exposure could be detected. PCB exposure via breast milk had no influence. Levels of PCB exposure in the USA are comparable to those in the Netherlands. It is important to realize that developmental tests and the developmental neurological examination measure different aspects of brain function. The Bayley - and the McCarthy Scales measure developmental levels at different ages quantitatively. The developmental neurological examination gives a qualitative appraisal of brain integrity, in which developmental age levels are unimportant.

In our study of newborns, we found that the combination of a high prenatal and high lactational exposure to PCBs and dioxins during the first 2 weeks after birth was associated with an increase in the prevalence of neurological non-optimality[5]. At 18 months, neurological differences could only be attributed to prenatal PCB exposure. It is difficult to relate the functions of the central nervous system in the neonatal period directly with those at 18 months, as they are generated by quite different brains. From the early stages of pregnancy until many years after birth, large morphological changes take place in the central nervous system, such as outgrowth and retraction of dendrites and axones, myelination, and synapse reorganisation. The effects of these maturational changes are reflected in the functional development of the child.

A possible explanation for the role of smoking of the father on the neurological condition at 18 months is that it is a passive smoking effect. We prefer, however, to view this variable as a proxy variable of characteristics of the relevant environment. Smoking behaviour of the mother was not significantly related to the neurological optimality score, but we recorded the smoking behaviour of the mother only during pregnancy. It is possible that mothers temporarily stop smoking because of their pregnancy, whereas it is less likely that fathers do.

The difference between the two study centres is probably due to the difference between the two observers. We also found differences between the two centres/observers in our study of neonates[5]. The regression analysis adjusts for these effects. Nevertheless, the issue of the reliability of methods of assessment of neurological condition in healthy young children needs further attention.

The neurochemical mechanisms responsible for the neurotoxic effects of PCBs are not well understood. In animals, PCBs and dioxins have been found to

affect dopamine[18-20] and thyroid hormonal metabolism[21,22]. In a subgroup of this study[23] and in another study concerning neonates[24], thyroid hormone status was not related to the neonatal neurological status[25,26].

An effect of lactational exposure to PCBs and dioxins could not be detected at 18 months. Despite the fact that greater amounts of PCBs and dioxins are transferred via nursing than via placental passage, we found indications of a beneficial effect of breast-feeding. These results support the findings of Rogan and Gladen[27], who reported that cognitive development in breast-fed children from two through to five years of age was slightly better than in formula-fed children. In the present study, a beneficial effect was found on the fluency of complex movement patterns. The fluency of movements is an indication of the quality of brain function rather than of the level of devlopment. This aspect of the quality of movements can be regarded as a reflection of the differentiation of cortex and basal ganglia function; cortical networks develop largely after birth, and also during the period of lactation.

Advantageous effects of breast-feeding on brain development are well documented[28-30], but the mechanism behind such beneficial effects remains unclear. Besides, the socio-behavioural aspect of breast-feeding, the composition of human milk might be responsible. Maternal hormones like the thyroid stimulating hormone and other biological active peptides reach the infant via breast milk. Long-chain polyunsaturated fatty acids seem to be essential for development of the brain[31-33]. They are present in breast milk, but are not in general added to term-infant formula milks.

In conclusion, transplacental PCB exposure is negatively related to the neurological condition in children at 18 months. No effect of postnatal exposure to PCBs and dioxins via breast milk could be detected. Despite the contamination of human milk with PCBs and dioxins, a beneficial effect of breast-feeding on the fluency of movements was found. This effect on the quality of brain function during development has not been reported before.

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9.6 References

1. Jensen AA. Polychlorobiphenyls (PCBs), polychlorodibenzo-p-dioxins (PCCDs) and polychlorodibenzofurans (PCDFs) in human milk, blood and adipose tissue. Sci Total Environ 1987;64:259-93.
2. Masuda Y, Kagawa R, Tokudome S, Kuratsune M. Transfer of polychlorinated biphenyls to the foetuses and offspring of mice. Food Cosmet Toxicol 1978;16:33-37.
3. Allen JR, Barsotti DA. The effects of transplacental and mammary movement of PCBs on infant rhesus monkeys. Toxicology 1976;6:331-40.

4. Jacobson JL, Fein GG, Jacobson SW, Schwartz PM, Dowler JK. The transfer of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) across the human placenta and into maternal milk. Am J Public Health 1984;74:378-9.
5. Huisman M, Koopman-Esseboom C, Fidler V, Hadders-Algra M, Paauw CG van der, Tuinstra LGMTh, *et al.* Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. Early Hum Dev 1995;41:111-127.
6. Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, *et al.* Neonatal effects of transplacental exposure to PCBs and DDE. J Pediatr 1986;109:335-41.
7. Rogan WJ, Gladen BC. PCBs, DDE, and child development at 18 and 24 months. Ann Epidemiol 1991;1:407-13.
8. Prechtl HFR. The optimality concept. Early Hum Dev 1980;4:201-205.
9. Touwen BCL, Huisjes HJ, Jurgens-van der Zee AD, Bierman-van Eendenburg, Smrkovsky M, Olinga AA. Obstetrical condition and neonatal neurological morbidity. An analysis with help of the optimality concept. Early Hum Dev 1980;4:207-28.
10. Prechtl HFR. The neurological examination of the full-term newborn infant, 2nd ed. Clinics in Developmental Medicine, No. 63. SIMP. London: Heinemann Medical Books, 1977.
11. Zorge JA van, Wijnen JH van, Theelen RMC, Olie K, Berg M van den. Assessment of the toxicity of mixtures of halogenated dibenzodioxins and dibenzofurans by use of TEF. Chemosphere 1989;19:1881-95.
12. Hempel MS. The neurological examination for toddler-age [dissertation]. Groningen (NL): Univ of Groningen, 1993.
13. Touwen BCL, Hempel MS, Westra LC. The development of crawling between 18 months and four years. Develop Med Child Neurol 1992;34:410-16.
14. Hempel MS. Neurological development during toddling age in normal children and children at risk of developmental disorders. Early Hum Dev 1993;34:47-57.
15. Prechtl HFR. Qualitative changes of spontaneous movements in fetus and preterm infant are a marker of neurological dysfunction. Early Hum Dev 1990;23:151-158.
16. Ferrari F, Cioni G, Prechtl HFR. Qualitative changes of general movements in preterm infants with brain lesions. Early Hum Dev 1990;23:193-231.
17. Gladen BC, Rogan WJ. Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. J Pediatr 1991;119:58-63.
18. Seegal RF, Bush B, Brosch KO. Comparison of effects of Aroclors 1016 and 1260 on non-human primate catecholamine function. Toxicology 1991;66:145-163.
19. Seegal RF, Shain W. Neurotoxicity of polychlorinated biphenyls: The role of ortho-substituted congeners in altering neurochemical function. In: Isaacson RL and Jensen KF, eds. The Vulnerable Brain and Environmental Risks, Plenum Press, New York 1992;2:169-195.

20. Shain W, Bush B, Seegal RF. Neurotoxicity of polychlorinated biphenyls: Structure-activity relationship of individual congeners. Toxicol Appl Pharmacol 1991;111:33-42.
21. Brouwer A, Berg KJ van den. Binding of a metabolite of 3,4,3',4'-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxin. Toxicol Appl Pharmacol 1986;85:301-312.
22. Berg KJ van den, Zurcher C, Brouwer A. Effects of 3,4,3',4'-tetrachlorobiphenyl on thyroid function and histology in marmoset monkeys. Toxicol Letters 1988;41:77-86.
23. Pluim HJ, Koppe JG, Olie K, Slikke JW van de, Kok JH, Vulsma T, *et al.* Effects of dioxins on thyroid function in newborn babies. Lancet 1992;339:1303.
24. Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, LutkeSchipholt IJ, Paauw CG van der, Tuinstra LGMTh, *et al.* Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatr Res 1994;36(4):468-73.
25. Koopman-Esseboom C, Huisman M, Morse DC, Touwen BCL, Paauw CG van der, Tuinstra LGMTh, *et al.* Newborns diagnosed as neurologically abnormal with relation to PCB/dioxin exposure and their thyroid hormone status. Submitted.
26. Pluim HJ. Dioxins - pre- and postnatal exposure in the human newborn [dissertation]. Amsterdam (NL): Univ of Amsterdam, 1993.
27. Rogan WJ, Gladen GC. Breast-feeding and cognitive development. Early Hum Dev 1993;31:181-93.
28. Fergusson DM, Beautrais AL, Silva PA. Breast-feeding and cognitive development in the first seven years of life. Soc Sci Med 1982;16:1705-08.
29. Lucas A, Morley R, Cole TJ, Lister G, Leeson-Payne C. Breast milk and subsequent intelligence quotient in children born preterm. Lancet 1992;339:261-64.
30. Lanting CI, Fidler V, Huisman M, Touwen BCL, Boersma ER. Neurological differences between 9-year-old children fed breast-milk or formula-milk as babies. Lancet 1994;344:1319-22.
31. Martinez M, Conde C, Ballabriga A. Some chemical aspects of human brain development: II phosphoglyceride fatty acids. Pediatr Res 1974;8:93-102.
32. Clandinin MT, Chappel JE, Leong S, Heim T, Swyer PR, Change GW. Extrauterine fatty acid accretion in infant brain: implications for fatty acid requirements. Early Hum Dev 1980;4:131-38.
33. Uauy RD, Birch DG, Birch EE, Tyson JT, Hoffman DR. Effect of omega-3 fatty acids on retinal function of very-low-birth-weight neonates. Pediatr Res 1990;28:485-92.

Table 9.1 Characteristics of the study group.

Variable	Breast-fed	Formula-fed
Number of children	209	209
Neonatal neurological diagnosis		
normal	197 (94%)	197 (94%)
mildly abnormal	10 (5%)	10 (5%)
abnormal	2 (1%)	2 (1%)
Neurological diagnosis at 18 months		
normal	205 (98%)	203 (97%)
mildly abnormal	3 (1%)	6 (3%)
abnormal	1 (1%)	0 (0%)
Neurological optimality score at 18 months		
P_5, P_{50}, P_{95}	41, 48, 53*	40, 47, 52
Fluency cluster score		
mean ± SD	10.2 ± 1.6*	9.5 ± 1.7
PCB/dioxin exposure†		
P_5, P_{50}, P_{95}		
ΣPCB_{cord} in μg/L	0.20, 0.43, 0.99*	0.16, 0.34, 0.80
$\Sigma PCB_{maternal}$ in μg/L	1.1, 2.2, 4.0*	0.95, 1.9, 3.6
TEQ_{PCB} in ng TEQ/kg milk fat	17, 33, 61*	below detection limit
TEQ_{dioxin} in ng TEQ/kg milk fat	15, 29, 52*	below detection limit
Education		
mother, higher education‡	132 (63%)*	50 (24%)
father, higher education	130 (62%)*	64 (31%)
Duration of exclusive breast-feeding (weeks)		
P_5, P_{50}, P_{95}	6, 13, 31	0
Parity		
first born	107 (51%)	94 (45%)
Gender		
male	118 (57%)	107 (51%)
Obstetrical optimality score		
P_5, P_{50}, P_{95}	59, 65, 69*	57, 64, 68
Birth weight (kg)		
mean ± SD	3.54 ± 0.46	3.49 ± 0.43
Maternal weight (kg)		
mean ± SD	64 ± 9	66 ± 11
Smoking		
mother, no smoking during pregnancy	175 (84%)*	135 (65%)
father, no smoking	126 (60%)	117 (56%)
Alcohol consumption during pregnancy		
mother, no alcohol consumption	131 (63%)*	171 (82%)

* *Significantly different from the formula-fed group ($p \leq 0.05$).*

† *ΣPCB: sum of the levels of PCB 118, 138, 153, 180; TEQ_{PCB}: toxic equivalent for planar, mono-ortho, and di-ortho PCBs; TEQ_{dioxin}: toxic equivalent for dioxins.*

‡ *Higher level secondary school or professional/university training.*

Table 9.2 Regression analysis: neurological optimality score*.

Variable	Regression coefficient (standard error)	p (two-tailed)
Constant	−2.039 (0.094)	
Education of the father (0 = low, 1 = high)	0.135 (0.046)	0.004
Parity (0 = first, 1 = not first born)	−0.159 (0.038)	0.000
Study centre (0 = Groningen, 1 = Rotterdam)	0.135 (0.038)	0.000
Log(ΣPCB_{cord}/0.08)	−0.149 (0.049)	0.003
Smoking of the father (0 = no, 1 = yes)	−0.402 (0.130)	0.002
Log(ΣPCB_{cord}/0.08) x Smoking of the father	0.200 (0.078)	0.011

* *transformation neurological optimality score into: −log(57.5 − neurological optimality score).*
n = 373, R^2 = 0.14

Table 9.3 Regression analysis: fluency cluster score.

Variable	Regression coefficient (standard error)	p (two-tailed)
Constant	8.539 (0.356)	
Type of feeding (0 = breast-feeding, 1 = formula-feeding)	−0.450 (0.177)	0.012
Log(ΣPCB_{cord})	−0.295 (0.175)	0.093
Parity (0 = first, 1 = not first born)	−0.394 (0.168)	0.020
Education of the father (0 = low, 1 = high)	1.352 (0.293)	0.000
Study centre (0 = Groningen, 1 = Rotterdam)	1.628 (0.336)	0.000
Education of the father x Study centre	−1.072 (0.388)	0.006

n = 373, R^2 = 0.15

APPENDIX

Criteria for the 57 items of the neurological optimality score at 18 months.

Items	Criteria for optimality
Prehension	
1. mode of grasping	pincer grasp present in left and right hand
2. posture arm/shoulder	normal, variable posture
3. quality of arm/shoulder movements*	smooth
4. posture hands/fingers	normal, variable posture
5. adjustment hand-opening	good
6. associated movements (hindering)	absent or if present they do not hinder
7. quality of hand mobility*	smooth
Sitting	
8. sitting (up)	can sit (up) without help
9. posture head/trunk/legs/feet/toes	normal, variable posture (head: centred and well-adapted posture)
10. trunk rotation, spontaneous*	trunk rotation present
11. trunk rotation, elicited*	trunk rotation $>45°$
12. fluency of trunk movements*	smooth
13. acceleration/deceleration*	smooth
Crawling	
14. symmetry of movements*	no asymmetry
15. posture head	centred and well-adapted
16. coordination of arm-leg movements	coordinated arm-leg movements
17. variability in speed	variable speed
18. fluency of trunk movements*	non-fluent/smooth
Standing	
19. standing up/free	can stand up without help with object in both hands / stands free
20. variability in standing up	various ways of standing up
21. posture head/arms/trunk/legs posture feet/toes	normal, variable and well-adapted
22. distance between feet	medium
23. balance without movements	no correction movements visible
24. balance with movements	no or small correction movements in the arms
25. trunk rotation, spontaneous*	trunk rotation present
26. trunk rotation, elicited	trunk rotation present
27. fluency of trunk movements*	non-fluent/smooth
28. reaction to push against shoulders	good balance

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

* *Included in the fluency cluster score.*

For descriptive details of the items see: Hempel MS. The neurological examination for toddler-age [dissertation]. Groningen (NL): Univ of Groningen, 1993.

CHAPTER 10

IMMUNOLOGIC EFFECTS OF BACKGROUND PRENATAL AND POSTNATAL EXPOSURE TO DIOXINS AND POLYCHLORINATED BIPHENYLS IN DUTCH INFANTS.

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IMMUNOLOGIC EFFECTS OF BACKGROUND PRENATAL AND POSTNATAL EXPOSURE TO DIOXINS AND POLYCHLORINATED BIPHENYLS IN DUTCH INFANTS.

Nynke Weisglas-Kuperus[1], Theo C.J. Sas[1], Corine Koopman-Esseboom[1], Cees W. Van der Zwan[2], Maria A.J. de Ridder[3], Auke Beishuizen[4], Herbert Hooijkaas[4], Pieter J.J. Sauer[1].

[1]Department of Paediatrics, Division of Neonatology, Erasmus University and University Hospital/Sophia Children's Hospital, Rotterdam.
[2]Department of Infectious Diseases Epidemiology, National Institute of Public Health and Environmental Protection, Bilthoven.
[3]Institute of Epidemiology and Biostatistics, Erasmus University Rotterdam.
[4]Department of Immunology, Erasmus University and University Hospital, Rotterdam, The Netherlands.

10.1 Abstract

Immunologic effects of pre- and postnatal polychlorinated biphenyl (PCB)/dioxin exposure in Dutch infants from birth to 18 mo of age are explored. The total study group consisted of 207 healthy mother-infant pairs, of which 105 infants were breast-fed and 102 children were bottle-fed.

Prenatal PCB exposure was estimated by the PCB sum (PCB congeners 118, 138, 153, and 180) in maternal blood and the total toxic equivalent (TEQ) level in human milk (17 dioxin and 8 dioxin-like PCB congeners). Postnatal PCB/dioxin exposure was calculated as a product of the total TEQ level in human milk multiplied by the weeks of breast-feeding.

The number of periods with rhinitis, bronchitis, tonsillitis, and otitis during the first 18 mo of life was used as an estimate of the health status of the infants. Humoral immunity was measured at 18 mo of age by detecting antibody levels to mumps, measles, and rubella. White blood cell counts (monocytes, granulocytes, and lymphocytes) and immunologic marker analyses $CD4^+$ T-lymphocytes, $CD8^+$ T-lymphocytes, activated T-lymphocytes ($HLA\text{-}DR^+CD3^+$), as well as T cell receptor (TcR) $\alpha\beta^+$, $TcR\gamma\delta^+$, $CD4^+CD45RA^+$ and $CD4^+CD45RO^+$ T-lymphocytes, B-lymphocytes ($CD19^+$ and/or $CD20^+$) and NK cells ($CD16^+$ and/or $CD56^+/CD3^-$) in cord blood and venous blood at 3 and 18 mo of age were assessed in a subgroup of 55 infants.

There was no relationship between pre- and postnatal PCB/dioxin exposure and upper or lower respiratory tract symptoms or humoral antibody production. A higher prenatal PCB/dioxin exposure was associated with an increase in the number of $TcR\gamma\delta^+$ T-cells at birth and with an increase in the total number of T-cells and the number of $CD8^+$ (cytotoxic), $TcR\alpha\beta^+$, and $TcR\gamma\delta^+$ T-cell at 18 mo of age. A higher prenatal as well as postnatal PCB/dioxin exposure was associated with lower monocyte and granulocyte counts at 3 mo of age.

In conclusion, our study suggests that background levels of PCB/dioxin exposure influences the human fetal and neonatal immune system.

10.2 Introduction

Prenatal and postnatal exposure to PCDDs, PCDFs, and PCBs produce a wide spectrum of toxic effects in animals, including body weight loss, hepatotoxicity, teratogenicity, carcinogenicity, neurotoxicity, reproductive toxicity, alterations in the thyroid hormone status[1], and immunotoxicity. Many animal studies have shown adverse effects of PCDDs, PCDFs (summarized as dioxins), and PCBs on the immune system. The most consistent finding in these studies is thymic atrophy.[2-5] *In utero* and lactational exposure is a more sensitive period for the immunotoxic effects than adult exposure, suggesting that during maturation the immune system is particularly sensitive to these compounds.

Most studies were conducted in mice in which the adult, as well as the fetus, is very sensitive to immunotoxic effects. There is evidence that perinatal TCDD exposure produces an alteration in the normal thymocyte maturation process.[6-10] In addition a direct effect on the fetal liver and neonatal bone marrow has been shown.[2,7,8] TCDD and TCDF may also interfere with B-cell maturation and with humoral antibody production.[11-14] In addition an increased activity of NK cells has been found.[15] A decrease in thymus weight and alterations in T cell differentiation, however, were also found in TCDD exposed rats[4,16] and a reduction in thymocytes in the offspring of PCB exposed pregnant minks.[17] Neubert et al.[18,19] found changes in the number of B cell, NK cell, and T cell subpopulations in venous blood of marmosets treated with low doses of TCDD. In seals fed on PCDD-, PCDF-, and PCB-polluted herrings, lower NK and T cell activity was found. In addition there were higher levels of circulating polymorphonuclear granulocytes, which may suggest an increase in the occurrence of bacterial infections.[20]

Data regarding the potential toxic effects of PCDDs, PCDFs, and PCBs on the immune system in human beings are scarce. *In vitro* studies of human venous blood and lymphocyte fractions incubated with low doses of TCDD demonstrated a decrease in B cells and $CD4^+$ (helper) T cells and an increase in $CD8^+$ (cytotoxic) T cells.[21] The first indication that PCBs and dioxins might be immunotoxic *in vivo* came from studies in accidentally exposed humans.[22-29] In highly industrialized, densely populated Western European countries, like the Netherlands, dioxin levels in human milk samples can be especially high (10-100 pg TEQ/g milk fat). Whether prenatal and postnatal exposure to these high background levels of PCDDs, PCDFs, and PCBs can alter the immune system in human infants and whether the health of the infant is adversely affected by these pollutants is not known. In this report we explore the immunologic effects of environmental prenatal and postnatal background exposure to PCDDs, PCDFs, and PCBs in infants from birth to 18 mo of age. This study is part of the Dutch PCB/Dioxin Study, a larger prospective longitudinal study on possible adverse health effects of these pollutants on human infants.

10.3 Methods

Subjects. The total study group consisted of 207 healthy mother-infant pairs, recruited between June 1990 and February 1992, living in the Rotterdam area and was described in detail in a previous paper.[30] All mother-infant pairs were of the Caucasian race. Pregnancy and delivery had to be completed without overt signs of serious illness or complications. Only infants born at term (37 to 42 wk of gestation) without congenital anomalies or diseases were included. A hundred and five infants were breast-fed for at least 6 wk, 102 infants were exclusively bottle-fed with Almiron M2 from one batch (Nutricia N.V., The Netherlands). A description of the characteristics of the total study group is presented in Table 10.1.

In a subgroup of infants, recruited between March 1991 and February 1992, white blood cell counts and immunologic marker analyses in cord and venous blood at 3 and 18 mo of age were done. Because fresh blood is needed for these measurements, only infants born on a weekday and living close to the hospital were included in this part of the study. A description of the characteristics of the subgroup in comparison to the total group is presented in Table 10.1. In 48 infants cord blood was analysed. At 3 mo of age, in 1 of the original 48 children the venipunction was not successful, and a randomly chosen child was added to the study group. At 18 mo of age fresh blood was available for analysis in 37 of the original 48 children, and 6 randomly chosen children were added. There were no significant differences between the total study group and the subgroup.

Measures of PCB/dioxin exposure. A blood sample was taken from the mothers in the last month of pregnancy (36 to 40 wk) and analysed by GC with electron capture detection for the measurement of four PCB congener levels (PCB 118, 138, 153 and 180). The four congener levels were added and summarized as the PCB-plasma-sum. One blood sample was missing for this analysis. In the second week after delivery, the breast-feeding mothers collected a 24-h representative human milk sample with a vacuum pump (KAWECO, Babyluxus 2, Stuttgart, Germany). Seventeen individual dioxin congener and 24 PCB congener levels were measured. Congener specific analyses of PCDDs, PCDFs, and PCBs were carried out using previously described methods.[31] The international toxic equivalence factor (I-TEF) approach was used for the PCDDs and PCDFs[32] and the WHO 1993 TEF approach for the PCBs.[33] According to the TEQ concept, a toxic equivalent factor (TEF) was assigned to the 17 dioxin and 8 dioxin-like (planar, mono-ortho and di-ortho) PCB congeners. By multiplying the concentration (pg/g milk fat) and the TEF value, a TEQ value of each congener was calculated (pg TEQ/g milk fat). The TEQ-sum of the 17 dioxin, 3 planar PCB (77, 126, 169), 3 mono-ortho PCB (105, 118, 156), and 2 di-ortho PCB (170, 180) congeners were summarized as the total TEQ level. Of the 105 human milk samples, 80 could be measured with sufficient accuracy for the total TEQ level. The other analytical measurements were inaccurate due to interferences in the chromatograms, or due to too small volumes of human milk samples and therefore excluded from further analyses. As a measure of prenatal exposure, the PCB-plasma-sum of all individual mother-infant pairs was used. For the breast-fed infants the total TEQ level in human milk was separately

studied as an estimate of the prenatal exposure. Postnatal PCB/dioxin exposure was calculated as a product of the total TEQ level in human milk, multiplied by weeks of breast-feeding.

Measures of immunologic effects. All parents were asked to complete a health questionnaire regarding their infant. The number of periods with rhinitis, bronchitis, tonsillitis, and otitis during the first 18 mo of life was counted and used as an estimate of the health status of the infants.

Vaccinations against mumps, measles, and rubella were given to 205 of the 207 children at approximately 14 mo of age as part of the National Immunisation Program. The vaccines were given at the local municipal health service. Humoral antibody production was measured at 18 mo of age by detecting antibody levels to mumps, measles, and rubella in plasma with an ELISA.[34]

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.[35] Using several monoclonal antibodies (MAb), absolute numbers of the following lymphocyte (sub)populations were determined: $CD4^+$ T-lymphocytes ($CD4^+CD3^+$), $CD8^+$ T-lymphocytes ($CD8^+CD3^+$), activated T-lymphocytes ($HLA\text{-}DR^+CD3^+$), as well as $TcR\alpha\beta^+$, $TcR\gamma\delta^+$, $CD4^+CD45RA^+$ and $CD4^+CD45RO^+$ 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 6.01. The relationship between immunologic parameters and PCB/dioxin exposure was studied in univariate analyses (t test, Chi-Square Test, and Spearman correlation coefficient). In a first analysis prenatal (PCB-plasma-sum and total TEQ) and postnatal (total TEQ multiplied by weeks of breast-feeding) PCB-dioxin exposure were studied in relation to the immunologic parameters. When the PCB-plasma-sum was significantly correlated ($p \leq 0.05$) with the outcome variable, analyses of the separate PCB congener levels 118, 138, 153, and 180 in maternal plasma were done. When the total TEQ was significantly correlated with the outcome variable, analyses of the dioxin and dioxin-like (planar, mono-ortho, and di-ortho) PCB congeners in human milk were done. Potential confounding variables at birth (birthweight, gestational age, sex, smoking and alcohol use during pregnancy, maternal education, and paternal occupation) and at 3 and 18 mo of age (sex, nutritional status, duration of breast-feeding, maternal education, and paternal occupation) were selected, according to clinical and immunologic knowledge. Potential confounding variables were analysed when the PCB/dioxin exposure was significantly correlated with the outcome variable.

The study protocol had been approved by the medical ethical committee of the University Hospital Rotterdam/Sophia Children's Hospital. Informed consent had been given by the parents.

10.4 Results

The mean PCB-plasma-sum in the total group was 2.25 ng/g plasma (SD 0.98, n = 206) and the mean total TEQ level was 66.59 pg TEQ/g fat (SD 24.35, n = 80). The mean PCB-plasma-sum in the subgroup was 2.10 ng/g (SD 0.87, n = 55) and the mean total TEQ was 64.20 pg TEQ/g fat (SD 19.08, n = 19). There were no significant differences in the mean PCB-plasma-sum or total TEQ level between the total group and the subgroup.

There were no significant correlations between the number of periods with rhinitis, bronchitis, tonsillitis, and otitis during the first 18 mo of life and prenatal (PCB-plasma-sum and total TEQ level) and postnatal (total TEQ level multiplied by the number of breast-feeding weeks) PCB/dioxin exposure. There were also no significant correlations between the specific antibody levels to mumps, measles, and rubella at 18 mo of age and pre- and postnatal PCB/dioxin exposure.

The results of the white blood cell counts and the immunologic marker analyses in cord blood and venous blood at 3 and 18 mo of age are presented in Table 10.2. The results are all within the normal ranges in age-matched children. Correlations between the different leucocyte (sub)populations and pre- and postnatal PCB/dioxin exposure are presented in Table 10.3.

At birth a higher total TEQ level was correlated with an increase in TcR$\gamma\delta^+$ T cells ($p = 0.03$). Correlation coefficients with the dioxin, planar, mono-ortho, and di-ortho PCB congeners only reached statistical significance for the dioxin TEQ level (Table 10.4, $p = 0.01$). There were no significant correlations between the number of TcR$\gamma\delta^+$ T cells and the potential confounders.

At 3 mo of age a higher total TEQ level was significantly correlated with a decrease in the number of monocytes ($p = 0.003$) and granulocytes ($p = 0.04$). A higher postnatal exposure was significantly correlated with a decrease in the total number of monocytes ($p = 0.03$), granulocytes ($p = 0.01$) and B-cells ($p = 0.05$). The monocyte count was significantly correlated with the dioxin TEQ level ($p = 0.01$), the mono-ortho ($p = 0.002$), and di-ortho ($p = 0.03$) PCB TEQ level. The granulocyte count was significantly correlated only with the total TEQ level; correlation coefficients with the dioxin, planar, mono-ortho, and di-ortho PCB congener levels did not reach statistical significance (Table 10.4). There was no significant correlation between the monocyte and granulocyte counts and the potential confounders. The number of CD19/20$^+$ cells was significantly correlated with the duration of breast-feeding ($r_s = 0.64$, $p = 0.003$).

At 18 mo of age higher total TEQ and PCB-plasma-sum levels were significantly correlated with an increase in the number of CD8$^+$ T cells (PCB-plasma-sum, $p = 0.01$, total TEQ, $p = 0.002$). In maternal plasma PCB 118 ($r_s = 0.33$, $p = 0.03$), PCB 138 ($r_s = 0.32$, $p = 0.04$), PCB 153 ($r_s = 0.37$, $p = 0.01$) and PCB 180 ($r_s = 0.80$, $p = 0.002$) were significantly correlated with the number of CD8$^+$ T cells. In human milk correlation coefficients with the dioxin, planar, mono-ortho and di-ortho PCB congeners reached statistical significance for the dioxin TEQ level ($p = 0.002$) and the planar ($p = 0.01$) and di-ortho ($p = 0.02$) PCB TEQ levels (Table 10.4). A higher total TEQ level was also significantly correlated with an increase in the number of TcR$\alpha\beta^+$ cells ($p = 0.05$). Correlation coefficients between the number of TcR$\alpha\beta^+$ cells and the dioxin, planar, mono-ortho, and di-

ortho PCB congeners reached statistical significance for the dioxin TEQ level ($p = 0.009$) and the di-ortho ($p = 0.04$) PCB TEQ level (Table 10.4). In addition a higher dioxin TEQ level was also significantly correlated with more $CD3^+$ ($r_s = 0.61$, $p = 0.04$) and $TcR\gamma\delta^+$ ($r_s = 0.70$, $p = 0.01$) T cells. There were no significant correlations of the T cell markers at 18 mo of age with postnatal PCB/dioxin exposure nor with the potential confounders.

Comparing the number of periods with respiratory tract infections and the leucocyte (sub)population at 18 mo of age, there was a significant relationship only between the number of periods with bronchitis and the $CD4^+CD45RA^+$ T-lymphocytes ($r_s = 0.33$, $p = 0.04$). Antibody levels to mumps were correlated with the total number of lymphocytes ($r_s = 0.32$, $p = 0.04$), the $CD8^+$ ($r_s = 0.33$, $p = 0.04$) and $TcR\gamma\delta^+$ ($r_s = 0.37$, $p = 0.02$) T- and the B-lymphocytes ($r_s = 0.32$, $p = 0.05$). Antibody levels to measles were significantly correlated with the number of $CD8^+$ ($r_s = 0.35$, $p = 0.03$) and $TcR\gamma\delta^+$ ($r_s = 0.34$, $p = 0.04$) T-lymphocytes and with the NK cells ($r_s = 0.32$, $p = 0.05$). Antibody levels to rubella were significantly correlated with the number of granulocytes ($r_s = 0.34$, $p = 0.04$) and the $TcR\gamma\delta^+$ ($r_s = 0.32$, $p = 0.05$) T-lymphocytes.

10.5 Discussion

In this study two different effects of PCB/dioxin background exposure on the developing immune system of human infants were found. Prenatal PCB/dioxin exposure was associated with changes in T cell subpopulations in the blood. These changes were mainly seen at 18 mo of age. At that age a higher prenatal PCB/dioxin exposure was associated with an increase in the total number of T cells as well as with an increase in the number of $CD8^+$ (cytotoxic), $TcR\alpha\beta^+$ and $TcR\gamma\delta^+$ T cells. These prenatal effects of PCB/dioxin exposure on changes in T cell subpopulations at a later age are consistent with findings in other human studies. In children born to mothers living in a TCDD-contaminated environment in Time Beach, MO, during and after pregnancy, a decrease in $CD4^+$ (helper) T cells and an increase of $CD8^+$ (cytotoxic) T cells has even been demonstrated at 9 to 14 years of age.[28] In one preliminary report from Northern Quebec, Inuit infants whose mothers have elevated levels of PCBs and dioxins in their breast milk, the $CD4^+$ (helper):$CD8^+$ (cytotoxic) T cell ratio was decreased at 6 and 12 mo of age, but not at 3 mo of age.[29] Our results are also in agreement with animal studies, where perinatal TCDD exposure produces an alteration in the normal thymocyte maturational process.[5,6] Moreover, *in vitro* studies of human venous blood and lymphocyte fractions incubated with TCDD demonstrated a decrease in $CD4^+$ (helper) T cells and an increase in $CD8^+$ (cytotoxic) T cells.[21] In contrast to the above studies, we did not find a decrease in $CD4^+$ (helper) T cells. All of these studies were, however, conducted *in vitro* or in higly exposed infants, whereas our study was conducted in background PCB/dioxin-exposed infants.

A higher prenatal as well as postnatal PCB/dioxin exposure was associated with lower monocyte and granulocyte counts only at 3 mo of age. The effects on the lymphocyte count fell short of statistical significance. Our findings are in agreement with animal studies where a direct effect on the fetal liver and

neonatal bone marrow was found after perinatal dioxin exposure.[2,7,8] In Taiwan, in the Yucheng incident, changes in the monocyte maturational process were found in PCB-poisoned patients.[23] Our results also agree with a previous study in Dutch infants where a negative correlation between dioxin concentrations in breast milk and the number of granulocytes was found at 1 wk of age.[36] Correlation coefficients of the monocyte and lymphocyte counts with prenatal PCB/dioxin exposure were higher than with postnatal exposure. Correlation coefficients of the granulocyte count with prenatal PCB/dioxin exposure were lower than with postnatal exposure. Unfortunately due to the nonparametric distribution of the white blood cell counts and the small numbers of cases left, multivariate analysis was impossible.

A higher postnatal PCB/dioxin exposure was associated with a decrease in the number of $CD19/20^+$ B cells. In the children, born to mothers living in TCDD-contaminated Time Beach, MO, a decrease in B cells has also been demonstrated.[28] Moreover, *in vitro* studies of human peripheral blood lymphocyte fractions incubated with TCDD showed a decrease of B cells.[21] In our study group, however, the correlation coefficient of the number of B cells with the duration of breast-feeding was higher than with postnatal PCB/dioxin exposure. We therefore presume that the decrease in B cells was mainly an effect of breast-feeding. Further studies on the effect of breast-feeding on the immune status of the infant are needed.

In our study there was no evidence of increased upper or lower respiratory tract symptoms or altered humoral antibody production in relation to PCB/dioxin exposure. Although there were differences in the leukocyte (sub)population between high and low PCB/dioxin-exposed infants, all values were within the normal range. Moreover, subtle changes in the number of blood leukocytes do not simply mirror alterations in the cell composition of lymphoid and nonlymphoid organs, nor do they simply reflect functional defects. In children born to accidentally highly exposed women, in the Yucheng incident, an increased incidence of respiratory symptoms was found.[26] The Inuit infants, whose mothers had elevated levels of PCBs and dioxins in their breast milk, experienced more episodes of acute otitis media at 3 to 6 mo of age.[29] In a prospective longitudinal study of background PCB exposure in the United States, however, there was no adverse effect on the frequency of physician visits for various illnesses.[37] The magnitude of the above described changes in the immune status of background-exposed infants associated with prenatal PCB/dioxin exposure, as compared with accidental high exposure, might be too subtle to induce these clinical symptoms. There are, however, some limitations to our health questionnaire. The number of periods with rhinitis, bronchitis, tonsillitis, and otitis was counted during the first 18 mo of life. No subdivision in shorter time periods was made. This might be the reason that we did not find a relationship between the number of respiratory infections and the leukocyte (sub)populations under study. There was, however, a significant relationship between the antibody levels and the number of $CD8^+$ (cytotoxic) and TcR$\gamma\delta$ T lymphocytes at 18 mo of age. Therefore, one might speculate that the lower numbers of monocytes and granulocytes at the age of 3 mo could have resulted in more (subclinical) infections during the first months of life and in an increase in the number of $CD8^+$ (cytotoxic) T cells thereafter.

The evaluation of PCB/dioxin exposure in human and environmental samples is difficult, because these compounds are complex mixtures of various related PCDD, PCDF and PCB congeners. Effects associated with one type of congeners may therefore actually be due to another type of congeners. Moreover, some PCB congeners may antagonize the TCDD-mediated immunotoxic effects.[38] Samples of blood or breast milk obtained from the mother can be presumed to reflect her lifetime exposure. Cord blood provides the more direct measure of fetal exposure but it is difficult to quantify the levels of the different PCB congeners because the fat content in cord blood is low. Given that PCBs and dioxins are lipophilic, the PCB levels in cordblood are low.[31] From a statistical standpoint, the poor reliability of measurement at these levels of exposure will tend to increase the likelihood of type II error and depress the magnitude of any effects associated with intra-uterine exposure to PCBs.[39] Breast milk and maternal plasma are easier to assess because of their higher lipid content. Because the PCB levels in maternal plasma correlate well with the PCB levels in umbilical cord plasma, and dioxin and PCB levels in human milk correlate well with the levels in adipose tissue,[32] PCB/dioxin exposure was estimated from the levels of PCBs in maternal plasma and the levels of dioxins and dioxin-like PCBs in human milk.

In conclusion, this exploratory study is the first to show that background levels of PCB/dioxin exposure influences the human fetal and neonatal immune system. Although there is no evidence of clinical symptoms or direct changes in the humoral immunity response in infancy and the results of the white blood cell counts and immunologic marker analyses were all within the normal range, the described changes in the T cell lymphocyte population could persist into later child- or adulthood and could presage difficulties, like immune suppression, allergy or autoimmunity.[40] Follow-up of these children to adulthood is therefore needed. In the meantime the prevention of further environmental and food chain contamination is essential, along with their monitoring in various commodities.

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10.6 References

1. Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lutkeschipholt IJ, van der Paauw CG, Tuinstra LGMT, Brouwer A, Sauer PJJ 1994. Effects on dioxins and polychlorinated biphenyls on thyroid hormone status og pregnant women and their infants. Pediatr Res 36(4):468-473.
2. Fine JS, Gasiewicz TA, Flore NC, Silverstone AE 1990. Prothymocyte activity is reduced by perinatal 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure. J Pharmacol Exp Ther Oct; 255(1):128-32.

3. Dencker L, Hassoun E, d'Argy R, Alm G 1985. Fetal thymus organ culture as an in vitro model for the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin and its congeners. Mol Pharmacol Jan;27(1):133-40.
4. Korte M, Stahlman R, Thiel R, Nagao T, Chaoud I, van Loveren H, Vos JG, Neubert D 1990. Effects of 2,3,7,8-terachlorodibenzo-p-dioxin on hepatic monooxygenases and resistance to trichinella spiralis infection in rat ofspring after perinatal exposure. In: Huntziger O, Fiedler H (eds) Organohalogen Compounds, Eco-Informa Press, Bayreuth, Dioxin I:89-94.
5. Holladay SD, Lindstrom P, Blaylock BL, Comment CE, Germolec DR, Heindell JJ, Luster MI 1991. Perinatal thymocyte antigen expression and postnatal immune development altered by gestational exposure to tetrachlorodibenzo-p-dioxin (TCDD). Teratology Oct;44(4):385-93.
6. Blaylock BL, Holladay SD, Comment CE, Heindel JJ, Luster MI 1992. Exposure to tetrachlorodibenzo-p-dioxin (TCDD) alters fetal thymocyte maturation. Toxicol Appl Pharmacol Feb;112(2):207-13.
7. Fine JS, Gasiewicz TA, Silverstone AE 1989. Lymphocyte stem cell alterations following perinatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Mol Pharmacol Jan;35(1):18-25.
8. Ackermann MF, Gasiewicz TA, Lamm KR, Germolec DR, Luster MI 1989. Selective inhibition of polymorphonuclear neutrophil activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol Dec;101(3):470-80.
9. Takagi Y, Aburada S, Otake T, Ikegami N 1987. Effect of polychlorinated biphenyls (PCBs) accumulated in the dam's body on mouse filial immunocompetence. Arch Environ Contam Toxicol May;16(3):375-81.
10. Tomar RS, Kerkvliet NI 1991. Reduced T-helper cell function in mice exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol Lett Jun;57(1):55-64.
11. Vecchi A, Sironi M, Canegrati MA, Recchia M, Garattini S 1983. Immunosuppressive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in strains of mice with different susceptibility to induction of aryl hydrocarbon hydroxylase. Toxicol Appl Pharmacol May;68(3):434-41.
12. Luster MI, Germolec DR, Clark G, Wiegand G, Rosenthal GJ 1988. Selective effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin and corticosteroid on in vitro lymphocyte maturation. J Immunol Feb 1;140(3):928-35.
13. Tucker AN, Vore SJ, Luster MI 1986. Suppression of B cell differentiation by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Mol Pharmacol Apr;29(4):372-7.
14. Clark GC, Blank JA, Germolec DR, Luster MI 1991. 2,3,7,8-Tetrachlorodibenzo-p-dioxin stimulation of tyrosine phosphorylation in B lymphocytes: potential role in immunosuppression. Mol Pharmacol Apr;39(4):495-501.
15. Funseth E, Ilback NG 1992. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on blood and spleen natural killer (NK) cell activity in the mouse. Toxicol Lett May; 60(3): 247-56.
16. Smialowicz RJ, Riddle MM, Williams WC, Diliberto JJ 1994. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on humoral immunity and lymphocyte subpopulations: differences between mice and rats. Toxicol Appl Pharmacol Feb;124(2):248-56.
17. Brunstrom B, Hakansson H, Lundberg K 1991. Effects of a technical PCB preparation and fractions thereof on ethoxyresorufin O-deethylase activity,

vitamin A levels and thymic development in the mink (Mustela vison). Pharmacol Toxicol Dec;69(6):421-6.
18. Neubert R, Jacob-Muller U, Stahlmann R, Helge H, Neubert D 1990. Polyhalogenated dibenzo-p-dioxins and dibenzofurans and the immune system. 1. Effects on peripheral lymphocyte subpopulations of a non-human primate (Callithrix jacchus) after treatment with 2,3,7,8-tetrachlorodi-benzo-p-dioxin (TCDD). Arch Toxicol 64(5):345-59.
19. Neubert R, Golor G, Stahlmann R, Helge H, Neubert D 1992. Polyhalogenated dibenzo-p-dioxins and dibenzofurans and the immune system. 4. Effects of multiple-dose treatment with 2,3,7,8-tetrachlorodi-benzo-p-dioxin (TCDD) on peripheral lymphocyte subpopulations of a non-human primate (Callithrix jacchus). Arch Toxicol 66(4):250-9.
20. de Swart R, Ross PS, Vedder LJ, Timmerman HH, Heisterkamp S, van Loveren H, Vos JG, Reijnders PJH, Osterhaus ADME 1994. Impairment of immune function in harbor seals (Phoca vitulina) feeding on fish from polluted waters. Ambio March;3(2):155-159.
21. Neubert R, Jacob-Muller U, Helge H, Stahlmann R, Neubert D 1991. Polyhalogenated dibenzo-p-dioxins and dibenzofurans and the immune system. 2. In vitro effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on lymphocytes of venous blood from man and a non-human primate (Callithrix jacchus). Arch Toxicol 65(3):213-9.
22. Shigematsu N, Ishimaru S, Saito R, Ikeda RT, Matsuba K, Sugiyama K, Masuda Y 1978. Respiratory involvement in polychlorinated biphenyls poisoning. Environmental Research 16:92-100.
23. Chang KJ, Hsieh KH, Lee TP, Tang SY, Tung TC 1982. Immunologic ecaluation of patients with polychorinated biphenyl poisoning: determination of phagocyte Fc and complement receptors. Environmental research 28:329-334.
24. Chang KJ, Hsieh KH, Lee TP, Tang SY, Tung TC 1981. Immunologic evaluation of patients with polychorinated biphenyl poisoning: determination of lymphocyte subpopulations. Toxicology and applied pharmacology 61:58-63.
25. Chang KJ, Hsieh KH, Tang SY, Tung TC, Lee TP 1982. Immunologic evaluation of patients with polychorinated biphenyl poisoning: hypersensitive response and its relation to clinical studies. J of Tox Env Health 9:217-223.
26. Rogan WJ, Gladen BC, Hung KL, Koong SL, Shih LY, Taylor JS, Wu YC, Yang D, Ragan NB, Hsu CC 1988. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science Jul 15;241(4863):334-6.
27. Hoffman RE, Stehr-Green PA, Webb KB, Evans RG, Knutsen AP, Schramm WF, Staake JL, Gibson BB, Steinberg KK 1986. Health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. JAMA Apr 18;255(15):2031-8.
28. Smoger GH, Kahn PC, Rodgers GC, Suffin S, McConnachie P 1993. In utero and postnatal exposure to 2,3,7,8-TCDD in Times beach Missouri; 1. Immunological effects; Lymphocyte phenotype frequencies. Short paper in: Dioxin '93. 13th International Symposium on Clorinated Dioxins and

Related compounds. Vienna, September:345-348.
29. Dewailly E, Bruneau S, Laliberte C, Belles Iles M, Weber JP, Ayotte P, Roy R 1993. Breast milk contamination by PCBs and PCDDs/PCDFs in Arctic Quebeq: Preliminary results on the immune status of Inuit infants. Short paper in: Dioxin '93. 13th International symposium on chlorinated dioxins and related compounds. Vienna, September:403-406.
30. Koopman-Esseboom C, Huisman M, Weisglas-Kuperus N, van der Paauw CG, Tuinstra LGMT, Boersma ER, Sauer PJJ 1994. PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 28(9);1721-1732.
31. Tuinstra LGMT, Traag WA, van Rhijn JA, van de Spreng P 1994. The Dutch PCB/dioxin study. Development of a method for the determination of dioxins, planar and other PCBs in human milk. Chemosphere 29: 1859-75.
32. NATO/CCMS 1988. Pilot study on international information exchange on dioxins and related compounds. International toxicity equivalency factor (I-TEF). Methods of Risk assessment for complex mixtures of dioxins and related compounds. Report number 176.
33. Ahlborg US, Bedring GC, Birnbaum LS 1994. Toxic Equivalency Factors for dioxin-like PCBs. Report on a WHO-ECEH and IPCS consultation, December 1993. Chemosphere 28(6):1049-1067.
34. Harmsen T, Jongerius MC, van der Zwan CW, Plantinga AD, Kraaijeveld CA, Berbers GA 1992. Comparison of a neutralization enzyme immunoassay and an enzyme-linked immunosorbent assay for evaluation of immune status of children vaccinated for mumps. J Clin Microbiol Aug;30(8):2139-44.
35. van de Merwe JP, van den Beemd MW, Hooijkaas H 1992. CD5+ B lymphocytes and other lymphocyte subsets in primary Sjogren's syndrome. Neth J Med Apr;40(3-4):158-64.
36. Pluim HJ 1993. Clinical laboratory manifestations of exposure to background levels of dioxins in the perinatal period. Dioxins, pre- and postnatal exposure in the human newborn, Thesis, Amsterdam University, Amsterdam, the Netherlands, 93-102.
37. Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, Tingelstad J, Tully M 1987. Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) in human milk: effects on growth, morbidity, and duration of lactation. Am J Public Health Oct;77(10):1294-7.
38. Davis D, Safe S 1989. Dose-response immunotoxicities of commercial polychlorinated biphenyls (PCBs) and their interaction with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Lett Jul;48(1):35-43.
39. Jacobson JL, Jacobson SW 1994. The effects of perinatal exposure to polychlorinated biphenyls and related contaminants. In Needleman HL, Bellinger D. Prenatal exposure to toxicants. Developmental consequences. John Hopkins University Press, Baltimore and London.
40. Schuurman HJ, Van Loveren H, Rozing J, Vos JG 1992. Chemicals trophic for the thymus: risk for immunodeficiency and autoimmunity. Int J Immunopharmacol Apr;14(3):369-75.

Table 10.1 Description of the characteristics and potential confounders of the total study group (n = 207) and the subgroup (n = 55)

Variables	Categories	Total group (n = 207)	Subgroup (n = 55)
Maternal and Socio-demographic Variables		%	%
- Maternal education	Low	19	13
	Medium	61	62
	High	20	25
- Paternal occupation	Low	59	64
	Medium	11	6
	High	30	30
- Smoking during pregnancy	No	77	84
	Yes	23	16
- Alcohol during pregnancy	No	83	84
	Yes	17	16
Infant variables			
- Sex	Male	54	60
	Female	46	40
- Type of feeding	Breast	52	50
	Formula	48	50
		Mean (SD)	Mean (SD)
- Duration of breast feeding (wks)		20.4 (13.6)	21.1 (13.1)
- Gestational age (wks)		40.5 (1.2)	40.2 (1.1)
- Birthweight (grams)		3465 (443)	3534 (338)
- Weight/length 3 months (gr/cm)		97.7 (9.7)	98.1 (8.2)
- Weight/lenght18 months (gr/cm)		145.1 (14.4)	145.6 (13.4)

Table 10.2 Results of the white blood cell counts and the immunological marker analyses in cordblood and venous blood at 3 and 18 months of age.

	Cordblood n=48 (percentiles, 10^6/l)			3 months n=48 (percentiles, 10^6/l)			18 months n=43 (percentiles, 10^6/l)		
	p25	p50	p75	p25	p50	p75	p25	p50	p75
White Blood Cell Counts									
Monocytes	925	1200	1675	600	700	900	500	700	800
Granulocytes	5500	7000	9625	2025	2550	3575	3000	3400	5100
Lymphocytes	3000	4000	4600	4425	5600	6575	3900	5300	6300
T-cell markers									
CD3+	1554	2088	2610	2749	3845	4358	2410	3340	4060
CD3+CD4+	1035	1448	1945	1995	2805	3509	1505	1950	2620
CD3+CD8+	265	480	730	643	940	1203	800	1110	1490
CD4+CD45RA+	450	760	1500	1488	1930	2645	1040	1420	1835
CD4+CD45RO+	40	60	190	130	160	228	180	290	350
TcR $\alpha\beta$+	1375	1960	2370	2545	3535	4180	2270	3030	3790
TcR $\gamma\delta$+	30	40	60	100	120	170	140	230	300
CD3+ HLA-DR+	40	50	108	160	225	328	180	290	410
B-cell marker									
CD 19/20+	314	550	773	1105	1448	1943	1050	1280	1875
NK-cell marker									
CD16+ and/or D56+/CD3-	533	785	1225	260	350	548	230	340	608

Table 10.3 Spearman rank correlation coefficients of the white blood cell counts and the immunological marker analyses in cordblood and venous blood at 3 and 18 months of age in relation with PCB/dioxin exposure.

	Cordblood (Spearman rank correl.coeff.) PCB/dioxin exposure		3 months (Spearman rank correl. coef.) PCB/dioxin exposure			18 months (Spearman rank correl. coef.) PCB/dioxin exposure		
	Prenatal (n=48)	Prenatal (n=19)	Prenatal (n=48)	Prenatal (n=19)	Postnatal (n=19)	Prenatal (n=43)	Prenatal (n=12)	Postnatal (n=12)
	PCB sum	Total TEQ	PCB sum	Total TEQ	TEQ x wks	PCB sum	Total TEQ	TEQ x wks
White Cell Blood Counts								
Monocytes	-.06	-.18	-.03	-.64**	-.49*	.06	.23	.12
Granulocytes	-.07	-.27	-.07	-.47*	-.55*	.02	.09	.27
Lymphocytes	-.03	-.06	-.10	-.42	-.40	.09	.36	.06
T-cell markers								
CD3+	.02	.07	.02	-.07	-.10	.19	.47	.20
CD3+CD4+	-.07	.01	-.02	-.05	-.05	.12	.43	.20
CD3+CD8+	.14	.17	.10	-.02	.03	.38*	.65*	.34
CD4+CD45RA+	-.17	.19	-.08	.01	.01	-.01	.33	.25
CD4+CD45RO+	-.05	.39	.07	.06	.01	.23	.19	-.22
TcR $\alpha\beta$+	-.01	.10	-.01	-.09	-.11	.20	.57*	.24
TcR $\gamma\delta$+	.18	.50*	.13	-.10	-.06	.22	.43	-.10
CD3 +HLA-DR+	-.18	-.03	.01	-.03	-.01	.16	.49	-.01
B-cell marker								
CD 19/20+	-.17	-.04	-.21	-.42	-.45*	-.12	.05	-.32
NK-cell marker								
CD16+ and/or CD56+/CD3-	.03	.06	.21	-.06	.04	.23	.03	-.12

* = $p \leq .05$, ** = $p \leq .01$

Table 10.4 Spearman rank correlation coefficients of the significant correlations ($p \leq .05$) between the immunological parameters and the total TEQ: dioxin TEQ, planar, momo-ortho and di-ortho PCB congener TEQ levels in breast-milk.

	Dioxin and dioxin-like PCB TEQ levels in breast-milk Spearman rank correlation coefficients				
	Dioxin TEQ	Planar PCB TEQ	Mono-ortho PCB TEQ	Di-ortho PCB TEQ	Total TEQ
White Cell Blood Counts					
Monocytes at 3 months	-.55**	-.37	-.67**	-.51*	-.64**
Granulocytes at 3 months	-.40	-.40	-.44	-.34	-.47*
T-cell markers					
CD3+CD8+ at 18	.80**	.71**	.52	.68*	.65*
months	.71**	.50	.44	.61*	.57*
TcR $\alpha\beta$+ at 18 months	.57**	.32	.40	.34	.50*
TcR $\gamma\delta$+ in cordblood					

* = $p \leq .05$, ** = $p \leq .01$

CHAPTER 11

SUMMARY AND CONCLUSIONS

11.1 Summary

In this thesis effects of perinatal exposure to polychlorinated biphenyls (PCBs) and dioxins on the early human development are described.

"The Dutch PCB and dioxin study" is a collaborative study between human clinical research workers and animal experimental research workers. The clinical part of the study involving human volunteers (mother-infant pairs) was done by the Department of Pediatrics of the Sophia Children's Hospital and Erasmus University Rotterdam, and by the Department of Obstetrics and Gynecology of the University Hospital Groningen. The animal experimental part of the study was done by the Department of Toxicology of the Agricultural University Wageningen, the Department of Biological Toxicology of TNO in Zeist and the Department of Neurotoxicology of MBL-TNO in Rijswijk. PCB measurements in human plasma were done by the Institute for Toxicology and Food of TNO in Zeist, the PCB and dioxin levels in human milk were analysed by the State Institute for Quality Control of Agricultural Products (RIKILT) in Wageningen. The study was supported by the Program Committee Toxicology (PCT) and the Dutch Health Research Promotion Program (SGO).

Both PCBs and dioxins are lipophilic stable toxic compounds which are widespread in the environment. Human adults are mainly exposed to these contaminants by consuming meat, fish, and dairy products and at the end of this food chain PCBs and dioxins are stored in the human adipose tissue. During pregnancy small amounts of PCBs and dioxins pass the placental barrier and the embryo and fetus are exposed during a critical period of organ growth and development. After birth the breast-fed infant is exposed to relatively high levels of these contaminants. The postnatal exposure of formula-fed infants is negligible since animal fat in formula is replaced by lipids of a vegetable origin.

In this study PCBs have been measured in maternal plasma and umbilical cord plasma as a measure of the intrauterine exposure of the infants. The postnatal exposure to PCBs and dioxins was estimated from the levels in human milk and formula. A prospective longitudinal follow-up study on the neurodevelopment was done in a cohort of 418 Dutch Caucasian fullterm infants in the second week after birth and at 18 months of age. This part of the clinical study was done in co-operation with the Department of Obstetrics and Developmental Neurology of the University of Groningen. Two hundred and seven mother-infant pairs lived in the Rotterdam area, an industrialized region in the western part of the Netherlands, the other 211 pairs lived in the Groningen area, a semi-urban region in the northern part of the Netherlands. Two hundred and nine infants were breast-fed for at least six weeks, the other 209 infants received formula from one batch as a reference. In addition, at the Sophia Children's Hospital/Erasmus University in Rotterdam, a subpopulation of 207 infants was also studied for their visual recognition memory at 3 and 7 months of age, and

for their mental and psychomotor development at 3, 7, and 18 months of age.

Chapter 2 describes the levels of PCB 118, 138, 153 and 180 in maternal plasma, sampled in the last month of pregnancy, as well as in umbilical cord plasma. These measurements are estimations of the prenatal exposure to PCBs. In human milk, sampled in the second week after birth, the levels of the 17 most toxic dioxin congeners are reported as well as 26 different PCB congener levels. It can be concluded that the PCB and dioxin levels in the Dutch population belong to the highest background levels in the world, and are comparable to the levels of other highly industrialized, densely populated countries in Western Europe. Correlation coefficients between the contaminant levels in maternal plasma, cord plasma, and human milk are highly significant. However, the 95% predictive interval is too wide to predict accurately the PCB and dioxin levels to which an individual infant is exposed in utero or postnatally by breast-feeding, from the PCB levels in maternal plasma. In a subpopulation of women PCB and dioxin levels were measured in human milk sampled in the second week after birth, as well as in the sixth week after birth. There was no significant decline in the dioxin, planar PCB or total-TEQ level in this period. However, the mono-ortho and di-ortho PCB level declined significantly.

Chapter 3 describes the different PCB and dioxin levels in samples from the Rotterdam area, a highly industrialized region in the western part of the Netherlands, compared with the levels in the samples from the Groningen area, a semi-urban region in the northern part of the Netherlands. After correction for covariates, we found significantly higher levels of PCB 118 in maternal plasma, and significantly higher levels of the dioxin-TEQ and ten individual dioxin and PCB congener levels in human milk from the western more industrialized area of the Netherlands, compared with the northern semi-urban part.

Chapter 4 describes the influences of PCB and dioxin exposure on the thyroid hormone status of a subgroup of 105 mother-infant pairs, living in the Rotterdam region. A higher PCB and dioxin exposure was significantly correlated with lower plasma levels of maternal TT3 and TT4, and with higher plasma levels of TSH in the infants in the second week and third month after birth. Infants exposed to higher levels of these contaminants had also lower plasma FT4 and TT4 levels in the second week after birth. In general, these effects resemble the measurements done in rats and monkeys. One of the proposed mechanisms by which PCBs and dioxins may alter the thyroid hormone status is the occupation of the T4 binding pocket on transthyretin (TTR), one of the plasma binding proteins for T4. Another mechanism might be the induction of hepatic uridine diphosphate-glucuronyltransferase by which the biliary excretion of T4-glucuronide is enhanced. The higher plasma TSH levels in the infants at 2 weeks and 3 months of age, might be a sustained reaction to remain euthyroid after the perinatal exposure to PCBs and dioxins. Sufficient levels of thyroid hormones are necessary for normal brain development. Small changes might be of influence on the development of the fetus and infant.

Chapter 5 describes the perinatal exposure to PCBs and dioxins in relation to neonatal neurological abnormalities in the second week after birth. The neonatal neurological examination technique, according to Prechtl, was applied to 418 newborns. Four neonates were diagnosed as neurologically definitively abnormal and twenty as mildly abnormal. There was no significant relationship

between the perinatal PCB or dioxin exposure and these clinically relevant neonatal neurological abnormalities. In the Rotterdam subgroup of 207 infants the thyroid hormone status was also measured. There was neither a significant relation between the infants' thyroid hormone status and their neonatal neurological outcome.

Chapter 6 describes the neonatal neurological optimality score of the 418 infants, evaluated with the Prechtl technique in the second week after birth, in relation to the perinatal exposure to PCBs and dioxins. With this technique exposure to higher levels of PCBs and dioxins in human milk was related to reduced neonatal neurological optimality scores and to a higher incidence of hypotonia. However, there was no relationship found between the perinatal PCB or dioxin exposure and clinically relevant neonatal neurological abnormalities as described in Chapter 5. Therefore, it is concluded that perinatal exposure to Dutch background levels of PCBs and dioxins gives subtle signs of neurological dysfunctioning without clinical relevance.

Chapter 7 describes the effects of perinatal exposure to PCBs and dioxins on the visual recognition memory at 3 and 7 months of age in the Rotterdam subpopulation of 207 infants. The Fagan Test of Infant Intelligence was used for these measurements. At 3 months of age there was no significant effect of the prenatal or postnatal PCB and dioxin exposure on this outcome. However, at 7 months of age breast-fed infants had significantly higher mean scores on the Fagan test, compared to formula-fed infants. Moreover, the visual recognition memory of the infants was positively correlated with the duration of breast-feeding. There were no deleterious effects of the perinatal exposure to PCBs and dioxins on the visual recognition memory outcome at this age. Although it is known from animal studies that low doses of dioxins may have a facilitating effect on memory functioning, it is more reasonable to believe that hormones, trophic factors, LCPUFAs or other lipophilic factors, present in human milk, are responsible for the better outcome of breast-fed infants.

Chapter 8 describes the effects of the perinatal exposure to PCBs and dioxins on the mental and psychomotor development estimated with the Bayley Scales of Infant Development at 3, 7, and 18 months of age in the Rotterdam subgroup of 207 infants. At 3 months of age, higher in utero exposure to PCBs was significantly associated with lower psychomotor scores: a doubling of the PCB load resulted in a decrease of 3 points. Breast-fed infants scored significantly higher on the psychomotor scale at 7 months of age, compared to formula-fed infants. However, the psychomotor score of the 66% highest exposed breast-fed infants (>756 pg total PCB-dioxin TEQ) was negatively influenced by this postnatal exposure, and comparable to the psychomotor score of the formula-fed infants. It is assumed that breast-feeding itself has a positive effect on the motor development due to hormones, trophic factors or other lipophilic substances which are absent in formula. However, when the PCB and dioxin load reaches a certain level they might have a deleterious effect on the psychomotor development so that the beneficial effects of human milk are nullified. Breast-fed infants scored higher on the mental scale at 7 months of age in a dose-dependent way. There was no significant influence of the perinatal PCB and dioxin exposure on the mental outcome at 3 and 7 months of age. At 18 months of age neither the mental nor the psychomotor score was related to

perinatal PCB or dioxin exposure, nor to the duration of breast-feeding. At this age environmental and socio-economical factors were more important for the outcome.

Chapter 9 describes the neurological optimality score of all 418 children at 18 months of age in relation to the perinatal exposure to PCBs and dioxins. After adjusting for covariates, transplacental exposure to PCBs was negatively related to the neurological condition at 18 months. There was no relationship with clinical relevant neurological abnormalities. A negative effect of lactational exposure to these contaminants on the neurological optimality score could not be detected. Besides, there was a beneficial effect of breast-feeding on the fluency of movements.

Chapter 10 describes the immunologic effects of the perinatal exposure to PCBs and dioxins in a subgroup of the Rotterdam study population. The number of periods with rhinitis, bronchitis, tonsillitis and otitis during the first 18 months of life was used as an estimate of the infant's health status. Humoral immunity was measured at 18 months of age by detecting antibody levels to mumps, measles and rubella after vaccination. White blood cell counts and immunologic markers were assessed in cord plasma and at 3 and 18 months of age in a subgroup of 55 infants. There was no significant relationship between perinatal exposure to PCBs or dioxins and upper or lower respiratory tract symptoms or altered humoral antibody production. A higher prenatal exposure to these contaminants was associated with an increase in the number of TcR$\gamma\delta$+ T cells at birth and with an increase in the total number of T cells and the number of CD8+, TcR$\alpha\beta$+ and TcR$\gamma\delta$+ T cells at 18 months of age. A higher prenatal as well as postnatal PCB and dioxin exposure was associated with lower monocyte and granulocyte counts at 3 months of age. In conclusion, Dutch background levels of PCB and dioxin exposure influence the human fetal and neonatal immune system.

11.2 Conclusions

Polychlorinated biphenyls (PCBs) and dioxins are widespread environmental hazardous compounds. Although the commercial PCB production was banned worldwide in the early 1980s, and the unwanted by-production of dioxins during combustion processes and manufacturing of organochlorine products was reduced by governments, all 418 mother-infant pairs in this study had detectable levels of both compounds in their body. Although the PCB and dioxin levels in human tissues show a tendency to decline over the last years, the levels we measured from 1990 until 1992 were comparable to the levels measured in 1983 and 1988 by the National Institute of Public Health and Environmental Protection (RIVM) in the Netherlands. This confirms that both compounds, which are stored in the human adipose tissue, are very stable with long half-lives. The levels in Western Europe belong to the highest background levels of the world. PCBs and dioxins cross the placental barrier, so the embryo and fetus is exposed to both toxic compounds during a critical period of organ growth and development. Since human milk contains relatively high levels of PCBs and dioxins, the breast-fed infant is also exposed postnatally to both compounds, a

period of further organ growth and differentiation.

We examined PCB and dioxin levels in mother-infant pairs living in the Rotterdam area, a highly industrialized region in the western part of the Netherlands, and compared them with the levels of pairs living in the Groningen area, a semi-urban region in the northern part of the Netherlands. After correction for covariates the total dioxin level in human milk of the Rotterdam population and some individual PCB and dioxin congener levels were significantly higher compared to levels in the Groningen population. However, the differences were small and it can be concluded that human exposure to PCBs and dioxins by dermal absorption or inhalation is small. It is well known that adults are mainly exposed to these hazardous compounds by dietary intake of meat, fish and dairy products, which are transported throughout the world. Because PCBs and dioxins can be spread by long-range atmospheric transport and since there are undetected sources of both compounds, governments worldwide must cooperate to detect these sources and diminish the PCB and dioxin output in the environment until zero. Governments must also strive for a further decline of the levels in consumption products. Trend measurements of the levels in the environment, alimentary products and human tissues must be continued worldwide.

We tried to predict the infant's postnatal exposure to PCBs and dioxins in human milk, by measuring PCB levels in maternal plasma before birth. Although the correlation coefficients between the levels in both compartments where highly significant for the whole group, the 95% predictive interval was too wide to predict accurately the PCB and dioxin levels to which an individual infant is exposed by breast-feeding. Dioxin measurements by gas chromatography-high-resolution mass spectrometry (GC-HRMS) are time-consuming and expensive. New methods should be developed to measure PCB and dioxin levels in an easier and cheaper way.

Although this study detected no serious, clinical relevant developmental abnormalities, even the Dutch background exposure to relatively low levels of PCBs and dioxins resulted in subtle signs of neurological dysfunctioning, a small delay in psychomotor development, alterations in the thyroid hormone status, and immunologic functions. The mechanisms by which these contaminants interfere are not clear at the moment. A negative effect on the neuro- and psychomotor development might be the result of effects on hormones, neurotransmitters or the developing muscle. The thyroid hormone level alterations detected in this study, lie within the normal ranges and are not directly associated with neurological dysfunctioning.

The mental development of the infants measured with the Bayley Scales of Infant Development and with the Fagan test of Infant Intelligence, was not negatively influenced by the exposure to PCBs and dioxins. Moreover, breast-feeding itself has a positive dose-dependent effect on the mental outcome parameters in this study. Other factors in human milk, like hormones, trophic factors and LCPUFAs are likely to give these positive effects. From the results of this study it is not necessary to discourage breast-feeding, parents can freely choose between breast-feeding and formula-feeding for their infant.

Since this study examined the children up till 18 months of age, follow-up studies on the mental and neurological development have to be done, since the organs and especially the brain continue to grow and develop until an older age.

SAMENVATTING EN CONCLUSIES

Samenvatting

In dit proefschrift worden effecten beschreven van blootstelling aan polygechloreerde biphenylen (PCBs) en dioxinen, voor en na de geboorte, op de vroege ontwikkeling van het kind.

"De Nederlandse PCB en dioxine studie", ook wel genoemd "Het Nederlandse moedermelk project", is een samenwerkingsverband tussen klinische en dierexperimentele onderzoekers. Het klinische deel van de studie dat werd verricht bij menselijke vrijwilligers, moeder-kind paren, werd uitgevoerd door de afdeling kindergeneeskunde van het Sophia Kinderziekenhuis en de Erasmus Universiteit te Rotterdam, en door de afdeling verloskunde/gynaecologie en ontwikkelings-neurologie van de Rijksuniversiteit te Groningen. Het dierexperimentele deel van de studie werd verricht door de afdeling toxicologie van de Landbouw Universitieit in Wageningen, de afdeling biologische toxicologie van TNO in Zeist en de afdeling neurotoxicologie van het Medisch Biologisch Laboratorium (MBL) van TNO in Rijswijk. PCB metingen in humaan bloed werden verricht door het Instituut voor toxicologie en voeding van TNO in Zeist, de PCB en dioxine niveaus in moedermelk werden geanalyseerd door het Rijks-Kwaliteitsinstituut voor land- en tuinbouwprodukten (RIKILT) in Wageningen. De studie werd geïnitieerd en gefinancierd door de Programmacommissie Toxicologie (PCT) en het Stimulerings-programma Geneeskundig Onderzoek (SGO).

Zowel PCBs als dioxinen zijn vetoplosbare, stabiele, toxische stoffen die wijdverspreid in het milieu voorkomen. Volwassen mensen worden voornamelijk aan deze verontreinigingen blootgesteld door het eten van vlees, vis en zuivelprodukten. De mens staat aan het einde van de voedselketen: PCBs en dioxinen worden in zijn vetweefsel opgeslagen. Tijdens de zwangerschap passeren kleine hoeveelheden PCBs en dioxinen de placenta zodat het embryo en de foetus aan deze stoffen worden blootgesteld tijdens een kritische periode van orgaan groei en ontwikkeling. Na de geboorte wordt het kind dat borstvoeding ontvangt, blootgesteld aan relatief hoge gehalten van deze contaminanten. De PCB en dioxine belasting van het kind dat flesvoeding krijgt na de geboorte, is verwaarloosbaar, aangezien dierlijke vetten in flesvoeding vervangen worden door plantaardige vetten, waarin de PCB en dioxine gehalten zeer laag zijn.

Bij dit onderzoek zijn PCB gehalten gemeten in moederlijk bloed en in navelstrengbloed als maat voor de blootstelling van de kinderen tijdens de zwangerschap. De blootstelling aan PCBs en dioxinen na de geboorte werd beoordeeld door het meten van de niveaus in moedermelk en flesvoeding. Een prospectieve longitudinale vervolg studie naar de neurologische ontwikkeling werd verricht bij een groep van 418 à term geboren Nederlandse kinderen, in de tweede week na de geboorte en op de leeftijd van 18 maanden. Dit gedeelte van de studie werd gedaan in samenwerking met de afdeling verloskunde/gynaecologie en ontwikkelingsneurologie van de Rijksuniversiteit in Groningen. Twee honderd en zeven moeder-kind paren woonden in Rotterdam en omgeving, dit is een geïndustrialiseerd gebied in het westelijke deel van Nederland. De andere 211 moeder-kind paren woonden in en rondom de stad Groningen, hetgeen een meer stedelijk gebied is in het noordelijk deel van Nederland. Twee honderd en negen kinderen kregen na de geboorte borstvoeding gedurende een periode van tenminste

6 weken, de andere 209 kinderen vormden de controle groep en kregen flesvoeding uit één batch. De afdeling kindergeneeskunde van het Sophia Kinderziekenhuis/ Erasmus Universiteit Rotterdam onderzocht bij 207 kinderen naast de neurologische ontwikkeling ook het visuele korte termijn geheugen op de leeftijd van 3 en 7 maanden, en de mentale en psychomotore ontwikkeling op de leeftijd van 3, 7 en 18 maanden.

Hoofdstuk 2 beschrijft de gehalten van de PCB pieken 118, 138, 153 en 180 in moederlijk bloed dat is afgenomen in de laatste maand van de zwangerschap, en in navelstrengbloed. Deze gehalten zijn een maat voor de kinderlijke blootstelling aan PCBs tijdens de zwangerschap. In moedermelk monsters, die verzameld werden in de tweede week na de geboorte van het kind, worden de gehalten van de 17 meest toxische dioxine pieken en van 26 verschillende PCB pieken gerapporteerd. Hieruit kan geconcludeerd worden dat de PCB en dioxine gehalten in de Nederlandse bevolking tot de hoogste achtergrond niveaus behoren die wereldwijd gemeten zijn. Ze zijn vergelijkbaar met de gehalten die gemeten zijn in andere geïndustrialiseerde West-Europese landen met een hoge bevolkingsdichtheid. De correlatie coëfficiënten tussen de gehalten van deze toxische stoffen in moederlijk bloed, navelstrengbloed en moedermelk zijn hoog significant. Het 95% betrouwbaarheidsinterval is echter te wijd om nauwkeurig de PCB en dioxine gehalten te kunnen voorspellen waaraan een individueel kind wordt blootgesteld tijdens de zwangerschap of tijdens de borstvoedingsperiode, vanuit de PCB gehalten die in moederlijk bloed werden gemeten. Bij een subpopulatie van de vrouwen die meewerkten aan het onderzoek werden PCB en dioxine gehalten gemeten in moedermelk monsters die verzameld werden in de tweede en zesde week na de geboorte van hun kind. Over deze periode van een maand bestond geen significante daling in dioxine, planair PCB of totaal-TEQ gehalte. Het mono-ortho en di-ortho PCB gehalte in moedermelk was echter wel significant gedaald binnen een maand.

Hoofdstuk 3 beschrijft de verschillen in PCB en dioxine gehalten in bloed en moedermelk monsters die verzameld werden in Rotterdam en omgeving, een geïndustrialiseerd gebied in het westelijk deel van Nederland, ten opzichte van de gehalten in de monsters die verzameld werden in de stad Groningen en omgeving, een stedelijk gebied in het noordelijk deel van Nederland. Nadat gecorrigeerd was voor verstorende factoren, bleek dat de PCB 118 gehalten in moederlijk bloed, het dioxine-TEQ gehalte en gehalten van 10 individuele dioxine en PCB pieken in moedermelk uit het Rotterdamse gebied, significant hoger waren dan in monsters uit het Groningse onderzoeksgebied.

Hoofdstuk 4 beschrijft de invloed van blootstelling aan PCBs en dioxinen op het schildklierhormoon metabolisme, gemeten bij een groep van 105 moeders en hun kinderen die in de omgeving van Rotterdam wonen. Een hogere blootstelling aan PCBs en dioxinen was significant gecorreleerd met lagere TT3 en TT4 gehalten in moederlijk bloed, en met hogere TSH gehalten in kinderlijk bloed dat verzameld werd in de tweede week en 3 maanden na de geboorte. Kinderen die blootgesteld werden aan hogere gehalten van deze toxische stoffen, hadden ook lagere FT4 en TT4 waarden in hun bloed dat verzameld werd in de tweede week na de geboorte. In het algemeen komen deze resultaten overeen met de effekten die gevonden zijn bij ratten en apen. Eén van de voorgestelde mechanismen waarlangs PCBs en dioxinen deze veranderingen in schildklierhormoon metabolisme zouden kunnen geven, is de bezetting van de thyroxine (T4) receptor op het bindingseiwit transthyretine. Een ander mechanisme waardoor deze veranderingen zouden kunnen ontstaan, betreft de inductie van het lever enzym uridine difosfaat-glucuronyltransferase, waardoor de

uitscheiding van T4-glucuronide via de gal wordt verhoogd. Het feit dat het TSH gehalte van de hoger belaste kinderen op de leeftijd van 2 weken en 3 maanden na de geboorte significant verhoogd is, kan een reactie zijn om een euthyreoïde situatie te handhaven nadat zij tijdens de zwangerschap en na de geboorte zijn blootgesteld aan bepaalde PCB en dioxine gehalten. Voldoende schildklierhormoon gehalten zijn nodig voor een normale ontwikkeling van de hersenen. Kleine veranderingen in deze gehalten zouden van invloed kunnen zijn op de ontwikkeling van de foetus en de zuigeling.

Hoofdstuk 5 beschrijft de relatie tussen blootstelling aan PCBs en dioxinen rondom de geboorte, en neurologische afwijkingen bij pasgeborenen in de tweede levensweek. Het neurologisch onderzoek volgens Prechtl werd verricht bij 418 zuigelingen. Hierbij bleken 4 kinderen neurologisch afwijkend te zijn. Twintig kinderen vertoonden lichte neurologische afwijkingen. Er bestond geen significant verband tussen de perinatale blootstelling aan PCBs en dioxinen, en deze klinisch relevante neurologische afwijkingen. Bij de Rotterdamse groep van 207 zuigelingen werd ook de werking van de schildklier beoordeeld aan de hand van schildklierhormoon gehalten in het bloed. Er bestond geen significante relatie tussen de schildklierhormoon waarden en de uitkomsten van het neonatale neurologisch onderzoek.

Hoofdstuk 6 beschrijft de neurologische optimaliteitsscore van 418 kinderen die werden onderzocht volgens het neurologisch onderzoek van Prechtl, in de tweede week na de geboorte, in relatie tot de blootstelling aan PCBs en dioxinen rondom de geboorte. Met deze techniek bleek dat blootstelling aan hogere PCB en dioxine gehalten gerelateerd was aan lagere neonatale neurologische optimaliteitsscores, en een hogere incidentie van hypotonie. Aangezien in hoofdstuk 5 beschreven werd dat er geen relatie bestond met klinisch relevante neurologische afwijkingen, kan geconcludeerd worden dat blootstelling aan PCBs en dioxinen tijdens de zwangerschap en kort na de geboorte, subtiele tekenen van neurologische dysfunctie geeft, echter zonder klinische relevantie.

Hoofdstuk 7 beschrijft de effekten van blootstelling aan PCBs en dioxinen op het visuele korte termijn geheugen van kinderen op de leeftijd van 3 en 7 maanden. Dit onderzoek werd verricht bij 207 kinderen, die in Rotterdam en omgeving wonen. Voor deze metingen werd de Fagan Test gebruikt die een actuele maat voor het visuele korte termijn geheugen geeft, en een voorspellende waarde voor latere cognitieve ontwikkeling. Op geen van beide leeftijden konden negatieve effecten op het visuele korte termijn geheugen gevonden worden ten gevolge van de blootstelling aan PCBs en dioxinen voor de geboorte, dan wel tijdens de periode van borstvoeding. Echter op de leeftijd van 7 maanden bleken de borstgevoede kinderen significant hogere scores te hebben met deze test in vergelijking met de flesgevoede kinderen. Bovendien bleek het visuele korte termijn geheugen van de kinderen op deze leeftijd positief gecorreleerd te zijn met de duur van de borstvoeding. Hoewel uit dierexperimenteel onderzoek bekend is dat lage doseringen dioxinen een faciliterend effekt kunnen hebben op geheugen funkties, is het meer aannemelijk om te veronderstellen dat vetten, zoals LCPUFAs of andere lipophiele stoffen als hormonen of trofische faktoren in moedermelk, verantwoordelijk zijn voor de betere uitkomsten van de borstgevoede kinderen.

Hoofdstuk 8 beschrijft de effekten van de perinatale blootstelling aan PCBs en dioxinen op de mentale en psychomotore ontwikkeling, gemeten met de Bayley Test op de leeftijd van 3, 7 en 18 maanden, bij de Rotterdamse groep van 207 kinderen. Op de leeftijd van 3 maanden bleek dat een hogere blootstelling aan PCBs tijdens de

zwangerschap significant geassocieerd was met lagere psychomotore scores: een verdubbeling van de PCB belasting van het kind resulteerde in een score daling van 3 punten. Borstgevoede kinderen scoorden significant hoger op de psychomotore schaal op de leeftijd van 7 maanden, in vergelijking met flesgevoede kinderen. Echter, de psychomotore score van de 66% hoogst belaste, borstgevoede kinderen (> 756 pg total PCB-dioxin TEQ) werd negatief beïnvloed door deze PCB en dioxine gehalten in moedermelk, en was vergelijkbaar met de psychomotore score van de flesgevoede kinderen. Aangenomen wordt dat moedermelk een positief effekt heeft op de motorische ontwikkeling omdat hierin stoffen aanwezig zijn zoals bepaalde vetten, hormonen en trofische factoren die ontbreken in flesvoeding. Echter, wanneer het PCB en dioxine gehalte in moedermelk een bepaald gehalte overschreidt, kunnen deze toxische stoffen een nadelig effekt hebben op de psychomotore ontwikkeling waardoor de positieve effekten van borstvoeding opgeheven worden. Borstgevoede kinderen scoorden hoger op de mentale schaal van de Bayley Test op de leeftijd van 7 maanden, dit effekt was dosis-afhankelijk. Aangenomen wordt dat ook deze positieve effekten bewerkstelligd worden door bepaalde stoffen die in borstvoeding aanwezig zijn, zoals vetten of andere lipophiele stoffen. Er was geen significante invloed van de blootstelling aan PCBs en dioxinen op de mentale ontwikkeling op de leeftijd van 3 en 7 maanden. Op 18 maanden bleek noch de mentale, noch de psychomotore ontwikkeling gerelateerd te zijn aan de perinatale blootstelling aan PCBs en dioxinen. Op deze leeftijd waren omgevings en sociaal-economische factoren meer van invloed op de uitkomsten.

Hoofdstuk 9 beschrijft de neurologische optimaliteitsscore van 418 kinderen gemeten op de leeftijd van 18 maanden, in relatie tot de perinatale expositie aan PCBs en dioxinen. Na correctie voor verstorende factoren, bleek dat de blootstelling aan PCBs gedurende de zwangerschap negatief gerelateerd was aan de neurologische conditie op de leeftijd van 18 maanden. Er bestond echter geen relatie met klinisch relevante neurologische afwijkingen. Op de neurologische optimaliteitsscore kon geen negatieve invloed gevonden worden van de blootstelling aan PCBs en dioxinen gedurende de borstvoedingsperiode. Daarnaast werd een positief effect gevonden van moedermelk op de "fluency" van de bewegingen.

Hoofdstuk 10 beschrijft effecten van perinatale expositie aan PCBs en dioxinen op de immunologische functies die gemeten werden bij een subgroep van de Rotterdamse kinderen. Het aantal perioden van verkoudheid, bronchitis, tonsillitis en middenoorontsteking gedurende de eerste 18 maanden werd gebruikt als een maat voor de gezondheidstoestand van het kind. De humorale immuniteit werd beoordeeld op de leeftijd van 18 maanden door het bepalen van antilichaam titers tegen het bof, mazelen en rodehond virus, als reactie op vaccinatie. Hoeveelheden witte bloed cellen en immunologische markers werden gemeten in navelstrengbloed en in bloed dat verzameld werd op de leeftijd van 3 en 18 maanden bij een groep van 55 kinderen. Er werd geen significant verband gevonden tussen de perinatale blootstelling aan PCBs en dioxinen, en ziekten van de bovenste of onderste luchtwegen, of veranderingen in de humorale antilichaam productie. Er bestond wel een relatie tussen een hogere expositie aan deze toxische stoffen tijdens de zwangerschap, en een toename in het aantal TcR$\gamma\delta$+ T cellen bij de geboorte, en het totale aantal T cellen, CD8+, TcR$\alpha\beta$+ en TcR$\gamma\delta$+ T cellen op de leeftijd van 18 maanden. Een hogere expositie aan PCBs en dioxinen zowel tijdens de zwangerschap als tijdens de borstvoedingsperiode was geassocieerd met lagere hoeveelheden monocyten en granulocyten op de leeftijd van 3 maanden. Hieruit kan geconcludeerd worden dat de Nederlandse achtergrond expositie aan PCBs en

dioxinen een invloed heeft op het foetale en neonatale immuunsysteem van de mens.

Conclusies

Polygechloreerde biphenylen (PCBs) en dioxinen zijn toxische stoffen die wijdverspreid in de omgeving voorkomen. Na het bekend worden van schadelijke effekten van deze stoffen, werd de commerciële produktie van PCBs grotendeels verboden, in het begin van de jaren tachtig. Daarnaast werd de ongewenste bijproduktie van dioxinen tijdens afvalverbrandingsprocessen en tijdens de vervaardiging van organische chloor produkten, door het ingrijpen van verschillende overheden, gereduceerd. Desondanks werden bij alle 418 moeders en hun kinderen die meewerkten aan dit onderzoek, detecteerbare hoeveelheden van beide stoffen in het lichaam aangetroffen. Hoewel de PCB en dioxine gehalten in menselijke weefsels een dalende trend vertonen over de laatste jaren, waren de gehalten die wij hebben gemeten tussen 1990 en 1992, vergelijkbaar met de gehalten die werden gemeten in Nederland door het RIVM tussen 1983 en 1988. Hiermee wordt bevestigd dat beide stoffen die met name worden opgeslagen in menselijk vetweefsel erg stabiel zijn en lange half-waarde tijden hebben. De PCB en dioxine gehalten die zijn gemeten in West-Europa behoren tot de hoogste achtergrondbelasting niveaus in de wereld. Zowel PCBs als dioxinen passeren de placenta, zodat het embryo en de foetus worden blootgesteld aan beide toxische stoffen, tijdens een kritieke periode van orgaan groei en differentiatie.

PCB en dioxine gehalten werden bepaald in bloed en moedermelk van 207 vrouwen en hun kinderen die in Rotterdam en omgeving wonen, hetgeen een gebied is met veel industrie. Deze uitkomsten werden vergeleken met de PCB en dioxine gehalten van 211 vrouwen en kinderen die in de omgeving van Groningen wonen, hetgeen een meer stedelijk gebied is. Na correctie voor verstorende factoren, bleek dat het totale dioxine gehalte en bepaalde individuele PCB en dioxine pieken in moedermelk en bloed van de Rotterdamse vrouwen, significant hoger waren in vergelijking met de gehalten van de Groningse vrouwen. De verschillen tussen beide regio's waren echter klein, zodat geconcludeerd kan worden dat menselijke blootstelling aan PCBs en dioxinen via huid absorptie of inhalatie van lucht gering is. Het is bekend dat volwassenen met name aan deze stoffen worden blootgesteld door het eten van vlees, vis en zuivelprodukten, die over de gehele wereld getransporteerd worden. Aangezien PCBs en dioxinen over een grote afstand verspreid kunnen worden via atmosferisch transport, en aangezien er vele nog niet ontdekte bronnen zijn van beide stoffen, moeten regeringen wereldwijd samenwerken om deze bronnen op te sporen en de PCB en dioxine uitstoot in de omgeving tot minimale gehalten te reduceren. Tevens moeten zij streven naar een verdere daling van de gehalten in consumptie produkten. Trend metingen van de gehalten in het milieu, etenswaren en menselijke weefsels moeten wereldwijd gecontinueerd worden.

Wij hebben geprobeerd om de blootstelling van de zuigeling aan PCBs en dioxinen tijdens de borstvoedingsperiode te voorspellen, door PCB gehalten te meten in het bloed van de moeder voor de geboorte. Hoewel de correlaties tussen deze gehalten in moederlijk bloed en moedermelk voor de hele groep hoog significant bleken te zijn, was het 95% betrouwbaarheidsinterval te wijd om nauwkeurig de PCB en dioxine gehalten te kunnen voorspellen waaraan het

individuele kind zou worden blootgesteld tijdens borstvoeding. Dioxine metingen met behulp van gas chromatografische-hoge-resolutie massa spectrometrie (GC-HRMS) zijn tijdrovend en duur. Er moeten nieuwe methoden ontwikkeld worden om PCB en dioxine gehalten op een eenvoudiger en goedkopere manier te kunnen bepalen.

Alhoewel deze studie geen ernstige, klinisch relevante afwijkingen in de ontwikkeling van het jonge kind heeft aangetoond, resulteerde de blootstelling van Nederlandse kinderen aan relatief lage achtergrond niveaus van PCBs en dioxinen toch in subtiele tekenen van neurologische dysfunktie, een geringe achterstand in psychomotore ontwikkeling, veranderingen in het schildklierhormoon metabolisme en immunologische funkties. De mechanismen waardoor deze toxische stoffen interfereren zijn op dit moment nog niet duidelijk. Een negatief effect op de neurologische en psychomotore ontwikkeling zou het resultaat kunnen zijn van effecten op hormoon of neurotransmitter gehalten, of effekten op de zich ontwikkelende spieren. De veranderingen in schildklierhormoon gehalten die in deze studie beschreven zijn, lagen binnen de normale grenzen en zijn niet direct geassocieerd met neurologische dysfunktie.

De mentale ontwikkeling van de kinderen, die werd beoordeeld met de Bayley Schalen en met de Fagan test, werd niet nadelig beïnvloed door de blootstelling aan PCBs en dioxinen. In tegendeel, borstvoeding zelf had een positief, dosis-afhankelijk effekt op de uitkomsten van de mentale testen in dit onderzoek. Het is aannemelijk dat bepaalde faktoren in moedermelk zoals hormonen, trofische faktoren of bepaalde vetten zoals lange-keten meervoudig onverzadigde vetzuren (LCPUFAs), of andere nog onbekende stoffen die afwezig zijn in flesvoeding, verantwoordelijk zijn voor deze positieve effekten.

Uitgaande van de resultaten van dit onderzoek is het niet nodig om het geven van borstvoeding in Nederland te ontraden. Ouders kunnen zelf de vrije keuze blijven maken of zij hun kind borstvoeding danwel flesvoeding willen geven.

Aangezien in deze studie kinderen zijn onderzocht tot de leeftijd van 18 maanden, moeten vervolgstudies worden gedaan naar de mentale en neurologische ontwikkeling op latere leeftijd, daar de organen en met name de hersenen nog verder groeien en differentiëren na de leeftijd van 18 maanden.

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CURRICULUM VITAE

Corine Koopman-Esseboom

5 november 1963	Geboren te Dordrecht.
1976 - 1982	Gymnasium ß opleiding aan Het Christelijk Lyceum te Dordrecht.
1982 - 1988	Studie Geneeskunde aan de Erasmus Universiteit te Rotterdam. Artsexamen september 1988.
mei - oktober 1988	Keuzeonderzoek: "Psychomotorische ontwikkeling van kinderen geboren in 1986 met een geboortegewicht van meer dan 1500 gram en beademd op de afdeling Neonatologie van het Sophia Kinderziekenhuis te Rotterdam", begeleider Dr. W.P.F. Fetter.
oktober 1988 - mei 1989	Assistent Kindergeneeskunde niet in Opleiding, Sophia Kinderziekenhuis te Rotterdam, Prof. Dr. H.K.A. Visser.
mei 1989 - november 1989	Assistent Kindergeneeskunde niet in Opleiding, Zuiderziekenhuis te Rotterdam, Dr. R.N. Sukhai
november 1989 - april 1994	Promotieonderzoek: "Effekten van perinatale blootstelling aan PCBs en dioxinen op de vroege ontwikkeling van de mens". Dierexperimentele en humane studie in samenwerking met de afdeling obstetrie en ontwikkelingsneurologie van de Rijks Universiteit te Groningen, de afdeling toxicologie van de Landbouw Universiteit te Wageningen, de afdeling biologische toxicologie en het instituut voor toxicologie en voeding van TNO te Zeist, de afdeling neurotoxicologie van MBL-TNO te Rijswijk en het RIKILT te Wageningen.
april 1990	Studiereis naar de USA voor overleg van de studie opzet met de onderzoeksgroepen van Dr. J.L. en S.W. Jacobson (Detroit), Dr. W.J. Rogan en Dr. B.C. Gladen (Research Triangle Park, NC) en Dr. J.F. Fagan (Cleveland).
april 1994 tot heden	Opleiding tot kinderarts, Sophia Kinderziekenhuis te Rotterdam, opleider Prof. Dr. H.K.A. Visser, tot 1 juli 1995.