Immunologic Effects of Background Exposure to Polychlorinated Biphenyls and Dioxins in Dutch Preschool Children

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Prenatal exposure to polychlorinated biphenyls (PCBs) and dioxins is associated with changes in the T-cell lymphocyte population in healthy Dutch infants. We investigated whether these changes persist into later childhood and whether background exposure to PCBs and dioxins is associated with the prevalence of infectious or allergic diseases and humoral immunity at preschool age. The total study group consisted of 207 healthy mother–infant pairs. We estimated prenatal exposure to PCBs and dioxins by the sum of PCBs 118, 138, 153, and 180 (ΣPCB) in maternal and cord plasma and in breast-fed infants by the dioxin, planar, and mono-ortho PCB toxic equivalent (TEQ) levels in human milk. At 42 months of age, current body burden was estimated by the ΣPCB in plasma. We assessed the prevalence of infectious and allergic diseases by parent questionnaire, and measured humoral immunity by antibody levels for mumps, measles, and rubella after primary vaccination. We performed immunologic marker analyses of lymphocytes in a subgroup of 85 children. Prenatal PCB exposure was associated with an increased number of lymphocytes, T-cells, and $CD3CD8^+$ (cytotoxic), $CD4^+CD45RO^+$ (memory), T-cell receptor (TcR) $\alpha\beta^+$, and $CD3^+HLA\text{-}DR^+$ (activated) T cells and lower antibody levels to mumps and measles at preschool age. Adjusted for confounders, prenatal PCB exposure was associated with less shortness of breath with wheeze, and current PCB body burden was associated with a higher prevalence of recurrent middle-ear infections and of chicken pox and a lower prevalence of allergic reactions. A higher dioxin TEQ was associated with a higher prevalence of coughing, chest congestion, and phlegm. We conclude that in Dutch preschool children the effects of perinatal background exposure to PCBs and dioxins persist into childhood and might be associated with a greater susceptibility to infectious diseases. Common infections acquired early in life may prevent the development of allergy, so PCB exposure might be associated with a lower prevalence of allergic diseases. *Key words*: allergic diseases, antibody levels, breast-feeding, infectious diseases, leucocyte (sub)populations, PCBs, PCDDs, PCDFs. *Environ Health Perspect* 108:1203–1207 (2000). [Online 17 November 2000]
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Polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) (the latter two are designated dioxins) are lipophilic stable toxic compounds that occur widely in the environment. They are presently a pollution problem because they resist chemical or biologic degradation and accumulate in the food chain. Persistent neurobehavioral, immunologic, reproductive, and endocrine alterations were observed in experimental animals after *in utero* and lactational exposure to PCBs and dioxins (*1*). Daily intake of PCBs and dioxins per kilogram body weight is 50 times higher in nursing infants than in adults (*2*); among Dutch preschool children, median plasma PCB levels are 4 times higher in previously breast-fed than in formula-fed children (*3*). PCBs and dioxins can cause a broad range of immunotoxic effects in animals, including decreased host resistance to infections and suppressed humoral and cell-mediated immune responses (*4*). Because the developing immune system is vulnerable, the potential immunotoxic effects of perinatal exposure to PCBs and dioxins are of particular concern. However, data regarding the potential toxic effects of *in utero* and lactational exposure to PCBs and dioxins in humans are scarce. In 1979, an episode of poisoning from ingestion of rice oil contaminated with PCBs and PCDFs occurred in central Taiwan (Yu-Cheng). Among children born to these highly exposed women, researchers found a higher incidence of respiratory symptoms during the first six months after birth (*5*) and of middle-ear diseases at school age (*6*). In Inuit infants, whose mothers have high levels of organochlorine compounds in their breast milk, perinatal organochlorine exposure was associated with more acute otitis media in the first year of life (*7*). In a previous paper (*8*) we concluded that prenatal exposure to PCBs and dioxins was associated with changes in the T-cell lymphocyte population in Dutch infants. No effect on the health status nor on humoral immunity was found. However, there is concern that such early subtle changes could persist into later childhood and could presage difficulties, such as immune suppression or allergy. In this paper we report on the immunologic effects of perinatal background exposure to PCBs and dioxins in Dutch preschool children. We investigated whether exposure to PCBs and dioxins is associated with the prevalence of infectious or allergic diseases, changes in antibody responses, or changes in lymphocyte phenotype determinations in Dutch children at preschool age.

Methods

Subjects. The total study group consisted of 207 healthy Caucasian mother–infant pairs, recruited between June 1990 and February 1992. Characteristics of the study group are described elsewhere (*3,9*). Pregnancy and delivery were uncomplicated. Only first- or second-born infants at term (37–42 weeks of gestation) without congenital anomalies, diseases, or perinatal complications were included. One hundred five infants were breast-fed for at least 6 weeks, and 102 infants were exclusively bottle-fed with formula from a single batch (Almiron M2; Nutricia NV, Zoetermeer, The Netherlands) until 7 months of age. In this formula concentrations of both PCBs and dioxins were not detectable. The study protocol was approved by the medical ethics committee of the University Hospital Rotterdam/Sophia Children's Hospital. Informed consent was given by the parents.

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Measures of exposure to PCBS and dioxins. We estimated prenatal exposure to PCBs and dioxins by the sum of four PCB congeners (PCB 118, 138, 153, and 180) in maternal and cord plasma (ΣPCB maternal and ΣPCB cord), and in breast-fed infants by the dioxin, planar, and mono-*ortho* PCB toxic equivalent (TEQ) levels in human milk. To express the total toxic potency of PCDDs, PCDFs, and dioxin-like PCBs, we used the toxic equivalent factor (TEF) approach according to the World Health Organization (*10*). We defined current PCB body burden as the sum of the four PCB congeners in plasma from 42-month-old children (ΣPCB 42 months). Methods of determination, laboratory validation, and quality control were described previously (*3,9*).

Measures of immunologic effects. At the end of the 42-month follow-up period, we sent a health questionnaire to all parents, covering topics regarding infectious and allergic diseases as well as potential confounding variables such as tobacco smoking, family history of atopy (asthma, bronchitis, hay fever, or eczema in first-degree relatives), and day care or nursery school attendance of the child. Parents were asked whether a doctor had ever given the child a diagnosis of otitis media, pneumonia, scarlatina, chicken pox, rubella, measles, mumps, pertussis, hepatitis, meningitis, or other infectious diseases. Additional questions included: Has your child ever had eczema or an allergic reaction? Has a doctor ever said to you that your child had asthma or bronchitis? Has your child had periods of coughing, chest congestion, or phlegm lasting for 10 days or attacks of shortness of breath with wheeze in the previous 12 months?

Primary vaccinations against mumps, measles, and rubella were given to 206 of the 207 children at approximately 14 months of age as part of the National Immunization Program. The vaccines were manufactured by the National Institute of Public Health and the Environment and given at the local municipal health service. When the children were 42 months of age, humoral antibody levels (IgG) in plasma were measured against measles, mumps, and rubella virus with an ELISA, as described previously (*11–13*).

Due to logistic reasons (fresh blood is needed for these measurements), in a subgroup of 85 children monocyte, granulocyte, and lymphocyte counts were determined by whole blood fluorescence-activated cell sorter analysis combined with the determination of the white blood cell count by a cell counter. Methods of determination were described previously (*14,15*). Analyses were performed with a FAC-Scan flow cytometer (Becton Dickinson, San Jose, CA, USA). Whole blood was incubated with a panel of optimally titrated fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies (MAbs) for single or double immunofluorescence staining. As a rule, irrelevant IgG2 FITC- or IgG1 PE-conjugated MAbs were used as negative controls. Before flow cytometric analysis, red blood cells were lysed with FACS lysing solution (Becton Dickinson). The MAbs used in this study, obtained from Becton Dickinson unless mentioned otherwise, were as follows: CD19 (Leu-12 PE) and CD20 (B1 FITC; Coulter Clone, Hialeah, FL, USA) to label B cells; CD3 (Leu-4 FITC), CD4 (Leu-3 FITC or Leu-3 PE), and CD8 (Leu-2 PE) to label T cells; TcR αβ-FITC (WT31) and TcR(γδ-FITC (11F2) to label TcR αβ and TcR γδ cells; CD16 (Leu-11c PE) and CD56 (Leu-19 PE) to label natural killer (NK) cells; anti-HLA-DR (L243 PE) to label activated T cells; and CD14 (My4 PE; Coulter Clone) and CD45 (HLe-1 FITC) to determine, in combination with the whole-blood forward and side-scatter patterns, the percentages of granulocytes, monocytes, and lymphocytes (Leucogate). Further data analyses were made of the lymphocyte population thus defined. This lymphocyte population represented at least 95% of the total number of lymphocytes and contained less than 3% contamination by monocytes. Thus absolute numbers of the following lymphocyte (sub)populations were determined: $CD3^+CD4^+$ (helper), $CD3^+CD8^+$ (cytotoxic), $CD3^+$HLA-DR (activated), TcR $\alpha\beta^+$ and TcR $\gamma\delta^+$, $CD4^+CD45RA^+$ (naive), and $CD4^+CD45RO^+$ (memory) T-lymphocytes, B-lymphocytes ($CD19^+$ and/or $CD20^+$), and NK cells ($CD16^+$and/or $CD56^+/CD3^-$).

Data analysis. We analyzed the data using the statistical software package SPSS win 7.5 (SPSS Inc., Chicago, IL). For dichotomous immunologic variables (health questionnaire), we used multiple logistic regression analysis, expressing the effects of PCB and dioxin levels on the prevalence of infectious or allergic disease as odds ratio (OR) per unit increase of levels of PCBs and dioxins, with 95% confidence interval and adjustment made for confounding variables. Based on clinical knowledge and literature (*16,17*), the confounding variables included in these analyses were sex, early feeding type (breast-fed or formula-fed), duration of breast-feeding during infancy (less or more than 16 weeks), parity (born first or second), maternal education (secondary school not finished or secondary school or more finished), and parental occupation (blue-collar or white-collar), tobacco smoking by one or both parents, family history of atopy in one or more parents, and day care or nursery school attendance for the child. For continuous immunologic variables (antibody levels and the immunologic marker analysis), we estimated Pearson correlation coefficients between the PCB and dioxin levels as well as the immunologic variables after logarithmic transformation of both variables involved. These analyses were performed separately for prenatal (ΣPCB maternal, ΣPCB cord, and dioxin, planar, and mono-*ortho* PCB TEQ levels) as well as current PCB body burden (ΣPCB 42 months). Results were considered statistically significant at the $p \leq 0.05$ level.

Results

At 42 months of age, 193 children were reexamined (*18*). Complete health questionnaires were returned by 175 of the 193 parents (90%). The characteristics of the study group and levels of ΣPCBs measured in plasma and of the planar, mono-*ortho*, and the dioxin TEQ in breast milk are presented in Table 1.

The prevalence of infectious and allergic diseases is presented in Table 2. According to parental report of doctors' diagnoses, 103 (58.9%) of the 175 children had 1 episode (50th percentile), and 21 (12.0%) had 6 (90th percentile, e.g., recurrent) episodes of middle-ear infections. Pneumonia was reported for 5 (2.9%), scarlatina for 13 (7.4%), chicken pox for 130 (74.3%), and other infectious diseases for 15 (8.6%) (1 meningitis, 1 cystitis, 1 gastroenteritis, 1 lymphadenitis, 3 measles, and 8 common viral diseases such as exanthema subitum or erythema infectiosum) of the 175 children. Hospital admission for infectious diseases was reported for 7 (4.0%) of the 175 children (3 respiratory infections, 1 meningitis, 1 scarlatina, and 2 other viral diseases). Of the 14 children with allergic reactions, 7 had shown an allergic reaction to food, 2 to dust, 2 to household pets, 2 to dust and household pets, and 1 to pollen, dust, and household pets. Adjusted for confounders (e.g., sex, early feeding type, duration of breast-feeding during infancy, parity, maternal education and parental occupation, tobacco smoking by one or both parents, family history of atopy in one or more parents, and day care or nursery school attendance for the child), a higher ΣPCB maternal was associated with less shortness of breath with wheeze. For the ΣPCB cord the results were in the same direction but not significant. Current PCB body burden was associated with a higher prevalence of recurrent middle-ear infections and chicken pox and a lower prevalence of allergic reactions (Table 2). In breast milk, the mono-*ortho* and planar PCB TEQ had a significant effect on recurrent middle-ear infections (mono-*ortho* PCB TEQ: OR 1.17 , 95% CI, 1.04–1.32, p = 0.01; planar PCB TEQ: OR 1.10, 95% CI, 1.00–1.20, p = 0.04) and the dioxin TEQ

had a significant effect on coughing, chest congestion, and phlegm (OR 1.06, 95% CI, 1.00–1.11, $p = 0.04$).

The effects of early feeding type and the duration breast-feeding on the prevalence of recurrent middle-ear infections, chicken pox and allergic reactions and current PCB body burden are described in Table 3. Although current PCB body burden was 3–4 times lower in formula-fed than in breast-fed children (median ΣPCB at 42 months was 0.21 vs. 0.75 μg/L), there was no difference in prevalence of recurrent middle-ear infections, chicken pox, or allergic reactions between these two groups. In the breast-fed group current PCB body burden was 2 times lower in children breast-fed for 16 weeks than in children breast-fed for more than 16 weeks (median ΣPCB at months 0.60 vs. 1.04 μg/L). The effect of current PCB exposure was counteracted by the effect of the duration of breast-feeding on the prevalence of recurrent middle-ear infections and chicken pox as well as on allergic reactions (Table 3).

Serum for antibody levels was available in 150 children at 42 months of age. There was no difference in characteristics between these 150 children and the whole study group, and the levels of ΣPCBs measured in plasma and of the dioxin, planar, and mono-*ortho* TEQ in breast milk were comparable. Four children showed no seroconversion for mumps, 3 for measles, and 2 for rubella at 18 months of age. These nonseroconverting antibody concentrations were excluded from further analysis. Median antibody levels were 94.3 U/mL (range 2.9–2334.1) for mumps; 1.2 IU/mL (range 0.08–12.0) for measles; and 47.0 IU/mL (range 4.3–220.0) for rubella. After logarithmic transformation of both variables, antibody levels to mumps were negatively correlated with ΣPCB maternal levels (Pearson correlation –0.17, $p = 0.04$) and antibody levels to rubella were negatively correlated with ΣPCB cord levels (Pearson correlation –0.19, $p = 0.03$). There were no significant correlations between antibody levels and the dioxin, planar, and mono-*ortho* PCB TEQ levels separately nor with the ΣPCB at 42 months of age.

White blood cell counts and immunologic marker analyses of the lymphocytes at 42 months of age were available for a subgroup of 85 children. Children breast-fed in infancy were accidentally underrepresented in this subgroup; consequently the ΣPCB 42 months in this subgroup was lower than in the whole group (Table 1). The results of the white blood cell counts and the immunologic marker analyses of the lymphocytes at 42 months of age in relation to exposure to PCBs and dioxins are presented in Table 4. These results were all within the normal ranges for age-matched children (*19*). After logarithmic transformation of both variables, significant positive correlations between prenatal PCB exposure and the number of lymphocytes, T cells, and $CD3^+CD8^+$ (cytotoxic), $CD4^+$ $CD45RO^+$ (memory), TcR $\alpha\beta^+$, and $CD3^+$HLA-DR^+ (activated) T cells were found for maternal as well as cord plasma. Results were significant in the formula-fed but not in the breast-fed group. There were no significant correlations between the results of the white blood cell counts and immunologic marker analyses and the

Table 1. Characteristics of the study group(s).

	Health questionnaire ($n = 175$)	Immunologic marker analyses ($n = 85$)
Sex (male)[a]	54	48
Early feeding type (breast-fed)[a]	52	36
Duration of breast-feeding (weeks)[b]	17 (6–72)	16 (6–72)
Parity (firstborn)[a]	50	41
Maternal education and parental occupation (low)[a]	6	6
Tobacco smoking by one or both parents (yes)[a]	39	46
Family history of atopy in one or both parents (yes)[a]	73	79
Day care or nursery attendance of the child (yes)[a]	85	90
Toxic compounds measured in plasma		
ΣPCB maternal (μg/L)[b]	2.07 (0.59–7.35) ($n = 174$)	1.81 (0.59–4.76) ($n = 85$)
ΣPCB cord (μg/L)[b]	0.40 (0.08–2.08) ($n = 158$)	0.35 (0.08–1.98) ($n = 78$)
ΣPCB at 42 months (μg/L)[b]	0.39(0.08–5.90) ($n = 158$)	0.26 (0.08–2.12) ($n = 84$)
Toxic compounds measured in breast milk		
Planar PCB TEQ (pg/g milk fat)[b]	14.9 (4.4–45.7) ($n = 81$)	17.1 (5.9–45.7) ($n = 30$)
Mono-*ortho* PCB TEQ (pg/g milk fat)[b]	14.0 (3.2–44.4) ($n = 85$)	14.0 (6.4–25.45) ($n = 30$)
Dioxin TEQ (pg/g milk fat)[b]	35.8 (10.2–87.2) ($n = 71$)	35.1 (15.6–66.6) ($n = 24$)

[a]Data are percentages. [b]Data are median (minimum–maximum)

Table 2. Prevalence of infectious and allergic diseases and effects of prenatal and current PCB body burden.

	Prevalence n (%) ($n = 175$)	Prenatal PCB exposure ΣPCB Maternal OR (95% CI)[a]	p-Value	Current PCB body burden ΣPCB at 42 months OR (95% CI)[a]	p-Value
Infectious diseases					
Middle-ear infections (1 or more episodes)	103 (58.9)	0.89 (0.65–1.23)	0.49	1.27 (0.61–2.64)	0.52
Recurrent middle-ear infections (6 or more episodes)	21 (12.0)	1.37 (0.87–2.17)	0.17	3.06 (1.17–7.98)	0.02*
Pneumonia	5 (2.9)	0.41 (0.10–1.63)	0.21	0.01 (0.01–29.68)	0.13
Scarlatina	13 (7.4)	1.00 (0.56–1.80)	0.98	0.59 (0.08–4.03)	0.59
Chicken pox	130 (74.3)	1.43 (0.92–2.24)	0.11	7.63 (1.21–48.54)	0.03*
Other infectious diseases	15 (8.6)	1.04 (0.60–1.82)	0.87	0.85 (0.27–2.67)	0.79
Hospital admissions for infectious diseases	7 (4.0)	1.04 (0.44–2.46)	0.93	0.68 (0.01–25.05)	0.37
Allergic diseases					
Eczema	42 (24.0)	1.18 (0.82–1.71)	0.37	0.92 (0.41–2.08)	0.84
Allergic reaction	14 (8.0)	0.62 (0.29–1.32)	0.22	0.01 (0.01–0.37)	0.01*
Asthma or bronchitis	30 (17.1)	0.87 (0.55–1.40)	0.56	0.38 (0.06–2.57)	0.32
Coughing, chest congestion, or phlegm lasting for 10 days or more[b]	48 (27.4)	1.08 (0.75–1.54)	0.69	1.12 (0.58–2.16)	0.74
Attacks of shortness of breath with wheeze[b]	17 (9.7)	0.44 (0.18–0.99)	0.05*	0.34 (0.02–4.49)	0.41

[a]Corrected for sex, early feeding type (breast-fed or formula-fed), duration of breast-feeding during infancy (less or more than 16 weeks), parity (firstborn or second born), maternal education and parental occupation (low), tobacco smoking by one or both parents (yes or no), family history of atopy in one or more parents (yes or no), and day care or nursery school attendance for the child (yes or no). [b]In the previous 12 months. *Significant at the ≤ 0.05 level.

dioxin, planar, and mono-*ortho* PCB TEQ levels separately nor with the ΣPCB at 42 months of age.

Discussion

Our exploratory study demonstrates for the first time that health effects may occur from "normal"' environmental PCB exposure in preschool children. Current PCB body burden is influenced mainly by lactational transfer (*3*) and associated with a higher prevalence of recurrent middle-ear infections and of a common viral disease like chicken pox. A higher dioxin TEQ was associated with a higher prevalence of coughing, chest congestion, and phlegm. In Inuit infants, whose mothers have high levels of organochlorine compounds in their breast milk, perinatal organochlorine exposure was also associated with more acute otitis media (*7*). In these highly exposed infants, however, the prevalence of at least one episode of middle-ear infection during the first year of life (80%) is much higher than in our study of Dutch preschool children (60%). Breast-feeding during 4 months or more has been associated with lower rates of middle-ear and other infectious diseases during infancy (*20*), and the first middle-ear infection occurred earlier in children who were weaned before 6 months of age (*21*). In our study, half of the children were already weaned before 4 months and 70 percent before 6 months of age; the positive effect of breast-feeding on recurrent middle-ear infections was counteracted by the negative effect of PCB exposure.

Prenatal PCB exposure was also associated with a lower prevalence of attacks of shortness of breath with wheeze, and current PCB body burden was associated with a lower prevalence of allergic reactions to food, pollen, dust, and/or household pets. Respiratory symptoms are frequent in very young children and the relation of these symptoms to later asthma is unknown. However, attacks of shortness of breath with wheeze through ages 3–4 are associated with the subsequent diagnosis of asthma (*22*). Among Japanese schoolchildren, a positive tuberculin response predicted a lower incidence of asthma (*23*); in African children measles infection possibly prevented the development of atopy (*24*); and in Italy the prevalence of atopy was low among Hepatitis A seropositive subjects (*25*). Common infections acquired early in life paradoxically might prevent the development of atopy (*26*), so PCB exposure may be associated with a greater susceptibility to infectious as well as a lower prevalence of allergic diseases.

In our study prenatal PCB exposure was associated with a higher number of $CD8^+$ (cytotoxic) and TcR $\alpha\beta^+$ T cells at 42 months of age. In the study of Inuit infants no association between organochlorine exposure and immunologic parameters was found (*7*). The cellular analyses of the Inuit study are not strictly comparable with our method, and our results are consistent with *in vitro* studies, where exposure to PCBs and dioxins may change the kinetics of thymocyte maturation and skew the thymocyte differentiation toward $CD8^+$ phenotypically more mature TcR $\alpha\beta^+$ T cells (*27*). Prenatal PCB exposure was also significantly correlated with more $CD4^+CD45RO^+$ (memory) and $CD3^+$HLA-DR^+ (activated) T cells as well as subtle changes in levels of antibody to mumps and rubella after primary vaccinations. These findings may indicate a greater susceptibility to infectious diseases in early childhood. In animals an impaired host resistance to infectious diseases in relation to exposure to PCBs and dioxins has also been found. In seals fed by contaminated Baltic herring, an impairment of natural killer (NK) cell activity, *in vitro* T-lymphocyte function, *in vivo* delayed-type

Table 3. Effects of early feeding type and the duration breast-feeding on the prevalence of middle-ear infections, chicken pox, and allergic reactions and current PCB body burden.

	Early feeding type (n = 175)				Duration of breast-feeding (n = 91)			
	Formula-fed (n = 84) Prevalence	Breast-fed (n = 91) Prevalence	Formula-fed vs. breast-fed OR (95% CI)[a]	p-Value	Short (n = 44) Prevalence	Long (n = 47) Prevalence	Short vs. long OR (95% CI)[b]	p-Value
Recurrent middle-ear infections	9 (10.7)	12 (13.2)	1.03 (0.29–3.61)	0.96	6 (13.6)	6 (12.8)	0.12 (0.01–1.07)	0.06
Chicken pox	58 (69.0)	72 (79.1)	0.67 (0.21–2.14)	0.51	36 (81.8)	36 (76.6)	0.23 (0.05–1.08)	0.06
Allergic reaction	8 (9.5)	6 (6.6)	0.86 (0.08–9.48)	0.90	2 (4.5)	4 (8.5)	26.9 (0.74–978.8)	0.07
ΣPCB at 42 months(μg/L) median (minimum–maximum)	0.21 (0.08–0.46)	0.75 (0.23–5.90)			0.60 (0.24–1.15)	1.04 (0.23–5.90)		

Prevalence is shown as number (percent). For duration of breast-feeding, short = 6–16 weeks and long = > 16 weeks.
[a]Corrected for ΣPCB at 42 months, sex, duration of breast-feeding during infancy (less or more than 16 weeks), parity (firstborn or second born), maternal education and parental occupation (low), tobacco smoking by one or both parents (yes or no) , family history of atopy in one or more parents (yes or no), and day care or nursery school attendance for the child (yes or no). [b]Corrected for ΣPCB at 42 months, sex, parity (firstborn or second born), maternal education and parental occupation (low), tobacco smoking by one or both parents (yes or no) , family history of atopy in one or more parents (yes or no), and day care or nursery school attendance for the child (yes or no).

Table 4. Results of the white blood cell counts and the immunologic marker analysis (n = 85) in relation to prenatal PCB exposure.

	Absolute counts Percentiles (10^9/L)			Prenatal PCB exposure			
				ΣPCB maternal		ΣPCB cord	
White blood cells	5th	50th	95th	Pearson correlation[a]	p-Value	Pearson correlation[a]	p-Value
Monocytes	0.3	0.5	0.9	0.04	0.73	0.09	0.43
Granulocytes	2.2	4.1	7.5	0.14	0.22	0.15	0.20
Lymphocytes	2.2	4.1	6.6	0.25	0.02*	0.22	0.05*
T-cell markers							
$CD3^+$	1.4	2.7	4.6	0.25	0.02*	0.21	0.07
$CD3^+CD4^+$	0.8	1.7	2.7	0.19	0.08	0.16	0.17
$CD3^+CD8^+$	0.4	0.9	1.7	0.27	0.01*	0.24	0.04*
$CD4^+CD45RA^+$	0.3	1.0	1.9	0.12	0.26	0.04	0.77
$CD4^+CD45RO^+$	0.2	0.4	0.6	0.25	0.02*	0.26	0.02*
TcR $\alpha\beta^+$	1.1	2.5	4.2	0.25	0.02*	0.20	0.08
TcR $\gamma\delta^+$	0.1	0.2	0.4	0.17	0.12	0.15	0.20
$CD3^+$HLA-DR^+	0.1	0.3	0.5	0.26	0.02*	0.31	0.005*
B-cell markers							
$CD\ 19/20^+$	0.4	0.9	1.7	0.12	0.28	0.15	0.20
NK-cell markers							
$CD16^+$ and/or $CD56^+/CD3^-$	0.1	0.3	1.1	0.13	0.23	0.11	0.31

[a]After logarithmic transformation of both variables involved. *Significant at the ≤ 0.05 level.

hypersensitivity, and antibody responses to ovalbumin was observed (*28*). In our human study T-lymphocyte functions and direct hypersensitivity responses were not tested and should be subject to further study.

The PCBs 118, 138, 153, and 180 are the four most abundant congeners, constituting 46 percent of the total PCBs (*29*) and representing a complex mixture of interrelated environmental xenobiotics. To get an indication about possible immunotoxicants other than PCBs and dioxins, we measured lead and cadmium at 18 months of age. Mean levels were low (Cd mean 0.5 μg/dL, Pb mean 4.8 μg/dL) and not related to the outcome variables. These measurements were therefore not repeated at 42 months of age. In our study, at 18 months of age the number of $CD8^+$ (cytotoxic) and TcR $\alpha\beta^+$ T cells correlated best with the dioxin TEQ levels, while at 42 months of age there was no significant relation with the dioxin TEQ levels (Pearson correlation 0.11 and 0.16). However, the number of $CD8^+$ (cytotoxic) and TcR $\alpha\beta^+$ T cells at 18 and 42 months of age was significantly correlated (Pearson correlation 0.64 and 0.63, $p < 0.0001$); at preschool age the ΣPCB maternal and ΣPCB cord were associated with an increased number of $CD8^+$ (cytotoxic), $CD4^+CD45RO^+$ (memory), TcR $\alpha\beta^+$, and $CD3^+$HLA-DR^+ (activated) T cells in the formula-fed group only. For logistic reasons immunologic marker analysis could be done in only 85 children. Breast-fed infants were accidentally underrepresented in this subgroup, so no relation with the dioxin, planar, and mono-*ortho* PCB toxic equivalent (TEQ) levels in human milk was found. Immunotoxicologic studies are usually performed in laboratory animals, exposing them to a range of concentrations of potentially immunotoxic compound. In the environment PCBs and dioxins are present as complex mixtures of various congeners, which may vary in metabolism and toxicity. Moreover, PCBs form persistent and abundant metabolites that accumulate in biota. Limited information is available on the immunotoxic effects of chronic background exposure to such complex mixtures of xenobiotics in the human food chain and, besides PCBs and dioxins, other related organochlorine compounds might also be responsible for the observed associations.

In conclusion, the effects of perinatal background exposure to PCBs and dioxins persist into childhood and might be associated with a greater susceptibility to infectious diseases. Common infections acquired early in life may prevent the development of allergy, and therefore PCB exposure might be associated with a lower prevalence of allergic diseases. In our study the negative effect of a higher postnatal PCB exposure was counteracted by the positive effect of a longer duration of breast-feeding in infancy. Moreover, as described previously, breast-fed children in our study did better in neurological (*30*) and cognitive outcome (*18*) than their formula-fed counterparts did. Our study does not provide data to discourage breast-feeding at present background PCB levels. Although most of the above-mentioned immunologic changes seen in preschool children may be subtle, these data indicate that human children might be susceptible to immunotoxic pollutants and that, due to present levels of PCBs and dioxins in the food chain, health effects may occur. These effects are important from a public health perspective because large population groups are exposed. Perinatal exposure to PCBs, dioxins, and related compounds should therefore be lowered by reducing the intake through the food chain at all ages, rather than by discouraging breast-feeding. Long-term follow-up studies of perinatally exposed cohorts should be conducted into later childhood, through puberty, and into adulthood to investigate the implications of our findings.

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