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 study group consisted of 207 healthy mother-child 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) αβ+, and CD3+HLA-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 in early 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, leukocyte (sub)populations, PCBs, PCDDs, PCDFs.

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This study is part of the Dutch PCB/Dioxin Study, a larger prospective longitudinal study on possible adverse health effects of these pollutants on human children, and was supported by the Dutch Toxicology Research Promotion Program and the European Commission for Environmental and Health Programs contract EVSV-CT92.0207.
Measures of exposure to PCBs and dioxins. We estimated prenatal exposure to PCBs and dioxins by the sum of the 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 smoking, asthma, 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: H as your child ever had eczema or an allergic reaction? H as a doctor ever said to you that your child had asthma or bronchitis? H as 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 the dioxin, planar, and mono-ortho PCB TEQ (ΣPCB 42 months). Results were considered statistically significant at the p ≤ 0.05 level.

Results
At 42 months of age, 193 children were re-examined (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 breastfeeding 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 higher 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 ΣPCB 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, 12 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 ΣPCB T EQ 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 CD 3⁺CD8⁺ (cytoxic), CD 4⁺ CD 45RA⁻ (memory), TCR αβ⁺, and CD 3⁻H LA-D⁺ (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 questionaire | Immunologic marker analyses |
|---------------------|-----------------------------|
| (n = 175)           | (n = 85)                    |
| Sex (male)³        |                             |
| Early feeding type (breast-fed)³ |                  |
| Duration of breast-feeding (weeks)³ |                |
| Parity (firstborn)³ |                             |
| Maternal education and parental occupation (low)³ |               |
| Tobacco smoking by one or both parents (yes)³ |             |
| Family history of atopy in one or both parents (yes)³ |          |
| Day care or nursery attendance of the child (yes)³ |          |
| Toxic compounds measured in plasma |                      |
| ΣPCB maternal (µg/L)³ | 2.07 (0.59–7.35)             |
| ΣPCB cord (µg/L)³   | 0.40 (0.08–2.08)             |
| ΣPCB at 42 months (µg/L)³ | 0.39(0.08–5.90)             |
| Toxic compounds measured in breast milk |                    |
| Planar PCB TEQ (pg/g milk fat)³ | 14.9 (4.4–45.7)              |
| Mono-ortho PCB TEQ (pg/g milk fat)³ | 14.0 (0.2–44.4)              |
| Dioxin TEQ (pg/g milk fat)³ | 35.8 (10.2–87.2)             |

*Data are percentages. *Data are median (minimum–maximum).

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

| Infectious diseases | Prevalence n (%) | Prenatal PCB exposure ΣPCB Maternal OR (95% CI) | p-Value | Current PCB body burden ΣPCB at 42 months OR (95% CI) | p-Value |
|---------------------|------------------|-----------------------------------------------|---------|-----------------------------------------------------|---------|
| 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 |
| Scarletina | 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 more⁴ | 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 wheeze⁴ | 17 (9.7) | 0.44 (0.18–0.99) | 0.05* | 0.34 (0.02–4.49) | 0.41 |

*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). *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 higher prevalence of coughing, chest congestion, and phlegm was also associated with a higher prevalence of shortness of breath with wheeze, 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 diseases 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 αβ+ 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 γδ+ 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-cell lymphocyte function, in vivo delayed-type hypersensitivity to Hepatitis A, and changes in the thymus-draining lymph node of mice were observed (23).

**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 | Breast-fed | Formula-fed vs. breast-fed (OR (95% CI))a | p-Value |
|                             | Prevalence | Prevalence | OR (95% CI) | p-Value |
| Recurrent middle-ear infections | 1.03 (0.29–3.61) | 0.96 |
| Chicken pox | 1.03 (0.29–3.61) | 0.96 |
| Allergic reaction | 0.86 (0.08–9.48) | 0.90 |
| ΣPCB at 42 months (µg/L) | 0.21 (0.08–0.46) | 0.75 (0.23–5.90) |
| Prevalence | 0.60 (0.24–1.15) | 1.04 (0.23–5.90) |

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

| White blood cells | Absolute counts (10⁹/L) | Prevalence | Pearson correlationa | p-Value | Pearson correlationa | p-Value |
|------------------|-------------------------|------------|---------------------|---------|---------------------|---------|
|                  | 5th Percentiles | 95th Percentiles | ΣPCB maternal | | ΣPCB cord | |
|                  | | | | | | |
| Monocytes | 0.3 | 0.5 | 0.9 | 0.04 | 0.73 | 0.09 | 0.43 | 0.20 |
| Granulocytes | 2.2 | 4.1 | 7.3 | 0.14 | 0.22 | 0.15 | 0.20 |
| Lymphocytes | 2.2 | 4.1 | 6.6 | 0.25 | 0.02* | 0.26 | 0.01* | 0.26 |
| 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 | 0.12* | 0.24 | 0.04* |
| CD4+CD45RA+ | 0.3 | 1.0 | 1.9 | 0.32 | 0.02* | 0.34 | 0.07 |
| CD4+CD45RO+ | 0.2 | 0.4 | 0.6 | 0.25 | 0.02* | 0.26 | 0.02* |
| TcR αβ+ | 1.1 | 2.5 | 4.2 | 0.25 | 0.02* | 0.20 | 0.08 |
| TcR γδ | 0.1 | 0.2 | 0.4 | 0.32 | 0.01 | 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 |

*aAfter 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-αβ+ T cells correlated best with the dioxin TEQ levels, while at 42 months of age there was no significant correlation with the dioxin TEQ levels (Pearson correlation 0.11 and 0.16). However, the number of CD8+ (cytotoxic) and TCR-αβ+ 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-αβ+, 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 neurologic (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, 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.

**References and Notes**

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