In this study, we investigated 17- to 18-year-old boys and girls to determine whether changes in humoral or cellular immunity or respiratory complaints were related to blood serum levels of polychlorinated biphenyls (PCBs) and dioxin-like compounds after lifetime exposure in Flanders (Belgium). We obtained blood samples from and administered questionnaires to 200 adolescents recruited from a rural area and two urban suburbs. Physicians recorded medical history and respiratory diseases. We measured immunologic biomarkers such as differential blood cell counts, lymphocyte phenotypes, and serum immunoglobulins. As biomarkers of exposure, we determined the serum concentrations of PCBs (PCB 138, PCB 153, and PCB 180) and dioxin-like compounds [chemical-activated luciferase expression (CALUX) bioassay]. The percentages of eosinophils and natural killer cells in blood were negatively correlated with CALUX toxic equivalents (TEQs) in serum (p = 0.009 and p = 0.05, respectively). Increased serum CALUX TEQs resulted in an increase in serum IgA levels (p = 0.05). Furthermore, levels of specific IgEs (measured by radioallergosorbent tests) of cat dander, house dust mite, and grass pollen were also significantly and negatively associated with the CALUX TEQ; with odds ratios (ORs) equal to 0.63 [95% confidence interval (CI), 0.42–0.96], 0.68 (0.5–0.95), and 0.70 (0.52–0.95), respectively. In addition, reported allergies of the upper airways and past use of antiallergic drugs were negatively associated with CALUX TEQs, with ORs equal to 0.66 (0.47–0.93) and 0.58 (0.39–0.85), respectively. We found a negative association between IgGs and marker PCBs in serum (p = 0.009). This study shows that immunologic measurements and respiratory complaints in adolescents were associated with environmental exposure to polyhalogenated aromatic hydrocarbons (PHAHs). The negative correlation between PHAHs and allergic responses in adolescents suggested that exposure may entail alterations in the immune status. Key words: biomonitoring, biomarkers, CALUX, immunotoxicity, polychlorinated biphenyls. Environ Health Perspect 110:595–600 (2002). [Online 26 April 2002] http://ehpnet1.niehs.nih.gov/docs/2002/110p595-600vandenheuvel/abstract.html

Polychlorinated aromatic hydrocarbons, including polychlorinated biphenyls (PCBs), polychlorinated dibenz-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), are industrial compounds that are contaminants in chemical manufacturing processes and by-products in the combustion of organic materials. They are widely found in the environment and in chemical-waste dump sites. Because of their lipophilic nature, halogenated aromatic compounds bioaccumulate in the food chain. Residues have been detected in foods and in human adipose tissue, milk, and serum fat (I).

The toxicity of dioxin-like compounds is mediated through binding to the aryl hydrocarbon receptor (AhR) (2). Upon receptor–ligand binding, the complex is translocated to the nucleus and binds to the dioxin-responsive elements of the DNA, which subsequently induces the transcription of genes, for instance, encoding for metabolic enzymes. More recently, interference of polyhalogenated aromatic hydrocarbons (PHAHs) or their metabolites with other hormone receptors has also been observed (3).

Polychlorinated aromatic xenobiotics elicit a broad spectrum of biologic and toxic responses. Toxic responses include dermal toxicity, immunotoxicity, carcinogenicity, and adverse effects on reproductive, neurobehavioral, and endocrine functions (4,5). Experiments in which laboratory animals and nonhuman primates have been exposed to PCDD/PCDFs and/or PCBs indicate that the immune system is perhaps the most sensitive target for PHAH-induced toxicity. Indeed, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) causes cellular and humoral immune suppression, increased susceptibility to various infectious diseases, thymus atrophy, and depressed antibody and lymphoproliferative responses (6–11). Moreover, in wildlife, PCBs/dioxins affect the survival of birds, seals, and beluga whales by diminishing host resistance and increasing incidence and severity of infections (12,13).

Accidental or occupational exposure as well as background exposure of the general population to PCBs and dioxins may affect the human immune system (7,8,14–18). There is suggestive evidence that dioxin-like compounds influence the immune response by changing the CD4/CD8 ratio, the ratio of other lymphocyte populations, or the antibody production by B cells (4,7,8,15,16,18,19). As in animals, an increased susceptibility to infectious diseases has been noted in adults and children (16,20,21).

In this study, we investigated whether lifetime exposure of Flemish adolescents to PCBs and dioxin-like compounds is associated with alterations in the immune system and immunologically mediated health effects.

Methods

Study area. Hoboken and Wilrijk, two adjacent suburbs of the city of Antwerp, Belgium, are located 11–13 km southeast of the chemical and petrochemical industry in Antwerp’s seaport. They are also the seat of several small and medium-sized enterprises, a large primary nonferrous smelter (mainly Hoboken), two waste incinerators (Wilrijk), and a crematory (Wilrijk). The two suburbs are traversed by highways with a traffic density >80,000 vehicles per day. The two waste incinerators near and in Wilrijk have been in operation since 1971 and 1980, respectively. In 1997, when they were shut down, they had annual turnovers of 23,000 and 110,000 tons (22). The dioxin levels in topsoil samples obtained in 1997 at a depth of 3–5 cm at 15 sites in a radius of 0.5–3.0 km around the incinerators ranged from 3.9 to 27.2 ng toxic equivalents (TEQ)/kg dry weight (mean, 9.8 ng (22)). In contrast, the town of
Blood cell counts on whole blood samples. We performed total and differential hematology (at room temperature) to the CALUX TEQ samples at 4°C; and we stored immunoglobulin and serum and blood for determination of individual PCBs; we stored immunoglobulins (2 mL), indicator PCB and dioxin incident (based on the International Classification of Diseases) (25), in particular, allergic complaints during the past year. Questionnaires were administered to assess lifestyle, dietary habits, smoking and drinking habits, intake of medications, and social class of the participants. Demographic and sociodemographic characteristics of the participants in the three areas are described elsewhere (24).

**Blood sample collection.** We collected blood samples in the morning and separated serum immediately. We divided serum into three parts for individual analysis of immunoglobulins (2 mL), indicator PCB congeners (3 mL), and chemical-activated luciferase expression (CALUX) TEQ (2.5 mL). We immediately froze samples of serum and blood for determination of indicator PCBs; we stored immunoglobulin and CALUX TEQ samples at 4°C; and we transported samples for phenotyping and hematometry (at room temperature) to the laboratories within 6 hr for further processing. We performed total and differential blood cell counts on whole blood samples.

**Immune phenotyping.** We performed two-color flow cytometric immunophenotyping using the lysed whole blood method (Becton-Dickinson, San Jose, CA, USA) to determine the following lymphocyte subsets: CD19+ B lymphocytes, CD3+ T lymphocytes, CD3+CD4+ T-helper lymphocytes, CD3+CD8+ T-suppressor lymphocytes, CD45+ leukocytes, and CD16+CD56+ natural killer (NK) cells. We used the following Simultest Kits from Becton-Dickinson: CD45-fluorescein isothiocyanate (FITC), CD3-FITC/CD4-phycocerythrin (PE), CD3-FITC/CD8-PE, CD3-FITC/CD19-PE, CD3-FITC/CD16+CD56-PE, and γ1-FITC/γ1-PE control. We stained CD-Chex PLUS from Becton-Dickinson with the same antibodies to serve as an intralab quality control.

We incubated 100 µL aliquots of whole blood with 20 µL antibody for 30 min at room temperature in the dark. After we lysed incubation erythrocytes using Facs lysis solution (Becton-Dickinson), we washed and subsequently fixed them with 1% paraformaldehyde. We performed anti-body staining and flow cytometry within 6 hr after blood sampling. We performed lymphocyte gating on forward/sideward scatter dot blots using CD45-FITC-labeled blood cells. Quality control criteria included that the gated population must contain 95% CD45+ cells.

We performed all analyses using a FacsStar Plus cytometer (Becton-Dickinson) equipped with a 488 nm argon air-cooled laser. We used CellQuest software (Becton-Dickinson) for data acquisition and data analyses. Lymphocyte subsets are expressed as percentage of the gated lymphocytes.

**Determination of serum immunoglobulins.** We used an ELISA method to measure IgA, IgG, IgM, and IgE levels in serum, and we tested hypersensitivity by specific IgE measurements [radioallergosorbent tests (RASTs)]. The antigens tested included house dust mite, cat dander, grass pollen, and birch. We considered the IgE RAST mixtures positive if their value was > 0.70 kU/L.

**Indicator PCBs.** As described elsewhere (26,27), we measured the lower (congeners 28, 52, and 101) and higher (congeners 138, 153, and 180) chlorinated PCBs in serum as biomarkers of exposure to PCBs. Briefly, the samples were vortexed with formic acid for homogenization, followed by two steps of solvent-extraction of PCBs (n-heptane) and purification of extracts by a silica gel column. We performed PCB analyses on a high-resolution gas chromatograph with electron capture detection equipped with two capillary columns of different polarity. We identified the PCBs by means of retention times and carried out quantification using Mirex as internal standard. The detection limit for each congener was 0.015 ng/mL. For internal quality control, we included a blank and a control sample in each series of measurements.

**CALUX bioassay.** Measurement of the serum dioxin concentration would have required an additional 50 mL of blood. We therefore estimated exposure to dioxin-like compounds via the CALUX bioassay (BioDetection Systems BV, Amsterdam, The Netherlands), an in vitro assay that requires only 2.5 mL of serum and is mechanistically based. In this assay, we assessed dioxin-like compounds via an in vitro activation of the AhR of cultured H4IIE cells (28–31).

The method involved n-hexane extraction of 2.5 mL of blood serum and removal of matrix components by passage through a 33% H2SO4 silica column. We partly evaporated the extract, quantitatively transferred it to a conical vial for further evaporation, and reconstituted it in dimethyl sulfoxide (Acros Organics, Geel, Belgium) for CALUX measurement using the rat hepatoma H4IIE cell line transfected with an AhR-controlled luciferase reporter gene construct (CALUX assay). We grew cells in 96-well plates in 100 µL of minimal essential medium (Gibco, NV Invitrogen SA, Merelbeke, Belgium) with 10% fetal calf serum (Gibco) at 37°C with 5% CO2. When the cell layer reached 70–80% confluency, we treated the cells with samples and TCDD standards in quadruplicate and incubated the cells for 24 hr. After removing the medium, we washed the cells with 100 µL phosphate-buffered saline without calcium and magnesium (Gibco) and added 30 µL of cell lysis reagent (Promega, Benelux BV, Leiden, The Netherlands). We then shook the well plates for at least 45 min and stored them at −80°C for at least 1 hr. For determination of luciferase activity, we thawed the cells on ice and added 100 µL of luciferin assay mix (Promega) at room temperature. We measured the light production using a Victor 2 Luminometer (EG&G Wallac, Oosterhout, The Netherlands). We calculated the CALUX-based TEQs by comparing the luciferase activity induced by the sample with a dose–response curve generated from TCDD concentration standards analyzed simultaneously.

**Statistical analysis.** Database management and statistical analysis were performed with SAS, version 6.12 (SAS Institute, Cary, NC, USA) and Statistica, version 99 (Statsoft, Tulsa, OK, USA). We log-transformed data that were not normally distributed and described continuous data by the arithmetic mean ± 95% confidence interval (CI) or the geometric mean with 95% CI. We used dichotomous classifications to code for the presence of allergic diseases and positive allergic tests. We used the Student’s t-test and Fisher’s exact test to compare means and proportions, respectively, between girls and boys. We identified confounding variables by stepwise multiple regression or logistic regression. The p-value for variables to enter and to stay in the model was set at 0.05. We checked the following covariables: sex, smoking habits, alcohol consumption, history of infectious or allergic diseases, familial
history of hay fever or asthma, maternal smoking habits during pregnancy, having been breast-fed, body mass index, social class of the parents, use of oral contraceptives, and mean atmospheric ozone concentrations and mean daily temperatures during the week before blood sampling (both obtained from the Royal Meteorological Institute, Brussels, Belgium).

We calculated dose–effect relations in individual subjects between the biomarkers of immunologic effects and those reflecting exposure to PHAHs, using multiple linear regression for continuous outcomes or logistic regression for categorical variables.

### Results

#### Characteristics of the participants

The 200 adolescents (mean age ± SD, 17.4 ± 0.8 years) included 120 girls (60%). Mean age (17.3 vs. 17.4 years), mean body mass index (21.2 kg/m² vs. 21.1 kg/m²), proportions of current smokers (25%), social class of parents (23% workers, 64% middle class, 12% educated professionals), and breast-fed subjects (56%) were similar in girls and boys. Compared with girls, more boys consumed alcohol (29% vs. 65%). Among the girls, 41% were on oral contraceptives.

#### Immunology

The red blood cell count and the total and differential white blood cell counts are shown in Table 1. The hematologic measurements were within the normal ranges.

The lower chlorinated PCB congeners (138, 153, and 180) accounted for 27% of the total. For all further analyses, we combined congeners 138, 153, and 180.

The mean serum concentrations of the marker PCBs were 0.99 and 1.67 nmol/L in girls and boys, respectively, whereas CALUX TEQ was similar in both sexes, 0.15 and 0.16 pg/mL, serum, respectively (Table 1).

The percentages of adolescents with positive RAST tests or positive personal or familial histories of allergic or bronchial disorders appear in Table 2.

### Table 1. Hematologic, immunologic, and exposure measurements in 200 adolescents.

| Measurement (unit) | Girls (n = 120) | Boys (n = 80) | p-Value* |
|--------------------|----------------|--------------|----------|
|                    | Mean           | 95% CI       | Mean     | 95% CI       |         |
| Red blood cells (E12/L) | 4.55           | 4.50–4.61    | 5.07     | 4.95–5.19    | < 0.0001|
| White blood cells (E9/L) | 6.32           | 6.05–6.59    | 5.82     | 5.61–6.18    | 0.03    |
| Lymphocytes (%)      | 31.56          | 30.23–32.89  | 33.53    | 31.77–35.30  | 0.07    |
| Monocytes (%)        | 6.87           | 6.56–7.19    | 7.72     | 7.04–8.05    | 0.0004  |
| Trombocytes (E9/L)   | 258.26         | 244.69–271.83| 223.70   | 207.66–239.74| 0.0014  |
| Eosinophils(%)       | 2.10           | 1.88–2.34    | 3.03     | 2.59–3.53    | 0.0001  |
| CD3 (%)              | 66.53          | 65.21–67.84  | 60.11    | 58.40–61.83  | < 0.0001|
| CD4 (%)              | 38.28          | 37.10–39.47  | 33.64    | 32.26–35.03  | < 0.0001|
| CD8 (%)              | 21.32          | 20.35–22.28  | 20.56    | 19.38–21.73  | 0.32    |
| CD19 (%)             | 13.15          | 12.35–14.00  | 14.57    | 13.63–15.50  | 0.03    |
| CD45 (%)             | 96.87          | 96.08–97.66  | 96.9     | 96.57–97.23  | 0.96    |
| CD4/CD8              | 1.87           | 1.76–1.97    | 1.74     | 1.62–1.87    | 0.15    |
| CD16/CD56 (%)        | 14.42          | 13.27–15.67  | 17.70    | 16.26–19.27  | 0.002   |
| IgA (mg/dL)          | 1.35           | 1.26–1.45    | 1.49     | 1.35–1.65    | 0.11    |
| IgG (mg/dL)          | 17.66          | 16.85–24.32  | 41.3     | 25.82–66.07  | 0.002   |
| IgM (mg/dL)          | 10.09          | 9.71–10.50   | 9.51     | 9.05–10.10   | 0.07    |
| CALUX TEQ (pg TEQ/g fat) | 0.15     | 0.13–0.17    | 0.16     | 0.14–0.19    | 0.45    |
| SUM marker PCBs (pmol/g fat) | 28.59 | 24.93–32.80  | 34.89    | 28.66–42.46  | 0.09    |
| SUM marker PCBs (nmol/g fat) | 0.99  | 0.90–1.09    | 1.67     | 1.51–1.83    | < 0.0001|
| SUM marker PCBs (pmol/g fat) | 189.67 | 172.19–208.45| 359.75   | 326.59–397.19| < 0.0001|

Values are arithmetic means with 95% CI except where indicated.

*Significance of the difference between girls and boys (Student’s t-test). Geometric means (logarithmically transformed distribution) with 95% CI.

The eosinophil count was negatively and independently correlated with the serum concentrations of dioxin-like compounds (r = 0.009; Table 3). Monocytes tended to decrease with increasing serum TEQ values (p = 0.055; Table 3). We observed a negative association, although at borderline significance (p = 0.055; Table 3). We found no significant associations between the other lymphocyte phenotypes and either serum TEQ values or the combined serum concentrations of PCB congeners 138, 153, and 180 (Table 3). We obtained similar results when we expressed lymphocyte subpopulations as absolute numbers (cells per milliliter).

The dioxin-like activity in the serum was negatively correlated with serum IgE levels (p = 0.02) but positively correlated with IgA concentrations (p = 0.05; Table 3). We found a negative correlation between IgG levels and the concentration of the combined marker PCBs (p = 0.009; Table 3).

After adjustment for sex and familial history of hay fever, serum TEQ values were negatively associated with the odds of having a positive RAST for house dust mites [odds ratio (OR) = 0.68; p = 0.01], cat dander (OR = 0.63; p = 0.03), and grass pollen (OR = 0.70; p = 0.02) (Table 4). A history of upper airway allergy was negatively associated with serum TEQ values (OR = 0.66; p = 0.02; Table 4).

Respiratory complaints (Table 4) were not confounded by meteorological conditions, such as mean daily temperature and ozone concentration. We found a negative association between the odds of bronchial wheezing and serum CALUX TEQ (OR = 0.25; p = 0.03). After adjustment for familial history of hay fever and/or asthma, the significance disappeared (OR = 0.72; p = 0.07). Before and after similar adjustments, a positive answer to the question “ever received medication against asthma” was negatively associated with serum CALUX TEQ (OR = 0.58; p = 0.005). “Ever asthma” was positively associated with the serum concentration...
of marker PCBs (OR = 2.12; p = 0.05) even after correction. The odds of suffering from hay fever increased with higher serum PCB concentrations (OR = 1.63; p = 0.04). However, after correcting for sex, significance disappeared, because in our study, hay fever was more frequently reported by boys (31%) than by girls (17%).

When we expressed the serum concentrations of PCBs or dioxin-like compounds per gram of fat rather than per volumetric unit, dose–effect and dose–response relationships were similar.

**Discussion**

In this study we report on the immune status of 200 Flemish adolescents in relation to their exposure to PCBs and dioxins. The serum concentration of dioxin-like compounds was negatively and independently correlated with a history of upper airway allergy and “ever received medication against asthma,” with eosinophil counts, with serum concentrations of IgEs, and with the odds of having a positive RAST for house dust mites, cat dander, and grass pollen. The serum concentration of dioxin-like compounds was negatively associated with the proportions of NK cells and monocytes but positively associated with serum IgA levels. The changes in the immune system may reflect a decreased susceptibility to allergic reactions, as suggested by Weisglas-Kuperus (18). In the present study we measured the serum concentrations of dioxin-like compounds by the mechanistically based CALUX bioassay, which measures all compounds in the serum that act via binding to the AhR. This direct toxicity measure of dioxin-like compounds was negatively associated with the proportions of NK cells and monocytes but positively associated with serum IgA levels. The changes in the immune system may reflect a decreased susceptibility to allergic reactions, as suggested by Weisglas-Kuperus (18).

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**Table 2. Percentage of adolescents with positive RASTs or positive personal or familial history of allergic or bronchial disorders.**

| RAST                  | Girls (n = 120) | Boys (n = 80) | p-Value* |
|-----------------------|----------------|--------------|----------|
| House dust mite       | 18.3           | 31.3         | 0.04     |
| Cat dander            | 5.9            | 13.7         | 0.08     |
| Grass pollen          | 19.2           | 37.5         | 0.005    |
| Birch                 | 4.2            | 20.0         | 0.0006   |
| Overall               | 27.5           | 46.3         | 0.01     |

**Table 3. Dose–effect relationships between various immunologic measurements and the biomarkers of exposure to polychlorinated hydrocarbons.**

| Biomarker of effect (unit) | Dioxin-like compounds in serum (pg CALUX TEQ/mL) | Combined marker PCBs (nmol/L) |
|----------------------------|-----------------------------------------------|------------------------------|
|                            | Partial correlation coefficient r Estimate (SE) p-Value | Partial correlation coefficient r Estimate (SE) p-Value |
| Red blood cells (E12/L)    | –0.11 –0.408 (0.24) 0.09                     | –0.03 –0.007 (0.02) 0.66     |
| White blood cells (E9/L)   | 0.10 1.177 (0.87) 0.18                       | 0.06 0.044 (0.06) 0.48       |
| Lymphocytes (%)            | –0.07 –4.368 (4.28) 0.31                     | –0.11 –0.399 (0.30) 0.18     |
| Monocytes (%)              | –0.13 –1.699 (0.88) 0.055                    | –0.10 –0.082 (0.06) 0.19     |
| Thrombocytes (E9/L)        | 0.015 8.80 (41.29) 0.83                      | –0.03 –1.04 (2.90) 0.72      |
| Eosinophils (%)            | –0.18 –4.008 (0.15) 0.009                    | 0.005 0.0038 (0.01) 0.95     |
| CD3 (%)                   | 0.09 5.87 (4.18) 0.16                        | 0.05 0.19 (0.28) 0.49       |
| CD4 (%)                   | 0.05 2.46 (3.60) 0.49                        | 0.10 0.35 (0.25) 0.16       |
| CD8 (%)                   | 0.03 1.31 (3.0) 0.66                         | –0.05 –0.13 (0.21) 0.54     |
| CD19 (%)                  | 0.02 0.81 (2.45) 0.74                        | 0.04 0.08 (0.17) 0.64       |
| CD45 (%)                  | 0.03 0.93 (2.0) 0.64                         | –0.05 –0.09 (0.14) 0.53     |
| CD16+CD56 (%)             | –0.13 –0.20 (0.1) 0.05                        | –0.08 –0.29 (0.26) 0.27     |
| CD4/CD8                   | 0.01 0.04 (0.33) 0.91                       | 0.11 0.03 (0.02) 0.16       |
| IgA (mg/dL)               | 0.14 0.20 (0.1) 0.05                        | 0.06 0.005 (0.007) 0.47     |
| IgE (kIU/L)               | –0.16 –1.08 (0.46) 0.02                     | –0.04 –0.015 (0.03) 0.65     |
| IgG (mg/dL)               | 0.04 0.03 (0.05) 0.56                       | –0.20 –0.009 (0.004) 0.009  |
| IgM (mg/dL)               | –0.01 –0.02 (0.1) 0.88                     | 0.002 0.0002 (0.007) 0.97   |

*Significance of the difference between girls and boys (Fisher’s exact test). Positive to any of the four RASTs.

We adjusted the partial correlation coefficients for sex and current smoking.

*Sum of congeners 138, 153, and 180.
burden or report only between-group differences instead of dose–effect or dose–response relationships. In the adolescents in this study, we found significant associations between biomarkers of immunologic status and biomarkers of internal PHAH exposure.

Changes in cell surface markers on T cells represent an apparently sensitive biomarker response to the effects of dioxin-like compounds in rodents and primates. Dioxin-like compounds can affect the primary immune response by changing the CD4/CD8 ratio or the ratio of other lymphocyte subpopulations (11,15,19,38–41). In the present study we found no associations between T-helper or T-suppressor cells and the serum PCB/CALUX TEQ levels. In our adolescents, the numbers of NK cells (CD16+CD56- cells), eosinophils, and monocytes showed negative correlations with the concentration of dioxin-like compounds in the serum. These findings are in agreement with a study in Dutch infants in which exposure was associated with lower monocyte counts (21) and with a Swedish study that indicated that consumers of persistent organochlorine compound-contaminated fish had lower proportions and numbers of NK cells (42).

Reduced numbers of monocytes and NK cells (CD16+CD56- cells) may be an indication of depressed cellular immunity (34). However, the present associations were of borderline significance and should be carefully interpreted. The body burden of dioxin-like substances in our young study group could have been too low to induce considerable alterations in the subpopulation of white blood cells. Alternatively, the immunosuppressive effect of dioxin-like compounds might be mediated by a decreased functionality of individual cells rather than by a reduction in absolute cell numbers in the peripheral blood.

In humans, dioxin-like compounds can act on B cells, resulting in an impairment of antibody production (40,43). In our study we measured the serum concentrations of immunoglobulins (IgA, IgM, IgG, IgE) as biomarkers of humoral immunity. We observed a positive correlation between serum IgA and serum TEQ/mL but a negative correlation between serum IgE and serum TEQ/mL. Both correlations were weak and only borderline significant. Furthermore, in experimental animals such as monkeys, PCBs affect the primary antibody response, as evidenced by the depressed antibody response to sheep red blood cells (44,45). However, in the present study none of the serum immunoglobulins correlated with the combined concentration of marker PCBs in serum.

Positive associations between serum IgA levels and TCDD exposure have been found in the residents of Missouri as well as in Vietnam veterans (40,46). The latter have been exposed to TCDD through use of the pesticide Agent Orange.

Because serum immunoglobulin levels do not necessarily reflect the specific immune responses to common respiratory allergens, we also performed RASTs. We noticed a negative association between the odds of having a positive response to house dust mites, cat dander, or grass pollen and the serum concentration of dioxin-like compounds. To the best of our knowledge, data on specific IgE levels in relation to PCB/TCDD exposure levels have not yet been reported. Reduced antigen-specific IgG antibody responses after mumps and rubella vaccination have been reported in perinatally exposed Dutch children (18).

Immune deficiency generally manifests as an increased susceptibility to infections. Increased infection rates (e.g., severe cases of the common cold) are difficult to ascertain in epidemiologic surveys (34,39). Wildlife populations show increased susceptibility to bacterial and viral infections after PCB/TCDD exposure (12,13). Mononuclear phagocytic cells of PCB-exposed experimental animals show reduced phagocytic activity (39). In animals, therefore, PCB exposure may be associated with a decreased clearance of pathogenic bacteria by the spleen and liver, a diminished resistance to viruses, and an increased sensitivity to bacterial endotoxins (7). One-year-old Inuits who were breast-fed with milk contaminated with PCBs (621 µg/kg) showed a 20-fold higher incidence of infectious diseases, such as measles, meningitis, and otitis media compared with age-matched controls (20). Yucheng children born between July 1978 and June 1987 to women accidentally exposed to PCBs/PCDFs through the consumption of contaminated rice bran oil showed a higher rate of bronchitis, compared with controls, in the first 6 months after birth and higher frequencies of respiratory tract infections and otitis media attacks in a 6-year follow-up (47). Higher prevalences of recurrent middle-ear infections and of chicken pox were positively associated with current PCB body burden in Dutch preschool children (18). A higher dioxin TEQ was also associated with a higher prevalence of coughing, chest congestion, and phlegm (78). The same Dutch study (18) showed a negative association between prenatal PCB exposure and shortness of breath with wheezing, whereas the current PCB burden was associated with a lower prevalence of allergic reactions. Suppression of the allergic immune response after TCDD exposure was recently observed in a rat model (48). In this study, history of allergic and respiratory complaints were also negatively associated with the serum concentration of dioxin-like activity.

In conclusion, we found in 17- to 18-year-old adolescents that biomarkers of internal exposure to PHAHs, in particular, dioxin-like compounds, were related to biomarkers of immune status. The effects of exposure to dioxin-like compounds in adolescents were associated with a lower prevalence of allergic diseases.

| Table 4. Dose–response relationship between the odds of a positive RAST or history of allergic or bronchial disease and the biomarkers of exposure to polychlorinated hydrocarbons. |
| --- |
| **Biomarker of effect** | **Dioxin-like compounds in serum (µg CALUX TEQ/mL)** | **Combined marker PCBs (nmol/L)** |
| **OR (95% CI)** | **p-Value** | **OR (95% CI)** | **p-Value** |
| Positive RASTb | | | |
| House dust mite | 0.68 (0.50–0.93) | 0.01 | 0.77 (0.45–1.31) | 0.33 |
| Cat dander | 0.63 (0.42–0.96) | 0.03 | 0.99 (0.44–2.22) | 0.98 |
| Grass pollen | 0.70 (0.52–0.95) | 0.02 | 1.17 (0.68–1.98) | 0.57 |
| Birch | 0.77 (0.51–1.14) | 0.19 | 0.91 (0.36–2.92) | 0.93 |
| Overall | 0.77 (0.58–1.01) | 0.06 | 1.03 (0.64–1.66) | 0.92 |
| History of allergic diseaseb | | | |
| Upper airways | 0.66 (0.47–0.93) | 0.02 | 1.18 (0.62–2.25) | 0.61 |
| Lower airways | 0.87 (0.52–1.47) | 0.61 | 0.94 (0.34–2.55) | 0.90 |
| Skin | 0.97 (0.62–1.51) | 0.88 | 0.48 (0.33–3.33) | 0.34 |
| Overall | 0.76 (0.57–1.03) | 0.08 | 1.25 (0.71–2.11) | 0.46 |
| History of infectious disease | | | |
| Bacterial infections | 1.86 (0.54–6.42) | 0.32 | 0.97 (0.88–1.07) | 0.54 |
| Viral infections | 1.38 (0.69–2.76) | 0.26 | 0.97 (0.93–1.00) | 0.09 |
| Overall infections | 1.30 (0.85–2.62) | 0.46 | 0.96 (0.93–1.01) | 0.15 |
| History of respiratory symptomsc | | | |
| Bronchial wheezing | 0.72 (0.51–1.03) | 0.07 | 1.18 (0.69–2.04) | 0.55 |
| Hay fever | 0.97 (0.71–1.33) | 0.86 | 1.63 (1.02–2.61) | 0.04 |
| Ever asthma | 0.75 (0.49–1.15) | 0.19 | 2.12 (1.01–4.46) | 0.05 |
| Ever medication against asthma | 0.58 (0.39–0.85) | 0.005 | 1.02 (0.56–1.87) | 0.95 |
| Breathless while wheezing | 0.90 (0.46–1.76) | 0.75 | 1.11 (0.46–2.67) | 0.81 |
| Wheezing during exertion | 0.69 (0.35–1.37) | 0.27 | 1.16 (0.52–2.62) | 0.70 |

*aSum of congeners 138, 153, and 180. *Adjusted for sex and family history of hay fever. *Adjusted for family history of hay fever and/or asthma.
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