Children's white blood cell counts in relation to developmental exposures to methylmercury and persistent organic pollutants

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ABSTRACT

Background: To explore possible markers of developmental immunotoxicity, we prospectively examined 56 children to determine associations between exposures to mercury and persistent organic pollutants since birth and the comprehensive differential counts of white blood cells (WBC) at age 5 years.

Materials and methods: Extended differential count included: neutrophils, eosinophils, basophils, lymphocytes (including T cells, NK cells, and B cells), and monocytes. Organochlorine compounds (OCs) including polychlorinated biphenyls (PCBs) and pesticides, five perfluoroalkyl substances (PFASs), and mercury (Hg) were measured in maternal (n=56) and children's blood at 18 months (n=42) and 5 years (n=56). We constructed latent functions for exposures at three different ages using factor analyses and applied structural equations models adjusted for covariates.

Results: Prenatal mercury exposure was associated with depleted total WBC, especially for lymphocytes, where a one standard deviation (SD) increase in the exposure was associated with a decrease by 23% SD (95% CI: -43, -4) in the cell count. Prenatal exposure to OCs was marginally associated with decreases in neutrophil counts. In contrast, the 5-year PFASs concentrations were associated with higher basophil counts (B= 46% SD, 95% CI: 13, 79). Significantly reduced subpopulations of lymphocytes such as B cells, CD4-positive T helper cells and CD4 positive recent thymic emigrants may suggest cellular immunity effects and dysregulation of T-cell mediated immunity.

Conclusion: Thus prenatal exposure to mercury and PFASs appears to have differential impacts on WBC counts.

1. Introduction

Exposure to environmental pollutants may significantly affect the immune system and cause immunologic suppression in children [1]. The developing immune system appears to be highly sensitive to toxic effects, and experimental animal studies and *in vitro* assays have shown immunomodulatory effects of persistent organic pollutants (POPs), especially polychlorinated biphenyls (PCBs) [2-5], organochlorine pesticides (OCPs) [5-7], and perfluoroalkyl substances (PFASs) [8-10], as well as mercury and other metal compounds [11, 12]. These immunotoxicants are ubiquitous, most are persistent, and they are widely detected in children and adults from the general population [13].

In humans, recent epidemiologic studies have reported immunomodulatory effects of mercury [14-16] and POPs [13, 17, 18] in children and adults. For example, higher exposures to PFASs were associated with a reduction of the humoral immune response to booster vaccination [17-19], whereas mercury exposures were associated with increased pro-inflammatory cytokines, antinuclear and antinucleolar autoantibodies and decreased anti-inflammatory cytokines [20]. Developmental exposure to PCBs and OCPs has also been shown to modulate the immune system in infants [21, 22], children [23], and adults [13]. Higher 6-month infant concentrations of PCB-153 and DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene] were strongly associated with lower 6-month BCG-specific antibody levels [24], and higher maternal and infant PCB concentrations have been associated with a reduced volume of the infant thymus, the site of T-cell maturation [25]. Moreover, early-life environmental PCB exposure was associated with fluctuations in major lymphocyte subsets [26], and affected the dynamics of cell surface lymphocyte receptor expression [27]. Overall, previous studies provide compelling evidence of the detrimental effects of exposure to environmental toxicants on the immune system,

including immune cell counts, cytokine responses, and levels of specific antibodies [28]; However, most of the human studies on immunotoxicity of POPs and mercury lack a prospective design with age-related exposure profiles, and no study has so far attempted to examine the potential joint effect of different POPs on the immune system development. Hence, the need for new statistical tools to detangle the role of multiple exposures on multiple immune system markers [29].

The white blood cell (WBC) count has been proposed as an immunologic end point to detect immunotoxic effects of environmental contaminants in prospective epidemiologic studies [30]. In humans, WBC counts have proven useful in signaling clinically relevant hematologic changes that may result in clinically identifiable immune disorders [31], with absolute numbers of WBC providing biologically more reliable information than percentages [32].

In the present study, we explored the associations between exposures to POPs and mercury measured at birth and at ages 18 months and 5 years in regard to extended WBC differential counts at age 5.

2. Materials and methods

2.1. Study population

The present study focuses on fifty-six children from a cohort of consecutive births formed in the Faroe Islands to include 490 children during an 18-month period between late 2007 and early 2009. Whole blood was taken from the cord and from the mother two weeks after parturition. In addition, maternal hair at parturition was also sampled. Blood and hair were obtained from the child at successive clinical examinations at ages 18 months and 5 years. At age 5 years, children

underwent detailed examinations including immune system biomarkers. On dates when fresh

blood samples could be transported to the immunological laboratory in Denmark, we obtained

maternal consent for a subgroup of 56 children to draw additional blood for the purposes of the

present study. Standard questionnaires were used to record past medical history, current health,

social factors, and nutritional habits during and before pregnancy. Relevant obstetric

information, including birth weight, parity and maternal age were abstracted from hospital's

medical records.

The study protocol was approved by the ethical review committee serving the Faroe

Islands and by the institutional review board at the Harvard T.H. Chan School of Public Health,

and written informed consent was obtained from all mothers.

2.2. WBC counts and lymphocyte subsets

The total number of WBCs, neutrophils, eosinophils, lymphocytes, and monocytes were

recorded by standard procedures using the ABX Pentra DX 120 (Horiba, United

Kingdom/Germany). Furthermore, T-cell (CD3), T-helper cells (CD4), T-cytotoxic cells (CD8),

B-lymphocytes (CD19), NK (CD16/56) cells and CD4+ recent thymic emigrants (CD4-RTE)

absolute counts were performed by a single-platform no-lyse-no-wash procedure. Fifty µl EDTA

anti-coagulated peripheral blood were incubated in TRUCount tubes (BD Biosciences, Denmark)

with a panel of conjugated monoclonal antibodies. The following combinations of antibodies

were used to characterize T cells as CD4 T cells (CD3-PerCP (clone SK7), CD4-FITC (clone

SK3), CD8 T cells (CD3-PerCP (clone SK7), CD8-PE (clone SK1)) and CD4-RTE (recent

thymic immigrant) cells as (CD3-ECD (clone UCHT1), CD4-PC7 (clone SFCT12T4D11),

CD31-PE (clone WM59), CD45RA-FITC (clone L48), and CD45RO-PC5 (clone UCHL1), NK

cells as (CD45-PerCP (clone 2D1), CD16/56-PE (clone B73.1 + NCAM16.2), and CD3-FITC

(clone SK7)), and B cells as (CD19-PE (clone 4G7), CD45-PerCP (clone 2D1)) (BD Biosciences, Beckman Coulter and AbD Serotec, Denmark). The samples were measured on FC500 flow cytometer (Beckman Coulter, Denmark). The laboratory participates in the quality assurance program by National External Quality Assessment Site (NEQAS).

2.3. Exposure assessment

PCBs, OCPs (dichlorodiphenyltrichloroethane [DDT], dichlorodiphenyldichloroethylene [DDE], and hexachlorobenzene [HCB]) and PFAS (perfluorohexane sulfonic acid [PFHxS], perfluorooctanoic acid [PFOA], perfluorooctane sulfonate [PFOS], perfluorononanoic acid [PFNA], and perfluorodecanoic acid [PFDA]) were measured in cord (n=56), maternal (n=56) and children's blood at 18 months (n=42) and 5 years (n=56). In addition, mercury (Hg) was measured in cord blood, maternal blood and hair, and in child blood and hair at 5 years.

Serum analyses of DDE, DDT, HCB, and PCBs were carried out by the same procedure for all samples using a gas chromatograph with electron capture detection at Department of Environmental Health, University of Southern Denmark [23]. To avoid problems with PCB congeners not assessed or below the detection limit, ΣPCB was calculated as the sum of major congeners 138, 153, and 180 multiplied by 2 [33]. *o,p'*-DDE at 18 months and 5 years, *p,p'*-DDT at 18 months, and *o,p'*-DDT at 5-years were detected in less than 50% of samples and were therefore not further considered. PFHxS, PFOA, PFOS, PFNA, and PFDA concentrations were measured using online solid-phase extraction and analyzed using high-pressure liquid chromatography with tandem mass spectrometry [34]. The accuracy and reliability of the data was ensured by including, in each analytical series, quality control serum samples, calibration standards, and reagent and serum blanks. Within-batch and between-batch coefficient of variations were better than 3.0% and 5.2% for all analytes. Total mercury analyses in hair and

blood were performed on a Direct Mercury Analyzer (DMA-80, Milestone Inc, Sorrisole, Italy), with imprecision below 4%. All POP results below the limit of detection (LOD) were replaced by the LOD divided by $\sqrt{2}$.

2.4. Statistical analyses

WBC, Hg, and POP concentrations were all log-transformed (base 2) before they entered the models to approximate a normal distribution. One child had very high WBC count (Total=16.4 $x10^9$ cells/L) due to a fever and was consequently removed from the analyses. Due to the large number of exposure variables, we used structural equation models (SEM) to assess the covariate-adjusted associations between prenatal, 18-month, and 5-year exposures and the extended WBC counts at 5 years.

We first conducted an exploratory factor analysis (EFA) without *a priori* information on the structure and correlations in the data, followed by a confirmatory factor analysis (CFA) to categorize exposures into a small number of factors, thereby reducing the extent of multiple comparisons. For prenatal exposures, this method resulted in three factors explaining 65% of the variance. Factor 1 included the 5 PFASs, factor 2 included mercury indicators (i.e., cord blood, maternal blood and hair concentrations), and factor 3 included HCB, PCB, *p,p′*-DDE, *o,p′*-DDT, and *p,p′*-DDT. For 18-month exposures, the method yielded 2 factors explaining 65% of the variance. Factor 1 included the 5 PFASs, whereas factor 2 included HCB, PCB, *o,p′*-DDT, and *p,p′*-DDE. At 5 years, three factors explained 56% of the variance. Factor 1 included the 5 PFASs; factor 2 included child hair-Hg and blood-Hg concentrations, whereas factor 3 included HCB, PCB, *p,p′*-DDE, and *p,p′*-DDT. For the sake of simplicity, factors 1, 2, and 3 will be referred to as PFAS, mercury (Hg), and organochlorine (OC) functions, respectively.

Correlations between the latent functions ranged between 0.07 (for 5-year Hg and OC) and 0.79 (for 18-month PFAS and OC). Although these correlations show good discriminant validity for the constructed factors and are considered reasonable to avoid multicollinearity issues in SEM analyses, the small sample size precluded the simultaneous analyses of all the factors in a single model. We therefore completed separate SEM analyses linking each latent exposure function with each WBC count. For each age, the exposure factors were regressed on the WBC counts, while adjusting for covariates. Models for prenatal exposures were adjusted for age at age-5 examination (months), sex, , parity (no older sibling / 1 or more older siblings), and maternal smoking during pregnancy; whereas models for 18-month and 5-year exposures were further adjusted for birth weight (g) and duration of breastfeeding ($<$ or $\geq$ 6 months). Missing data for breastfeeding (n=2) were compensated by Full Information Maximum Likelihood estimation.

3. Results

3.1. Descriptive results

The characteristics of the study population are presented in Table 1. The mean age of children was 60 months, with fewer boys than girls (39% vs 61%). Most of the children had an older sibling (71%), and 41% were breastfed for 6 months or longer (Table 1). Except for one child with a total WBC count below 4 x 10^9 cells/L, all remaining children were within a normal range (4-11 x 10^9 cells/L) [35]. The total WBC count did not differ in regard to age, sex, parity, maternal smoking during pregnancy, birth weight, or breastfeeding duration.

3.2. Exposures

Among the PFASs, PFOS showed the highest serum concentrations at all ages, followed by PFOA and PFHxS, while PFNA and PFDA occurred in the lowest concentrations (Table 2).

Between-PFAS correlation coefficients for maternal serum or child at age 18-month and 5-year ranged from 0.29 to 0.87 and were higher at 18 months and lower at 5 years. Within-PFAS correlation coefficients ranged between 0.28 and 0.79, and were generally higher for the child's 18-month and 5-year serum samples and lower between maternal and child at either age (Figure 1). For mercury exposures, the higher cord blood concentrations were closely correlated with those in maternal blood (0.92). Blood- and hair-Hg concentrations were also well correlated on each occasion (0.73 for maternal concentrations and 0.89 for child concentrations at age 5). Regarding POPs, PCBs showed the highest within correlation coefficient between serum concentrations at 18 months and 5 years. PCBs also showed high correlations with the other POPs and PFASs at each age (Figure 1). PFAS concentrations at all ages were generally lower in mothers with a previous childbirth, whereas POPs at 18 months were lower in children breastfed for 6 months or more.

3.3. WBC counts in relation to exposure levels

The CFA models exhibited a good to very good fit to the data. All measured exposures showed significant and high correlations to their latent functions (Table 3) and therefore appeared as appropriate indicators. The variance in observed exposures explained by the latent exposure functions ranged between about 50 and 100%.

SEMs showed an acceptable (age-5 models) to a very good fit to the data. Table 4 shows the resulting associations between the latent exposure functions for PFAS, Hg, and OC at each age in regard to the WBC counts at 5 years. Higher maternal Hg exposure was consistently associated with lower WBC counts. For instance, a one standard deviation (SD) increase in maternal Hg concentrations was associated with a 23% SD decrease in lymphocytes (95% confidence intervals [CI]: -43, -4%) and a 30% SD decrease in total WBC count (95% CI: -56, -

5%). Maternal OC concentrations were marginally associated with decreases in neutrophil and total WBC counts, with a 1-SD increase in maternal OC concentrations associated with a 27% SD decrease in total WBC count (95% CI: -57, 4%). Further, a 1-SD increase in 5-years OC concentrations was also marginally associated with a 23% SD decrease in total WBC count (95% CI: -47, 1%). Finally, higher PFAS concentrations at all ages were associated with higher basophil counts (B=28 % SD; 95% CI: -2, 57%, B=34 % SD; 95% CI: 0, 68%, and B=46 % SD; 95% CI: 13, 79%, respectively for maternal, 18-month, and 5-year PFAS).

In regard to the specific lymphocyte subpopulations, maternal Hg concentrations were significantly associated with decreased CD3$^+$ T cells, CD4$^+$ cells, CD4-RTE and CD19$^+$ B Cells concentrations. For instance, a 1-SD increase in maternal Hg concentrations was associated with a 23% SD (95% CI: -40, -6%) and a 24% SD (95% CI: -47, -1%) decrease in CD4$^+$ T cell and 19$^+$ B cell concentrations, respectively. No associations were observed with 18-month and 5-year exposures (Table 5). Attempts to incorporate all exposure information in a single model failed, as the fit indices were poor, and signs of multicollinearity issues were observed in the resulting estimates, with unstable parameter estimates and negative variances.

4. Discussion

In this prospective study with age-related mercury and POPs exposure profiles, we found an association between prenatal mercury concentrations and depleted total white blood cell counts, especially for monocytes, basophils and CD3$^+$ T lymphocytes, CD4$^+$ T lymphocytes, CD4$^+$ recent thymic emigrants (CD4-RTE), and CD19$^+$ B lymphocytes. Further, prenatal and concurrent concentrations of organochlorine compounds were marginally associated with decreased neutrophil and total WBC counts. Finally, higher 18-month and 5-year PFAS concentrations were associated with increased basophil levels in 5-year children.

Very few studies investigated WBC count in relation to developmental exposure to mercury and POPs. Of greatest interest, our study suggests a negative impact on recent thymic immigrant cells, as well as on mature lymphocyte subsets. The results are in line with other human studies showing decreased lymphocyte [16, 36], and monocyte [37] counts in relation to mercury exposures, but contradict a recent cross-sectional study showing a positive correlation of the serum-mercury concentration – likely reflecting inorganic mercury exposure – with lymphocyte count [15]. In the Faroes, total mercury concentrations in blood and hair primarily indicate exposures to methylmercury [38], thus suggesting that different chemical speciations may affect the mercury-related toxicity. Mercury-induced cytotoxicity has been demonstrated in cells of the immune system, including B cells, T cells, and monocytes [39, 40], and two *in vitro* studies demonstrated that Hg exposure causes mitochondrial dysfunction and enhances the apoptosis of neutrophils and mononuclear cells including T cells [41, 42]. However, mercury has also been shown to exert immunomodulatory and anti-apoptotic effects in animals [43] and to be associated with autoimmune diseases in both animals and humans [44]. Although no clear picture has emerged so far, the differences between studies may mainly be attributable to differences in concentrations and mercury species, i.e., whether inorganic mercury or methylmercury [45].

Our study also found that OCs tended to have similar effects as mercury, though not significantly so. Previous studies have reported similar findings in various settings. Higher PCB concentrations were associated with lower neutrophil and lymphocyte percentages, and a lower total WBC count [13, 46]. Further, Inuit infants whose mothers had elevated levels of PCBs and dioxins in their breast milk had decreased $CD4^+/CD8^+$ T cell ratio at 6 and 12 months of age [47]. A joint effect of higher exposures of DDT and PCBs on $CD16^+$ NK cells was also observed

in Japanese infants [22]. Finally, neutrophil counts were inversely related to OC pesticides concentrations in the general U.S. population form NHANES 1999-2002 [48]. Similar findings have also been reported in animal and *in vitro* studies [49-52]. The strong correlations between PCBs and other OCs exposures observed in this population make it difficult to distinguish between effects of specific OC substances. It is also worth mentioning that although we were able to collect serum samples and examine OC concentrations at different time points, with apparent different associations with neutrophils and total WBC count, it is still challenging to state about the exact timing of greatest susceptibility. Given the long half-lives of OCs, the serum concentrations at 18 months and 5 years are likely still affected by prenatal and lactational transfer from the mother.

We also observed an association between PFAS exposure and increased basophil count. Although the role that basophils play in the immune system has not been thoroughly investigated in the past, recent studies revealed their crucial role in various immune responses such as allergy and anti-parasitic protective immunity [53]. Importantly, elevated IgE concentrations correlate with increased numbers of circulating basophils both in mice and in humans [54], and recent evidence from epidemiologic and animal studies suggests that PFASs may be associated with allergy and asthma-related outcomes, perhaps as a result of a shift in the host's immune state toward a more TH2-like state [55-59].

Based on the results from the present study, it is hard to infer any increased risk of disease outcome. The observed changes in the WBC counts warrant further investigation, and the clinical significance of these changes is unclear at this point. However, these results are indicative of immunotoxic and/or immunomodulatory effects of mercury and POPs, and the demonstrated effects on the cellular and humoral immune responses might lead to increased

susceptibility to various infectious diseases or to immune dysfunction with an impaired immune tolerance. Interestingly, our findings point to developmental toxicity on the thymus, as demonstrated by the lower CD4-RTE concentration, associated with perinatal Hg exposure. One may speculate that a compromised thymic function could lead to insufficient T cell helper activity for developing B-cell based immunity and in the longer term perhaps also an increased risk of autoimmune disease, as may be seen in immune deficiency conditions.

A major limitation of this study is the small sample size. Although the use of structural equation models allowed a reduction of multiple comparisons and a gain in statistical power due to the by reduced measurement error, the small sample size did not allow for mutual adjustment of the three families of exposures, nor mutual adjustment for pre- and postnatal exposures.

To our knowledge, this is the first study to investigate associations of both prenatal and postnatal exposure to multiple POPs and mercury and children's WBC counts. Despite the small sample size, we were able to adjust for several potentially relevant confounders, and their effect on the results was marginal. The potential influence of any unmeasured determinant of WBC appears minimal, especially when taking into regard the homogeneity of the Faroese population in terms of socio-demographic, lifestyle, and genetic characteristics. Thus, these findings should inspire further study of the possible effects of developmental exposure to environmental chemicals on children's immune functions.

5. Conclusions

In this study of children from the Faroe Islands, we report an inverse association between prenatal mercury and organochlorine exposures and total WBC counts. The finding of significantly reduced subpopulations of lymphocytes such as B-lymphocytes, CD4 positive T helper cells and CD4 positive recent thymic emigrants suggest an effect of prenatal mercury

exposure on cellular immunity and a dysregulation of T-cell mediated immunity. Prenatal OC exposure was marginally associated with decreases in neutrophil counts, while postnatal PFAS concentrations appeared to be associated with increased basophil counts. These observations suggest a differential effect of developmental exposures to immunotoxicants on specific circulating leukocyte populations in childhood. Importantly, this study provides new evidence of the potential immunomodulatory effects of persistent organic pollutants and mercury.

Funding

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TABLES:

Table 1: Characteristics of the study population (n=55)

Population characteristic	Mean (SD) or n (%)
Age, months	60.1 (0.8)
Birth weight, grams	3684 (537)
Maternal age at delivery, years	30.6 (5.7)
Sex	
Boys	22 (40)
Girls	33 (60)
Parity	
No siblings	16 (29)
≥ 1 siblings	39 (71)
Maternal smoking during pregnancy	
No	44 (80)
Yes	11 (20)
Breastfeeding duration	
< 6 months	32 (60)
≥6 months	21 (40)
Missing	2
Differential White blood cells count (10^9 Cells/L)	
Neutrophils	3.13 (1.75)
Basophils	0.10 (0.13)
Lymphocytes	2.57 (0.84)
CD3	2.00 (0.62)
CD4	1.11 (0.40)
CD8	0.75 (0.26)
CD4-RTE	0.55 (0.24)
B Cells	0.56 (0.24)
NK Cells	0.16 (0.07)
Monocytes	0.43 (0.15)
Eosinophils	0.31 (0.25)
Total WBC	6.54 (2.12)

Table 2: Descriptive statistics of maternal and child's exposures (μg/L)

	Maternal exposures (n=55)				18-month exposures (n=41)				5-year exposures (n=53)			
	# < LOD	GM	GSE	Range	# < LOD	GM	GSE	Range	# < LOD	GM	GSE	Rar
PFOA	0	1.470	0.100	0.46 - 4.13	0	3.580	0.391	0.85 - 22.52	0	2.596	0.128	1.44 -
PFNA	0	0.789	0.056	0.30 - 4.35	0	1.244	0.135	0.23 - 7.10	0	1.358	0.095	0.38 -
PFDA	0	0.298	0.020	0.12 - 0.91	0	0.347	0.033	0.1 - 3.29	0	0.357	0.024	0.11 -
PFHxS	1	0.240	0.022	<LOD - 0.86	3	0.247	0.045	<LOD - 3.77	0	0.372	0.032	0.14 -
PFOS	0	9.075	0.495	3.67 -19.31	0	8.252	0.761	1.95 - 28.30	0	5.058	0.261	2.17 -
∑PCB	0	0.470	0.051	0.07 - 1.90	0	0.734	0.108	0.07 - 3.07	0	0.465	0.067	0.02 -
HCB	0	0.018	0.001	0.005 - 0.08	6	0.032	0.003	<LOD - 0.11	0	0.025	0.002	0.008
Blood-Hg	0	3.066	0.255	0.53 - 19.96	-	-	-	-	0	2.328	0.242	0.21 -
Hair-Hg	0	0.748	0.073	0.14 - 3.88	-	-	-	-	0	0.611	0.084	0.02 -
Cord blood-Hg	0	4.649	0.371	0.77 - 21.06	-	-	-	-	-	-	-	-
o,p'-DDE	15	n/a	n/a	<LOD - 0.02	38	n/a	n/a	<LOD -0.11	47	n/a	n/a	<LOD
p,p'-DDE	0	0.142	0.020	0.02 - 1.38	3	0.271	0.053	<LOD - 1.95	0	0.229	0.032	0.02 -
o,p'-DDT	2	0.009	0.001	<LOD -0.14	11	0.033	0.004	<LOD - 0.23	29	n/a	n/a	<LOD
p,p'-DDT	2	0.007	0.001	<LOD - 0.09	31	n/a	n/a	<LOD - 0.09	3	0.001	0.000	<LOD

Table 3. Results of the confirmatory factor analysis for prenatal, 18-month, and 5-year exposures.

	Factors	Indicators	Loading	SE	p-value	Standardized estimate
Prenatal	PFAS	PFOA	1.00	0.00	NA	0.65
		PFNA	1.22	0.25	<0.001	0.78
		PFDA	1.43	0.30	<0.001	0.94
		PFOS	1.04	0.20	<0.001	0.83
		PFHxS	1.29	0.38	0.001	0.62
	OC	HCB	1.00	0.00	NA	0.92
		PCB	1.34	0.17	<0.001	0.84
		p,p′-DDE	1.80	0.19	<0.001	0.87
		o,p′-DDT	1.44	0.25	<0.001	0.65
		p,p′-DDT	1.33	0.20	<0.001	0.7
	Hg	hair Hg	1.00	0.00	NA	0.87
		blood Hg	0.91	0.13	<0.001	0.91
		CB-Hg	0.95	0.11	<0.001	0.99
18 months	PFAS	PFOA	1.00	0.00	NA	0.89
		PFNA	1.03	0.10	<0.001	0.92
		PFDA	0.88	0.11	<0.001	0.9
		PFOS	0.86	0.12	<0.001	0.9
		PFHxS	1.56	0.23	<0.001	0.82
	OC	HCB	1.00	0.00	NA	0.94
		PCB	1.83	0.28	<0.001	0.99
		p,p′-DDE	1.62	0.61	0.008	0.65
		o,p′-DDT	1.27	0.11	<0.001	0.65
5 years	PFAS	PFOA	1.00	0.00	NA	0.54
		PFNA	1.74	0.48	<0.001	0.67
		PFDA	1.32	0.39	0.001	0.53
		PFOS	1.59	0.52	0.002	0.85
		PFHxS	1.95	0.52	<0.001	0.62
	OC	HCB	1.00	0.00	NA	0.97
		PCB	1.85	0.33	<0.001	0.84
		p,p′-DDE	1.80	0.27	<0.001	0.83
		p,p′-DDT	1.05	0.22	<0.001	0.5
	Hg	hair Hg	1.00	0.00	NA	0.89
		blood Hg	0.84	0.35	0.02	1

Table 4. Associations between PFAS, Hg, and POPs exposure and WBC concentrations at 5 years of age. Estimate is expressed as the change in leukocytes SD associated with a one SD change in the exposure.

Leukocytes	Exposures	Maternal exposures				18-month exposures				5-year exposures			
		Std Estimate	Lower CI	Upper CI	p-value	Std Estimate	Lower CI	Upper CI	p-value	Std Estimate	Lower CI	Upper CI	p-value
Neutrophils	PFAS	-0.14	-0.44	0.17	0.37	0.09	-0.19	0.37	0.53	-0.06	-0.44	0.31	0.73
	Hg	-0.26	-0.54	0.02	0.07	-	-	-	-	0.13	-0.05	0.30	0.16
	OC	-0.30	-0.64	0.03	0.07	-0.14	-0.48	0.20	0.41	-0.21	-0.43	0.01	0.07
Basophils	PFAS	0.28	-0.02	0.57	0.07	0.34	0.00	0.68	0.05	0.46	0.13	0.79	0.01
	Hg	-0.09	-0.32	0.14	0.46	-	-	-	-	-0.17	-0.59	0.24	0.41
	OC	0.21	-0.08	0.50	0.15	0.25	-0.13	0.62	0.19	0.18	-0.24	0.59	0.40
Eosinophils	PFAS	-0.07	-0.32	0.17	0.56	0.14	-0.20	0.48	0.43	-0.06	-0.38	0.27	0.73
	Hg	-0.14	-0.48	0.19	0.41	-	-	-	-	-0.11	-0.35	0.13	0.38
	OC	-0.11	-0.39	0.17	0.45	0.16	-0.17	0.48	0.35	-0.08	-0.31	0.15	0.48
Lymphocytes	PFAS	-0.04	-0.30	0.22	0.78	0.16	-0.13	0.45	0.27	-0.11	-0.36	0.14	0.39
	Hg	-0.23	-0.43	-0.04	0.02	-	-	-	-	-0.01	-0.20	0.18	0.90
	OC	-0.10	-0.35	0.14	0.41	0.20	-0.11	0.51	0.20	-0.18	-0.46	0.10	0.22
Monocytes	PFAS	-0.10	-0.38	0.19	0.51	0.16	-0.12	0.43	0.26	0.05	-0.34	0.44	0.80
	Hg	-0.23	-0.46	0.00	0.05	-	-	-	-	-0.07	-0.30	0.16	0.56
	OC	-0.17	-0.46	0.11	0.23	-0.08	-0.37	0.21	0.59	-0.06	-0.28	0.15	0.56
Total WBC	PFAS	-0.11	-0.40	0.19	0.47	0.15	-0.13	0.44	0.28	-0.06	-0.38	0.25	0.70
	Hg	-0.30	-0.56	-0.05	0.02	-	-	-	-	0.06	-0.11	0.23	0.46
	OC	-0.27	-0.57	0.04	0.08	0.00	-0.34	0.34	1.00	-0.23	-0.47	0.01	0.06

Table 5. Associations between PFAS, Hg, and POP exposures and specific lymphocyte counts at 5 years of age. Estimates are expressed as the relative change in regard to the leukocyte SD associated with a one SD increase in the exposure.

Lymphocytes	Exposures	Maternal exposures				18-month exposures				5-year exposures			
		Std Estimate	Lower CI	Upper CI	p-value	Std Estimate	Lower CI	Upper CI	p-value	Std Estimate	Lower CI	Upper CI	p-value
CD3 T cells	PFAS	-0.12	-0.36	0.13	0.36	0.09	-0.31	0.49	0.66	-0.15	-0.40	0.10	0.24
	Hg	-0.22	-0.41	-0.04	0.02	-	-	-	-	0.00	-0.08	0.09	0.95
	OC	-0.11	-0.34	0.12	0.37	0.13	-0.20	0.47	0.42	-0.19	-0.51	0.14	0.26
CD4 T cells	PFAS	-0.12	-0.37	0.13	0.35	0.07	-0.29	0.43	0.71	-0.18	-0.44	0.07	0.16
	Hg	-0.23	-0.40	-0.06	0.01	-	-	-	-	0.04	-0.16	0.24	0.72
	OC	-0.10	-0.33	0.13	0.41	0.12	-0.20	0.44	0.46	-0.22	-0.56	0.11	0.19
CD8 T cells	PFAS	-0.12	-0.36	0.12	0.32	0.16	-0.27	0.59	0.46	-0.08	-0.33	0.17	0.53
	Hg	-0.16	-0.38	0.06	0.15	-	-	-	-	-0.05	-0.26	0.17	0.68
	OC	-0.13	-0.37	0.10	0.27	0.17	-0.20	0.53	0.37	-0.09	-0.40	0.21	0.55
CD4-RTE	PFAS	-0.05	-0.32	0.22	0.70	0.23	-0.08	0.53	0.14	-0.12	-0.35	0.11	0.31
	Hg	-0.26	-0.45	-0.08	0.01	-	-	-	-	0.00	-0.07	0.08	0.91
	OC	-0.10	-0.34	0.14	0.41	0.20	-0.28	0.69	0.42	-0.05	-0.40	0.29	0.76
NK cells	PFAS	-0.12	-0.41	0.18	0.43	-0.20	-0.50	0.09	0.18	0.16	-0.16	0.48	0.33
	Hg	-0.11	-0.33	0.11	0.32	-	-	-	-	-0.02	-0.10	0.06	0.63
	OC	0.11	-0.13	0.35	0.38	-0.22	-0.66	0.22	0.33	-0.13	-0.40	0.15	0.36
B cells	PFAS	-0.06	-0.33	0.21	0.65	0.19	-0.08	0.47	0.17	-0.12	-0.42	0.17	0.41
	Hg	-0.24	-0.47	-0.01	0.04	-	-	-	-	0.05	-0.16	0.26	0.62
	OC	-0.10	-0.38	0.17	0.46	0.09	-0.22	0.40	0.59	-0.14	-0.42	0.14	0.33

FIGURES

Figure 1: Correlation heat map and hierarchical clustering of maternal, 18-month, and 5-year
POPs and Hg exposures. Red represents positive correlations whereas blue represents negative ones. The
intensity of the color is a function of the correlation.