DO BACKGROUND *IN UTERO* EXPOSURE TO NON-DIOXIN-LIKE PCBs AND p,p´-DDE INFLUENCE NUMBERS AND PERCENTAGES OF WHITE BLOOD CELLS IN INFANTS?

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Introduction

Persistent and lipid-soluble organochlorine compound (OCCs), such as the industrial chemicals polychlorinated biphenyls (PCBs), the contaminants dioxins (PCDD/DFs) and the pesticide DDT, are ubiquitously present in the environment as a complex mixture. Levels of PCBs and DDT have decreased in the Swedish environment since the ban of use and production was initiated in the 1970s.[1,2] Similarly, the levels of dioxins have decreased, although in the last decades the decrease appears to have levelled off in some areas.[1] The current body burdens of OCCs among pregnant and lactating women in Sweden are considerably lower than those detected in the early 1970s. Breast milk studies have shown that body burdens of PCBs, PCDD/DFs and *p,p´*-DDE have steadily declined 3%-10% per year since the mid 1990s.[3] The body burdens are, however, still close to those found in populations where influences of OCCs on immune function in infants have been indicated.[4,5] We here report associations between background *in utero* OCC exposure and numbers/percentages of B-leucocytes in 3-months-old infants.

Table 1. Characteristics of the participating mother/child pairs.

Variable[a]	N	Median (range)		
Mother´s age (yr)	88	29 (21-36)		
Infant´s age (days)	88	93 (76-112)		
LPCB *in utero* (ng/g lipid)	88	4 (3-427)		
Di-*ortho in utero* (ng/g lipid)	88	127 (44-342)		
p,p´-DDE *in utero* (ng/g lipid)	88	85 (24-622)		
Mono-*ortho in utero* (pg/g lipid)	88	4 (1-11)		
PCDD/DF *in utero* (pg/g lipid)[b]	48	10 (5-23)		
	N	N	N	N
Smoking pregnancy	88	Never:66	Before:10	During:12
Alcohol pregnancy	88	No:72	Yes:16	
Education years	88	≤13:45	14-16:23	>16:20
Infection before sampling	88	No:63	Yes:25	

[a]*In utero* exposure: mother´s serum levels in late pregnancy (week 32-34). LPCB=CB 28, CB 52, CB 101; Di-*ortho* PCBs=CB 138, CB 153, CB 180; Mono-*ortho* PCBs=CB 105, CB 118, CB 156, CB 167; PCDD/DF=sum of the 17 congeners with an assigned WHO TEF[6]

[b]Breast milk levels of PCDD/DF TEQ were used since results from serum analysis was not available

Materials and Methods

Between 1996 and 1999, 323 primiparous women from Uppsala County were recruited in late pregnancy. The women donated a blood sample for organochlorine analysis in late pregnancy (week 32-34).[7] A subgroup of 211 women also donated breast milk, sampled during the third week after delivery, for OCC analysis.[8] Interviews about life-style and medical history were performed in early (week 6-11) and late pregnancy (week 32-34). After delivery the women answered an extensive food frequency questionnaire and questions about delivery and the nursing period up to 3 months after delivery. Three months after delivery blood was sampled from 88 randomly selected infants of mothers that had donated breast milk, and differential leukocyte counts were performed by standard methods at the University Hospital in Uppsala (Table 1 and 2). The local Ethics Committee in Uppsala approved the study design and informed consent was obtained from the study participants.

Table 2. Differential counts of leukocytes in 3 months old infants.

	N	No. ($x10^9$) of cells/L	N	% of B-leukocytes
B-leukocytes	81	8 (5-15)		
Neutrophils	80	1.6 (0.6-5.7)	85	21 (8-47)
Eosinophils	80	0.28 (0.06-0.97)	85	4 (0.5-10)
Lymphocytes	80	5.4 (2.9-9.5)	85	70 (37-86)
Monocytes	80	0.34 (0.08-1.37)	85	4 (1-15)

Median (range)

In the statistical analysis, organochlorine compounds were grouped according to both toxicological activity and to strength of correlations between levels of different compounds. CB 28, CB 52 and CB 101 were grouped together as the exposure variable LPCB, CB 138, CB 153 and CB 180 were grouped together as di-*ortho* PCBs, CB 105, CB 118, CB 156 and CB 167 as mono-*ortho* PCB TEQs, and the seventeen toxic PCDD/DFs as PCDD/DF TEQs (breast milk levels). *p,p´*-DDE was treated separately in the statistical analysis. Serum organochlorine concentrations were lipid adjusted, as well as breast milk PCDD/DF levels when used as a measure of the mother´s body burden during pregnancy.

Associations between *in utero* OCC exposure and leukocyte counts and percentages were first analysed by simple regression. Multiple regression was then performed in order to adjust associations for potential confounders (mother´s age, smoking and alcohol during pregnancy, mother´s education and upper respiratory infections in infants). The levels of LPCB were categorized since more than 40% of the women had levels below the limit of quantification. The distributions of OCC levels were log-normal and the data were therefore ln-transformed.

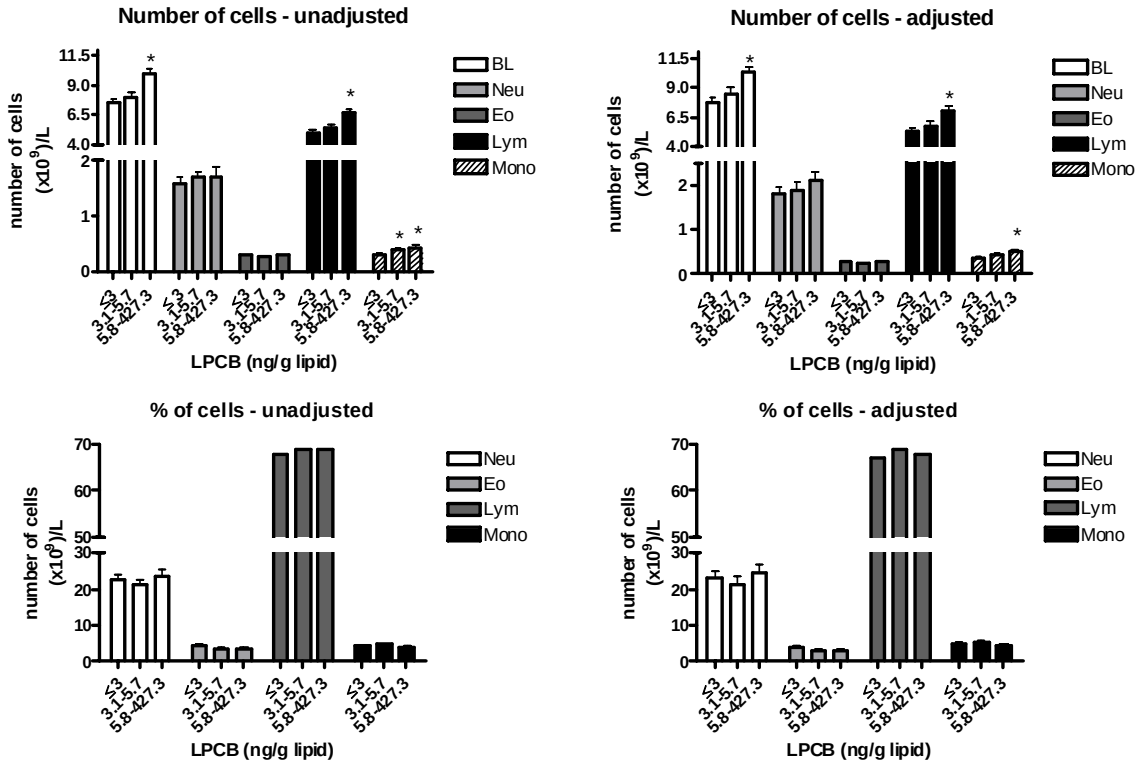

Figure 1. Unadjusted and adjusted means (SE) of numbers and percentages of B-leukocytes in 3 months old infants after *in utero* exposure to the PCB congeners CB 28, CB 52 and CB 101 (LPCB). For adjustment see Materials and Methods. BL=B-leukocytes, Neu=neutrophils, Eo=eosinophils, Lym=lymphocytes, Mono=monocytes. *p<0.05, N=74-85.

Results and Discussion

In utero exposure of the infants was estimated from serum levels of organochlorines in mothers in late pregnancy. Among the PCB congeners, the sum of CB 28, CB 52 and CB 101 (LPCB) showed the lowest median levels in serum from the mothers in late pregnancy (Table 1). Variation in levels was, however, large (>100-fold). The high levels in a few women are not likely of dietary origin, since levels of CB 28, CB 52 and CB 101 are low in Swedish food in comparison to di-*ortho* PCBs[9]. A possible source of exposure could be PCB-containing building material in houses and workplaces[10]. Median levels of di-*ortho* PCBs (CB 138, CB 153 and CB 180) and *p,p´*-DDE were much higher than those of LPCB, but the variation was lower (8- and 26-fold). The median PCDD/DF level, expressed as TEQs, was 2-fold higher than the median mono-*ortho* PCB TEQ level. Variation was 10-fold or less (Table 2).

The simple regression analysis showed that infants with the highest exposure to LPCB had higher numbers of B-leukocytes compared with the group with the lowest exposure (Fig. 1). Among different types of B-leukocytes numbers of lymphocytes and monocytes increased with increasing LPCB exposure (Fig. 1). Adjustment with potential confounders did not markedly change the results. No significant associations between *in utero* exposure to LPCB and percentages of B-leukocytes were found. (Fig. 1). *p,p´*-DDE exposure was negatively associated with the number and percentage of eosinophils both in the simple and multiple regression (Table 3). Leukocyte and lymphocyte numbers were positively associated with *p,p´*-DDE exposure after adjustment with potential confounders but not in the simple regression. Otherwise no statistically significant associations were found.

Table 3. Associations between organochlorine exposure *in utero* and white blood cell counts[a]

	Di-*ortho* PCB	*p,p´*-DDE	Mono-*ortho*	PCDD/DF
Unadjusted[b]				
B-leukocyte no	-0.01±0.54	0.49±0.36	0.28±0.44	-0.49±0.81
Neutrophil no	0.04±0.18	-0.03±0.11	0.08±0.14	-0.48±0.30
Neutrophil %	0.99±2.10	-0.74±1.5	0.33±1.7	2.4±3.8
Eosinophil no	0.01±0.04	-0.06±0.03*	0.02±0.03	-0.01±0.07
Eosinophil %	0.10±0.55	-1.0±0.4*	0.06±0.45	-0.68±0.94
Lymphocyte no	-0.06±0.42	0.50±0.28	0.14±0.35	-0.06±0.65
Lymphocyte %	-0.93±2.2	1.3±1.6	0.08±1.8	1.6±3.7
Monocyte no	0.02±0.05	0.04±0.03	0.01±0.04	0.11±0.08
Monocyte %	0.38±0.51	0.32±0.36	-0.08±0.42	1.3±0.8
Adjusted[c]				
B-leucocytes	0.90±0.81	1.1±0.45*	1.1 ±0.60	0.06±1.4
Neutrophil no	0.26±0.27	0.01±0.16	0.27±0.20	-0.52±0.50
Neutrophil %	2.7±3.2	-0.95±2.0	0.89±2.4	-2.0±5.8
Eosinophil no	-0.01±0.06	-0.09±0.03*	0.02±0.05	-0.01±0.11
Eosinophil %	0.09±0.82	-1.8±0.5*	0.04±0.61	-0.13±1.4
Lymphocyte no	0.41±0.65	0.95±0.37*	0.64±0.48	0.64±1.1
Lymphocyte %	-3.4±3.3	1.4±2.1	-0.62±2.5	-0.27±5.7
Monocyte no	0.05±0.07	0.08±0.04	-0.01±0.05	0.17±0.13
Monocyte %	0.06±0.74	0.24±0.47	-0.66±0.55	0.80±1.1

[a]*In utero* exposure: mother´s serum levels in late pregnancy (week 32-34). N=74-85; PCDD/DF: N=36-44.
[b]Regression coefficients from simple regression analysis (mean±SE)
[c]Partial regression coefficients (mean±SE) adjusted for age of the mother, smoking and alcohol during pregnancy, mother's education, infant´s upper respiratory infections
*$p \leq 0.05$.

Our results indicate that *in utero* exposure to the sum of CB 28, CB 52 and CB 101 may influence the number of B-leukocytes in 3-months-old infants, by increasing the number of lymphocytes and monocytes. It is, however, not possible to draw firm conclusions about causality of the associations, but the possibility of causality is strengthened by the tendency of a dose response (Fig. 1). Moreover the associations were not markedly changed

after adjustment for potential confounders. The picture is the same for the association between increased *p,p´*-DDE exposure and decreased number and percentage of eosinophils.

To our knowledge this is the first study of how non-dioxin-like PCBs CB 28, CB 52 and CB 101 influences markers of immune function in infants. It is from our results not possible to draw conclusions about health consequences of a slight shift in the lymphocyte and monocyte populations within the normal range. A few studies have reported associations between other OCCs and immune markers and disease incidence in infants.[4,5,11,12] Among Inuit infants an increased risk of otitis media was associated with increasing levels of *p,p*-DDE in breast milk.[5,12] Associations between *p,p´*-DDE and differential counts were not analysed.[5,12] Average *p,p*-DDE body burdens among the Inuit mothers were on average 3- to 10-fold higher than those found in our study.[5, 12] A few other studies have indicated that OCCs may both affect levels of immune markers and incidence of infections, allergies and respiratory problems in pre-school and school children.[13-17] Further follow-up studies on our cohort are needed in order to find out if children health is affected by the exposure levels found.

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