Effects of perinatal PCB and dioxin exposure and early feeding mode on child development
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Chapter 5

Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age

ABSTRACT

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

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

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

Conclusions. In utero exposure to background PCB concentrations is associated with poorer cognitive functioning in preschool children. Children of mothers at the upper end of exposure are especially at risk. Therefore, maternal PCB body burden should be reduced, rather than discouraging breast-feeding.
INTRODUCTION

Polychlorinated biphenyls (PCBs), chlorinated dibenzo-p-dioxins (PCDDs) and dibenzo-furans (PCDFs) are environmentally persistent organic pollutants and believed to be neurotoxic\(^1\)\(^2\). The highly lipophilic PCBs and dioxins (PCDDs and PCDFs) tend to partition into soil and sediment, bioconcentrate from water to aquatic animal, and accumulate in the food chain\(^3\). Humans are high on the food chain; exposure to PCBs and dioxins is mainly from meat, dairy products and fish\(^2\). After ingestion, these compounds accumulate in adipose tissue. Their elimination depends on metabolic degradation - slight or nil for most congeners - and on the rate of excretion which is almost via the faeces. As a result, the half life is very long, in the order of a decade\(^2\). The human fetus is exposed to PCBs and dioxins through placental transport\(^4\) and larger quantities are transferred to the infant during breast-feeding\(^5\)\(^6\).

Rice oil contaminated with PCBs, PCDFs and a small amount of PCDDs was used in cooking and ingested by over 1850 individuals in Japan in 1968 (Yusho)\(^7\) and over 2000 persons in Taiwan in 1979 (Yu-Cheng) in remarkably similar incidents\(^8\). These exposures caused illness similar to those documented in animal studies\(^2\). Rice oil-poisoned mothers reported lower birthweight, hyperpigmentation, conjunctivitis, nail changes and natal teeth in their offspring and delay in developmental milestones\(^8\). Follow-up studies of Yu-Cheng children at school age showed lower IQs\(^9\), behavioral effects\(^10\) and growth delay\(^11\).

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

Contamination of breast milk with PCBs and dioxins in The Netherlands belong to the highest measured in the world\(^19\). A prospective follow-up study was launched by the Dutch government in 1989, the ‘Dutch PCB/Dioxin-Breast Milk Study’, to investigate possible adverse effects of PCBs and dioxins on growth and development of healthy term born babies. The follow-up at preschool age was funded by an European
collaborative study. Previous results showed that prenatal PCB exposure was related to a lower birth weight\textsuperscript{20}, lower growth rate\textsuperscript{20}, and lower psycho-motor scores at 3 months of age\textsuperscript{21}, as well as a poorer neurological condition at birth\textsuperscript{22} and 18 months\textsuperscript{23}. Postnatal PCB and dioxin exposure was related to lower psychomotor development at 7 months\textsuperscript{21}. The effects of environmental PCB and dioxin exposure on cognitive abilities assessed at 42 months of age are presented in this paper.

METHODS

Subjects
From June 1990 until June 1992, healthy pregnant women living in Rotterdam and Groningen were asked by their obstetrician or midwife to participate in a prospective, longitudinal neuro-developmental study. The Rotterdam area is a highly industrialized and densely populated region situated in the western part of The Netherlands, and the Groningen area is a semi-urban region in the north. To study the effects of pre- as well as postnatal PCB and dioxin exposure, women were included who intended to breast-feed their child for at least 6 weeks (breast-fed group) in addition to women who intended to use formula-feeding (formula-fed group). All formula-fed infants received formula from a single batch (Almiron M2, Nutricia NV, The Netherlands) from birth until 7 months of age. In this formula, concentrations of both PCBs and dioxins were not detectable. Further inclusion criteria were: 1) Pregnancy and delivery had to be without complications or serious illnesses. Instrumental deliveries or caesarian sections were excluded. 2) First or second- born infants. 3) Born at term, 37-42 weeks of gestation. 4) No congenital anomalies or diseases. 5) Caucasian race. The Medical Ethics Committees of both University Hospitals approved the study protocol. Informed consent was given by participating parents. Children were examined for their growth and neuro-development at the ages of 2 weeks, 3-, 7-, 18- and 42 months. Details of the study design, chemical analysis, PCB and dioxin concentrations, as well as results up to 18 months have been published elsewhere\textsuperscript{6, 20-24}.

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

At two weeks after delivery a 24-hour representative breast milk
sample was collected from each breast-feeding mother, and analyzed for
17 dioxins (PCDDs and PCDFs), 6 dioxin-like PCBs (IUPAC Nos. 77, 105,
118, 126, 156, and 169) and 20 non-dioxin like PCBs (IUPAC Nos. 28, 52,
66, 70, 99, 101, 128, 137, 138, 141, 151, 153, 170, 177, 180, 183, 187,
194, 195, and 202). To express the toxic potency of the mixture of dioxins
and dioxin-like PCBs, the toxic equivalent factor (TEF) approach was used
according to the latest WHO-meeting, June 1997\textsuperscript{25}. The toxic equivalents
(TEQ) were calculated by multiplying the concentration of each congener
by its TEF value. PCBs and dioxins measured in breast milk shortly after
birth are an index of the maternal PCB and dioxin body burden. Breast
milk PCB and dioxin concentrations are therefore an indirect measure of
prenatal exposure\textsuperscript{5}.

Prenatal PCB exposure was estimated from \(\sum_{PCB}\) concentrations
in maternal plasma and cord plasma. In the breast-fed group, prenatal
exposure to dioxin-TEQs, dioxin-like PCB-TEQs and non-dioxin like PCBs
(sum of 20 PCB congeners) was assessed from breast milk
concentrations. Three measures of lactational exposure were calculated by
multiplying breast milk concentrations with the number of weeks of breast-
feeding. 1) TEQ exposure, the sum of dioxin-TEQs and PCB-TEQs. 2) The
sum of PCB 118, 138, 153, and 180 (\(\sum_{PCB}\)). 3) The sum of 20 non-dioxin
like PCBs. Current PCB body burden was estimated from the \(\sum_{PCB}\)
measured in 42-month plasma samples.

Cognitive abilities at 42 months
The child’s level of intellectual functioning at 42 months of age was
measured with the Dutch version of the Kaufman Assessment Battery for
Children (Dutch K-ABC)\textsuperscript{26}. The Dutch K-ABC consists of 11 subtests and is
standardized for a large sample of normal preschool children in the 2.5 to
4.5 year range. Raw scores of each subtest are transformed into
normalized standard scores with a mean (± standard deviation (SD)) of 10
± 3. Factor analysis of the 11 subtests yielded two scales, both considered
to be equally vital to intellectual functioning. 1) the sequential processing
scale - the average of the Hand Movements, Number Recall, Arithmetic,
Gross and Fine motor skills subtests. 2) The simultaneous processing scale - the average of the Magic Window, Face Recognition, Gestalt Closure, Vocabulary, Faces & Places and Riddles subtests. The standard scores were summed and a scale score was calculated. The combined scale score of the sequential and simultaneous processing scale form the overall cognitive scale score. All three scale scores are normalized to a mean (± SD) of 100 ± 15.

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

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

Covariables
The K-ABC was administered by two trained examiners (S.P. and C.I.L.), one in each study centre. They were both not aware of the prenatal, lactational, and current exposure values, nor were they aware of the feeding mode given during infancy. To adjust for inter-observer and between-centre differences, study centre/examiner was included as a covariable. Covariables known to be related to child development were selected from a list containing data on socio-economic background, obstetrical and neonatal history, maternal age, parents’ education level, parity, gender, fetal exposure to alcohol and cigarette smoking, type of feeding during infancy, and the breast-feeding period. Since small numbers of subjects were in the categories of smoking or alcohol use during pregnancy, dichomotizing these covariables into no/yes was justified. The child’s home environment was assessed by the Dutch version of the Home Observation for Measurement of the Environment (HOME). The verbal IQ of the parent, most often with the child (usually
the mother), was assessed by 2 subtests; Information and Vocabulary, from the Dutch Wechsler Adult Intelligence Scale (WAIS)\(^\text{29}\). These two subtests exhibit good correlations with the verbal IQ scale\(^\text{29}\).

**Statistical analysis**

To compare groups for a single variable we used the chi-square test, the Student's t-test and the Mann-Whitney test. Plasma PCB values were positively skewed and therefore normalized by natural logarithm (ln\(\sum\)PCB). The effects of prenatal, lactational, and current exposure to PCBs and dioxins, on cognitive abilities at 42 months of age were studied by multiple linear regression analyses, adjusted for covariables. Dependent variables were scores on the overall cognitive scale, the sequential and simultaneous processing scale of the K-ABC, and the verbal comprehension scale of the RDLS. Each outcome variable was analyzed with each exposure variable separately in a regression analysis. Results were considered to be significant if \(p \leq 0.05\).

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

The effect of prenatal PCB exposure measured from maternal ln\(\sum\)PCB and cord ln\(\sum\)PCB on outcome variables, were examined in the whole group as well as in the formula-fed group. The formula-fed group represents children who were predominantly exposed to PCBs prenatally. In addition, the effect of prenatal exposure to dioxin-TEQs, dioxin-like PCB-TEQs as well as non-dioxin like PCBs measured from breast milk concentrations was studied in the breast-fed group. The effect of lactational exposure to PCB- and dioxin-TEQs, \(\sum\)PCB in breast milk and non-dioxin like PCBs was also studied in the breast fed group. The effect of 42-month PCB body burden on cognitive abilities was investigated in the whole group as well as both feeding groups separately.
RESULTS

From the original cohort of 418 children, 209 were breast-fed (BF) and 209 were formula-fed (FF) during infancy. Two hundred and seven subjects are from the Rotterdam area and 211 subjects from the Groningen area. At 42 months of age, 395 (94%) subjects were re-examined for their neuro-developmental follow-up. Six percent (n=23) was lost to follow up, due to lack of interest (n=19) and emigration (n=4). Another 15 children failed to cooperate with the testing procedure, refusing to respond to some of the 11 subtests, and were excluded from the final analysis. The excluded children did not differ from the others in the sample in terms of PCB and dioxin concentrations. The RDLS was completed in all children from the Rotterdam cohort. Between the two study centers - Rotterdam (n=193) and Groningen (n=202) - no differences were found with respect to PCB concentrations measured in maternal-, cord-, and 42-month-old plasma, nor in breast milk PCB and dioxin concentrations.

In table 1, exposure variables are listed. Three maternal plasma samples were missing. In 382 cord plasma samples, concentrations of PCB IUPAC nos. 138, 153 and 180 were analyzed. Nine cord samples were missing for the analysis of PCB 118. In human milk, dioxin-TEQ, mono-ortho PCB-TEQ, and planar PCB-TEQ concentrations were available for 176, 195, and 194 milk samples, respectively. Blood samples for PCB analysis at 42 months were available in 299 (76%) children. Median $\sum$PCB concentration at 42 months of age in the BF group is nearly 4 times higher than median $\sum$PCB concentration in the FF group.

In table 2, covariables are presented according to prenatal PCB exposure estimated from maternal plasma $\sum$PCB concentrations, divided 5 groups of exposure. Maternal age, parental education, verbal IQ, and HOME score are higher in the highest exposed group. In table 3, the mean scores $\pm$ SD on the three scales of the K-ABC and verbal comprehension scale of the RDLS are given for the whole group as well as both feeding groups. All scores are within or higher than the mean $\pm$ SD population score of 100 $\pm$ 15. Children in the BF group had higher mean scores on the K-ABC and the RDLS when compared with the FF group (t-test, all p-values <0.01, table 3). After adjustment for covariables, the outcome scores were not significantly different between the two feeding groups.
Table 1: Concentrations of polychlorinated biphenyls and dioxins measured in plasma and in breast milk samples.

<table>
<thead>
<tr>
<th>Concentration percentiles</th>
<th>P5</th>
<th>P50</th>
<th>P95</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma (µg/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΣPCB maternal* (n=415)</td>
<td>1.00</td>
<td>2.04</td>
<td>3.81</td>
</tr>
<tr>
<td>ΣPCB cord* (n=373)</td>
<td>0.18</td>
<td>0.38</td>
<td>0.86</td>
</tr>
<tr>
<td>ΣPCB 42 months* (n=299)</td>
<td>0.11</td>
<td>0.35</td>
<td>1.54</td>
</tr>
<tr>
<td>-breast-fed group (n=154)</td>
<td>0.29</td>
<td>0.78</td>
<td>1.90</td>
</tr>
<tr>
<td>-formula-fed group (n=145)</td>
<td>0.10</td>
<td>0.20</td>
<td>1.49</td>
</tr>
<tr>
<td><strong>Measured in breast milk (breast-fed group, n=209)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΣPCB* (µg/kg milk fat) (n=193)</td>
<td>205</td>
<td>405</td>
<td>723</td>
</tr>
<tr>
<td>Non-dioxin like PCBs‡ (µg/kg milk fat)(n=193)</td>
<td>269</td>
<td>545</td>
<td>914</td>
</tr>
<tr>
<td>Mono-ortho PCB-TEQ† (ng/kg fat) (n=195)</td>
<td>6.8</td>
<td>14.2</td>
<td>24.8</td>
</tr>
<tr>
<td>Planar PCB-TEQ† (ng/kg fat) (n=194)</td>
<td>7.1</td>
<td>14.4</td>
<td>31.7</td>
</tr>
<tr>
<td>Dioxin-TEQ† (ng/kg fat) (n=176)</td>
<td>17.2</td>
<td>33.5</td>
<td>59.5</td>
</tr>
</tbody>
</table>

* sum of polychlorinated biphenyls (ΣPCBs) IUPAC nos. 118, 138, 153, and 180 measured in plasma and breast milk. † TEQ toxic equivalents according to the 1997 WHO TEF values for mono-ortho PCBs IUPAC nos. 105, 118, and 156; planar PCBs IUPAC nos. 77, 126, and 169; seventeen 2,3,7,8 substituted polychlorinated dibenzo-dioxins (PCDDs) and furans (PCDFs), ‡ sum of 20 non-dioxin like PCBs in breast milk.

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

Adjusted mean scores and standard errors of the mean (SEM) for the cognitive scale on the K-ABC at 42 months of age for the whole group according to prenatal PCB exposure are presented in figure 1a. Five cut off points are given for maternal plasma $\Sigma$PCB, based upon the range of plasma $\Sigma$PCB concentration. The mean score on the cognitive scale in the highest exposed group ($\Sigma$PCB maternal $\geq$ 3 µg/l) is 4 points lower compared to the lowest exposed group ($\Sigma$PCB maternal < 1.5 µg/l). In figures 1b and 1c, similar dose-response relationships are presented for the sequential and simultaneous processing scale. A 4-point deficit on both processing scales is also calculated for the highest exposed group.

In the BF group, effects of prenatal exposure to dioxin-TEQs, dioxin-like PCB-TEQs and non-dioxin like PCBs was examined. After adjustment for the same set of covariables, no negative effects of prenatal TEQ exposure nor prenatal PCB exposure on performance of the K-ABC and the RDLS were found. Lactational exposure to non-dioxin like PCBs, dioxin like PCB-TEQs, and dioxin-TEQs were also not related to performance on the K-ABC and the RDLS (results not presented). Current PCB body burden, measured from ln$\Sigma$PCB in 42-month-old plasma samples, was not related with cognitive abilities in the whole group (table 4) as well as the two feeding groups separately.
Table 2: Characteristics of the study population according to PCB concentrations measured in maternal plasma during pregnancy.

<table>
<thead>
<tr>
<th>Characteristics*</th>
<th>&lt;1.5 µg/l</th>
<th>1.5-1.99 µg/l</th>
<th>2.0-2.49 µg/l</th>
<th>2.5-2.99 µg/l</th>
<th>≥3.0 µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>At birth</td>
<td>n=90</td>
<td>n=105</td>
<td>n=86</td>
<td>n=66</td>
<td>n=68</td>
</tr>
<tr>
<td>Study center, Rotterdam</td>
<td>47 (52%)</td>
<td>46 (44%)</td>
<td>42 (59%)</td>
<td>31 (47%)</td>
<td>40 (59%)</td>
</tr>
<tr>
<td>Feeding type, breast-fed</td>
<td>28 (31%)</td>
<td>47 (45%)</td>
<td>49 (57%)</td>
<td>41 (62%)</td>
<td>42 (62%)</td>
</tr>
<tr>
<td>Breast-feeding period (wks)</td>
<td>22 (6-78)</td>
<td>17 (6-56)</td>
<td>22 (9-54)</td>
<td>16 (6-56)</td>
<td>20 (6-62)</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>27±4</td>
<td>28±4</td>
<td>29±3</td>
<td>31±3</td>
<td>32±3</td>
</tr>
<tr>
<td>Maternal smoking during pregnancy, yes</td>
<td>30 (33%)</td>
<td>27 (26%)</td>
<td>18 (21%)</td>
<td>18 (27%)</td>
<td>15 (22%)</td>
</tr>
<tr>
<td>Maternal alcohol use during pregnancy, yes</td>
<td>15 (17%)</td>
<td>19 (18%)</td>
<td>19 (22%)</td>
<td>30 (45%)</td>
<td>33 (45%)</td>
</tr>
<tr>
<td>Gender, male</td>
<td>46 (51%)</td>
<td>44 (42%)</td>
<td>40 (42%)</td>
<td>31 (47%)</td>
<td>34 (50%)</td>
</tr>
<tr>
<td>Birth order, first born</td>
<td>45 (50%)</td>
<td>52 (50%)</td>
<td>45 (52%)</td>
<td>30 (45%)</td>
<td>28 (41%)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>40.5±1.0</td>
<td>40.4±1.2</td>
<td>40.4±1.1</td>
<td>40.4±1.2</td>
<td>39.9±1.3</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>3567±456</td>
<td>3533±429</td>
<td>3519±402</td>
<td>3468±418</td>
<td>3470±523</td>
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<tr>
<td></td>
<td>n=83</td>
<td>n=103</td>
<td>n=82</td>
<td>n=61</td>
<td>n=65</td>
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<td>--------------------------------</td>
<td>------</td>
<td>-------</td>
<td>------</td>
<td>------</td>
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</tr>
<tr>
<td><strong>Maternal education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low</td>
<td>39 (46%)</td>
<td>25 (24%)</td>
<td>11 (13%)</td>
<td>6 (10%)</td>
<td>5 (8%)</td>
</tr>
<tr>
<td>medium</td>
<td>31 (37%)</td>
<td>47 (46%)</td>
<td>25 (30%)</td>
<td>18 (29%)</td>
<td>21 (32%)</td>
</tr>
<tr>
<td>high</td>
<td>15 (18%)</td>
<td>31 (30%)</td>
<td>47 (57%)</td>
<td>38 (61%)</td>
<td>39 (60%)</td>
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<tr>
<td><strong>Paternal education</strong></td>
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<tr>
<td>low</td>
<td>42 (50%)</td>
<td>31 (31%)</td>
<td>13 (16%)</td>
<td>11 (18%)</td>
<td>10 (15%)</td>
</tr>
<tr>
<td>medium</td>
<td>18 (21%)</td>
<td>30 (30%)</td>
<td>23 (28%)</td>
<td>14 (22%)</td>
<td>10 (15%)</td>
</tr>
<tr>
<td>high</td>
<td>24 (29%)</td>
<td>40 (39%)</td>
<td>47 (57%)</td>
<td>37 (60%)</td>
<td>45 (70%)</td>
</tr>
<tr>
<td>HOME</td>
<td>38±4</td>
<td>39±3</td>
<td>39±4</td>
<td>40±2</td>
<td>40±3</td>
</tr>
<tr>
<td><strong>Parental verbal IQ</strong></td>
<td>111±18</td>
<td>114±15</td>
<td>123±16</td>
<td>123±12</td>
<td>125±13</td>
</tr>
</tbody>
</table>

*Values are numbers (percentages), means ± standard deviations or medians (range). Maternal and paternal education: Low, Primary school finished or secondary school not finished; Medium, secondary school finished; High, high school finished or professional/university training. Parental verbal IQ: two subtests of the Wechsler Adult Intelligence Scale assessed from the parent. HOME, home observation for the measurement of the environment. ∑PCB, sum of the polychlorinated biphenyl congeners IUPAC nos. 118, 138, 153, and 180 measured in maternal plasma during last month of pregnancy.*
Table 3: In utero exposure to polychlorinated biphenyls (lnΣPCBmaternal*) in relation to cognitive abilities at 42 months.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>mean±SD</th>
<th>n</th>
<th>regression coefficient (SE)§</th>
<th>p-value</th>
<th>R² whole model</th>
<th>Residual SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-ABC†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cognitive developmental scale</td>
<td>111±14</td>
<td>373</td>
<td>-4.56(1.62)</td>
<td>&lt;0.01</td>
<td>0.42</td>
<td>10.7</td>
</tr>
<tr>
<td>-Sequential processing subscale</td>
<td>109±14</td>
<td>373</td>
<td>-4.16(1.79)</td>
<td>0.02</td>
<td>0.32</td>
<td>11.9</td>
</tr>
<tr>
<td>-Simultaneous processing subscale</td>
<td>109±14</td>
<td>384</td>
<td>-3.82(1.60)</td>
<td>0.02</td>
<td>0.38</td>
<td>10.9</td>
</tr>
<tr>
<td>RDLS‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal comprehension scale</td>
<td>105±12</td>
<td>190</td>
<td>-3.36(1.91)</td>
<td>0.08</td>
<td>0.40</td>
<td>9.4</td>
</tr>
<tr>
<td>Breast-fed group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-ABC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cognitive developmental scale</td>
<td>114±12</td>
<td>195</td>
<td>-2.20(2.14)</td>
<td>0.30</td>
<td>0.33</td>
<td>10.6</td>
</tr>
<tr>
<td>-Sequential processing subscale</td>
<td>111±13</td>
<td>195</td>
<td>-1.49(2.46)</td>
<td>0.54</td>
<td>0.25</td>
<td>12.1</td>
</tr>
<tr>
<td>-Simultaneous processing subscale</td>
<td>112±12</td>
<td>198</td>
<td>-2.45(2.18)</td>
<td>0.26</td>
<td>0.28</td>
<td>10.8</td>
</tr>
<tr>
<td>RDLS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal comprehension scale</td>
<td>108±11</td>
<td>100</td>
<td>-0.20(2.74)</td>
<td>0.94</td>
<td>0.38</td>
<td>9.3</td>
</tr>
</tbody>
</table>
Table 3 continued

**Formula-fed group**

<table>
<thead>
<tr>
<th>K-ABC</th>
<th>Cognitive developmental scale</th>
<th>-Sequential processing subscale</th>
<th>-Simultaneous processing subscale</th>
<th>Standard error</th>
<th>Regression coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDLSVerbal comprehension scale</td>
<td>101±12</td>
<td>90</td>
<td>-6.13(2.79)</td>
<td>0.03</td>
<td>0.40</td>
<td>9.6</td>
</tr>
</tbody>
</table>

*p* prenatal exposure was defined as the ln∑PCB of 4 congeners measured in maternal plasma during the last month of pregnancy, † Dutch version of the Kaufmann Assessment Battery for Children, ‡ Dutch version of the Reynell Language developmental Scales only in the Rotterdam cohort, § regression coefficient and standard error (SE) for prenatal PCB exposure from multiple linear regression analyses, t-test, significantly higher in the breast-fed group. All the regressions are adjusted for study centre (except RDLS), HOME, birth order, maternal age, parental verbal IQ, maternal and paternal education, gender, maternal cigarette and alcohol use during pregnancy. Feeding type and breast-feeding period were additionally entered as covariates in the whole group and period of breast-feeding was an additional covariate in the breast-fed group. Note: If the ∑PCB increases by 10%, the expected additive change in outcome variable approximates 10% of the regression coefficient of ln∑PCB.
Discussion

We report that prenatal exposure to background PCB concentrations, is associated with poorer performance on cognitive tests in Dutch children at 42 months of age. No associations between lactational exposure to PCBs and dioxins nor current PCB body burden, and cognitive abilities at 42 months of age are found, suggesting that the developing fetal brain is particularly sensitive to these compounds. Our results are in agreement with the reported cognitive deficits in the Yu-Cheng 'poisoning' study\textsuperscript{8, 9} and the Michigan 'fish exposure' study\textsuperscript{14, 15}. In the Michigan cohort, adverse effects of prenatal PCB exposure were found on short-term memory on both verbal and numerical tests at 4 years, and on the full scale and verbal IQ scores at 11 years of age. In our cohort effects are found on overall cognitive functioning as well as on the sequential- and simultaneous processing scale, including short- and long-term memory tasks.

Table 4: Effects of contemporary exposure* to PCBs in relation to cognitive abilities at 42 months in the whole group.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>n</th>
<th>regression coefficient (SE)†</th>
<th>p-value</th>
<th>R\textsuperscript{2} whole model</th>
<th>Residual SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-ABC‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall cognitive scale</td>
<td>286</td>
<td>1.16 (1.56)</td>
<td>0.46</td>
<td>0.44</td>
<td>10.9</td>
</tr>
<tr>
<td>-Sequential scale</td>
<td>286</td>
<td>1.82 (1.73)</td>
<td>0.30</td>
<td>0.34</td>
<td>12.1</td>
</tr>
<tr>
<td>-Simultaneous scale</td>
<td>296</td>
<td>0.28 (1.48)</td>
<td>0.85</td>
<td>0.39</td>
<td>11.0</td>
</tr>
<tr>
<td>RDLS§</td>
<td>171</td>
<td>1.82 (1.55)</td>
<td>0.24</td>
<td>0.40</td>
<td>9.3</td>
</tr>
</tbody>
</table>

* contemporary exposure, ln$\sum$PCB of 4 congeners measured in child’s plasma at 42 months of age, † regression coefficient and standard error (SE) for ln$\sum$PCB at 42 months from multiple linear regression analyses, ‡ Dutch version of the Kaufmann Assessment Battery for Children, § Dutch version of the Reynell Language developmental Scales only in the Rotterdam cohort. All the regressions are adjusted for study centre (not for RDLS), HOME, birth order, maternal age, parental verbal IQ, parental education, gender, maternal cigarette and alcohol use during pregnancy. Note: If the ln$\sum$PCB level in plasma increases by 10%, the expected additive change in outcome variable approximates 10% of the regression coefficient of ln$\sum$PCB.
Exposure data are difficult to compare, due to differences in analytical methods used in the Yu-Cheng, US, and Dutch studies. In the Yu-Cheng study children from exposed mothers were compared with matched controls. In Michigan, exposure was defined from total PCB levels determined in cord and maternal blood as well as breast milk, by summing 10 Webb-McCall peaks. In North Carolina two Webb McCall peaks were quantified, using packed column gas chromatography. Although the Webb-McCall method was state-of-the-art at that time, it is crude by today’s standards and provides no information of individual PCB congeners. In the Dutch study, PCB concentrations were measured by GC-ECD and provided information of 4 individual PCB congeners. In the Michigan study, pooled serum samples in 4 year old children were also analyzed for individual PCB congeners, and it appears that the four PCBs (IUPAC nos. 118, 138, 150, and 180) constitute 46% of the total PCBs. Assuming that the PCB congener mix in The Netherlands is similar to that in Michigan, doubling the maternal plasma PCB values from the Dutch study (mean ∑PCB=2.2 µg/l) gives PCB levels which are roughly comparable to those measured in Michigan (mean ∑PCB=4.7 µg/l). In the North Carolina study developmental effects were found in the first 2 years of life and not at 3, 4, and 5 years of age. There is reason to suspect that exposure levels are lower in the North Carolina study. The Dutch cohort comes from a more industrialized and densely populated area, which could result into higher background PCB levels in The Netherlands.

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

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

The development of the central nervous system both in utero and during childhood is a continuous process in which many morphological changes take place. Due to variability in rate of development, especially in the younger age group, it is difficult to detect subtle neuro-developmental deficits. Adverse effects of prenatal PCB exposure on the neurological condition found at birth22 and at 18 months of age23, could not be detected at 42 months of age34, however the 42-month cognitive abilities (this paper) were negatively associated with in utero exposure. This difference can be explained by the different testing procedures. The K-ABC measures the child's cognitive abilities in a quantitative fashion, whereas the neurological examination34 is a qualitative measure of brain development.

In conclusion, in utero exposure to background PCB concentrations as found in The Netherlands is associated with poorer cognitive functioning in preschool children. Children of mothers at the upper end of exposure are especially at risk. Although lactational exposure to PCBs and dioxins is much higher compared to transplacental exposure, no negative effects were found in children breast-fed during infancy. Moreover, breast-fed children performed better on cognitive tests and are from a more advantaged socio-economic environment than their formula-fed counterparts. Fetal exposure to PCBs and related compounds should be
lowered by reducing maternal body burden, rather than discouraging breast-feeding. Our data demonstrate the continuation of a toxic impact received in utero on cognitive functioning at toddler age. Studies at school age are needed to investigate whether long-term implications for later intellectual functioning exist.

ACKNOWLEDGEMENTS
We would like to thank Dr. C. Koopman-Esseboom and Dr. M. Huisman for the recruitment of all mother-infant pairs. We thank Prof. J. L. Jacobson and Prof. G. Winneke for their critical review of this paper, and all the families who have participated in this follow-up study.
Figures 1a, b.

![Bar chart 1](image1)

- **Cognitive Scale**
- **Sum PCB Level in Maternal Plasma (μg/l)**
- **n** values: 78, 98, 74, 58, 65

![Bar chart 2](image2)

- **Sequential Processing Scale**
- **Sum PCB Level in Maternal Plasma (μg/l)**
- **n** values: 78, 98, 74, 58, 65
Figures 1a, b, c: Five cut-off points are given for the ΣPCB levels measured in maternal plasma, based upon the range and distribution of the ΣPCB levels. The bars represent the dose-response relationship of the mean score and standard error of the mean (SEM) on the cognitive scale of the Dutch version of the Kaufman Assessment Battery for Children (K-ABC) and maternal ΣPCB levels adjusted for covariates (maternal age, parity, gender, parental education and verbal IQ, HOME score, maternal alcohol use and cigarette smoking during pregnancy, feeding type in infancy, breast-feeding period, and study centre). The score on the cognitive scale in the highest-exposed group (ΣPCB in maternal plasma >3 μg/L), was 4 points lower compared to the lowest-exposed group (ΣPCB in maternal plasma <1.5 μg/L) [Figure 1a]. Similar dose-response relationships are given for the sequential and simultaneous processing subscales of K-ABC [figures 1a and 1c, respectively].
Chapter 5

References


27. van Eldik MCM, Schlichting JEPB, Lutje Spelberg HC, van der Meulen BF, van der Meulen S
Chapter 5


