

Immune cell counts and risks of respiratory infections among infants exposed pre- and post-natally to organochlorine compounds: a prospective study

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## Abstract

**Background.** Early-life exposure to chemicals may influence immune system development, subsequently affecting child health. We investigated the immunomodulatory potential of polychlorinated biphenyls (PCBs) and *p,p'*-DDE in infants.

**Methods.** Pre-natal exposure to PCBs and *p,p'*-DDE was estimated from maternal serum concentrations during pregnancy, and post-natal exposure was calculated from the concentrations of compounds in mother's milk, total number of nursing days, and percentage of full nursing each week during the 3 month nursing period. Number and types of infections among infants were registered by the mothers (N=190). Blood was sampled from a subgroup of the infants at 3 months of age (N=86), and white blood cell (WBC) counts were analyzed. Lymphocyte subsets were analyzed in 52 of these infants.

**Results.** Infants with the highest pre-natal exposure to PCB congeners CB 28, CB 52 and CB 101 had an increased risk of respiratory infections during the 3 month study period. The odds ratio for infections decreased with increasing pre-natal mono-*ortho* PCB exposure and post-natal di-*ortho* PCB exposure. Similarly, the reference groups with the lowest pre-natal di-*ortho* PCB exposure and post-natal mono-*ortho* PCB and *p,p'*-DDE exposure had a higher odds ratio than the groups of infants with higher exposures. Altogether, a negative relationship was indicated between infections and the total organochlorine compound exposure during the whole pre- and post-natal period. Pre-natal exposure to PCB congeners CB 28, CB 52 and CB 101 was positively associated with numbers of lymphocytes and monocytes in infants 3 months after delivery. Moreover, a negative association between prenatal exposure to mono- and di-*ortho* PCBs and percentage of CD8<sup>+</sup> cytotoxic T-cells was observed, whereas pre-natal exposure to *p,p'*-DDE was negatively associated with the percentage of eosinophils.

**Conclusion.** This hypothesis generating study suggest that background exposure to PCBs and *p,p'*-DDE early in life may modulate the development of the human immune system. Both stimulation and suppression of the immune system was indicated, which may affect child health later in life.

## Background

Persistent and lipophilic organochlorine compounds, such as the industrial chemicals polychlorinated biphenyls (PCBs), the pesticide DDT, and dioxin-like contaminants polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), are immunotoxic to animals [1-3]. Studies on human populations with high exposures to these types of substances have strongly suggested that the early development of the immune system is susceptible to high exposure. Children from Taiwan, who were accidentally exposed to high levels of both non-dioxin-like and dioxin like PCBs and polychlorinated dibenzofurans (PCDFs) pre-natally, had higher rates of bronchitis, upper respiratory infections and middle ear infections than normally found in reference populations with low exposure [4,5]. Suppression of the humoral and cellular immune system is one of the most sensitive endpoints after pre-natal exposure of animals to the highly toxic dioxin-like compound 2,3,7,8-tetrachloro dibenzo-*p*-dioxin (TCDD) [6,7].

A few studies have reported that exposure of the fetus and/or infant to background levels of non-dioxin-like and dioxin-like PCBs, the DDT metabolite *p,p'*-DDE, and PCDD/Fs are associated with alterations in markers of immune function, such as white blood cell counts and numbers of lymphocyte subsets during childhood [8-13]. Furthermore, studies on neonates born in an area with high environmental load of both non-dioxin-like and dioxin-like PCBs in Eastern Slovakia have shown that high pre-natal PCB exposure is associated with a decreased thymus size [14]. PCB exposure early in life has been positively associated with acute *otitis media* and negatively associated with incidence of asthma or allergies later in life in children [10,11,15]. Studies of pre-school and school children have also suggested that exposure to *p,p'*-DDE early in life may be a risk factor for acute *otitis media* and asthma later during childhood [13,16,17]. A decreased antibody response after tetanus and diphtheria vaccination was found among children exposed to high levels of PCBs early in life [18].

In order to better understand how PCB compounds and *p,p'*-DDE may influence children health, we studied the associations between pre- and post-natal PCB and *p,p'*-DDE exposure and numbers and percentage of WBCs and lymphocyte subsets in three months old infants. Moreover, associations between organochlorine compound exposure and upper/lower respiratory infections in infants were studied during the first three months of life. Compounds were assigned to one of four compound groups depending on biological activity and sources of exposure: non-dioxin-like tri- to pentachlorinated PCBs (CB 28, CB 52 and CB 101), non-dioxin-like di-*ortho* PCBs (CB 138, CB 153 and CB 180), dioxin-like mono-*ortho* PCBs (CB 105, CB 118, CB 156 and CB 167), and *p,p'*-DDE.

## Methods

***Study population and sampling.*** Between 1996 and 1999, 325 primiparous women, living and seeking prenatal care in Uppsala County, Sweden, agreed to donate a serum sample (82% participation rate) in late pregnancy (week 32-34) for chemical analysis of organochlorines [19]. The results of the PCB and DDE analyses of serum sampled from the mothers in late pregnancy were used as an estimate of pre-natal PCB and DDE exposure of the infants, and 190 mother/child pairs had complete data for statistical analyses of associations between pre-natal PCB and DDE exposure and risk of infections (Table 1).

During the third week after delivery mothers from the group of 190 participants sampled milk while nursing their infants, using a manual breast pump and/or a passive mother's milk sampler. Mothers were asked to daily sample milk for seven days, at different time points during the nursing sessions. The mother's milk was kept frozen in acetone-washed glass bottles and newly sampled milk was poured on top of the frozen milk. The results of the PCB and DDE

analyses of mother's milk was used in the assessment of post-natal exposure (see below) and 175 mother/infant pairs had complete data for statistical analyses of associations between post-natal PCB and DDE exposure and risk of infections.

Within this group of 175 mother/infant pairs, blood was sampled from 86 infants 3 months after delivery for analysis of numbers and percentages of WBCs and lymphocyte subsets. The whole group of 175 infants could not be sampled due to lack of financial resources. Among these 86 mother/infants pairs data on numbers of WBCs were finally available from 81 infants and percentage data from 85 infants. Lymphocyte subsets could only be analyzed in 52 infants from this subgroup of 86 infants, due to limited volumes of blood available. Among the infants with data on lymphocyte subsets, data on lymphocyte numbers and percentages were available for 47 and 52 infants, respectively. The study was approved by the Ethics Committee of the Medical Faculty at Uppsala University. All participating women gave their informed consent prior to the inclusion in the study group.

***Interviews and questionnaires.*** At 6-12 and 32-34 completed gestational weeks, in-person interviews regarding maternal characteristics were performed, using a structured questionnaire [19,20]. Data on maternal characteristics included age, height, body weight before pregnancy, body weight at interview, years of education, home address, country of birth, and alcohol consumption and smoking before and during pregnancy. Blood samples for cotinine analysis (used as indicator of smoking habits) were taken at both interview occasions.

After delivery, the mothers were visited by a midwife when the infant was 3 months old. In an in-person interview, using a structured questionnaire, the mothers answered questions about sex of the infant, vaccination, nursing habits, and infant health during the first 3 months. Women gave information about the extent of nursing for each week of the 3 month period (full nursing,

partial nursing and no nursing). The participants also gave information about the health of the children each week from delivery to the date of the interview. The question about type of infection or other disease was open ended, and the number of days of disease was recorded for each disease occasion.

**Organochlorine compound analysis.** The lipid portion of serum samples was analyzed for the DDT metabolite *p,p'*-DDE, and 10 PCB congeners (IUPAC nos. 28, 52, 101, 105, 118, 138, 153, 156, 167 and 180). Procedures for extraction, sample clean-up and analysis, and quality control is described in Glynn et al. [19]. In mother's milk the compounds were analyzed using a method and quality assurance described by Glynn et al. [21]. When concentrations were below the limit of quantification (LOQ) they were set to 50% of LOQ in the statistical analysis.

**Immune cell analysis.** The hematological tests were performed at the Department of Clinical Chemistry, University Hospital of Uppsala. Capillary blood samples were collected in microtainer tubes (Sarstedt, Sweden). Differential counts were carried out by an automated instrument, Celldyn 4000 (Abbott Scandinavia AB, Solna, Sweden) based on a combination of optical characteristics and histochemical reactions. Number of total WBCs, and number and percentages of neutrophils, eosinophils, lymphocytes, and monocytes were recorded.

Analysis of lymphocyte subpopulations was performed on mononuclear cells prepared by Ficoll-Paque centrifugation and stained as described in Gräske et al. [22]. The following subpopulations were evaluated by flow cytometry as described in Gräske et al. [22]: CD3<sup>+</sup> cells (T-lymphocytes), CD19<sup>+</sup> cells (B-lymphocytes), CD4<sup>+</sup> cells (T-helper cells), CD8<sup>+</sup> cells (cytotoxic cells), and CD56<sup>+</sup> cells (NK-cells).

**Calculations and statistics.** Serum lipid organochlorine concentrations of the mothers in late pregnancy were used as a measure of pre-natal exposure of the fetus [23-25]. Post-natal exposure (PE) (ng or pg/g\*days) was calculated from the organochlorine concentration (ng or pg/g fresh weight) in mother's milk three weeks after delivery (Oconc), the number of days of nursing (Nd), and the percentage of full nursing during the study period (%N) using the equation

$$\text{PE (ng or pg/g*days)} = \text{Oconc} * \text{Nd} * (\%N/100).$$

In the exposure analysis tri- to penta-chlorinated CB 28, CB 52 and CB 101, with no dioxin-like biological activity, were grouped together (CB 28+52+101) because they showed low correlations with levels of other PCB congeners and chlorinated pesticides/metabolites in serum lipid among the women [19]. These three congeners have been detected in elevated levels in indoor air and in the blood of residents of buildings containing PCB-laden building materials [26-29]. CB 138, CB 153 and CB 180 (di-*ortho* PCBs) were grouped together because of high correlations between serum concentrations of these congeners [19], and because of their lack of dioxin-like biological activity [30]. Dioxin-like PCBs consisted of CB 105, CB 118, CB 156 and CB 167 (mono-*ortho* PCBs) [30]. *p,p'*-DDE was treated separately in the statistical analysis.

Statistical analysis was performed using MINITAB® For Windows, 14. Spearman's rank correlation analysis was used in analysis of correlations between different exposure variables. The association between immune cell numbers/percentages and organochlorine exposure was explored by linear regression analysis. Regression analysis was performed on logarithmically transformed organochlorine compound exposure data, since the distributions of data closely followed a log-normal distribution. However, in the case of pre-natal exposure to PCB 28+52+101 many women had serum levels below LOQ and this exposure variable was therefore

categorized. Some women did not nurse their infants at all and post-natal exposure of these infants was set to zero. This made ln-transformation impossible and post-natal exposure was therefore categorized.

In the statistical analysis of immune cell results the significance level was set to  $p \leq 0.01$ , since multiple comparisons were made. In cases when a statistically significant association between immune cells and organochlorine compound exposure was found in simple regression analysis, multiple regression analysis was used to adjust the associations for potential confounders. In linear regression analysis a few observations with a standardized residual  $\geq 3.0$  were omitted from the data sets due to a large impact on the regression results.

In the analysis of infection results odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression. Respiratory infection (influenza, a common cold, or a common cold with cough) (N=54) was the only health problem that was frequent enough to allow statistical analysis. Other health problems reported were stomach problems (N=1), navel infection (N=1), cows' milk intolerance/allergy (N=1), urinary infection (N=1), eye infection (N=2) and middle ear infection (N=2). In the regression analysis all independent variables were categorized in order to handle outlier problems. First, ORs were calculated in regressions with the exposure to each organochlorine compound group as the only independent variable. In the next step the ORs were adjusted for possible confounders of immune-related diseases.

We also analyzed the associations between respiratory infections and the total exposure to PCBs and *p,p'*-DDE during the whole pre-natal and post-natal period. In this analysis the pre- and post-natal PCB and *p,p'*-DDE exposure were separately categorized in four categories for each compound group, with the lowest exposure in the first category (categories 1-4, 1-3 for prenatal CB 28+52+101). The categorized exposures were thereafter summed up for each infant and the results were subsequently categorized into four new categories of total exposure. An

effort was made to have equal numbers of study participants in the different exposure categories. The level of statistical significance in logistic regression analysis was set to  $p \leq 0.05$ .

## **Results**

**Personal characteristics.** Table 1 gives the personal characteristics of the study participants with complete information about pre-natal organochlorine compound exposure and infections (N=190), the subgroup of mother/infant pairs that donated blood for WBC counts (N=86), and pairs within this subgroup that donated enough blood for lymphocyte subset analyses. A comparison of personal characteristics between mother/child pairs among the 190 study participants that did not donate infant blood for differential count and lymphocyte subset analyses (N=104) with those donating infant blood (N=86) showed no significant differences in age of the mother (t-test,  $p=0.29$ ), age of the infant (t-test,  $p=0.82$ ), smoking during pregnancy (Chi-square test,  $p=0.06$ ), alcohol consumption during pregnancy (Chi-square test,  $p=0.89$ ), years of education (Chi-square test,  $p=0.57$ ), nursing (Chi-square test,  $p=0.28$ ), and respiratory infections (Chi-square test,  $p=0.52$ ). Furthermore, pre- and post-natal organochlorine compound exposure did not differ between the groups (t-test,  $p>0.05$ ). However, there were more girls than boys in the subgroup with blood samples whereas the reverse was evident among mother/infant pairs that did not donate infant blood (Chi-square test,  $p=0.002$ ). Moreover, among infants with blood samples fewer had been vaccinated at the end of the study period (Chi-square test,  $p=0.038$ ).

**Pre- and post-natal exposure.** Among the PCB congeners, the median pre-natal exposure of the sum of CB 28, CB 52 and CB 101 was low (Table 2). Over 40% of the serum samples of the mothers in the whole study group had concentrations of all the three substances below the LOQ.

Concentrations  $\geq 10$  ng/g lipid were however found in 15% of the study participants. Median concentrations of di-*ortho* PCBs (CB 138, CB 153 and CB 180) and *p,p'*-DDE were similar, and the concentrations varied 8- to 26-fold. The concentrations of mono-*ortho* PCB TEQs varied 9-fold or more (Table 2).

Correlations between serum concentrations of PCB 28+52+101 and the other organochlorines in the total study group (infants) were weak, with Spearman correlation coefficients ranging from 0.13 to 0.24 (N=206). Concentrations of  $\Sigma$ di-*ortho* PCBs and mono-*ortho* PCB TEQs were strongly correlated ( $r=0.93$ ), whereas correlations between these two PCB groups and *p,p'*-DDE were less strong ( $r=0.66-0.75$ ).

Variation in organochlorine exposure from mother's milk during the first 3 months of infancy was large (Table 2). The correlation between post-natal exposure to di-*ortho* PCBs and mono-*ortho* TEQs was strong ( $r=0.95$ ). Other correlations between the studied compound groups were less strong ( $r=0.40-0.82$ ). Pre- and post-natal exposure were significantly correlated, with correlation coefficients for PCB 28+52+101, mono-*ortho* PCB, di-*ortho* PCB and *p,p'*-DDE ranging from 0.75-0.78.

***Organochlorine compound exposure and WBCs.*** The numbers and percentages of WBCs are presented in Table 3. In both simple and multivariate regression analysis, infants with the highest PCB 28+52+101 exposure had significantly higher mean numbers of total WBCs, lymphocytes and monocytes than infants in the reference category with the lowest exposure ( $p \geq 0.01$ ) (Figure 1). Moreover, the percentage of eosinophils was negatively associated with pre-natal *p,p'*-DDE exposure (Table 4). Otherwise, no significant associations were found (Figure 1, Table 4).

Numbers and percentages of WBCs were not significantly associated with post-natal exposure to any of the analyzed organochlorines (see additional files: Table A and Table B).

***Organochlorine exposure and lymphocyte subsets.*** We found a statistically significantly negative association between percentage of CD4<sup>+</sup>CD8<sup>+</sup> cells and pre-natal exposure to di-*ortho* and mono-*ortho* PCBs in the univariate and multivariate analyses (Table 4). Otherwise numbers and percentages of different lymphocyte subsets were not associated with pre-natal exposure (Figure 2, Table 4). Post-natal exposure to the organochlorines was not significantly associated with numbers and percentages of lymphocyte subsets (see additional file: Table C and and Table D).

***Respiratory infections.*** Infants exposed to the highest levels of PCB 28+52+101 pre-natal had a significantly increased odds ratio for respiratory infections during the first three months after birth compared with infants in the reference group with the lowest exposure (Table 5). This finding did not change in multivariate-adjusted logistic regression analysis. Moreover, a lowered odds ratio was found for infants in the second to fourth exposure category of pre-natal di-*ortho* PCB exposure, although the difference between the highest exposure category and the reference category only showed borderline statistical significance (Table 5). Similarly, a lowered odds ratio was found for infants in the highest exposure category of pre-natal mono-*ortho* PCB exposure in the multivariate-adjusted analysis (Table 5). For *p,p'*-DDE the reference category of infants with the lowest pre-natal exposure the odds ratio was higher than for infants with higher *p,p'*-DDE exposures, but the difference never reached statistical significance.

In the analysis of post-natal exposure, the OR for respiratory infections among infants in the highest exposure category of di-*ortho* PCB exposure was significantly lower than that of the

reference category in the un-adjusted analysis (Table 5). Multivariate analysis did not change this difference markedly. Significantly lowered ORs were observed for the second exposure category of *p,p'*-DDE and mono-*ortho* PCB, suggesting a U-shaped relationship (Table 5). An U-shaped relationship was also suggested for PCB 28+52+101, but the differences in odds ratio between the reference category and the other exposure categories were not statistically significant.

ORs for respiratory infections among infants in the second, third and fourth categories of total organochlorine compound exposure during the whole pre- and post-natal period were 0.74 (95% confidence interval:0.29-1.88), 0.36 (0.13-0.96) and 0.68 (0.28-1.68) respectively. The difference between the reference category with the lowest exposure and the third exposure category was statistically significant.

## **Discussion**

In this exploratory study of associations between early life exposure to organochlorine compounds and risks of respiratory infections among 3 month old infants, diverging associations between exposure to organochlorines compounds and infection risk were found. Our results suggest the pre-natal background exposure to the PCB congeners CB 28, CB 52 and CB 101 may cause immunosuppression, as indicated by an increased risk of respiratory infections among infants with high pre-natal exposures to PCB 28+52+101. Immunoactivation was suggested among infants with high pre-natal exposure to mono- and di-*ortho* PCBs, and post-natal di-*ortho* PCB exposure. Immunoactivation by post-natal mono-*ortho* PCB and *p,p'*-DDE exposure, but the dose-response was not as clear as for di-*ortho* PCBs. A similar immunostimulatory effect of the total PCB/DDE exposure during the whole pre- and post-natal period was indicated.

Our study was small and the results should therefore be interpreted with caution. Moreover, it may have been difficult for the mothers to correctly report their children's health history during the first three months after delivery. Bias could have been introduced in the diagnosis of disease, since the disease diagnosis was not confirmed from medical records. It is, however, not likely that such confirmation would have been possible in all cases, since many of the mothers probably did not seek medical help when their infants only had common colds. The women did not get information about their body burdens of organochlorine compounds, avoiding bias in the reporting of diseases due to knowledge about the degree of organochlorine compound exposure of the infant. There are many factors that influence the risk of respiratory infections among infants. Even though the results were adjusted with several potential confounders, unknown factors may still be involved in the associations observed. The results therefore have to be considered as hypothesis generating.

Our results do not allow for firm conclusions about possible mechanisms behind the diverging results. Two of the studied groups of PCB compounds, the PCB 28+52+101 and di-*ortho* PCB groups, were composed of non-dioxin-like congeners, whereas dioxin-like PCBs were grouped in the mono-*ortho* PCB group. Animal studies suggest that dioxin-like compounds modulate the immune system function through the aryl hydrocarbon receptor (AhR) pathway during exposure early in life [31,32]. Non-dioxin-like PCBs, may act through AhR-receptor independent pathways. For instance phagocytosis by human neutrophils and monocytes was reduced after *in vitro* exposure to di-*ortho* CB 138, CB 153 and CB 180, whereas the most toxic dioxin-like compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) did not alter phagocytosis [33]. Moreover, CB 153 induced DNA fragmentation in spleen cells from AhR knockout mice and no such effect of the dioxin-like PCB 126 was observed [34].

The strong correlation between dioxin-like mono-*ortho* PCB and non-dioxin-like di-*ortho* PCB exposures among the Uppsala infants make it difficult to suggest which of the two PCB groups that contribute most to the observed decline in infection risk at higher mono- and di-*ortho* PCB exposure levels. Furthermore, among the Uppsala mothers, body burdens of PCDD/Fs were strongly correlated with body burdens of mono- and di-*ortho* PCBs [21], making it even more complicated to draw conclusions about the contribution of non-dioxin-like and dioxin-like compounds to the observed effects. Animal studies have indicated that parts of the immune system may be activated at low exposure levels of TCDD and suppressed at higher exposures [35-37]. We speculate that dioxin-like effects may be the reason behind an activation of parts of the immune system in infants with the highest pre-natal exposure of mono- and di-*ortho* PCBs, thus causing a declined infection risk.

High pre-natal PCB 28+52+101 exposure appeared to enhance the susceptibility of the infants against respiratory infections. Correlations between infant exposures to PCB 28+52+101 and the other two PCB compound groups studied by us were weak, probably due to different sources of exposure of the mothers. Food is the major source of exposure of the mothers to mono- and di-*ortho* PCBs [38]. More than 40% of the mothers had serum concentrations of PCB 28+52+101 below the LOQ, showing that the general PCB 28+52+101 exposure from food is low in Sweden. PCB 28+52+101 concentrations of 10 ng/g lipid or higher were however detected in 15% of the mothers. Elevated blood concentrations of CB 28, CB 52 and CB 101 congeners have been detected among individuals spending long time periods in buildings containing PCB-laden building materials in Sweden [28]. In a study of children of school age with significantly elevated blood levels of CB 28, CB 52 and CB 101, no statistically significant difference in health complaints were observed in comparison with complaints reported for a control group of children from a non-contaminated school [29]. No study have reported associations between

infection risks among infants and pre- and post-natal exposure to PCB congeners CB 28, CB 52 and CB 101.

Only a few earlier studies have looked at associations between background mono- and di-*ortho* PCB exposure early in life and infant risk of respiratory infections. Among Dutch infants no significant associations were found between risks of upper/lower respiratory infections and pre- or post-natal exposure to the sum of congeners CB 118, CB 138, CB 153 and CB 180 at the age of 3 months [12]. Similarly, two studies of 3-6 months old Inuit infants found no significant trends of increased or decreased risks of respiratory infections/bronchopulmonary diseases with increased pre-natal exposure to PCB [13,39]. Pre-natal PCB exposure was in these two studies estimated from levels of CB 153 in blood serum of the mothers [39], and from the sum levels of CB 138, CB 153 and CB 180 [13]. In one of the Inuit studies a significant positive trend was found between pre-natal PCB exposure and all infections, including respiratory and gastrointestinal tract infections and otitis media [39].

It is difficult to determine the reasons behind the diverging results between studies. Pre- and post-natal exposure levels were higher among the Dutch and Inuit infants than among infants from Uppsala. For instance, the mean level of the sum of di-*ortho* congeners CB 138, CB 153 and CB 180 was 375-620 ng/g lipid in plasma/milk from Dutch and Inuit mothers (assuming a lipid content of 0.6% of blood plasma) [12,13], whereas it was 143 ng/g lipid in blood serum among the Uppsala women. Mean levels of the PCB marker congener CB 153 in cord blood/mother's plasma in the other Inuit study was 2-fold higher than the mean level found in mother's serum in the Uppsala cohort [19,39]. Mean body burdens of *p,p'*-DDE among Inuit mothers were 3- to 11-fold higher than among the Uppsala mothers [13,39]. Moreover, the results may have been influenced by the general health status of the infants. Acute otitis media was common among the Inuit infants. In one study it was found that 48% of all infants had one or

more episodes of otitis media during the first 3 months of life [13]. Among the Uppsala infants, middle ear infection was reported by 2 out of 190 participating mothers (approximately 1%).

No significant trends of decreased or increase risk of respiratory infections with increased *p,p'*-DDE exposure were found among the Inuit infants [13,39]. Similarly we did not find significant associations between pre-natal *p,p'*-DDE exposure and infection risk among the Uppsala infants, although there was a non-significant tendency of decreased risks at higher exposures. Post-natal *p,p'*-DDE exposure tended to decrease the risk of infections at medium high exposure levels, suggesting a U-shaped relationship. This indicates that relatively low exposure levels of *p,p'*-DDE early in life, similarly as in the case of mono- and di-*ortho* PCBs, may cause immunostimulation of parts of the immune system involved in the defense against respiratory infections. Taken together, the total exposure to the studied PCBs and *p,p'*-DDE during the whole pre- and post-natal period appeared protect the infants to some extent from respiratory infections during the first 3 months of life.

WBC and lymphocyte subset analyses were performed at the end of the 3 month study period. Therefore some of the observed associations with organochlorine compound exposure may be a consequence of the respiratory infections the infants had experienced during the study period. However, adjustment of the immune cell results for respiratory infections during the study period did not alter the observed associations. The immune system is very complex and we only studied a few immune markers. It is consequently difficult to draw conclusions about the relation between immune cell and infection results.

Nevertheless, similarly as in the case of respiratory infections, the WBC count and lymphocyte sub-population analyses suggested that PCB 28+52+101 influenced the immune system of the infants in a different manner than mono- and di-*ortho* PCBs. High pre-natal PCB 28+52+101 exposure was associated with increased numbers of lymphocytes and monocytes,

whereas pre-natal exposure to mono- and di-*ortho* PCBs was negatively associated with the percentage of CD8<sup>+</sup> cytotoxic T-cells. Both animal and human studies suggest that early life exposure to dioxin-like compounds may modulate the numbers/percentages of CD8<sup>+</sup> cytotoxic T-cells. Among Dutch infants a positive association was found between CD8<sup>+</sup> cell numbers in infants at 18 months of age and pre-natal exposure to mono-*ortho* PCBs, di-*ortho* PCBs and other dioxin-like PCBs and PCDD/Fs, but no association was found at 3 months of age [12]. In mice and rat offspring the population of CD8<sup>+</sup> cytotoxic T-cells was increased after pre- and post-natal TCDD exposure [2,40,41]. The low exposure of the Uppsala infants to dioxin-like PCBs and PCDD/Fs may have contribute to diverging results among Uppsala and Dutch infants. In mother's milk the mean total PCB/PCDD/PCDF TEQ concentration was 18 pg TEQ/g lipid and 64 pg TEQ among the Uppsala and Dutch mothers, respectively [12,37].

Apart from the Dutch studies of infants, a few other studies have reported associations between numbers and percentages of immune cells in early infancy and background pre- and post-natal exposure to mono- and di-*ortho* PCBs, and *p,p'*-DDE [8,12,13]. The observed associations between exposure and immune cell numbers/percentages differed between studies. Nevertheless, as in our study, the few observed shifts in immune cell numbers/percentages associated with organochlorine compound exposure were generally within the normal range [42,43], making it difficult to determine the clinical consequences of the observed shifts.

The WBC count and the analysis of lymphocyte subsets were performed on only 50-80 infants. A comparison of the personal characteristics of the participating mother/infant pairs that did not donate infant blood with those that donated blood showed no significant differences in most cases. There were however proportionally more girls in the WBC subgroup than among the rest of the study participants and fewer of the infants in the WBC group had been vaccinated. The immune cell results can therefore not be directly extrapolated to the whole study group.

Moreover, the immune cell numbers and percentages were only measured at one time point at the end of the 3 month study period, and we do not know if the results are representative for the other parts of the study period. Many statistical comparisons were made and it can therefore not be excluded that the results were due to chance. We used a strict significance level ( $p \leq 0.01$ ) and the results did not change significantly after adjustment for potential confounders, which reduces the possibility of chance findings.

We found no evidence of influence of pre- and post-natal  $p,p'$ -DDE exposure on numbers of different types of lymphocytes, which is in accordance with the results reported for Inuit infants [13]. A negative association between pre-natal  $p,p'$ -DDE exposure and percentage of eosinophilic granulocytes was however observed among the Uppsala infants. A study on German children in the ages 7-10 years reported a reduced eosinophilic granula content of eosinophilic granulocytes among children with the highest body burdens of  $p,p'$ -DDE [44]. Taken together the results indicate that eosinophilic granulocytes may respond to background exposures to  $p,p'$ -DDE among infants and children.

## **Conclusions and perspectives**

Our results indicate that organochlorine compound exposure early in life may influence infant susceptibility to respiratory infections. If causal, the observed effects may have consequences for health development among the children later in life. Respiratory infections early in life may be risk factors for development of asthma and middle ear infections during childhood [45-47]. Studies of children from the Netherlands, Canada, Spain and Germany have suggested that early life exposure to PCB and  $p,p'$ -DDE may modulate the risks of having respiratory infections, otitis media, and asthma during childhood [15,17,39]. Further follow-up of the children in the Uppsala

cohort will improve the possibilities to draw conclusions about the influence of the studied organochlorine compounds on the development of child health.

### **Competing interests**

The authors declare that they have no competing interests.

### **Authors' contribution**

The planning of the study, data collection and data analyses were performed by AG, AT, MA, AJ, POD and SC. MA was responsible for organochlorine compound analysis. AJ and GR was responsible for the immune cell analyses. AG wrote the first draft of the manuscript. All authors participated in the preparation of the final manuscript and approved the submission.

### **Acknowledgements**

We thank the participating women for patience and dedication. Midwives Irma Häggbom, Ragnhild Cnattingius, Margareta Aveskog, Ingela Wessén, Astrid Bengtsson, Marianne Leimar, and laboratory personnel Ingalill Gadhasson, Lena Hansson, Lotta Larsson and Elvy Netzel are thanked for good collaboration. The study was in part funded by the Swedish Cancer Society and the Swedish EPA.

## References

- [1] Banerjee BD: **The influence of various factors on immune toxicity assessment of pesticide chemicals.** *Toxicol Lett* 1999, **107**: 21-31.
- [2] Gehrs BC, Riddle MM, Williams WC, Smialowicz RJ: **Alterations in the developing immune system of the F344 rat after perinatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin: II. Effects on the pup and the adult.** *Toxicology* 1997, **122**: 229-240.
- [3] Kerkvliet NI, Baecher-Steppan L, Smith BB, Youngberg JA, Henderson MC, Buhler DR: **Role of the Ah locus in suppression of cytotoxic T lymphocyte activity by halogenated aromatic hydrocarbons (PCBs and TCDD): structure-activity relationships and effects in C57Bl/6 mice congenic at the Ah locus.** *Fundam Appl Toxicol* 1990, **14**: 532-541.
- [4] Guo YL, Lambert GH, Hsu CC, Hsu MM: **Yucheng: health effects of prenatal exposure to polychlorinated biphenyls and dibenzofurans.** *Int Arch Occup Environ Health* 2004, **77**: 153-158.
- [5] Yu ML, Hsin JW, Hsu CC, Chan WC, Guo YL: **The immunologic evaluation of the Yucheng children.** *Chemosphere* 1998, **37**: 1855-1865.
- [6] SCF: **Opinion of the SCF on the risk assessment of dioxins and dioxin-like PCBs in food.** *Opinion of the Scientific Committee on Foods.* Brussels, Belgium: European Commission; 2000.
- [7] JECFA: **Safety evaluation of certain food additives and contaminants. Polychlorinated dibenzodioxins, polychlorinated dibenzofurans, and coplanar polychlorinated biphenyls.** *IPCS INCHEM Report.* Geneva, Switzerland, WHO; 2002.

- [8] Belles-Isles M, Ayotte P, Dewailly E, Weber JP, Roy R: 2002. **Cord blood lymphocyte functions in newborns from a remote maritime population exposed to organochlorines and methylmercury.** *J Toxicol Environ Health, Pt A* 2002, **65**: 165-182.
- [9] Nagayama J, Tsuji H, Iida T, Hirakawa H, Matsueda T, Okamura K, Hasegawa m, Sato K, Ma HY, Yanagawa T, Igarashi H, Fukushige J, Watanabe T: **Postnatal exposure to chlorinated dioxins and related chemicals on lymphocyte subsets in Japanese breast-fed infants.** *Chemosphere* 1998, **37**: 1781-1787.
- [10] ten Tusscher GW, Steerenberg PA, van Loveren H, Vos JG, von dem Borne AE, Westra M, van der Slikke JW, Olie K, Pluim HJ, Koppe JG: **Persistent hematologic and immunologic disturbances in 8-year-old Dutch children associated with perinatal dioxin exposure.** *Environ Health Perspect* 2003, **111**: 1519-1523.
- [11] Weisglas-Kuperus N, Patandin S, Berbers GA, Sas TC, Mulder PG, Sauer PJ, Hooijkaas H: **Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children.** *Environ Health Perspect* 2000, **108**: 1203-1207.
- [12] Weisglas-Kuperus N, Sas TC, Koopman-Esseboom C, van der Zwan CW, De Ridder MA, Beishuizen A, Hooijkaas H, Sauer JP: **Immunologic effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in Dutch infants.** *Pediatr Res* 1995, **38**: 404-410.
- [13] Dewailly E, Ayotte P, Bruneau S, Gingras S, Belles-Isles M, Roy R: **Susceptibility to infections and immune status in Inuit infants exposed to organochlorines.** *Environ Health Perspect* 2000, **108**: 205-211.

- [14] Park H.-Y, Hertz-Picciotto I, Petrik J, Palkovicova L, Kocan A, Trnovec T: **Prenatal PCB exposure and thymus size at birth in neonates in eastern Slovakia.** *Environ Health Perspect* 2008, **116**: 104-109.
- [15] Weisglas-Kuperus N, Vreugdenhil HJ, Mulder PG: **Immunological effects of environmental exposure to polychlorinated biphenyls and dioxins in Dutch school children.** *Toxicol Lett* 2004, 149:281-285.
- [16] Karmaus W, Kuehr J, Kruse H: **Infections and atopic disorders in childhood and organochlorine exposure.** *Arch Environ Health* 2001, **56**: 485-492.
- [17] Sunyer J, Torrent M, Munoz-Ortiz L, Ribas-Fito N, Carrizo D, Grimalt J, Anto JM, Cullinin P: **Prenatal dichlorodiphenyldichloroethylene (DDE) and asthma in children.** *Environ Health Perspect* 2005, **113**: 1787-1790.
- [18] Heilmann C, Grandjean P, Weihe P, Nielsen F, Budtz-Jorgensen E: **Reduced antibody responses to vaccinations in children exposed to polychlorinated biphenyls.** *PLOS Medicine* 2006, **3**:1352-1359.
- [19] Glynn A, Aune M, Darnerud PO, Cnattingius S, Bjerselius R, Becker W, Lignell S: **Determinants of serum concentrations of organochlorine compounds in Swedish pregnant women: a cross-sectional study.** *Environ Health* 2007, **6**: 1-14.
- [20] Clausson B, Granath F, Ekbohm A, Lundgren S, Nordmark A, Signorello LB, Cnattingius S: **Effect of caffeine exposure during pregnancy on birth weight and gestational age.** *Am J Epidemiol* 2002, **155**: 429-436.
- [21] Glynn AW, Atuma S, Aune M, Darnerud PO, Cnattingius S: 2001. **Polychlorinated biphenyl congeners as markers of toxic equivalents of polychlorinated biphenyls, dibenzo-p-dioxins and dibenzofurans in breast milk.** *Environ Res* 2001, **86**: 217-228.

- [22] Graske A, Thuvander A, Johannisson A, Gadhasson I, Schutz A, Festin R, Glynn AW: **Influence of aluminium on the immune system--an experimental study on volunteers.** *Biometals* 2000, **13**: 123-133.
- [23] Ayotte P, Muckle G, Jacobson JL, Jacobson SW, Dewailly E: **Assessment of pre- and postnatal exposure to polychlorinated biphenyls: lessons from the Inuit Cohort Study.** *Environ Health Perspect* 2003, **111**: 1253-1258.
- [24] Covaci A, Jorens P, Jacquemyn Y, Schepens P: **Distribution of PCBs and organochlorine pesticides in umbilical cord and maternal serum.** *Sci Total Environ* 2002, **298**: 45-53.
- [25] Jaraczewska K, Lulek J, Covaci A, Voorspoels S, Kaluba-Skotarczak A, Drews K, Schepens P: **Distribution of polychlorinated biphenyls, organochlorine pesticides and polybrominated diphenyl ethers in human umbilical cord serum, maternal serum and milk from Wielkopolska region, Poland.** *Sci Total Environ* 2006, **372**: 20-31.
- [26] Gabrio T, Piechotowski I, Wallenhorst T, Klett M, Cott L, Friebel P, Link B, Schwenk M: **PCB-blood levels in teachers, working in PCB-contaminated schools.** *Chemosphere* 2000, **40**: 1055-1062.
- [27] Johansson N, Hanberg A, Bergek S, Tysklind M: **PCB in sealant is influencing the levels in indoor air.** *Organohalogen Compounds* 2001, **52**: 436-440.
- [28] Johansson N, Hanberg A, Wingfors H, Tysklind M: **PCB in building sealant is influencing PCB levels in blood of residents.** *Organohalogen Compounds* 2003, **63**: 381-384.
- [29] Liebl B, Schettgen T, Kerscher G, Broding HC, Otto A, Angerer J, Drexel H: **Evidence for increased internal exposure to lower chlorinated polychlorinated biphenyls (PCB) in pupils attending a contaminated school.** *Int J Hyg Environ Health* 2004, **207**: 315-324.
- [30] Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Håkansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Trisher A,

- Toumisto J, Tysklind M, Walker N, Peterson RE: **The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds.** *Toxicol Sci* 2006, **93**: 223-241.
- [31] Hogaboam JP, Moore AJ, Lawrence BP: **The aryl hydrocarbon receptor affects distinct tissue compartments during ontogeny of the immune system.** *Toxicol Sci* 2008, **102**:160-170.
- [32] Teske S, Bohn AA, Hogaboam JP, Lawrence BP: **Aryl hydrocarbon receptor targets pathways extrinsic to bone marrow cells to enhance neutrophil recruitment during influenza infection.** *Toxicol Sci* 2008, **102**:89-99.
- [33] Levin M, Morsey B, Mori C, Nambiar PR, De Guise S: **Non-coplanar PCB-mediated modulation of human leukocyte phagocytosis: a new mechanism for immunotoxicity.** *J Toxicol Environ Health, Pt A* 2005, **68**:1977-1993.
- [34] Jeon YJ, Youk EO, Lee SH, Suh J, Na YJ, Kim HM: **Polychlorinated biphenyl-induced apoptosis of murine spleen cells is aryl hydrocarbon receptor independent but caspases dependent.** *Toxicol Appl Pharmacol* 2002, **181**: 69-78.
- [35] Fan F, Wierda D, Rozman KK: **Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on humoral and cell-mediated immunity in Sprague-Dawley rats.** *Toxicology* 1996, **106**: 221-228.
- [36] Fan F, Yan B, Wood G, Viluksela M, Rozman KK: **Cytokines (IL-1beta and TNFalpha) in relation to biochemical and immunological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rats.** *Toxicology* 1997, **116**: 9-16.
- [37] Neubert R, Golor G, Stahlmann R, Helge H, Neubert D: **Polyhalogenated dibenzo-p-dioxins and dibenzofurans and the immune system. 4. Effects of multiple-dose treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on peripheral**

- lymphocyte subpopulations of a non-human primate (*Callithrix jacchus*).** *Arch Toxicol* 1992, **66**: 250-259.
- [38] Ankarberg E, Aune M, Concha G, Darnerud PO, Glynn A, Lignell S, Törnkvist A: **Risk assessment of persistent chlorinated and brominated environmental pollutants in food.** *NFA Report 9/07*. Uppsala, Sweden: Swedish National Food Administration; 2007. [http://www.slv.se/upload/dokument/rapporter/kemiska/2007\\_livsmedelsverket\\_9\\_risk\\_assessment\\_chlorinated\\_brominated\\_pollutants.pdf?epslanguage=EN-GB](http://www.slv.se/upload/dokument/rapporter/kemiska/2007_livsmedelsverket_9_risk_assessment_chlorinated_brominated_pollutants.pdf?epslanguage=EN-GB)
- [39] Dallaire F, Dewailly E, Vézina C, Muckle G, Weber JP, Bruneau S, Ayotte P: **Effect of prenatal exposure to polychlorinated biphenyls on incidence of acute respiratory infections in preschool Inuit children.** *Environ Health Perspect* 2006, **11**: 1301-1305.
- [40] Gehrs BC, Smialowicz RJ: **Alterations in the developing immune system of the F344 rat after perinatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin I. [correction of II]. Effects on the fetus and the neonate.** *Toxicology* 1997, **12**: 219-228.
- [41] Holladay SD, Lindstrom P, Blaylock BL, Comment CE, Germolec DR, Heindell JJ, Luster MI: **Perinatal thymocyte antigen expression and postnatal immune development altered by gestational exposure to tetrachlorodibenzo- p-dioxin (TCDD).** *Teratology* 1991, **44**: 385-393.
- [42] Bellamy GJ, Hinchliffe RF, Crawshaw KC, Finn A, Bell F: **Total and differential leucocyte counts in infants at 2, 5 and 13 months of age.** *Clin Lab Haematol* 2000, **22**: 81-87.
- [43] Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, Wara DW, Douglas SD, Luzuriaga K, McFarland EJ, Yogev R, Rathore MH, Levy W, Graham BL, Spector SA: **Lymphocyte subsets in healthy children from birth through 18 years of**

- age: the Pediatric AIDS Clinical Trials Group P1009 study.** *J Allergy Clin Immunol* 2003, **112**: 973-980.
- [44] Karmaus W, Brooks KR, Nebe T, Witten J, Obi-Osius N, Kruse H: **Immune function biomarkers in children exposed to lead and organochlorine compounds: a cross-sectional study.** *Environ Health* 2005, **4**: 1-10.
- [45] Daly KA, Brown JE, Lindgren BR, Meland MH, Le CT, Giebink GS: **Epidemiology of otitis media onset by six months of age.** *Pediatrics* 1999, **103(6 Pt 1)**: 1158-1166.
- [46] Dik N, Anthonisen NR, Manfreda J, Roos LL: **Physician-diagnosed asthma and allergic rhinitis in Manitoba: 1985-1998.** *Ann Allergy Asthma Immunol* 2006, **96**: 69-75.
- [47] Lemanske RF, Jr Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA, Kirk CJ, Reisdorf D, Roberg KA, Anderson EL, Carlson-Dykes KT, Adler KJ, Gilbertson-White S, Pappas TE, Dasilva DF, Tisler CJ, Gern JE: Rhinovirus illnesses during infancy predict subsequent childhood wheezing. *J Allergy Clin Immunol* 2005, **116**: 571-577.
- [48] Van den Berg M, Birnbaum L, Bosveld AT, Brunstrom B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegaw R, Kennedy SW, Kubiak T, Larsen JC, Van Leeuwen FX, Liem AK, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T: **Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife.** *Environ Health Perspect* 1998, **106**: 775-792.

## Figure legends

Figure 1. Unadjusted and adjusted means ( $\pm$ SE) of white blood cell numbers and percentages in three months old infants pre-natally exposed to the sum of CB 28, CB 52 and CB 101. Serum lipid concentrations of the PCB compounds measured in the blood of the mothers in late pregnancy (week 32-34) were used as a measure of pre-natal exposure. Adjusted means were calculated in cases when statistically significant results were found in the un-adjusted analysis. Results were adjusted for age of the mother, smoking and alcohol during pregnancy, mother's education, vaccination of the infant, nursing of the infant, age of the infant, infant's respiratory infections. \*Significantly different from the group with the lowest exposure (reference category) (N= 81-85,  $p \leq 0.01$ ).

Figure 2. Unadjusted means ( $\pm$ SE) of numbers and percentages of lymphocyte subsets in three months old infants pre-natally exposed to the sum of CB 28, CB 52 and CB 101. Serum lipid concentrations of the PCB compounds, measured in the blood of the mothers in late pregnancy (week 32-34), were used as a measure of pre-natal exposure.  $CD4^+/CD8^+$ : the ratio between numbers of  $CD4^+CD8^-$  and  $CD4^-CD8^+$  lymphocytes. \*Significantly different from the group with the lowest exposure (reference category) (N=47-52,  $p \leq 0.01$ ).

Table 1. Characteristics of the participating mother/child pairs<sup>a</sup>.

Variable	Infections (N=190)			White blood cells (N=86)			Lymphocyte subsets (N=52)		
Mother's age (yr)	28 (21-41)			29 (21-36)			29 (22-35)		
Infant's age (days)	92 (75-123)			93 (76-112)			93 (76-112)		
	Percent			Percent			Percent		
Education yrs	≤13:50	14-16:24	>16:26	≤13:50	14-16:27	>16:23	≤13:43	14-16:30	>16:27
Smoking preg.	No:82	Yes:18		No:86	Yes:14		No:89	Yes:11	
Alcohol preg.	No:82	Yes:18		No:83	Yes:17		No:77	Yes:23	
Nursing	Whole:81	Partial/no:19		Whole:84	Partial/no:16		Whole:91	Partial/no:9	
Infant's sex	Girl:44	Boy:56		Girl:58	Boy:42		Girl:57	Boy:43	
Vaccination	No:69	Yes:31		No:76	Yes:24		No:85	Yes:15	
Resp. infection	No:72	Yes:28		No:71	Yes:29		No:78	Yes:22	

<sup>a</sup>Mother's and infant's age: median (range). The partial nursing also includes women that did not nurse their infant at all. White blood cells were analyzed in a subgroup of 86 infants, and within this subgroup lymphocyte subsets were analyzed among 52 infants with enough blood left after white blood cell count analysis.

Table 2. Organochlorine concentrations in mother's serum lipids in late pregnancy and infant exposure from mother's milk<sup>a</sup>.

Serum <sup>b</sup>	Infections <sup>d</sup>	White blood cells <sup>d</sup>	Lymphocyte subsets <sup>d</sup>
PCB 28+52+101	4 (3-427)	4 (3-427)	4 (3-19)
Di-ortho PCB	131 (44-362)	127 (44-342)	129 (44-342)
Mono-ortho PCB TEQ	4 (1-20)	4 (1-11)	4 (1-9)
<i>p,p'</i> -DDE	88 (21-622)	85 (24-622)	83 (29-622)
Mother's milk exposure <sup>c</sup>			
PCB 28+52+101	8 (0-155)	8 (0-76)	9 (0-39)
Di-ortho PCB	364 (0-1666)	351 (0-830)	372 (0-830)
Mono-ortho PCB TEQ	11 (0-54)	11 (0-26)	11 (0-26)
<i>p,p'</i> -DDE	311 (0-2199)	289 (0-2199)	306 (0-2199)

<sup>a</sup>Median (range). Di-ortho PCB=CB 138, CB 153, CB 180; Mono-ortho PCB TEQ=CB 105, CB 118, CB 156, CB 167 [48].

<sup>b</sup>ng/g lipid. Mono-ortho PCBs: pg TEQ/g lipid. Serum sampled week 32-34.

<sup>c</sup>ng/g fresh weight\*days. Mono-ortho PCBs: pg TEQ/g fresh weight\*days. Calculated as organochlorine concentration in mother's milk on a fresh weight basis\*days of nursing\*(%of full nursing/100).

<sup>d</sup>Infections:Pre-natal exposure, N=190; Post-natal exposure, N=175. Differential count:N=86. Lymphocyte subsets:N=52.

Table 3. Numbers and percentages of white blood cells and lymphocyte subsets in 3 month-old infants<sup>a</sup>.

Differential count	N	No. of cells x 10 <sup>9</sup> /L	N	% of white blood cells
White blood cells	81	8 (5-15)		
Neutrophils	80	1.6 (0.6-5.7)	85	21 (8-47)
Eosinophils	80	0.3 (0.1-1.0)	85	4 (0.5-10)
Lymphocytes	80	5.4 (2.9-9.5)	85	70 (37-86)
Monocytes	80	0.3 (0.1-1.4)	85	4 (1-15)
Lymphocyte subsets				
CD19 <sup>+</sup>	47	0.8 (0.1-2.0)	51	16 (2-34)
CD3 <sup>+</sup>	47	3.7 (1.5-7.3)	52	70 (38-85)
CD4 <sup>+</sup> CD8 <sup>-</sup>	47	3.1 (1.0-5.3)	52	55 (24-72)
CD4 <sup>-</sup> CD8 <sup>+</sup>	47	0.7 (0.2-2.1)	52	13 (5-23)
CD56 <sup>+</sup>	47	0.05 (0-0.1)	52	0.8 (0-2.2)

<sup>a</sup>Median (range)

Table 4. Regression coefficients for associations between organochlorine exposure prenatally and numbers and percentages of numbers white blood cells and lymphocyte subsets<sup>a</sup>

	Di-ortho PCB	p,p'-DDE	Mono-ortho PCB TEQ
White blood cell count <sup>b</sup>	-0.01±0.54	0.49±0.36	0.28±0.44
Neutrophil numbers <sup>b</sup>	0.04±0.18	-0.03±0.12	0.08±0.14
Neutrophil % <sup>b</sup>	0.99±2.10	-0.74±1.47	0.33±1.73
Eosinophil numbers <sup>b</sup>	0.01±0.04	-0.06±0.03	0.02±0.03
Eosinophil % <sup>b</sup>	0.09±0.55	<b>-1.04±0.36*</b>	0.06±0.45
Eosinophil % <sup>c</sup>		<b>-1.68±0.43*</b>	
Lymphocyte numbers <sup>b</sup>	-0.06±0.42	0.50±0.28	0.14±0.35
Lymphocyte % <sup>b</sup>	-0.93±2.23	1.33±1.56	0.08±1.84
Monocyte numbers <sup>b</sup>	0.02±0.05	0.04±0.03	0.001±0.04
Monocyte % <sup>b</sup>	0.59±0.50	0.41±0.35	0.16±0.42
CD19 <sup>+</sup> numbers <sup>b</sup>	-0.04±0.16	0.02±0.11	-0.05±0.15
CD19 <sup>+</sup> % <sup>b</sup>	0.05±2.6	-0.81±1.7	0.87±2.3
CD3 <sup>+</sup> numbers <sup>b</sup>	-0.02±0.46	0.42±0.30	-0.14±0.42
CD3 <sup>+</sup> % <sup>b</sup>	-3.4±4.0	-1.2±2.8	-3.6±3.5
CD4 <sup>+</sup> CD8 <sup>-</sup> numbers <sup>b</sup>	0.14±0.36	0.29±0.24	0.06±0.33
CD4 <sup>+</sup> CD8 <sup>-</sup> % <sup>b</sup>	0.01±3.78	-0.61±2.61	-0.16±3.37
CD4 <sup>-</sup> CD8 <sup>+</sup> numbers <sup>b</sup>	-0.24±0.09	-0.15±0.06	-0.19±0.08
CD4 <sup>-</sup> CD8 <sup>+</sup> % <sup>b</sup>	<b>-4.02±1.29*</b>	-2.25±0.98	<b>-3.35±1.14*</b>
CD4 <sup>-</sup> CD8 <sup>+</sup> % <sup>c</sup>	<b>-5.61±1.96*</b>		<b>-6.24±1.84*</b>
CD4 <sup>+</sup> /CD8 <sup>+</sup> <sup>b</sup>	0.91±0.61	0.44±0.41	0.71±0.55
CD56 <sup>+</sup> numbers <sup>b</sup>	0.01±0.01	0.003±0.003	0.003±0.008
CD56 <sup>+</sup> % <sup>b</sup>	0.19±0.18	0.06±0.12	0.11±0.16

<sup>a</sup>Pre-natal exposure: mother's serum lipid concentrations in late pregnancy (week 32-34). Di-ortho PCB=CB 138, CB 153, CB 180; Mono-ortho PCB TEQ=CB 105, CB 118, CB 156, CB 167 [48]. White blood cell counts: N=80-85. Lymphocyte subsets: N=47-52.

<sup>b</sup>Regression coefficients from simple regression analysis (mean±SE)

<sup>c</sup>Partial regression coefficients (mean±SE) adjusted for age of the mother, smoking and alcohol during pregnancy, mother's education, vaccination of the infant, nursing of the infant, age of the infant, and infant's respiratory infections.

\*p≤0.01.

Table 5. Odds ratios (95% CI) for associations between respiratory infection during the first 3 months after birth and pre- or post-natal exposure to organochlorine compounds<sup>a</sup>

<b>Pre-natal</b>		Model <sup>b</sup>	Category 1	Category 2	Category 3	Category 4 <sup>c</sup>
PCB 28+52+101	Unadjusted	1.0	1.5 (0.66-3.3)	<b>2.6 (1.2-5.6)</b>		
	Multivariate	1.0	1.7 (0.71-4.1)	<b>3.4 (1.4-7.8)</b>		
Di-ortho PCB	Unadjusted	1.0	<b>0.35 (0.14-0.86)</b>	<b>0.34 (0.14-0.83)</b>	0.49 (0.21-1.2)	
	Multivariate	1.0	<b>0.28 (0.10-0.79)</b>	<b>0.23 (0.07-0.71)</b>	0.29 (0.08-1.0)	
<i>p,p'</i> -DDE	Unadjusted	1.0	0.67 (0.27-1.6)	0.73 (0.30-1.78)	0.80 (0.34-1.9)	
	Multivariate	1.0	0.64 (0.24-1.7)	0.69 (0.25-1.9)	0.74 (0.25-2.2)	
Mono-ortho PCB TEQ	Unadjusted	1.0	0.71 (0.30-1.7)	0.54 (0.23-1.3)	0.46 (0.19-1.2)	
	Multivariate	1.0	0.58 (0.22-1.6)	0.34 (0.10-1.1)	<b>0.23 (0.06-0.91)</b>	
<b>Post-natal</b>						
PCB 28+52+101	Unadjusted	1.0	0.51 (0.19-1.4)	0.72 (0.28-1.9)	1.4 (0.58-3.4)	
	Multivariate	1.0	0.44 (0.14-1.4)	0.65 (0.23-1.8)	1.4 (0.53-3.8)	
Di-ortho PCB	Unadjusted	1.0	0.48 (0.19-1.2)	0.53 (0.21-1.3)	<b>0.31 (0.12-0.83)</b>	
	Multivariate	1.0	<b>0.27 (0.08-0.87)</b>	<b>0.26 (0.08-0.85)</b>	<b>0.14 (0.04-0.50)</b>	
<i>p,p'</i> -DDE	Unadjusted	1.0	<b>0.25 (0.09-0.71)</b>	0.53 (0.21-1.3)	0.69 (0.29-1.7)	
	Multivariate	1.0	<b>0.18 (0.06-0.60)</b>	0.40 (0.14-1.2)	0.52 (0.17-1.5)	
Mono-ortho PCB TEQ	Unadjusted	1.0	<b>0.36 (0.14-0.97)</b>	0.53 (0.21-1.3)	0.69 (0.29-1.7)	
	Multivariate	1.0	<b>0.23 (0.07-0.79)</b>	0.37 (0.12-1.2)	0.33 (0.10-1.1)	

<sup>a</sup>Pre-natal exposure: mother's serum lipid concentrations in late pregnancy (week 32-34). Post-natal exposure: mother's milk concentrations on a fresh weight basis\*days of nursing\*(%of full nursing/100). Di-ortho PCB=CB 138, CB 153, CB 180; Mono-ortho PCB TEQ=CB 105, CB 118, CB 156, CB 167 [48]. Pre-natal exposure:N=190. Post-natal exposure:N=175. Bold odds ratios significantly different from the odds ratio of the reference category ( $p \leq 0.05$ ).

<sup>b</sup>Multivariate model also included the independent variables age of the mother, smoking and alcohol during pregnancy, mother's education, vaccination of the infant, nursing of the infant, age of the infant, infant's respiratory infections.

## **Additional files**

File name: Table A, Table B, Table C and Table D

File format: MS Word document.

Title of data:

White blood cell and lymphocyte subset numbers/percentages in 3 months old infants exposed to PCB and *p,p'*-DDE postnatally.

Description of data:

4 Tables

Table A. Unadjusted means ( $\pm$ SE) of white blood cell numbers ( $\times 10^9$ ) in 3-month-old infants exposed to organochlorines post-natally<sup>a</sup>

Table B. Unadjusted means ( $\pm$ SE) of white blood cell percentages in 3-month-old infants exposed to organochlorines post-natally<sup>a</sup>

Table C. Unadjusted means ( $\pm$ SE) of lymphocyte subset numbers ( $\times 10^9$ ) in 3-month-old infants exposed to organochlorines post-natally

Table D. Unadjusted means ( $\pm$ SE) of lymphocyte subset percentages in 3-month-old infants exposed to organochlorines post-natally<sup>a</sup>

Figure 1

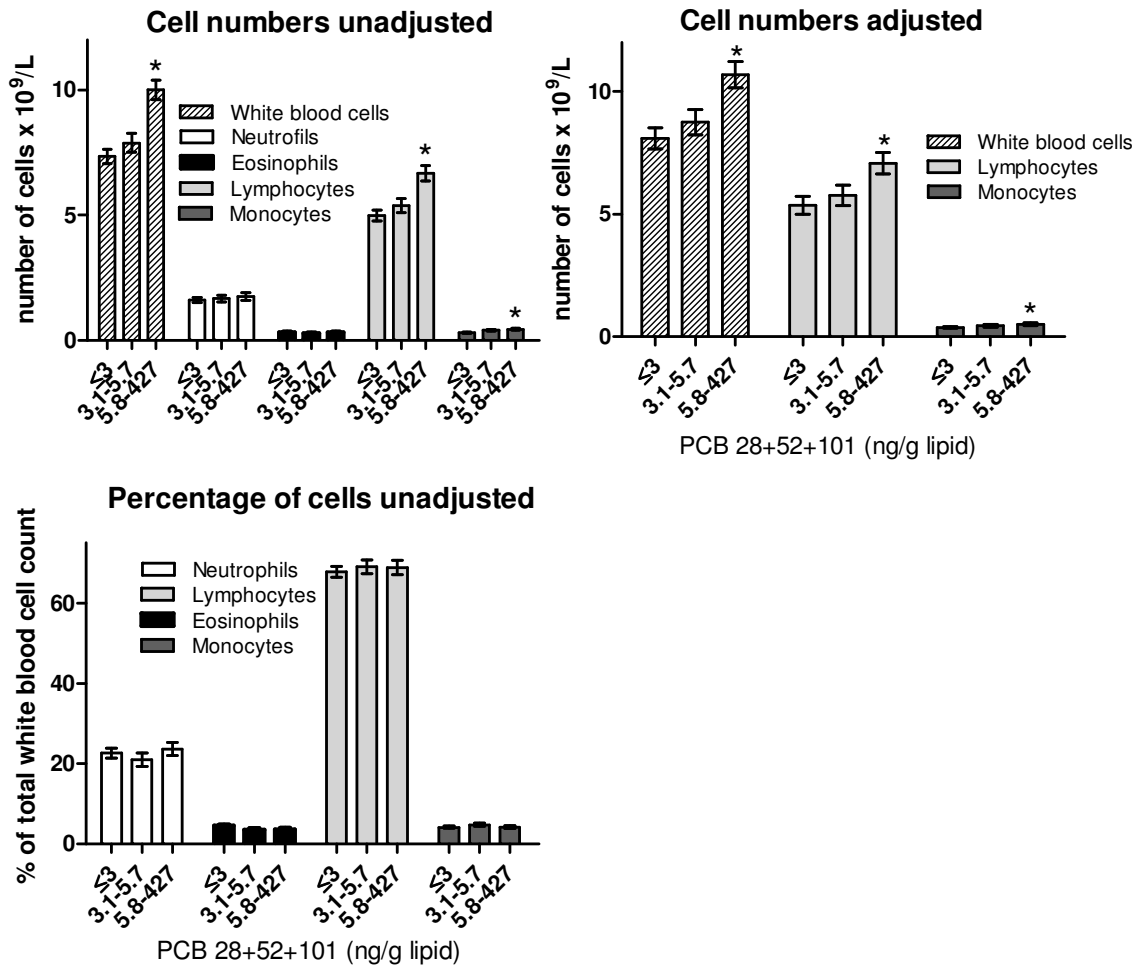
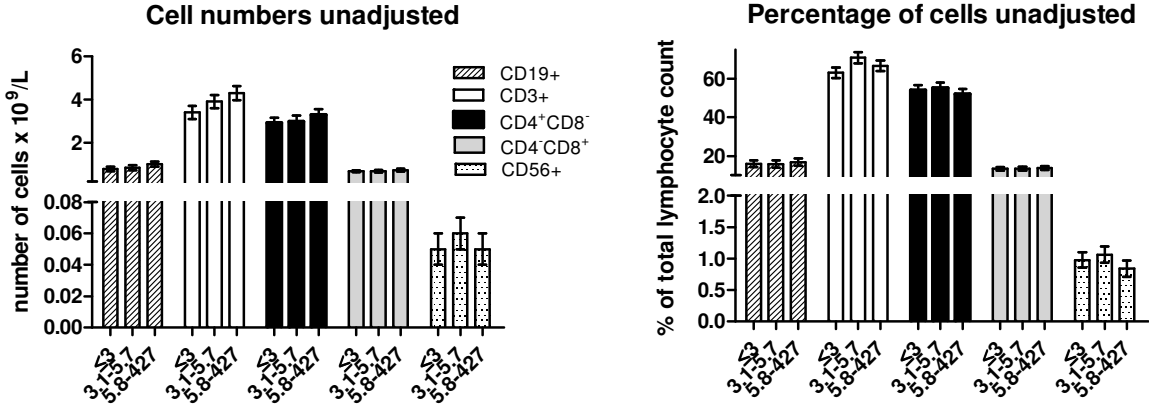


Figure 2



**Additional files provided with this submission:**

Additional file 1: table a.pdf, 15K

<http://www.ehjournal.net/imedia/9777796621633448/supp1.pdf>

Additional file 2: table b.pdf, 15K

<http://www.ehjournal.net/imedia/1661584882216334/supp2.pdf>

Additional file 3: table c.pdf, 16K

<http://www.ehjournal.net/imedia/8801515072163344/supp3.pdf>

Additional file 4: table d.pdf, 16K

<http://www.ehjournal.net/imedia/7014698632163344/supp4.pdf>