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Associations between exposure to metals, chlorinated pesticides, and PCBs and differential leukocyte profiles in Flemish adolescents

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ABSTRACT

Although environmental pollutants are known to affect the immune system, the impact of chemical mixtures on adolescent immune function remains understudied. Adolescence is a critical period for immune maturation, and disruptions during this stage may have implications for long-term health. Leukocytes, key components of the immune system, serve as indicators of immune status, with altered levels reflecting potential inflammation or immunosuppression. The present study examined the associations of 14 chemical exposure biomarkers measured in blood with counts of total leukocytes and leukocyte subtypes in 980 adolescents (13–16 years old) from the Flemish Environment and Health Studies 2012–2020 (FLEHS III and IV). The exposure biomarkers included 5 metals, 3 chlorinated pesticides, and 6 polychlorinated biphenyls (PCBs). We used four different statistical approaches: multiple linear regression, elastic net, Bayesian model averaging, and Bayesian kernel machine regression. Total leukocyte and neutrophil counts were negatively associated with PCBs and manganese (Mn), and positively associated with copper (Cu), whereas lymphocyte count was negatively associated with thallium (Tl). The neutrophil-lymphocyte ratio (NLR) was also negatively associated with Mn. An analysis excluding smokers additionally showed that higher cadmium (Cd) concentrations were associated with lower leukocyte count. Our study suggests immunosuppressive effects of PCBs, non-essentials metals Tl and Cd, and the essential metal Mn. Due to the cross-sectional design, we cannot rule out the possibility of reverse causation. The current study provides epidemiological evidence that exposure to metals and PCBs may have adverse effects on the immune system at concentrations detected in a general population of adolescents.

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Glossary

BKMR =	Bayesian kernel machine regression
BMA =	Bayesian model averaging
BMI =	body mass index
CHOL =	cholesterol
CI =	confidence interval
CrI =	credible interval
Cu =	copper
ENET =	elastic net
FLEHS =	Flemish Environment and Health Studies
GC-ECNI/MS =	gas chromatography with mass spectrometry
GMR =	geometric mean ratio
HR-ICP-MS =	High Resolution Inductively Coupled Plasma Mass Spectrometry
IQFc =	interquartile fold change
ISCED =	International Standard Classification of Education
LOD =	limit of detection
LOQ =	limit of quantification
Mn =	manganese
mpMLR =	multi-pollutant multiple linear regression
NLR =	neutrophil–lymphocyte ratio
OXC =	oxychlordane
P25 =	25th percentile
P75 =	75th percentile
PARC =	European Partnership for the Assessment of Risks from Chemicals
Pb =	lead
PCB =	polychlorinated biphenyl
PFER =	per-family error rate
PIP =	marginal posterior inclusion probability
POP =	persistent organic pollutants
spMLR =	single-pollutant multiple linear regression
SumPCB =	sum of PCBs 138, 153, 170, 180, and 187
TG =	triglycerides
Tl =	thallium
TL =	total blood lipid concentration
TN =	trans-Nonachlor
VIF =	variance inflation factor

1. Introduction

People are exposed to chemical mixtures through contaminated food and drinks, chemicals in consumer products, and through other sources such as polluted air and dust. Exposure to chemical pollution has been linked to a wide range of health outcomes, including both acute and chronic diseases affecting various systems such as the immune, central nervous, cardiovascular, renal, dermal, and reproductive systems (Naidu et al., 2021). Metal pollution is a growing global public health problem (Mishra et al., 2019). Metals enter the surroundings through natural pathways and human activities, and many accumulate in the food chain and body, leading to chronic exposure and potential long-term health effects (Jaishankar et al., 2014). Another group of hazardous chemicals are persistent organic pollutants (POPs), including chlorinated pesticides, polychlorinated biphenyls (PCBs), and unintentional by-products of industrial processes, such as dioxins. POPs are considered silent killers due to their persistence, mobility, bioaccumulation, and toxicity, and they have been linked to a variety of adverse health outcomes (Alharbi et al., 2018; Carpenter, 2006; Jayaraj et al., 2016). Both metal and POP exposure have been suggested to affect human health through transient or permanent alterations of the immune system (Mokarizadeh et al., 2015; Popov Aleksandrov et al., 2021; Zheng et al., 2023). Epidemiological studies have shown that exposure to non-essential heavy metals such as lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) can suppress the immune system in children, particularly by reducing T lymphocyte counts (Zheng et al., 2023). These metals have also been associated with increased levels of innate immune cells, which may raise the risk of infections, inflammation, and allergic reactions, as well as with elevated inflammatory cytokine levels (Zheng et al., 2023). In addition, Pb, As, and Hg have been found to impair vaccine responses, resulting in reduced antibody protection in children (Zheng et al., 2023). Epidemiologic evidence for immunotoxic effects of other metals is more limited. Essential metals such as copper (Cu) and manganese (Mn) serve crucial biological functions but can disrupt metal homeostasis and have toxic effects if present in excess (Jomova et al., 2022; Wu et al., 2021). Experimental studies indicate that excess Cu induces oxidative stress, which disrupts mitochondrial function and triggers cell death pathways, ultimately leading to immune system dysregulation (Wang et al., 2025). Studies on welders exposed to Mn fumes suggest that high-level Mn exposure can reduce lymphoid cell populations and impair innate immune responses (Wu et al., 2021). Exposure to POPs such as polychlorinated biphenyls (PCBs) and organochlorine pesticides has also been associated with immune alterations, including changes in immune cell profiles and increased susceptibility to infectious diseases (Glynn et al., 2008; Haase et al., 2016; Nagayama et al., 1998; Weisglas-Kuperus et al., 2000), as well as reduced antibody responses to routine vaccinations (Heilmann et al., 2010; Timmermann et al., 2022).

Adolescence is a critical developmental window marked by rapid changes in physiology, hormones, and immune function, including shifts in leukocyte populations and cytokine regulation (UNICEF, 2025). Environmental exposures during this life stage may disrupt these processes, potentially leading to long-term alterations in immune competence and disease susceptibility (MacGillivray and Kollmann, 2014). Despite this heightened vulnerability, most epidemiological studies have focused on early childhood or adulthood. Moreover, the majority of studies have examined exposure to one chemical at a time, thereby ignoring potential confounding by other substances present and not accounting for potential mixture compositions in which the presence of certain compounds may alter the activity of others (Zheng et al., 2023). A better understanding of how exposure to chemical mixtures may affect the immune system during adolescence is needed to clarify potential health risks and inform preventive strategies.

Leukocytes, also referred to as white blood cells, are central to the immune system and contribute to both innate and adaptive immune responses, defending the body against infection and disease. An elevated leukocyte count is typically considered as a biomarker of infection, while a low count may indicate reduced immunity and increased susceptibility to infection (Parkin and Cohen, 2001). There are five types of leukocytes that differ in function, count, size, and structure: neutrophils, lymphocytes, monocytes, eosinophils, and basophils (Horne et al., 2005). Neutrophils, the most abundant type, are key cells of innate immunity as they are the first responders to pathogens (Scapini and Cassatella, 2014). Lymphocytes (including T-cells, B-cells, and natural killer cells) are critical to adaptive immunity through antibody production (Larosa and Orange, 2008). The neutrophil-to-lymphocytes ratio (NLR) is emerging as a biomarker of inflammation in diseases such as cardiovascular disorders (Angkananard et al., 2018) and cancers (Faria et al., 2016).

This study aimed to investigate the association between exposure to metals, PCBs, and chlorinated pesticides and immune function, represented by leukocyte and subtype counts, in a general population of adolescents. The chemicals were selected based on the availability and detection frequency of exposure biomarkers in the study population.

2. Materials and methods

2.1. Study population

Data of 1018 adolescents (13–16 years old) from two successive cycles of the Flemish Environment and Health Studies: FLEHS III (2012–2015) and IV (2016–2020). FLEHS was established in 2002 as the Flemish Government's environmental health surveillance program (Baeyens et al., 2014; Buekers et al., 2021; Den Hond et al., 2013; Koppen et al., 2002; Schoeters et al., 2012, 2022). FLEHS III recruited 208 adolescents from the general Flemish population through schools, aiming to include a representative sample with respect to sex and geographical area and with efforts being made to include participants from different socio-economic statuses. In addition, 200 adolescents were recruited in an industrial hot spot area. In FLEHS IV, 610 adolescents were recruited: 182 newborns that participated in FLEHS I (2002–2006) and reached adolescence at the time of FLEHS IV recruitment, and 428 adolescents newly recruited through schools. The newborns from FLEHS I were recruited in 8 geographical areas reflecting different types of pollution pressure: two urban areas (Ghent and Antwerp), four areas with different types of industry (harbor, non-ferrous smelter, chemical industry, waste incinerator) and two rural areas of which one had intensive fruit cultivation. Details of recruitment and sampling have been reported in previous studies (Schoeters et al., 2017, 2022).

Extensive questionnaires were used to collect information on socio-demographics (e.g., age and education) and lifestyle (e.g., smoking, alcohol, and drug use). Highest educational level of the household was categorized based on the International Standard Classification of Education (ISCED) developed by the United Nations Educational, Scientific and Cultural Organization (UNESCO) (UNESCO Institute for Statistics, 2011): low education was defined as no secondary to lower secondary education (ISCED level 0–2), medium education as having attained upper secondary to post-secondary non-tertiary education (ISCED level 3–4), and high education as having attained tertiary education or higher (ISCED level $\geq$5). Missing values for highest educational level of the household (1.5 % in FLEHS III, 1.3 % in FLEHS IV) were imputed by study with the R package mice, through single polynomial regression imputation using the following predictor variables: age, sex, height, weight, smoking, sporting, alcohol consumption, equivalent household income, degree of urbanization, and maternal smoking. Participants' height and weight were measured during field work at school by trained staff, and body mass index (BMI) scores were calculated. BMI classes (underweight, normal weight, and overweight) were defined based on age- and sex-specific Belgian growth curves (Roelants et al., 2009).

The studies were conducted in accordance with the principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of the University Hospital Antwerp and the University of Antwerp (Approval Number: B300201316515 for FLEHS III and B300201732753 for FLEHS IV). All participants provided written informed consent before participation in the study.

2.2. Exposure biomarkers

For this study, we selected exposure biomarkers measured in all adolescent participants of both FLEHS cycles. Only the biomarkers that were above the limit of detection (LOD) or limit of quantification (LOQ) in at least 70 % of the samples within each cycle were considered, resulting in a total of 14 exposure biomarkers for further statistical analysis: (a) five metals/trace elements, i.e. cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb), and thallium (Tl); (b) three chlorinated pesticides, i.e. dichlorodiphenyldichloroethylene (DDE), oxychlordane (OXC) and trans-Nonachlor (TN); (c) six polychlorinated biphenyls (PCBs), i.e. PCB 118, 138, 153, 170, 180, and 187. Metals and trace elements were measured in whole blood through High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS) (Schroijen et al., 2008). Chlorinated pesticides and PCBs were measured in blood serum through gas chromatography with mass spectrometry (GC-EC-NI/MS) (Covaci and Voorspoels, 2005). Exposure biomarker measurements below the LOD or LOQ were imputed by single random imputation from a censored lognormal distribution within each study. This was the case for OXC, DDE, TN, PCB 118, PCB 170. The proportion of samples that needed imputation for these biomarkers was similar between study cycles and was well below 5 %, except for TN and PCB 187 (13.9 % and 7.1 %, respectively) (Supplementary Table S1). Lipid-soluble serum biomarkers (chlorinated pesticides and PCB's) were standardized by total blood lipid concentration (TL), which was calculated from triglycerides (TG) and total cholesterol (CHOL) using the formula proposed by Bernert et al. (2007) and Phillips et al. (1989): $TL = 2.27 * CHOL + TG + 62.3$ (mg/dL). Whole blood and blood serum samples were available for 990 (out of 1018) participants, but TG and CHOL measurements were missing for two of these, resulting in 988 (97.1 %) participants with complete exposure biomarker information.

2.3. Effect biomarkers

Total leukocyte count and leukocyte subtype distribution (proportion of neutrophils, lymphocytes, monocytes, eosinophils, and basophils) were assessed using the Sysmex Xe-2100 (Sysmex Corp. Kobe, Japan) for hematology analysis, which combines flow cytometry with fluorescence detection. Counts of leukocyte subtypes were subsequently calculated by multiplying the subtype proportion with the total leukocyte count. Assessment of the NLR was conducted as it is an emerging marker of disease and serves as an indicator of immune system homeostasis (Buonacera et al., 2022). Seven out of 988 participants with complete exposure biomarker information (0.7 %) had missing leukocyte data. One participant with very low leukocyte, lymphocyte, and monocyte count was excluded from the final analyses as it appeared to be an influential observation with outlying residuals in single-pollutant multiple regression models. This resulted in a final sample size of 980 individuals for statistical analysis (see Supplementary Fig. S1 for the flow chart detailing the sample selection process).

2.4. Statistical analysis

Differences in population characteristics, exposure biomarkers and effect biomarkers between FLEHS III and IV were assessed by chi-square tests (categorical variables) and Kruskal-Wallis tests (continuous variables). Correlations between exposure biomarkers were calculated within and across study cycles using pairwise Spearman rank correlations. The association between exposure biomarkers and differential leukocyte counts was assessed through four statistical methods (the motivation for these methods is given in the discussion): multiple linear regression (MLR) for single- and multiple-pollutant analysis, elastic net (ENET) (Zou and Hastie, 2005), Bayesian model averaging (BMA) (Clyde et al., 2011), and Bayesian kernel machine regression (BKMR) (Bobb et al., 2015). In the main analysis, we assessed seven outcome variables, i.e., counts of total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, and basophils, and the NLR. Because of their skewed distribution, outcome variables and exposure biomarkers were log-transformed for statistical analyses. To ensure that exposure biomarkers with larger absolute values or greater variability do not disproportionately influence the results, they were additionally centered (subtracting the mean) and scaled (dividing by the standard deviation).

We used single-as well as multiple-pollutant regression models (spMLR and mpMLR), by entering concentrations of different exposure biomarkers in separate models and in the same model, respectively. Collinearity in the mpMLR was assessed by estimating variance inflation factors (VIFs), with a VIF greater than five considered to indicate a problem of (multi-)collinearity (Kleinbaum et al., 1998).

ENET combines the strengths of Lasso and Ridge regression, which are both regularization techniques that address the problem of

overfitting by adding a penalty term to the ordinary least squares objective function (Tay et al., 2023; Zou and Hastie, 2005). Ridge regression penalizes the sum of squared coefficients (L2 penalty), whereas Lasso penalizes the sum of their absolute values (L1 penalty), thereby performing variable selection as some coefficients exactly become zero (which is never the case in Ridge regression). The penalty term in ENET is a linear combination of the L1 and L2 norms, where the mixing parameter alpha controls the balance between the two penalties. Alpha was set to 0.5 and 10-fold cross-validation was used to select the regularization parameter (λ) which achieved the smallest cross-validation error. Stability selection was performed based on resampling using a selection probability cutoff of 0.8 and controlling the upper limit of the per family error rate (PFER)at 0.5.

Conditioning on a single selected model ignores model uncertainty, leading to underestimation of uncertainty when making inferences. BMA addresses this problem by averaging over all possible models (i.e. combinations of predictors) [43]. BMA was implemented using the Bayesian adaptive sampling (BAS) algorithm described in Clyde et al. (Clyde et al., 2020). Unlike Markov Chain Monte Carlo (MCMC), BAS is guaranteed to enumerate the space of models if the number of iterations is equal to the dimension of the model space but uses a stochastic sampling algorithm when enumeration is not feasible. The Jeffreys-Zellner-Siow (JZS) prior was used for the prior distribution of the regression coefficients and a uniform (flat) prior was used on the model space (Liang et al., 2008). We used 50000 iterations and calculated the marginal variable inclusion probability (PIP) for each exposure biomarker by summing the posterior sampling probabilities for models in which it was included. The median probability model, consisting of variables with a PIP of at least 0.5, is often the optimal predictive model, so a PIP threshold of 0.5 or higher is often used as a cut-off for variable selection (Barbieri and Berger, 2004). Estimates and 95 % Bayesian credible intervals were calculated based on the full posterior distribution of all regression coefficients.

BKMR uses a flexible nonparametric approach to model the high-dimensional exposure-response surface with a kernel function, considering potential interactions and possible nonlinear associations between exposures and outcome (Bobb et al., 2018). We used component-wise variable selection and a Gaussian kernel function. The model was fitted using four parallel MCMC chains of 50000 iterations each. Similar to BMA, PIPs are estimated to provide a measure of variable importance, with a threshold of 0.5 or higher often used to identify important exposures. To explore potential nonlinear and interaction effects, we generated predictor-response and interaction plots. In these visualizations, co-exposures were fixed at the 10th (P10), 50th (P50), and 90th (P90) percentiles to represent low, medium, and high exposure levels. This choice increases contrast between exposure levels, helping reveal effects that may be less visible within moderate ranges like P25–P75.

All models included the following covariates: study cycle (FLEHS III versus IV), sex, age (in months), BMI class, and highest educational level of the household as a proxy for socioeconomic status (SES). This basic set of potential confounders was selected based on literature from similar epidemiological studies investigating associations between chemical exposures and immune parameters (Heilmann et al., 2010; Knudsen et al., 2018; Ma et al., 2022; Oulhote et al., 2017; Serdar et al., 2014; Zheng et al., 2023). To address potential residual confounding, we considered additional covariates such as lifestyle habits and dietary variables in sensitivity analyses. Covariates were forced into the model by keeping them unpenalized (ENET) or by including them in the minimal model (MLR/BMA/BKMR).

In a secondary analysis, we modelled the leukocyte subtypes expressed as the proportion of total leukocyte counts (untransformed). We also performed sex-stratified analyses to explore potential differences in associations between boys and girls. To assess the robustness of our findings, we conducted several sensitivity analyses. First, we performed an analysis with additional adjustment for sporting, smoking, and alcohol use. Second, we repeated the analyses after excluding 64 smoking adolescents (6.5 %). Third, to account for potential differences between study populations within study cycles, we additionally adjusted for the following indicator variable: FLEHS III reference, FLEHS III hotspot, FLEHS IV participants that participated in FLEHS I, and FLEHS IV newly recruited. Fourth, we conducted an analysis excluding the FLEHS III hotspot participants (N = 198) to examine whether associations were driven by this subgroup. Fifth, to account for potential confounding by dietary habits, we performed separate sensitivity analyses with additional adjustment for consumption of meat, fish, local fruit, local vegetables, and local eggs during the past year. These variables were categorized based on questionnaire responses into frequency-based groups (e.g., meat: ≤3 times/week, 4–6 times/week, ≥1 time/day; fish: never, <weekly, ≥weekly; fruit: never, ≤2 pieces/week, >2 pieces/week; vegetables: never, ≤3 portions/week, 4–10 portions/week, >10 portions/week; eggs: never, ≤1/month, 1–4/month, >1/week). Sixth, we performed a sensitivity analysis stratifying by FLEHS campaign to assess whether associations differed between study cycles. Finally, to assess the impact of imputation, we excluded (in separate analyses) participants with TN or PCB187 values below the LOQ. These biomarkers had the highest < LOQ proportions: 14.0 % and 7.2 %, respectively.

Data processing and statistical analyses were done in R version 4.3.0 (R Foundation for Statistical Computing, Vienna, Austria), using R packages glmnet (Friedman et al., 2010) and stabs (Hofner et al., 2015) for ENET, BAS (Clyde et al., 2020) for BMA, and bkmrhat (Bobb et al., 2018) for BKMR. For log-transformed outcomes (leukocyte and subtype counts and the NLR), estimated regression coefficients were presented as the ratio of the geometric mean values (further referred to as geometric mean ratio [GMR]) for an interquartile fold change in exposure biomarker concentration (IQFc; the fold change of the 75th percentile over the 25th percentile in exposure), with 95 % confidence intervals (CI) for MLR and 95 % credible intervals (CrI) for BMA and BKMR. For untransformed outcomes (leukocyte subtype proportions), estimates represent the unit change in the outcome per IQFc of the exposure.

3. Results

3.1. Descriptives

Study population characteristics of adolescents from FLEHS III and IV are summarized in Table 1. A total of 980 adolescents were included in the analyses, consisting of 510 boys (52 %) and 470 girls (48 %). The median age of the study population was 14 years (177 months), with a range of 13–16 years. The median BMI for the pooled sample was 19.6 kg/m^2. BMI differed significantly between study cycles, with a lower proportion of underweight (13.2 % versus 18.7 %) and a higher proportion of overweight participants (13.4 % versus 8.2 %) in FLEHS IV than FLEHS III. There was also a difference in the distributions of highest educational level of the household and smoking between cycles, with a greater proportion of high education (65.2 % versus 50.2 %) and fewer smokers (3.6 % versus 10.7 %) in FLEHS IV than in FLEHS III. The median leukocyte count was 6370 cells per microliter (cells/μL). The median counts for leukocyte subtypes were as follows: (1) neutrophils: 3284 cells/μL, (2) lymphocytes: 2168 cells/μL, (3) monocytes: 532 cells/μL, (4) eosinophils: 142 cells/μL, and (5) basophils: 23 cells/μL. The median NLR was 1.5. Although leukocytes and subtypes had been measured by the same laboratory and using the same analytical method, counts were higher in FLEHS IV than in FLEHS III, except for neutrophils. We also observed some differences between study populations within study cycles (results not shown): eosinophil and basophil counts were higher in the hotspot sample than in the reference sample of FLEHS III and total leukocytes, neutrophils, and basophils were higher in the newly recruited sample of FLEHS IV than in participants that participated in FLEHS I.

Summary statistics for exposure biomarkers are presented in Table 2. Concentrations of all biomarkers except for TN and PCB118 were

Table 1
Population characteristics of adolescents (13–16 years) from the Flemish Environment and Health Studies (FLEHS III and IV, N = 980, Flanders).

Characteristic	N (%) or median (P25-P75)			*p*-value[a]
	FLEHS III (N = 402)	FLEHS IV (N = 578)	Total (N = 980)	
Age (years)	14 (14–15)	14 (14–15)	14 (14–15)	0.031
Age (months)	177 (173–183)	177 (173–181)	177 (173–182)	0.058
Sex (%)				
Male	209 (52.0)	301 (52.1)	510 (52.0)	1.000
Female	193 (48.0)	277 (47.9)	470 (48.0)	
BMI (kg/m^2)	19.0 (17.7–21.0)	20.1 (18.3–22.4)	19.6 (18.0–21.8)	<0.001
BMI (%)				
Underweight	75 (18.7)	54 (9.3)	129 (13.2)	<0.001
Normal weight	294 (73.1)	426 (73.7)	720 (73.5)	
Overweight & Obese	33 (8.2)	98 (17.0)	131 (13.4)	
Household education				
Low (ISCED 0–2)	40 (10.0)	28 (4.8)	68 (6.9)	<0.001
Medium (ISCED 3–4)	160 (39.8)	173 (29.9)	333 (34.0)	
High (ISCED 5–8)	202 (50.2)	377 (65.2)	579 (59.1)	
Sport (%)				
Rarely	58 (14.4)	77 (13.3)	135 (13.8)	0.065
1–2 times/ week	173 (43.0)	212 (36.7)	385 (39.3)	
>2 times/week	171 (42.5)	289 (50.0)	460 (46.9)	
Smoker (%)				
No	359 (89.3)	557 (96.4)	916 (93.5)	<0.001
Yes	43 (10.7)	21 (3.6)	64 (6.5)	
Alcohol consumption (%)				
Never	210 (52.2)	379 (65.6)	589 (60.1)	<0.001
<1 time/month	100 (24.9)	124 (21.5)	224 (22.9)	
≥1 time/month	92 (22.9)	75 (13.0)	167 (17.0)	

P25 = 25th percentile; P75 = 75th percentile; ISCED = International Standard Classification of Education.
[a] Chi-square test for categorical variables and Kruskall-Wallis test for continuous variables.

significantly lower in FLEHS IV than in FLEHS III, in line with findings from a previous study comparing exposure biomarker levels in FLEHS IV with earlier FLEHS studies (Schoeters et al., 2022). PCBs with the highest and lowest median concentrations were PCB153 (10.0 ng/g lipid) and PCB187 (1.2 ng/g lipid), respectively. There were also some differences between study populations within study cycles (results not shown): Cd and Mn were lower whereas PCBs were higher in the hotspot sample than in the reference sample of FLEHS III, and Pb, chlorinated pesticides and PCBs were higher in the FLEHS IV sample that participated in FLEHS I than in the newly recruited sample. Correlations of metals with chlorinated pesticides and PCBs were low ($|r| < 0.25$) (Supplementary Fig. S2). Correlations between the three chlorinated pesticides ranged from 0.45 (OXC and DDE) to 0.79 (OXC and TN), and correlations between chlorinated pesticides and PCBs ranged from 0.53 (DDE and PCB118) to 0.74 (TN and PCB153). Correlations between PCBs were all above 0.85, except for correlations with PCB118 (ranging from 0.64 to 0.77). To reduce the influence of multicollinearity, the sum of PCB 138, 153, 170, 180 and 187 was calculated and used in statistical models (hereafter referred to as SumPCB), whereas PCB 118 was kept as a separate variable. This aligns with the chemical properties of PCBs, as these 5 highly correlated PCBs fall within the category of non-dioxin-like PCBs, while PCB 118 is considered dioxin-like (37). Correlations between SumPCB and the individual non-dioxin-like PCBs were ≥0.94, whereas the correlation with PCB 118 was 0.72. Correlations between exposure biomarkers calculated within study cycles or within samples of the same study cycle (e.g. FLEHS III reference versus hotspot) were similar (results not shown).

Table 2
Descriptives of effect and exposure biomarkers measured in adolescents (13–16 years) from the Flemish Environment and Health Studies (FLEHS III and IV, N = 980, Flanders).

Biomarker	Median (P25-P75)			*p*-value[a]
	FLEHS III (N = 402)	FLEHS IV (N = 578)	Total (N = 980)	
Leukocytes and differential counts (cells/μL, except for NLR)				
Leukocytes	6050 (5275–7260)	6555 (5480–7730)	6370 (5400–7522)	<0.001
Neutrophils	3223 (2583–4191)	3326 (2591–4471)	3284 (2588–4383)	0.266
Lymphocytes	2009 (1712–2420)	2272 (1908–2613)	2168 (1800–2570)	<0.001
Monocytes	500 (409–612)	561 (454–674)	532 (431–652)	<0.001
Eosinophils	136 (80–226)	149 (92–261)	142 (89–242)	0.033
Basophils	22 (17–33)	27 (19–38)	23 (18–37)	0.008
NLR	1.6 (1.2–2.1)	1.5 (1.1–2.0)	1.5 (1.2–2.1)	0.014
Leukocyte differential proportions (%)				
Neutrophils	54.7 (47.5–60.9)	52.0 (46.3–59.5)	52.8 (46.8–59.9)	0.011
Lymphocytes	33.6 (28.7–39.5)	35.0 (29.5–40.1)	34.5 (29.0–39.9)	0.038
Monocytes	8.2 (6.7–9.5)	8.4 (7.1–10.0)	8.3 (7.0–9.9)	0.024
Eosinophils	2.2 (1.3–3.6)	2.4 (1.4–3.9)	2.3 (1.4–3.8)	0.394
Basophils	0.40 (0.20–0.50)	0.40 (0.30–0.50)	0.40 (0.30–0.50)	0.509
Metals in blood (μg/L, except for Tl which is expressed ng/L)				
Cd	0.16 (0.13–0.23)	0.18 (0.14–0.22)	0.17 (0.14–0.22)	0.04
Cu	870 (799–965)	790 (719–882)	827 (748–922)	<0.001
Mn	10.4 (8.7–12.5)	9.4 (7.8–11.3)	9.8 (8.2–11.8)	<0.001
Pb	9.1 (6.9–12.2)	7.9 (6.1–10.3)	8.4 (6.4–11.1)	<0.001
Tl	28.5 (24.7–33.1)	26.9 (24.0–31.6)	27.4 (24.1–32.0)	0.006
Chlorinated pesticides in serum (ng/g lipid)				
OXC	1.3 (0.9–1.8)	1.1 (0.7–1.6)	1.2 (0.8–1.7)	<0.001
DDE	36.5 (23.1–68.4)	32.4 (20.9–57.6)	34.3 (22.0–61.5)	0.007
TN	0.7 (0.5–1.1)	0.8 (0.5–1.1)	0.7 (0.5–1.1)	0.772
Polychlorinated biphenyls in serum (ng/g lipid)				
PCB118	1.9 (1.4–2.8)	2.0 (1.5–2.7)	1.9 (1.4–2.7)	0.335
PCB138	6.8 (4.5–10.0)	6.0 (4.2–8.8)	6.4 (4.3–9.5)	0.003
PCB153	11.4 (7.3–17.9)	9.0 (5.8–14.3)	10.0 (6.3–15.9)	<0.001
PCB170	2.7 (1.6–4.7)	1.9 (1.2–3.2)	2.2 (1.3–3.8)	<0.001
PCB180	5.7 (3.4–10.1)	4.0 (2.5–7.0)	4.6 (2.8–8.1)	<0.001
PCB187	1.3 (0.8–2.3)	1.0 (0.6–1.7)	1.2 (0.7–1.9)	<0.001
SumPCB[b]	28.9 (17.9–45.0)	22.2 (14.5–35.5)	24.7 (15.6–39.3)	<0.001

P25 = 25th percentile; P75 = 75th percentile; NLR = neutrophil–lymphocyte ratio.
[a] Kruskall-Wallis test.
[b] Sum of PCB 138, 153, 170, 180, 187.

3.2. Main analysis

Fig. 1 presents the effect estimates (with 95 % CI or CrI) for the associations between exposure biomarkers and differential leukocyte counts from the different models. Supplementary Table S2 additionally provides *p*-values and PIPs. There was no evidence for multicollinearity in mpMLR models (all VIF <2). Plots of ENET stability selection and BKMR (univariate exposure-response curves, estimates obtained at different levels of other exposures and their difference, and bivariate exposure-response curves) are given in Supplementary Figs. S3 and S4, respectively. Higher concentrations of Cu were associated with higher leukocyte and neutrophil counts, whereas Mn and SumPCB were negatively associated with these outcomes (significant in spMLR and mpMLR models and PIP>0.5 in BMA). The association between Mn and neutrophil count was additionally confirmed by stability selection of ENET. For neutrophils, the estimated GMR per IQFc in Cu ranged from 1.02 (95 % CI: 1.00, 1.05; BMA) to 1.03 (1.00, 1.06; mpMLR). Corresponding estimates for Mn ranged from 0.96 (0.93, 1.00; BMA) to 0.95

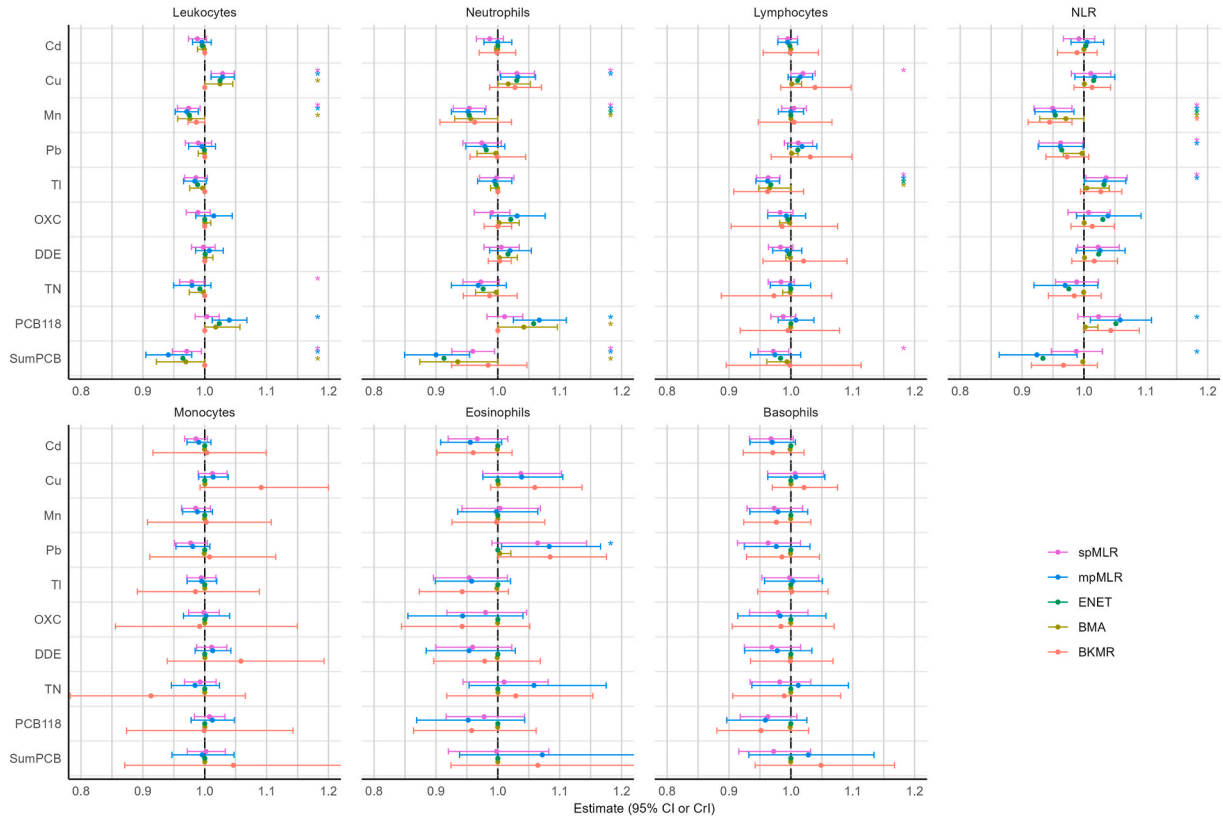

Fig. 1. Associations between exposure biomarker concentrations and differential leukocyte counts in adolescents (13–16 years) from the Flemish Environment and Health Studies (FLEHS III and IV, N = 980, Flanders), estimated by the different statistical methods. Estimates (with 95 % CI or CrI) represent the GMR per IQFc in exposure biomarker concentrations, adjusted for other exposure biomarkers (except in the spMLR), study cycle, sex, age, BMI class, and highest educational level of the household. BKMR results are the estimates obtained when fixing other exposure biomarkers at their median value. Relevant associations are indicated with a star: exposures with a *p*-value <0.05 in spMLR and mpMLR, exposures selected by ENET and surviving stability selection testing with a PFER value of 0.50 and a selection probability of 0.80, and exposures with a PIP >0.5 in BMA and BKMR. Abbreviations: NLR = neutrophil–lymphocyte ratio; Cd = cadmium; Cu = copper; Mn = manganese; Pb = lead; Tl = thallium; OXC = oxychlordane; TN = trans-Nonachlor, PCB = polychlorinated biphenyl; SumPCB = sum of PCBs 138, 153, 170, 180, and 187; spMLR = single-pollutant multiple linear regression; mpMLR = multi-pollutant multiple linear regression, ENET = elastic net; BMA = Bayesian model averaging; BKMR = Bayesian kernel machine regression; CI = confidence interval; CrI = credible interval; GMR = geometric mean ratio; IQFc = interquartile fold change; PFER = per-family error rate; PIP = marginal posterior inclusion probability.

(0.93, 0.98; mpMLR), and for SumPCB from 0.96 (0.93, 0.99; spMLR) to 0.90 (0.85, 0.95; mpMLR). PCB118, however, was positively associated with neutrophil counts, with an estimated GMR of 1.07 (1.03, 1.11) in mpMLR, 1.04 (1.00, 1.10) in BMA, and 1.06 in ENET (although the selection probability in the latter was only 0.1). Estimated effects for total leukocytes were similar because leukocyte and neutrophil counts were highly correlated (r = 0.92). Higher Tl concentrations were associated with lower lymphocyte counts in spMLR, mpMLR, ENET and BMA, with estimated GMRs ranging from 0.97 (ENET) to 0.96 (0.94, 0.98; mpMLR). Although BKMR models did not show evidence for nonlinear or interaction effects (Supplementary Fig. S4), none of the above associations was picked up by BKMR (PIPs close to zero). The NLR was negatively associated with Mn concentrations in all models, with GMRs ranging from 0.97 (0.93, 1.00; BMA) to 0.94 (0.91, 0.98; BKMR). Both spMLR and mpMLR models indicated that the NLR was negatively associated with Pb and positively with Tl, but these associations were not confirmed by other models. We did not observe associations for monocytes, eosinophils, and basophils, except for a positive association between eosinophils and Pb in the mpMLR model.

3.3. Secondary and sensitivity analyses

The secondary analysis using leukocyte subtype proportions instead of counts, showed similar results to the primary analysis, i.e. inverse associations between Tl and lymphocytes, and between Mn and neutrophils (Supplementary Table S2 and Fig. S5). Additionally, we found a positive association between Mn and lymphocyte proportions, with estimates ranging from 0.49 % (0.00 %, 1.35 %; BMA) to 0.96 % (0.34 %, 1.58 %; spMLR), and a positive association between SumPCB and monocyte proportions in spMLR (0.28 %, [0.06 %, 0.49 %]) and mpMLR models (0.49 %, [0.13 %, 0.84 %]). This analysis also revealed a negative association between PCB118 and basophils with estimates ranging from −0.03 % (ENET) to −0.06 % (−0.10, −0.03; BKMR).

Sex-stratified analyses showed that the direction of associations for Mn, Tl, and PCBs was generally consistent between boys and girls, with negative associations observed for Mn and SumPCB with leukocytes and/or neutrophils, and for Tl with lymphocytes (Supplementary Figs. S6 and S7). However, selection thresholds were not always reached, which might be due to reduced statistical power in these stratified analyses. Notably, Cu showed positive associations with leukocyte, neutrophil, and lymphocyte counts in boys, while no evidence for these associations was observed in girls.The exclusion of smoking adolescents (6.5 %) in a sensitivity analysis yielded similar results to the main analysis, except for an additional negative association between Cd and total leukocyte count (Supplementary Table S3 and Fig. S6). Estimated GMRs ranged from 0.987 (0.961, 1.000; BMA) to 0.970 (0.952, 0.989; spMLR). Sensitivity analyses with additional adjustment for lifestyle factors (sporting activity, smoking, and alcohol use) and refined correction for study population sample (four distinct groups instead of two) yielded results nearly identical to the main

analysis (results not shown). Sensitivity analyses excluding FLEHS III hotspot participants and adjusting for dietary variables (meat, fish, local fruit, vegetables, and eggs) showed the same general patterns, although exposures were less frequently selected across models (results not shown). Nonetheless, the same exposures (Cu, Mn, Tl, and SumPCB) repeatedly emerged, and the overall direction of associations was preserved, indicating that dietary habits and hotspot status were not major confounders. Results from the sensitivity analysis stratifying by study cycle showed that the pooled associations observed for Mn appeared to be driven by FLEHS III, while the associations for Cu were only observed in FLEHS IV (Supplementary Figs. S9 and S10). For Tl and SumPCB, both FLEHS cycles showed similar patterns of inverse associations with lymphocyte and neutrophil counts, respectively, although selection thresholds for Tl were not reached in FLEHS IV. Finally, results for TN and SumPCB did not change when excluding participants with TN and PCB 187 values below the LOQ, respectively (results not shown), supporting the robustness of findings despite imputation.

4. Discussion

4.1. Main findings

This study investigated associations of metals, PCBs and chlorinated pesticides with differential leukocyte profiles, representing early biomarkers of the immune response. To ensure robust interpretation, we focused on associations supported by multiple statistical methods. The results discussed below were generally consistent across spMLR, mpMLR, and BMA approaches, and, to a lesser extent, ENET. BKMR identified fewer associations, which is discussed in detail below. Based on this multi-method approach, we found that exposure to non-dioxin-like PCBs, the non-essential metals Tl and Cd, and the essential metal Mn were associated with immunosuppression. Specifically, the sum of five non-dioxin-like PCB congeners and Mn concentrations were negatively associated with leukocyte and neutrophil counts, Tl concentrations with lymphocytes, and Cd concentrations with leukocytes (the latter only after exclusion of smokers). PCB118 exposure was negatively associated with basophil proportions but positively associated with neutrophil counts. Also Cu concentrations were positively associated with leukocyte and neutrophil counts. Observations for leukocytes are mainly driven by neutrophils as they account for more than half of the leukocyte count (Spearman correlation = 0.92). We did not observe consistent associations between exposure biomarkers and monocyte or eosinophil counts.

While declines in leukocyte counts are interpreted as indicative of immunosuppression, the absolute magnitudes of the estimated effects in our study were modest. Estimated GMRs for an IQFc increase in the exposure biomarker ranged from 0.90 to 0.98, and these changes occurred within the clinical normal range for leukocyte subtypes in our sample. Nevertheless, even modest shifts at the population level may be biologically relevant, particularly if they reflect persistent perturbations of immune homeostasis during adolescence, a developmentally sensitive window. Such changes could potentially translate into altered vaccine responses or increased infection susceptibility, as suggested by prior epidemiological studies linking metal and POP exposure to reduced antibody responses and higher infection rates (Gascon et al., 2013; Winans et al., 2011; Zheng et al., 2023).

4.2. Comparison with previous studies

The negative association of non-dioxin-like PCBs with leukocyte and neutrophil counts observed in our study is in line with some studies conducted in humans (Knudsen et al., 2018; Oulhote et al., 2017; Serdar et al., 2014) and animals (Wang et al., 2022; Yu et al., 2012). Leukocyte, neutrophil, lymphocyte, and eosinophil counts were inversely associated with the sum of 14 PCBs in Greenlandic pregnant women (Knudsen et al., 2018). In a study of 56 children in the Faroe Islands, leukocyte and neutrophil counts (at the age of 5 years) were marginally negatively associated with prenatal as well as concurrent (at the age of 5 years) concentrations of organochlorine compounds (including PCBs and pesticides) (Oulhote et al., 2017). A cross-sectional assessment of 17 non-dioxin-like and 19 dioxin-like PCBs measured in participants (aged 12 years and older) of NHANES 2003–2004 showed that the group with the highest PCB concentrations had the lowest leukocyte and neutrophil counts, whereas TN and OXC were found to be positively correlated with leukocytes (Serdar et al., 2014). Interestingly, that study observed a stronger decline in leukocytes for non-dioxin-like than for dioxin-like PCBs. Our results also showed a negative association for the aggregate of non-dioxin-like PCBs, but a positive association for the dioxin-like PCB 118, indicating the need for further exploration of the diverse characterizations of dioxin-like and non-dioxin-like PCBs and their effects on the immune system. Mechanistically, PCBs can exert immunosuppressive effects by activating the aryl hydrocarbon receptor (AhR) and generating oxidative stress, disrupting intracellular calcium homeostasis and inducing apoptosis in immune cells, and impairing leukocyte proliferation and cytokine regulation, processes that are consistent with reduced leukocyte counts and diminished immune competence (Tan et al., 2003; Wang et al., 2019; Winans et al., 2011).

As far as we know, epidemiological evidence for the association between Tl and leukocytes is limited, and mechanisms of Tl-induced toxicity in living organisms have not been fully elucidated. Our study provides evidence for a potential adverse effect of Tl on the immune system by showing that higher Tl concentrations were associated with lower lymphocytes. This negative association is supported by experimental and mechanistic evidence. In Nile tilapia, Tl exposure elicited a decline in the leukocyte count and an alteration of the immunity indexes including serum lysozyme and immunoglobulin M (Farag et al., 2022). In mice, Tl exposure increased B lymphocyte cell apoptosis and a reduced B cells generation in the bone marrow (Li et al., 2023). Tl accumulates intracellularly and is known to have cytotoxic and mutagenic effects through several pathways, including induction of DNA damage, oxidative stress, mitochondrial dysfunction, and apoptosis, collectively reducing cell viability and proliferation (Avendaño-Briseño et al., 2025; Sánchez-Chapul et al., 2023). Consistent with these findings, genotoxicity assessments in human cells indicate that Tl compounds diminish the viability of peripheral lymphocytes and elevate cell mortality (Rodríguez-Mercado et al., 2019).

Results of our study also indicate immunosuppressive effects of Cd exposure. The negative association between Cd concentrations and leukocytes was only observed after exclusion of smoking adolescents, which is intriguing given that smoking (both active and passive) is considered as an important source of human exposure to Cd (Krivohlavek et al., 2021). Our findings are in line with an experimental study showing that Eurasian carp exposed to low concentrations of Cd had lower leukocyte counts than the control group, with the decrease depending on exposure time (Ghiasi et al., 2010). An inverse association between Cd exposure and leukocyte count was also found in a study using NHANES 2003–2010 data, but this study focused on urinary Cd levels, which reflects chronic exposure (Colacino et al., 2014). Another study of NHANES 1999–2016 data, however, found that increased serum levels of Cd were associated with increased levels of leukocytes and other inflammatory factors, and with an elevated risk of cardiovascular disease (Ma et al., 2022). In the general population of southern Taiwan, Cd concentrations in urine were positively associated with eosinophil counts, but not with total leukocyte count (Huang et al., 2022). Mechanistic evidence indicates that Cd can impair immune function through multiple pathways, including oxidative stress, apoptosis, altered cytokine signalling, epigenetic modifications, and activation of immune-relevant signalling pathways such as NF-κB and MAPK (Wang et al., 2021b). While these mechanisms predominantly suggest immunosuppression, Cd-induced oxidative stress may also trigger compensatory inflammatory responses, potentially explaining the elevated leukocyte counts reported in some studies. Discrepancies

across studies may stem from differences in exposure conditions (e.g., dose, frequency, duration, timing), sources of exposure, biological matrices (e.g., blood vs. urine), population characteristics, and methodological approaches, including study design and adjustment for confounders.

The positive association between Cu and leukocyte and neutrophil counts observed in our study is in line with previous findings. Korean studies showed positive associations between serum Cu concentrations and leukocyte counts in adolescents (Choi and Kim, 2005), and between Cu concentration in hair and the NLR in overweight or obese adults (Jeong et al., 2021). Similarly, elevated levels of Cu in urine were significantly linked to increased counts of leukocytes and eosinophils in adults with a mean age of 55 in southern Taiwan (Huang et al., 2022). In previous animal models, increased Cu concentrations were associated with higher neutrophil counts in the trans-mammary epithelium (Wang et al., 2021a). Cu can enhance immune responses by promoting T-lymphocyte proliferation, increasing pro-inflammatory cytokine production (IL-12p70, IFN-γ, IL-4, IL-5), and modulating neutrophil activity (Rouaen et al., 2024; Tulinska et al., 2022), which may contribute to higher leukocyte and neutrophil counts. Notably, reverse causation cannot be excluded in our study as elevated Cu concentrations may be the consequence rather than the cause of the inflammatory response (Pereira et al., 2016).

The association between Mn exposure and leukocytes has been studied mostly in occupational and experimental studies, showing inconsistent results. Similar to our findings, a study on workers exposed to welding fumes in Thailand reported a lower leukocyte count in the group with high Mn exposure compared to the group with low exposure (Li and Taneepanichskul, 2021). Although not specifically looking at Mn, another study found increased leukocyte and neutrophil counts associated with welding fume exposure (Kim et al., 2005), which has been postulated as a potential mechanism underlying the elevated risk of cardiovascular disease in occupational welders (Ibfelt et al., 2010; Sjögren et al., 2002). Some animal studies reporting a positive association between Mn exposure and leukocytes are in accordance with this mechanism (Chandel and Jain, 2016; Sani and Abdullahi, 2019), but others are not (Khan et al., 1997). Mechanistic evidence indicates that Mn can influence immune function through multiple pathways: it can activate pro-inflammatory cytokine production via NF-κB and p38MAPK signaling in macrophages and epithelial cells (Gandhi et al., 2025; Mokgobu et al., 2015), while in lymphocytes it has been shown to inhibit cytokine expression (Lu et al., 2015) and induce apoptosis (Schrantz et al., 1999). These opposing effects may help explain the inconsistencies observed across studies.

4.3. Strengths and limitations

The harmonized data collection and analysis methods in FLEHS III and IV enabled a pooled data analysis, thereby increasing the sample size and thus the reliability of the results. Rather than concentrating on the impact of a single chemical exposure, this study investigated the influence of a mixture of chemicals. We did not, however, estimate overall (total) mixture effects because the studied mixture was rather heterogeneous, encompassing different chemical groups, and the direction of observed associations was not always the same (e.g. leukocyte counts were negatively associated with Mn and non-dioxin-like PCBs, but positively associated with Cu). The focus of this study was on the identification of important mixture components, and the estimation of their effect, using a complementary set of statistical methods. Unlike spMLR, multiple-pollutant methods account for co-exposures and can reduce confounding by correlated exposures. However, they may also amplify bias if unmeasured confounding is present (Weisskopf et al., 2018). mpMLR provides interpretable estimates in a standard linear framework, but in small datasets with many (highly) correlated exposures, multicollinearity can result in imprecise and unreliable estimates. ENET addresses this by applying penalization to perform variable selection and shrinkage, helping to identify the most relevant exposures, though it may bias coefficients downward and does not provide precision estimates. BKMR allows exploration of nonlinear relationships and interactions, while BMA accounts for model uncertainty across multiple plausible exposure combinations. Together, these methods combine interpretability, selection, flexibility, and robustness, offering a more comprehensive analysis of mixture effects than any single approach alone. When there are no strong nonlinear or interaction effects, one would expect that BKMR results are similar to those from linear models, but many of the associations picked up by the other methods, were not confirmed by BKMR in this study. This likely reflects differences in model structure and sensitivity. BKMR's flexible, nonparametric framework captures complex exposure-response relationships but may attenuate weak associations due to its hierarchical modelling and smoothing. Its conservative estimation may limit its ability to detect subtle associations, particularly in smaller samples.

It is worth noting that there were several limitations in this study. Due to the cross-sectional study design, we cannot draw a conclusion on the causality of the observed associations. Yet, some results of this study are supported by other epidemiological studies, experimental animal models, and well-known biological mechanisms, enhancing the plausibility of our findings. Second, the immune parameters in the current study were limited to a single measurement of leukocytes and their subtypes, while the clinical relevance of the observed effects is still unclear considering the values were within normal ranges. However, our results indicate potential early physiological changes in immune responses that link to immune-dysregulation or -suppression, which might lead to increased susceptibility to diseases in adolescence or later in life. Finally, given the ubiquitous presence of diverse types of chemicals in consumer products and the home environment, we cannot exclude the possibility of unmeasured or residual confounding by other exposures such as per- and polyfluoroalkyl substances (PFAS) and phthalates, or by other covariates such as medical history or dietary habits. However, although detailed dietary data were not available, sensitivity analyses adjusting for meat, fish, local fruit, local vegetable, and local egg consumption suggested that residual confounding from diet is unlikely to have substantially influenced the findings.

5. Conclusions

Our study showed that higher blood serum levels of non-dioxin-like PCBs, Tl, Cd, and Mn were associated with lower leukocyte counts in a general population of 13- to 16-year-old adolescents. Despite the uncertainty of causality in this cross-sectional study, our findings of immunosuppression at biologically relevant doses of chemical exposure warrant further investigation given the crucial role of leukocytes and the immune system in the vulnerable developmental phase of adolescence and in later life.

CRediT authorship contribution statement

Fen Zhang: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Lützen Portengen:** Writing – review & editing, Methodology. **Hamid Y. Hassen:** Writing – review & editing. **Laura Rodriguez Martin:** Writing – review & editing, Data curation. **Madeline Carsique:** Writing – review & editing, Project administration, Funding acquisition. **Amélie Crépet:** Writing – review & editing, Project administration, Funding acquisition. **Jasper Engel:** Writing – review & editing. **Jacob Van Klaveren:** Writing – review & editing, Project administration, Funding acquisition. **Nicolas Van Larebeke:** Writing – review & editing. **Willy Baeyens:** Writing – review & editing, Conceptualization. **Stefaan De Henauw:** Writing – review & editing. **Tim S. Nawrot:** Writing – review & editing. **Adrian Covaci:** Writing – review & editing. **Elly Den Hond:** Writing – review & editing. **Greet Schoeters:** Writing – review & editing. **Veerle J. Verheyen:** Writing – original draft, Conceptualization. **Eva Govarts:**

Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Bianca Cox:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

Conflict of interest statement

The authors declare no conflict of interest.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.123188.

Data availability

The authors do not have permission to share data.

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