Prenatal exposure to polychlorinated biphenyls and breastfeeding: opposing effects on auditory P300 latencies in 9-year-old Dutch children

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Effects of perinatal exposure to polychlorinated biphenyls (PCBs) on auditory P300 latencies and amplitudes were evaluated in children from a Rotterdam cohort. From this cohort of healthy, term babies, the 26 lowest and 26 highest prenatally PCB-exposed children from the breastfed and the formula-fed groups (n=104) were invited for P300 assessment when they were 9 years of age. For P300 assessment an auditory simple odd-ball paradigm was used. In the 83 participating children, 60 assessments (32 males, 28 females) satisfied the measurement criteria and were included in the data analyses. After adjusting for confounding variables, children with high prenatal exposure were found to have longer P300 latencies than children with low prenatal exposure. Lactational exposure to PCBs through breastfeeding milk was not related to P300 latencies. P300 latencies were shorter in children breast-fed for at least 16 weeks than in children breastfed for 6 to 16 weeks and formula-fed children. P300 amplitudes were not related to perinatal PCB exposure nor breastfeeding. Results of this exploratory study suggest that prenatal exposure to environmental levels of PCBs and related compounds delays mechanisms in the central nervous system that evaluate and process relevant stimuli, whereas breastfeeding accelerates these mechanisms.

See last page for list of abbreviations.

Polychlorinated biphenyls (PCBs) and dioxins are toxic compounds that are detectable in human milk and tissues and are present because of background exposure to these environmental pollutants. The foetus is exposed to maternal levels of these compounds through placental transport. Additionally, a breastfed infant is exposed to relatively large amounts of PCBs and dioxins in breast milk (Patandin et al. 1997). These compounds are well known for their neurotoxic properties, although the neurotoxic mechanisms of PCBs and dioxins remain largely unknown. Many systems and levels of the developing central nervous system (CNS) have been reported to be involved in the complex mechanism of the neurotoxic action of PCBs and dioxins (Brouwer et al. 1995, Tilson and Kodavanti 1998). These include neuronal and glial cells (Seegal and Shain 1992, Morse et al. 1996), brain neurotransmitters (Seegal et al. 1989, 1991; Mariussen and Fonnum 2001), and several hormone systems (Weisglas-Kuperus 1998, Brouwer et al. 1999), depending on the type of congener and its metabolites.

Human epidemiological studies have provided accumulating evidence for the neurotoxic effects of predominantly prenatal exposure to PCBs by showing relations between exposure levels and neurodevelopmental outcome. In these cohort studies it was suggested that there were delayed effects of prenatal exposure to PCBs on general cognitive and motor development (Jacobson et al. 1990, Jacobson and Jacobson 1996, Koopman-Esseboom et al. 1996, Patandin et al. 1999, Walkowiak et al. 2001, Vreugdenhil et al. 2002), processing speed and attention (Jacobson et al. 1992, Jacobson and Jacobson 1996, Vreugdenhil et al. 2004), memory (Jacobson et al. 1990), verbal comprehension (Jacobson and Jacobson 1996, Patandin et al. 1999), and planning skills (Vreugdenhil et al. 2004).

Neurophysiological techniques might provide a more direct evaluation of CNS function than neurodevelopmental tests. Moreover, the measurement of event-related brain potentials (ERPs), and especially the cognitive P300 component, is a useful tool for investigating cognitive function (Pritchard 1981, Magliero et al. 1984, Polich and Herbst 2000). ERPs result from intracortical currents induced by excitatory and inhibitory postsynaptic potentials that are triggered by the release of neurotransmitters. The P300 component is a positive ERP that occurs with a latency of about 300ms when a person is actively processing ('attending to') incoming stimuli (Sutton et al. 1965). The latency of the P300 is considered to be an indicator of the neural activity underlying the processes of attention allocation and immediate memory (Polich and Herbst 2000) and a measure of stimulus classification speed (Kutas et al. 1977, Polich 1986). The amplitude of the P300 is assumed to reflect the quality with which incoming information is processed when it is incorporated into its memory representations and the context in which the stimulus occurs (Polich and Herbst 2000).

In adults, the amplitude and latency of the P300 can discriminate brain pathology from control conditions, including occupational exposure to neurotoxic chemicals such as organic solvents (Morrow et al. 1992, 1998; Steinhauer et al. 1997), specific neuropathological states such as Alzheimer's disease (Neshige et al. 1988), closed-head injury (Papanicolaou et al. 1984, Keren et al. 1998), psychiatric disorders such as schizophrenia (Ford et al. 1999, Blackwood 2000), and depression (Bruder et al. 1995, Yanai et al. 1997).

In children, P300 abnormalities have been associated with

several pathologies including cognitive dysfunction (Finley et al. 1985, Kaneko et al. 1996), attention-deficit disorders (Holcomb et al. 1985, Satterfield et al. 1990), and dyslexia (Taylor and Keenan 1990). Moreover, the latency and amplitude of the P300 decrease and increase respectively with age, until adolescence, reflecting maturation processes in the CNS (Martin et al. 1988, Polich et al. 1990, Sangal et al. 1998).

Effects of prenatal exposure to PCBs and dioxins on the P300 have been addressed in the Yu Cheng, Taiwan cohort (Chen and Hsu 1994), consisting of children born to mothers that were accidentally exposed to high levels of PCBs and polychlorinated dibenzofurans. In the prenatally exposed children, auditory P300 latencies were prolonged, and amplitudes were lower than in non-exposed matched controls. In that study, visual and short-latency somatosensory evoked potentials were not different for the groups (Chen and Hsu 1994).

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

The aim of the present study was to gain more insight into the neurotoxic mechanism of perinatal exposure to PCBs by exploring effects on a more direct measurement of CNS functioning: the P300 ERPs.

Method

PARTICIPANTS AND STUDY DESIGN

The original study population consisted of 207 healthy Caucasian mother–infant pairs who were recruited from June 1990 to February 1992 in the area of Rotterdam, a highly industrialized and densely populated area in the Netherlands. The study design and recruitment process, chemical analysis, and measurement of PCBs and dioxin concentrations have been described in detail elsewhere (Koopman-Esseboom et al. 1994a). Pregnancy and delivery were uncomplicated. Only first or second healthy children born at term were included. One hundred and five children were breastfed for at least 6 weeks and 102 children were formula-fed during infancy. All formula-fed infants received formula from a single batch (Almiron M2; Nutricia NV, Zoetermeer, the Netherlands) from birth until 7 months of age. Concentrations of PCBs and dioxins were undetectable in this formula.

We invited 104 nine-year-old children of the Rotterdam cohort, the 26 lowest and 26 highest prenatally exposed children (based on the sum of PCBs in maternal plasma [$\sum PCB_{maternal}$]) from each feeding group, to participate in a follow-up assessment in the Sophia Children's Hospital in Rotterdam. Children were not eligible for selection if they had not participated in the follow-up at 3 years 6 months or 7 years of age, or if they had moved from the Rotterdam area, because families had to visit the hospital for the assessment.

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

AUDITORY EVENT-RELATED POTENTIALS

An auditory simple odd-ball paradigm was used to elicit the P300 component. Two different sinusoidal tone bursts of two frequencies (1kHz tone, 70dB normal hearing level, 50ms duration, 5ms rise/fall time [non-target]; or 1.5kHz tone, 70dB normal hearing level, 66.7ms duration, 6.7ms rise/fall time [target]), using a fixed 1.25-second interstimulus interval, were presented binaurally through earphones in pseudo-randomized order. Twenty per cent of these tones were targets (1.5kHz) and 80% were non-targets (1kHz); the software package used was Nicolet Viking (version 4.7.1b). Children were required to lie on a bed and press a hand-held button as quickly as possible in response to target stimuli. ERPs were recorded with Ag/AgCl electrodes placed over the midline frontal (Fz), central (Cz), and parietal (Pz) positions referred to linked ears, with forehead ground. Eye movements and blink artefacts were differentially recorded by two electrodes, one lateral inferior to the right eye and the other superior to the left eye. Raw potentials were filtered, with the bandpass set at 0.5 to 30Hz. Artefact rejection at 9μV was used.

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

ERP-WAVEFORM ANALYSIS

The ERP waveforms were labelled conventionally. For the purpose of this study, the P300 peak was identified in the individual recordings, generally in the first ERP assessment, by two raters who were unaware of the child's exposure levels and type of feeding during infancy. The P300 was identified as the largest positive peak in the area of 250 to 450ms (Fig. 1). The latency and amplitude of the P300 peak at the Fz, Cz, and Pz positions were used as outcome variables. For each exposure group, separate grand-average ERP waveforms were calculated for the three electrode recordings (Fz, Cz, and Pz).

Table I: Characteristics of children with complete and incomplete event-related potentials (ERPs) assessments and of non-participants

Characteristic	*ERP complete (n=60)*	*ERP incomplete (n=23)*	*Non-participants (n=21)*
$\sum PCB_{maternal}$, median (range) μg/l	2.54 (0.59–4.71)	1.71 (0.80–5.08)	2.63 (0.73–7.35)
Nr of $\sum PCB_{low}$, *n* (%)	28 (46.7)	14 (60.9)	10 (57.1)
Nr of BF, *n* (%)	32 (53.3)	12 (52.2)	9 (42.9)

$\sum PCB_{maternal}$, sum of PCB congeners (International Union of Pure and Applied Chemistry numbers 118, 138, 153 and 180) in maternal plasma; $\sum PCB_{low}$, number of children with low levels of prenatal PCB exposure.

ASSESSMENT OF EXPOSURE VARIABLES

Plasma samples were collected from the mothers during the last month of pregnancy and cord plasma samples were collected directly after birth. These samples were analyzed for four PCB congeners: International Union of Pure and Applied Chemistry (IUPAC) numbers 118, 138, 153, and 180. Two weeks after delivery a 24-hour representative breast-milk sample was collected from the mothers who were breastfeeding their children. Breast-milk samples were analyzed for 17 dioxins (polychlorinated dibenzodioxins and polychlorinated dibenzofurans), 6 dioxin-like PCBs (IUPAC numbers 77, 105, 118, 126, 156, and 169), and 20 non-dioxin-like PCBs (IUPAC numbers 28, 52, 66, 70, 99, 101, 128, 137, 138, 141, 151, 153, 170, 177, 180, 183, 187, 194, 195, and 202). The toxic potency of the mixture of dioxins and dioxin-like PCBs was expressed by using the toxic equivalent factor approach (Van den Berg et al. 1998).

In the present study, we compared the outcome of a group exposed to low levels of PCBs with that of a group exposed to high levels, based on the sum of the four PCB congeners measured in maternal plasma, ($\sum PCB_{maternal}$).

ASSESSMENT OF CONFOUNDING VARIABLES

Variables that may influence child neurodevelopment were assessed, they included birthweight, duration of gestation, foetal exposure to alcohol and cigarette smoke, parity, type of feeding during infancy, duration of breastfeeding, sex, and parental education level. The child's home environment was assessed by the Home Observation for Measurement of the Environment (HOME; Caldwell and Bradley 1984) during the home visit for the follow-up at 7 years of age. Verbal IQ of the parent who spent the most time with the child (usually the mother) was assessed by the Information and Vocabulary subtests from the Dutch version of the Wechsler Adult Intelligence Scale (Stinissen et al. 1970).

DATA ANALYSIS

To compare the groups for a single variable we used Student's *t*-test, the Mann–Whitney *U* test, or the χ^2 test for continuous variables with a Gaussian-shaped distribution, categorical ordinal variables, or categorical nominal variables. The difference in outcome between the groups with low and high prenatal exposures was studied by means of multiple linear regression analyses (SPSS, version 10). Variables that were likely to affect P300 outcome (latency or amplitude) were included in the regression model as a fixed set of variables. These variables were as follows: sex (0/1: male/female); highest education level of either parent (0/1/2: primary school, secondary school not finished/secondary school finished/high school finished, professional and university training); type of feeding and duration of breastfeeding (captured in two dummy variables for formula-feeding, BF_{short}: 6 to 16 weeks of breastfeeding, BF_{long}: ≥16 weeks of breastfeeding); and age at examination. Additionally, confounding variables, namely variables

Table II: Parent' characteristics and characteristics of children with low ($\sum PCB_{low}$) and high ($\sum PCB_{high}$) prenatal exposure to PCBs with complete event-related brain potentials assessment

Characteristic	$\sum PCB_{low}$ *(n=28)*	$\sum PCB_{high}$ *(n=32)*
Nr of mothers who smoked during pregnancy, *n* (%)	7 (25)	8 (25)
Nr of mothers who used alcohol during pregnancy, *n* (%)[a]	2 (7)	9 (28)
Birthweight, mean (SD) kg	3406 (404)	3344 (535)
Gestational age, mean (SD) wk	40.2 (1.1)	39.7 (1.3)
Nr breastfed, *n* (%)	13 (46)	19 (59)
Duration of breastfeeding, median (range) wk	16 (6–40)	16 (6–62)
Nr of males, *n* (%)	13 (46)	19 (59)
Nr of first born, *n* (%)	15 (54)	16 (50)
Maternal age, mean (SD) y[b]	27.3 (3.0)	31.7 (3.3)
Parental education level[b]		
Low (primary school, secondary school unfinished), *n* (%)	8 (29)	1 (3)
Medium (secondary school finished), *n* (%)	12 (43)	13 (41)
High (high school finished, professional and/or university training), *n* (%)	8 (29)	18 (56)
Parental Verbal IQ, mean (SD)[b]	117 (16.6)	127.2 (14.8)
HOME score at 7 years, mean (SD)	47.8 (2.6)	48.5 (2.8)
Age of child at assessment, mean (SD) y	9.2 (0.2)	9.2 (0.2)
Exposure variables		
$\sum PCB_{maternal}$, median (range) μg/L[b]	1.4 (0.59–1.93)	3.2 (2.51–4.71)
$\sum PCB_{cord}$, median (range) μg/L[b]	0.3 (0.08–0.63)	0.58 (0.29–1.98)
$\sum PCB_{milk}$, median (range) μg/kg fat[b]	242.5 (173.7–371.1)	572.4 (333.6–804.5)
$\sum PCB_{20\ non\text{-}dioxin\text{-}like}$, median (range) μg/kg fat[b]	255.2 (204.6–466.1)	608.5 (347.2–858.1)
Total TEQ, median (range) ng/kg fat[b]	43.8 (28.06–88.20)	84.05 (58–111.41)

Significance levels: [a]$p<0.05$, [b]$p<0.01$ (Student's *t*–test, Mann-Whitney *U*, or χ^2 test). Parental Verbal IQ was taken as score on two subtests of Wechsler Adult Intelligence Scale, Information and Vocabulary (Stinissen et al. 1970), assessed on one parent; HOME, Home Observation for the Measurement of the Environment; $\sum PCB_{maternal}$, $\sum PCB_{cord}$, $\sum PCB_{milk}$, sum of PCB congeners (International Union of Pure and Applied Chemistry [IUPAC] numbers 118, 138, 153, and 180 in mother, cord plasma, and in breast milk; $\sum PCB_{20\ non\text{-}dioxin\text{-}like}$, sum of 20 non-dioxin-like PCBs in breast milk; Total TEQ, sum of toxic equivalents according to 1997 WHO toxic equivalency factors for mono-ortho-PCBs (IUPAC numbers 105, 118, and 156) planar PCBs (IUPAC numbers 77, 126, and 169) and seventeen 2,3,7,8-substituted polychlorinated dibenzodioxins and polychlorinated dibenzofurans.

that were correlated ($p<0.2$), adjusted for the fixed set of variables with the exposure variable ($\sum PCB_{low/high}$) and with one of the outcome variables, were added to the regression model. Candidate confounders were alcohol use (0/1: no/yes) and smoking during pregnancy (0/1: no/yes), duration of gestation, birthweight, parity (0/1: first/second born), parental verbal IQ, and HOME score. This procedure resulted in the following set of explanatory variables included in the regression model for P300 outcome variables: $\sum PCB_{low/high}$, alcohol use during pregnancy, sex, type of feeding and duration of breastfeeding, parental education level, and age at assessment. Results were considered significant at $p \leq 0.05$.

Results

From the invited children ($n=104$), 83 (80%) were willing to participate (ages 8 years 9 months to 9 years 7 months; mean 9 years 2 months, standard deviation [SD] 0.2). Parents of 21 children were not motivated to participate in this follow-up for which they had to visit the hospital. Exposure levels in participating and non-participating children were comparable. From the 83 children in whom ERP assessments were performed, 60 measurements were complete (i.e. 48 accepted target stimuli) and were included in the data analyses. In Table I prenatal exposure levels, the number of children with low and high exposure, and the type of feeding are presented for the included and excluded children, as well as the children who were not willing to participate in this study. The three groups did not show statistical differences in these variables.

The characteristics of the children with low and high exposure whose ERP measurements were included in the data analyses are presented in Table II. As described in more detail previously (Vreugdenhil et al. 2002), parental education level and Verbal IQ were significantly higher in the group of children with high exposure than in the low-exposure group. All prenatal exposure measurements of PCBs and dioxins were significantly higher in the high-exposure group, which is inherent in the study design. In Table III, the mean latency and amplitude of the P300 are shown for the groups with low and high prenatal exposures and for the breastfed and formula-fed groups of children.

Grand averages of the ERP waveforms are presented for the two exposure groups, not adjusted for confounding differences between the exposure groups, in Figure 1. The grand-average waveform for the low-exposure group showed a better peak pronunciation than the grand-average waveform for the high-exposure group, especially in the parietal and central (data not shown) recordings. The P300 latency of high-exposure children was prolonged in comparison with that of the low-exposure children.

Results of multiple regression analyses on the P300 peak latencies are presented in Table IV. Especially for the Cz and the Pz recordings, the P300 latencies were significantly longer in children with high prenatal exposure than in those with low exposure, after adjustment for confounding variables. Moreover, for the Fz, Cz, and Pz recordings, children who were breastfed for a long period (BF_{long}) had significantly shorter P300 latencies than children that were breastfed for a short period (BF_{short}). For the Pz recording, BF_{long} children also had a shorter P300 latency than formula-fed children. P300 amplitudes were not statistically different for children with low and high exposures, nor for the three feeding groups, when adjusted for confounding variables.

To estimate effects of postnatal exposure through lactation, the group of breast-fed children was divided into four groups based on prenatal exposure levels (high or low) and duration of breastfeeding (BF_{short}: less than 16 weeks; BF_{long}: 16 weeks

Table III: Mean (SD) P300 latencies and amplitudes for low-exposure ($\sum PCB_{low}$) and high exposure ($\sum PCB_{high}$) children breastfed and formula fed

Variables	*Frontal position*		*Central position*		*Parietal position*	
	Latency (ms)	*Amplitude (μV)*	*Latency (ms)*	*Amplitude (μV)*	*Latency (ms)*	*Amplitude (μV)*
$\sum PCB_{low}$	333 (28)	5 (4)	327 (29)	6 (4)	327 (27)	8 (4)
$\sum PCB_{high}$	339 (40)	7 (4)	341 (41)	8 (3)	339 (41)	8 (4)
Breastfed	334 (35)	6 (4)	332 (36)	7 (4)	330 (35)	8 (3)
Formula-fed	338 (34)	5 (4)	337 (37)	7 (4)	337 (36)	8 (4)

Table IV: Adjusted mean differences between groups resulting from multiple regression analysis of P300 latencies (ms) measured at midline frontal (Fz), central (Cz), and parietal (Pz) positions

Position	$\sum PCB_{high}$ *versus* $\sum PCB_{low}$			BF_{short} *versus FF*			BF_{long} *versus FF*			BF_{long} *versus* BF_{short}		
	Diff.	*SE*	*p*	*Diff.*	*SE*	*p*	*Diff.*	*SE*	*p*	*Diff.*	*SE*	*p*
P300Fz	14.3	9.5	0.140	15.0	10.5	0.160	–19.8	10.8	0.073	–34.7	12.1	0.006
P300Cz	25.6	9.6	0.011	13.0	10.6	0.229	–20.2	10.9	0.070	–33.2	12.2	0.009
P300Pz	22.0	9.4	0.023	12.0	10.3	0.251	–22.5	10.6	0.039	–34.5	11.9	0.005

Values are adjusted for foetal exposure to alcohol, sex, parental education, and age at assessment. Differences were estimated in essentially same regression model by reparameterizing effects of three categories for duration of breastfeeding (formula-fed [FF], breastfed (BF) for 6 to 16 weeks [BF_{short}], or breastfed for 16 weeks or longer [BF_{long}]). Diff., difference; SE, standard error; $\sum PCB_{low}$,/$\sum PCB_{high}$, children with low/high exposure to PCBs.

or longer). In Figure 2 the mean adjusted latencies measured on Pz are presented for these four groups and for the formula-fed groups with low and high exposure; the significant differences in mean adjusted latencies between the six feeding groups are indicated. Low-exposure BF_{long} children (E) had significantly shorter P300 latencies than their high-exposure (Fz, $p=0.013$; Cz, $p=0.002$; Pz, $p=0.005$) (E′) and low-exposure (Fz, $p=0.040$; Cz, $p=0.046$; Pz, $p=0.114$) BF_{short} counterparts. In the high-exposure BF_{long} group (F), latencies were also generally shorter than in BF_{short} children with high exposure (Fz, $p=0.061$; Cz, $p=0.086$; Pz, $p=0.021$) (F′).

Discussion

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

In the Yu Cheng cohort (Chen and Hsu 1994), delayed P300 latencies have been reported in 7- to 12-year-old children who were accidentally exposed to relatively high prenatal levels of PCBs and polychlorinated dibenzofurans. Although the exposure levels we describe are expected to be much lower than in the Yu Cheng study, the difference in P300 latency between the exposed group and the control group in the Yu Cheng study (Cz, 26.7ms; Pz, 25.2ms; Chen and Hsu 1994) and between PCB_{high} and PCB_{low} (Cz, 25.6ms; Pz, 22.0ms) in the present study are equal within the measurement error. In the Lake Michigan cohort at 11 years of age (Jacobson and Jacobson 1996; an American cohort in which neurodevelopmental effects of perinatal exposure to environmental levels of PCBs were addressed), the magnitude of effects of prenatal exposure to PCBs on IQ was also comparable to the difference seen in exposed and non-exposed children in the Yu Cheng study.

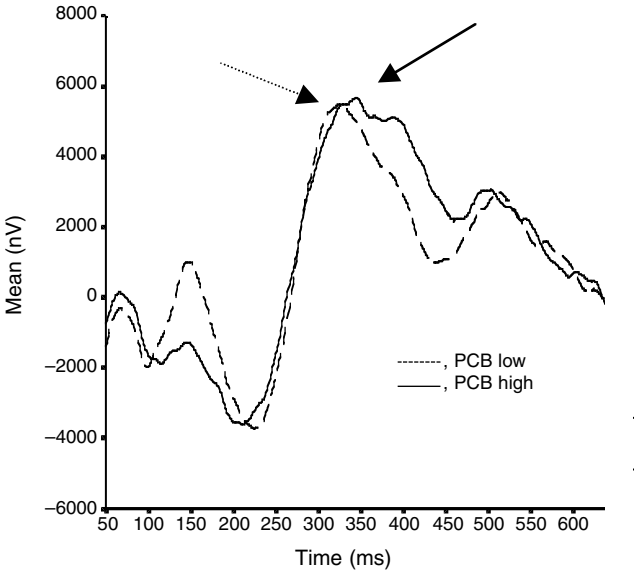

Figure 1: *Grand-average event-related brain potentials at Pz for children with prenatal low (broken line) and high (solid line) exposures to PCB. Broken arrow, P300 for low-exposure group; solid arrow, P300 for high-exposure group.*

In contrast to the Yu Cheng study, in the present study the P300 amplitude was not statistically different for the two exposure groups. The latency of the P300 is considered to be an indicator of the neural activity underlying the processes of attention allocation and immediate memory (Polich and Herbst 2000) and a measure of stimulus classification speed (Kutas et al. 1977, Polich 1986). The amplitude of the P300 is assumed to reflect the quality with which incoming information is processed when it is incorporated into its memory representations and the context in which the stimulus occurs (Polich and Herbst 2000). The amplitude is, among other other things, considered to be related to the discrepancy between the expected and actual stimulus properties, whereas the latency reflects the duration of the stimulus-evaluation process. Specific neuropathological states and their cognitive deficits seem to be more often related to prolonged latency of P300 (Neshige et al. 1988; Polich 1989, 1991; Morrow et al. 1992, 1996), whereas decrements in P300 amplitude are more often associated with the presence of psychiatric disorders such as schizophrenia (Ford 1999, Jeon and Polich 2001) and depression (Blackwood et al. 1987, Bruder 1992, Yanai et al. 1997). We hypothesize that the difference in the observed effects of prenatal exposure to PCBs on the P300 amplitude in the Yu Cheng cohort and in the Dutch PCB/dioxin cohort might reflect differences in exposure levels and mixture content or subtle differences in the assessment of the P300. For example, the interstimulus interval that was applied in the Yu Cheng study was larger (2.5 seconds vs 1.25 seconds in the Dutch study), which might have caused larger P300 amplitudes.

Correlation analysis of the P300 outcome variables and neuropsychological outcome variables that were assessed during the same follow-up session (namely the Rey Complex Figure Task, the Auditory–Verbal Learning Test, Simple Reaction Time Task, and the Tower of London Test; Vreugdenhil et al. 2004) showed no statistically significant interrelationships. However, ERPs are believed to measure only a fraction of the neural activity associated with stimulus processing and do not measure the more elaborated neuronal processes of cognitive processes (Rugg and Coles 1995).

The effect of a longer duration of breastfeeding on the P300 latency might suggest positive effects of substances in breast milk, such as long-chain polyunsaturated fatty acids, that stimulate brain development. The brain is 60% structural lipid and uses arachidonic acid and docosahexaedonic acid, which are deposited in the non-myelin membranes of the developing nervous system and are believed to be essential for the growth, function, and integrity of the CNS (Uauy et al. 2001, Uauy and Mena 2001). These acids were not available for formula-fed children, and children who were breastfed for a shorter period may have received smaller amounts of these compounds than children who were breastfed for a longer period. These results illustrate the complexity of risk assessment of exposure to environmentally persistent compounds, especially with regard to breastfeeding. Assessment of more specific cognitive functions might help to refine our knowledge of the neurotoxic effects of early exposure to PCBs and dioxins at

different stages of development.

In the present study we compared groups of children with low and high prenatal exposures, based on $\sum PCB_{maternal}$ during the last month of pregnancy. Levels of these compounds assessed in maternal blood were highly correlated with the levels of these compounds in breast milk as well as with toxic equivalents of dioxins in breast milk (Koopman-Esseboom et al. 1994b). In the environment, PCBs, their metabolites, and related compounds, such as dioxins, are present as complex mixtures of various congeners that can vary in metabolism and toxicity. Hence specific effects of either group of compounds are methodologically difficult to detect. We believe, therefore, that the difference in outcome between the low and high exposure groups could be related to differences in exposure levels of other PCB congeners, dioxins, and related compounds and differences in their metabolites.

Results of this study suggest a negative effect of prenatal exposure to environmental levels of PCBs and dioxins on P300 latency in a cohort of normally developing children with mean age around 9 years. Prenatal exposure to PCBs and dioxins are suggested to slow down mechanisms in the CNS that evaluate and process relevant stimuli. No evidence was seen for effects on the P300 of postnatal exposure to PCBs and dioxins through lactation. Moreover, an accelerating effect of a longer duration of breastfeeding on P300 latencies was found. Given these results, which suggest a positive effect of a longer duration of breastfeeding in addition to the general decline in contamination of breast milk with PCBs and dioxins in the Netherlands, we conclude that breastfeeding for a long duration might be beneficial and should not be discouraged. However, these results indicate that at the time of this study, the level of prenatal exposure to PCBs and dioxins was high enough to make neurophysiological effects noticeable in children of school age. Results of this study, therefore, emphasize efforts to reduce environmental levels of PCBs and dioxins and related compounds, to reduce maternal levels of PCBs and dioxins.

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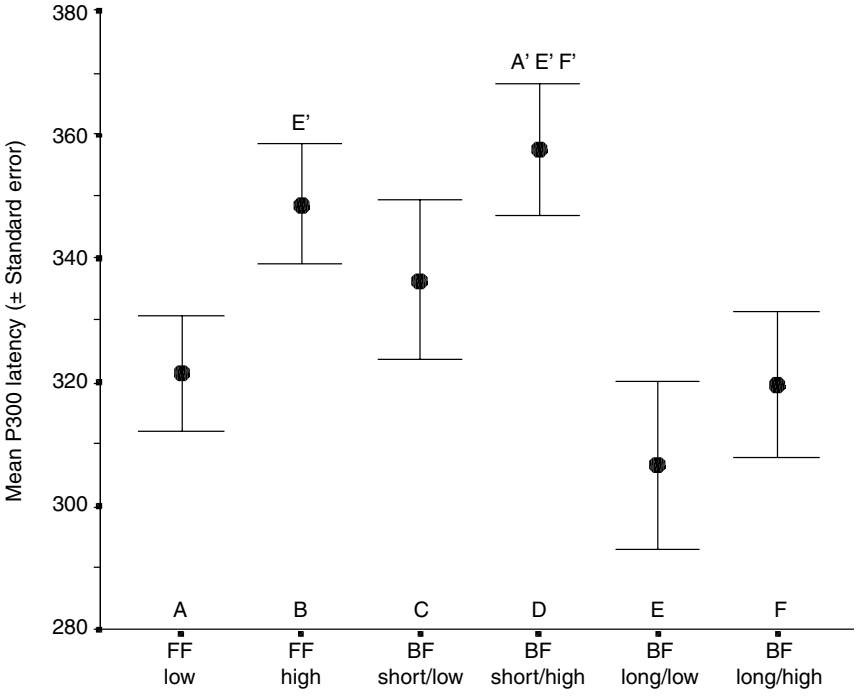

Figure 2: *Adjusted mean P300 latencies (ms) at Pz. A′, significantly different (p≤0.05) from A; E′, significantly different (p≤0.05) from E; F′, significantly different (p<0.05) from F. Error bars show standard errors.Black dots show adjusted mean P300 latencies. FF low, formula-fed, low PCB exposure; FF high, formula-fed, high PCB exposure; BF short/low, breast-fed 6 to 16 weeks, low PCB exposure; BF short/high, breast-fed 6 to 16 weeks, high PCB exposure; BF long/low, breast-fed ≥ 16 weeks, low PCB exposure; BF long/high, breast-fed ≥ 16 weeks, high PCB exposure.*

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List of abbreviations

BF	Breastfed
$BF_{short/long}$	Breastfed for 6 to 16/longer than 16 weeks
CNS	Central nervous system
ERPs	Event-related brain potentials
FF	Formula-fed
HOME	Home Observation for the Measurement of the Environment
IUPAC	International Union of Pure and Applied Chemistry
PCBs	Polychlorinated biphenyls
$\sum PCB_{low/high}$	Low or high prenatal PCB levels
$\sum PCB_{maternal}$	Sum of PCBs in maternal plasma