Environmental Pollution 246 (2019) 587–596

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Cardiovascular endothelial inflammation by chronic coexposure to lead (Pb) and polycyclic aromatic hydrocarbons from preschool children in an e-waste recycling area☆

Xiangbin Zheng [a], Xia Huo [b], Yu Zhang [a, c], Qihua Wang [a], Yuling Zhang [a], Xijin Xu [a, d, *]

[a] Laboratory of Environmental Medicine and Developmental Toxicology, Guangdong Provincial Key Laboratory of Infectious Diseases and Molecular Immunopathology, Shantou University Medical College, Shantou, 515041, Guangdong, China
[b] Laboratory of Environmental Medicine and Developmental Toxicology, Guangdong Key Laboratory of Environmental Pollution and Health, School of Environment, Jinan University, Guangzhou, 510632, Guangdong, China
[c] Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, 9713, GZ, the Netherlands
[d] Department of Cell Biology and Genetics, Shantou University Medical College, Shantou, 515041, Guangdong, China

ARTICLE INFO

Article history:
Received 4 June 2018
Received in revised form
22 November 2018
Accepted 17 December 2018
Available online 19 December 2018

Keywords:
E-waste
Pb
Polycyclic aromatic hydrocarbons
Children
Cardiovascular endothelial inflammation

ABSTRACT

Lead (Pb) and polycyclic aromatic hydrocarbon (PAH) exposure is positively associated with cardiovascular disease (CVD), and the possible potential mechanism may be caused by damage to the endothelium by modulation of inflammatory processes. No comprehensive research shows co-exposure of Pb and PAH on cardiovascular endothelial inflammation in electronic waste (e-waste) exposed populations. Given this, the aim of this study is to provide evidence for a relationship between Pb and PAH co-exposure and cardiovascular endothelial inflammation, in an e-waste-exposed population, to delineate the link between a potential mechanism for CVD and environmental exposure. We recruited 203 preschool children (3–7 years) were enrolled from Guiyu (e-waste-exposed group, n = 105) and Haojiang (reference group, n = 98). Blood Pb levels and urinary PAH metabolites were measured. Percentages of T cells, $CD4^+$ T cells and $CD8^+$ T cells, complete blood counts, endothelial inflammation biomarker (serum S100A8/A9), and other inflammatory biomarkers [serum interleukin (IL)-6, IL-12p70, gamma interferon-inducible protein 10 (IP-10)] levels were evaluated. Blood Pb, total urinary hydroxylated PAH (ΣOHPAH), total hydroxynaphthalene (ΣOHNap) and total hydroxyfluorene (ΣOHFlu) levels, S100A8/A9, IL-6, IL-12p70 and IP-10 concentrations, absolute counts of monocytes, neutrophils, and leukocytes, as well as $CD4^+$ T cell percentages were significantly higher in exposed children. Elevated blood Pb, urinary 2-hydroxynaphthalene (2-OHNap) and ΣOHFlu levels were associated with higher levels of IL-6, IL-12p70, IP-10, $CD4^+$ T cell percentages, neutrophil and monocyte counts. Mediator models indicated that neutrophils exert the significant mediation effect on the relationship between blood Pb levels and S100A8/A9. IL-6 exerts a significant mediation effect on the relationship between blood Pb levels and IP-10, as well as the relationship between urinary ΣOHFlu levels and IP-10. Our results indicate that children with elevated exposure levels of Pb and PAHs have exacerbated vascular endothelial inflammation, which may indicate future CVD risk in e-waste recycling areas.

1. Introduction

Lead (Pb) and polycyclic aromatic hydrocarbons (PAHs) are the major industrial and environmental contaminants, and have high toxicity (Alabi et al., 2012; Garcia-Leston et al., 2012; Guo et al., 2012; Xu et al., 2015; Zeng et al., 2016a). Pb exposure is thought to be an important factor contributing to the development of cardiovascular disease (CVD), including hypertension, arteriosclerosis and coronary heart disease (Ding et al., 2016; Navas-Acien et al., 2007; Prokopowicz et al., 2017; Vaziri, 2008). In addition, an increasing number of studies show that PAH exposure is positively associated with CVD, such as peripheral arterial disease, fatal

☆ This paper has been recommended for acceptance by Prof. Wen-Xiong Wang.

* Corresponding author. Laboratory of Environmental Medicine and Developmental Toxicology, Shantou University Medical College, 22 Xinling Rd., Shantou, 515041, Guangdong, China.
E-mail address: xuxj@stu.edu.cn (X. Xu).

https://doi.org/10.1016/j.envpol.2018.12.055
0269-7491/

ischemic heart disease and arteriosclerosis (Burstyn et al., 2005; Hu et al., 2018; Xu et al., 2013a). However, the underlying mechanism of the positive association of Pb or PAH exposure and CVD remain unclear.

Several pieces of evidence have indicated that Pb can stimulate mouse macrophages, human endothelial cells and peripheral blood mononuclear cells to enhance the release of various pro-inflammatory cytokines (Cheng et al., 2006; Guo et al., 1996; Villanueva et al., 2000; Zeller et al., 2010). Possible underlying mechanisms involving Pb include excessive Pb-induced reactive oxygen species (ROS), which can activate numerous intracellular signaling pathways, such as mitogen-activated protein kinases and protein kinase C pathways, converging on nuclear factor-κB (NF-κB) and inducing transcriptional events (Cheng et al., 2006; Liu et al., 2012). Concomitantly, PAHs can bind to the aryl hydrocarbon receptor (AhR), a transcription factor involved in mediating the toxicity of environmental toxins (Schmidt and Bradfield, 1996; Veldhoen et al., 2008). Previous studies reported PAHs differentially regulate cytokine production by activated leukocytes along the inflammatory process involving both AhR-dependent and independent pathways (Goulaouic et al., 2008; Ple et al., 2015).

Inflammatory cytokines play a role in inflammatory cell and inflammatory biomarker accumulation (Moser and Willimann, 2004; Nicaise et al., 2017; Taub et al., 1993), which may aggravate endothelial inflammation and damage in CVD (Peng et al., 2013). Gamma interferon-inducible protein 10 (IP-10), an important inflammatory chemokine of the CXC chemokine family, is secreted by immune cells (e.g. neutrophils, eosinophils, monocytes) and nonimmune cells (e.g. endothelial, epithelial, and fibroblasts) in response to such inflammatory stimuli as interferon-γ (IFN-γ), interleukin (IL)-1, and tumor necrosis factor (TNF)-α (Lee et al., 2009; Liu et al., 2011). IP-10 has been found to be the main chemoattractant for T lymphocytes and monocytes as well as potentiates T cell adhesion to endothelium (Taub et al., 1993, 1996). The S100A8/A9 heterodimer (also called calprotectin), is a vascular-specific inflammatory biomarker, is secreted from myeloid cells stimulated by inflammatory cytokines, and can be released during the interaction of activated phagocytes with activated endothelium (Eue et al., 2000; Frosch et al., 2000; Pruenster et al., 2015; Rammes et al., 1997; Suryono et al., 2003; Tardif et al., 2015). S100A8/A9 can be a useful biomarker of cardiovascular endothelial inflammation, and has been implicated in endothelial cell apoptosis (Viemann et al., 2007), endothelial dysfunction (Bhattacharjee et al., 2012; Viemann et al., 2005; Wang et al., 2014), leukocyte recruitment and adhesion (Croce et al., 2009; Ehlermann et al., 2006; Eue et al., 2000; Ryckman et al., 2003; Viemann et al., 2005), and intimal hyperplasia (Croce et al., 2009; Hokamura et al., 2010; Inaba et al., 2009), which is among the early steps leading to atherosclerosis. Taken collectively, current evidence shows possible potential mechanism of Pb- and PAH-induced CVD may be caused by the damage to the endothelium through modulation of inflammatory processes.

Guiyu is one of the largest electronic waste (e-waste) destinations and recycling areas in the world (Wu et al., 2010). Informal e-waste dismantling and recycling result in release of Pb and PAHs into the air, sediment and soil (Deng et al., 2006; Wong et al., 2007; Zheng et al., 2016). Our previous studies found elevated Pb levels in umbilical cord blood, placenta and child blood (Huo et al., 2014; Lu et al., 2018; Xu et al., 2016), as well as high PAH concentrations in umbilical cord blood and child venous blood (Xu et al., 2013b, 2015). Moreover, our recent research has shown that environmental PM_{10}, $PM_{2.5}$, NO_2, SO_2, and CO are significantly higher in Guiyu, and air pollution has a clear adverse effect on heart rate and plasma norepinephrine in children (Cong et al., 2018). At the same time, another recent study by our laboratory has shown increased child blood Pb levels are positively associated with elevated serum TNF-α, IL-6, IL-8, and plasma lipoprotein-associated phospholipase A2 concentrations, aberrant blood pressure and disordered lipid parameters in Guiyu children (Lu et al., 2018). However, co-exposure of Pb and PAHs on cardiovascular endothelial inflammation in e-waste-exposed populations has yet to be demonstrated. We predict that the vascular endothelial function in children living in e-waste recycling areas may be affected as a result of elevated Pb and PAH co-exposure by modulation of endothelial inflammatory processes. Therefore, the goal of the present study is to provide evidence for a relationship between Pb and PAH co-exposure and cardiovascular endothelial inflammation in e-waste exposed populations, which may be a potential mechanism for CVD that have been linked to environmental exposure.

2. Methods

2.1. Study area and participants

We recruited 203 preschool children 3–7 of age from Guiyu (n = 105), an e-waste recycling town (Li et al., 2018; Wu et al., 2012), and Haojiang (n = 98) from November to December 2016. Haojiang was chosen as a reference area because it lies approximately 31.6 km to the east of Guiyu and has no record of e-waste recycling, but is similar to Guiyu in terms of cultural background, lifestyle, socioeconomic status and population (Liu et al., 2018; Zeng et al., 2016b; Zhang et al., 2018). Guardians or parents of all children administered a detailed questionnaire regarding information on potential routes of exposure to e-waste, Pb and PAH, as well as general demographic information. The questionnaire covered child demographic (e.g. age, gender, family member daily smoking), parental socioeconomic (e.g. education, and monthly household income), child dietary habits (e.g. eating daily products, and canned food), child behavior habits (e.g. biting pencils or erasers, washing hands before eating and contact with e-waste), family dwelling environment (e.g. distance from home to a road, and home close to e-waste within 50 m) and family medical and health histories. The subjects included in this study met the following criteria: 1) residence in the local areas for more than 1 year, 2) no absentee record for infectious diseases, any immune related diseases, and CVD within 1 month before sample collection, and 3) no consumption of drugs or antibiotics within 1 month before sample collection. This study procedure was approved by the Human Ethics Committee of Shantou University Medical College (SUMC-2015-19) and informed consent was obtained from guardians or parents of the participants of all studies.

2.2. Sample collection

A 2.5 mL sample of peripheral venous blood was drawn from each child into a Pb-free tube that contained EDTA as an anticoagulant and was utilized for routine blood testing by an automated Sysmex XT-1800i hematology analyzer (Sysmex Corporation, Kobe, Japan) and blood Pb testing by graphite furnace atomic absorption spectrometry (GFAAS, Jena Zeenit 650, Germany). Another 2.5 mL blood was collected, in a separate Vacutainer tube (Chengdu Rich Science Industry Co, China), for clotting and separation of serum. The 1.5 mL of blood was collected in a Vacutainer tube containing EDTA for flow cytometry analysis. Serum was stored at −70 °C until assay for cytokines, chemokines and S100A8/A9. Urine samples (the first urine after getting up in the morning) measuring 15 mL were collected into a polypropylene conical centrifuge tube (BD, New Jersey, USA) and stored at −20 °C until analysis for PAH metabolites.

2.3. Measurement of blood Pb concentrations

Blood Pb levels were determined by graphite furnace atomic absorption spectrometry (Jena Zenit 650, Germany). Details for detection methods were described according to our previous publications (Yang et al., 2013; Zhang et al., 2017). The limit of detection (LOD) of this method was 0.051 μg/dL. The accuracy of the method was verified by recoveries between 95% and 107% from the spiked blood samples.

2.4. Measurement of PAH metabolites in urine

For details of chemicals and standard, sample preparation, see Supplementary Methods. Urinary PAH metabolites [1-hydroxynaphthalene (1-OHNap), 2-hydroxynaphthalene (2-OHNap), 9-hydroxyfluorene (9-OHFlu), 2-hydroxyfluorene (2-OHFlu), 4-hydroxyphenanthrene (4-OHPhe), 9-hydroxyphenanthrene (9-OHPhe), 2-hydroxyphenanthrene (2-OHPhe) and 1-hydroxypyrene (1-OHPyr)] were determined by a gas chromatography/mass spectrometry GC/MS (7890A-5975C Agilent Technologies), with electron ionization used in selected ion monitoring mode. Methods for QA/QC were based on our previously published methods with minor modifications (Guo et al., 2012; Xu et al., 2015). In Table S1, recoveries of the spiking blanks for individual PAHs varied from 84.56% to 117.38%. Relative standard deviation (RSD) insured the reproducibility of recovery results and ranged (%) from 0.71% to 4.74%. Nine-concentration (0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64 μg/L) calibration curves showed excellent linearity ($r^2 > 0.995$).

2.5. Blood cell examination and flow cytometry analysis

Blood cell counts were immediately determined by an automated Sysmex XT-1800i hematology analyzer (Sysmex Corporation, Kobe, Japan). The percentage of $CD3^+$ (T cells), $CD3^+CD4^+CD8^-$ ($CD4^+$ T cells) and $CD3^+CD8^+CD4^-$ ($CD8^+$ T cells) was determined using monoclonal antibodies: CD3-APC-CY7, CD4-PE-CF594, CD8-BV510 (BD Bioscience, USA), as detailed in previous studies by our laboratory (Cao et al., 2018; Zhang et al., 2016). Cells were analyzed by flow cytometry (FACS Navio FC, Beckman Coulter, Fullerton, CA, USA), and data were analyzed using Kaluza 3.0 analysis software (Beckman Coulter) (Fig. S1).

2.6. Cytokine, chemokine and S100A8/A9 assays

Multiple cytokine/chemokine concentrations in serum were determined by a ProcartaPlex Human Cytokine & Chemokine Panel 1A (eBioscience, USA). All analytical processes were performed according to instructions of the manufacturer. Data were collected with a Luminex 200 device (Luminex, USA). The sensitivity of IL-6, IL-12p70 and IP-10 was 0.4, 0.04, and 0.3 pg/mL, respectively. Serum S100A8/A9 levels were measured using a Human S100A8/S100A9 Heterodimer Quantikine ELISA kit (R&D Systems Inc., USA) according the manufacturer's protocol. The minimum detectable dose (MDD) of human S100A8/S100A9 ranged from 0.005 to 0.215 ng/mL. The mean MDD was 0.086 ng/mL. The accuracy of the results was validated by determination of S100A8/A9 in the certified reference standard (Human S100A8/S100A9 Heterodimer Controls, R&D Systems, Catalog # QC223).

2.7. Statistical analysis

All statistical analyses were conducted with the Statistical Package for the Social Sciences (SPSS), version 19.0 statistical software (IBM Corporation, NJ, USA), PROCESS (Hayes) and GraphPad Prism 5.0 software (GraphPad, San Diego, CA). The independent-sample *t*-test, Mann-Whitney *U* test, and chi-square test were used to compare two groups. Median and interquartile range (IQR) were used to describe blood Pb levels, urinary PAH metabolite concentrations, serum levels of S100A8/A9, inflammatory cell (neutrophil, monocyte, and leukocyte) counts and cytokine/chemokine concentrations. Mean and standard deviation (SD) were used to depict the percentage of $CD4^+$ T cells and $CD8^+$ T cells. The relationship between two sets of data (e.g. blood Pb levels, urinary PAH metabolite concentrations, S100A8/A9 levels, inflammatory cells, and cytokines/chemokines) was analyzed by the Spearman's correlation test. In addition, Spearman rank correlation analyses were used to evaluate the relationship between blood Pb and urinary PAH metabolite concentrations, and the potential risk factors in the questionnaire. Multiple linear regression analyses for the contributions of Pb, PAH, and mixed Pb and PAHs to inflammatory biomarkers in children have been performed. To examine the effects of mediators on the associations between blood Pb levels/urinary PAH metabolites and inflammatory biomarkers, multiple mediator analyses were carried out as previously described (Hayes et al., 2011; Preacher and Hayes, 2008). A $P < 0.05$ was considered statistically significant.

3. Results

3.1. General characteristics of the study population

Tables 1 and S2 summarize the demographic characteristics of the children in this study. Weight, height and body mass index (BMI) of the children in the exposed group were lower than those in the reference group (16.68 ± 2.48 kg vs. 18.81 ± 3.78 kg, 104.70 ± 7.08 cm vs. 108.71 ± 6.97 cm, 15.17 ± 1.18 kg/m^2 vs. 15.80 ± 2.03 kg/m^2, respectively, all $P < 0.01$). Family income, smoking by a family member, child contact with e-waste, father or mother's work related to e-waste, and e-waste contamination occur within 50 m around the living house were statistically different between the two groups ($P < 0.05$). However, no significant difference in the mean age and gender distribution of the children was observed in the two groups ($P > 0.05$).

3.2. Blood Pb levels and urinary PAH metabolites and related factors

The median blood Pb level was significantly higher in children from the exposed group than that from the reference group (7.232 μg/dL vs. 3.912 μg/dL) ($P < 0.01$, Table 1). The median blood Pb level in e-waste-exposed children (7.232 μg/dL) exceeded the 5.0 μg/dL threshold limit determined by the U.S. Center for Disease Control and Prevention (CDC) (Betts, 2012). Furthermore, we found blood Pb levels exceeded 5 μg/dL in 86.5% (90/104) of the children from the exposed group but only in 20.8% (20/96) of the children from the reference group. Spearman correlation analysis was used to evaluate correlations between blood Pb levels and the investigated factors (Table S3). The child being born in the local area and child contact with e-waste had the highest correlation with child blood Pb levels ($r_s = 0.358$, and $r_s = 0.308$, both $P < 0.01$).

PAH metabolite concentrations in urine of children from the two groups are presented in Tables 1 and S4. Children from the exposed group had significantly higher concentrations of 2-OHNap, 9-OHFlu, 2-OHFlu, total hydroxylated PAHs (ΣOHPAHs), total hydroxynaphthalene (ΣOHNap) and total hydroxyfluorene (ΣOHFlu) in urine compared with the reference group (all $P < 0.01$). However, the concentration of 9-OHPhe in the urine of exposed children was lower. Child contact with e-waste and use of the house as a family workshop had the highest correlation with child urinary PAH metabolite concentrations (Table S3).

Table 1
General characteristics of the study population in the exposed and reference groups.

Characteristics	N	Reference group	N	Exposed group	P-value
Age (years, Mean ± SD)	98	4.78 ± 0.90	105	4.64 ± 0.87	0.248[a]
Gender [n (%)]	98		105		0.607[b]
Male		53 (54.1)		53 (50.5)	
Female		45 (45.9)		52 (49.5)	
BMI (kg/m^2, Mean ± SD)	97	15.80 ± 2.03	105	15.17 ± 1.18	0.008[a]
Blood Pb levels (μg/dL) [median (IQR)]	96	3.912 (3.058–4.717)	104	7.232 (5.670–8.885)	0.000[c]
>5 μg/dL		20 (20.8)		90 (86.5)	0.000[b]
<5 μg/dL		76 (79.2)		14 (13.5)	
ΣOHNap (μg/L) [median (IQR)]	98	3.040 (2.360–4.725)	103	4.020 (2.820–6.120)	0.004[c]
ΣOHFlu (μg/L) [median (IQR)]	98	0.853 (0.703–0.928)	103	1.020 (0.822–1.214)	0.000[c]
ΣOHPhe (μg/L) [median (IQR)]	98	0.994 (0.918–1.155)	103	0.980 (0.912–1.072)	0.160[c]
ΣOHPAHs (μg/L) [median (IQR)]	98	5.789 (4.675–7.129)	103	6.322 (5.104–9.092)	0.005[c]

BMI, body mass index; IQR, interquartile range; ΣOHNap, combined sum of the two examined naphthalene metabolites; ΣOHFlu, combined sum of the two examined fluorene metabolites; ΣOHPhe, combined sum of the three examined phenanthrene metabolites; ΣOHPAHs, combined sum of the eight examined PAH metabolites.
[a] Analysis by independent-sample *t*-test for differences between the exposed and reference groups.
[b] Analysis by chi-square test for differences between two groups.
[c] Analysis by the Mann-Whitney *U* test. $P < 0.05$ were considered statistically significant.

In order to gain a better understanding of the correlation between blood Pb levels and urinary PAH metabolites, the Spearman correlation coefficient matrix was calculated (Table S5). The concentrations of blood Pb levels were positively associated with urinary 2-OHNap, 9-OHFlu, 2-OHFlu, ΣOHNap and ΣOHFlu levels ($r_s = 0.149$, $r_s = 0.151$, $r_s = 0.289$, $r_s = 0.141$, $r_s = 0.253$, respectively; all $P < 0.05$).

3.3. Indices of inflammatory biomarkers in peripheral blood

Monocytes, neutrophils, leukocytes, and T cell subsets were estimated and are expressed in Fig. 1. Higher median absolute values were observed for neutrophils, monocytes, and leukocytes of children in the exposed group compared with the reference group (3.95×10^9/L vs. 3.37×10^9/L, 0.50×10^9/L vs. 0.43×10^9/L, 8.65×10^9/L vs. 7.42×10^9/L, all $P < 0.01$). There were higher CD4$^+$ T cell percentages of children in the exposed group (mean 56.52%) than those in the reference group (mean 52.53%) ($P < 0.01$).

Serum IL-6, IL-12p70 and IP-10 levels were determined (Fig. 2A). We found that serum IL-6, IL-12p70 and IP-10 concentrations of children were significantly higher in the exposed group compared to the reference group (10.00 pg/mL vs. 1.61 pg/mL, 0.35 pg/mL vs. 0.09 pg/mL, 51.52 pg/mL vs. 36.50 pg/mL, all $P < 0.01$). As shown in Fig. 2B, the serum S100A8/A9 level was significantly higher in exposed children than in reference children (1067.51 ng/mL vs. 843.86 ng/mL) ($P < 0.05$).

3.4. Association between Pb, PAH exposure and inflammatory biomarkers

Inflammatory cells (neutrophils and monocytes) and inflammatory cytokines/chemokines (IL-6, IL-12p70, and IP-10) were correlated with blood Pb levels and urinary PAH metabolite concentrations (Fig. 3). Blood Pb levels were positively related to serum IL-6, IL-12p70, IP-10 concentrations, and neutrophil and monocyte counts ($r_s = 0.600$, $r_s = 0.616$, $r_s = 0.470$, $r_s = 0.148$ and $r_s = 0.195$, respectively; all $P < 0.05$). Urinary ΣOHFlu levels were positively associated with serum IL-6, IL-12p70 and IP-10 concentrations ($r_s = 0.289$, $r_s = 0.297$, $r_s = 0.219$, respectively; all $P < 0.01$). Urinary 2-OHNap levels were positively correlated with CD4+T cell percentages ($r_s = 0.188$, $P < 0.05$, data not shown).

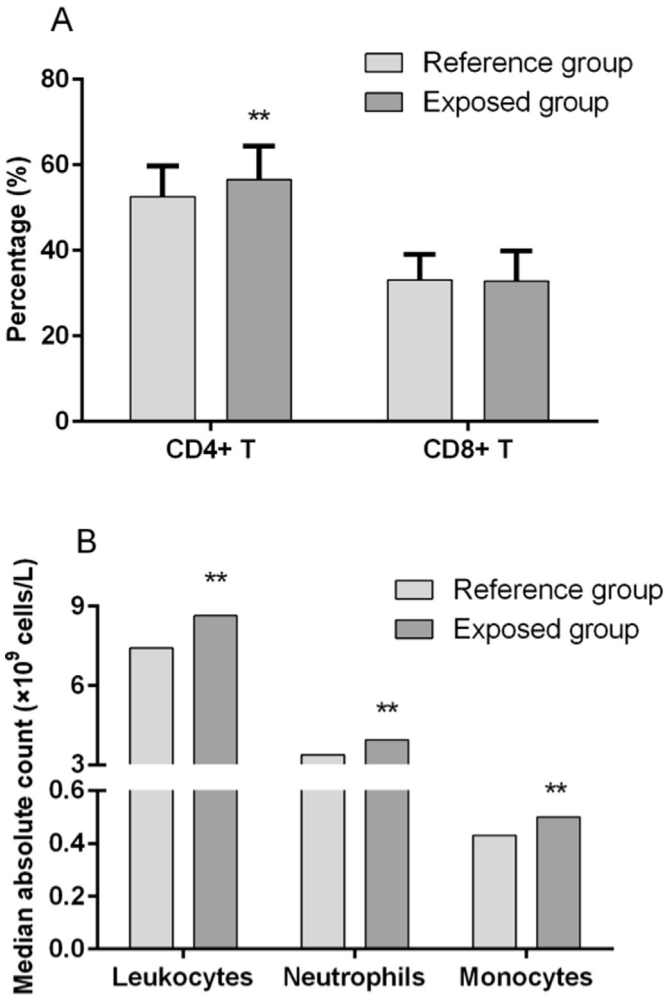

Fig. 1. Immune cell counts and percentages in child peripheral blood between the two groups. A, show the percentage of CD4$^+$ and CD8$^+$ T cells; B, represent innate immune cell counts. A: exposed group, n = 65; reference group, n = 67; B: exposed group, n = 105; reference group, n = 97. A: results are presented as mean ± SD; analysis by independent-sample *t*-test; B: results are presented as median; analysis by the Mann-Whitney *U* test. Values of $^{**}P < 0.01$ were considered statistically significant.

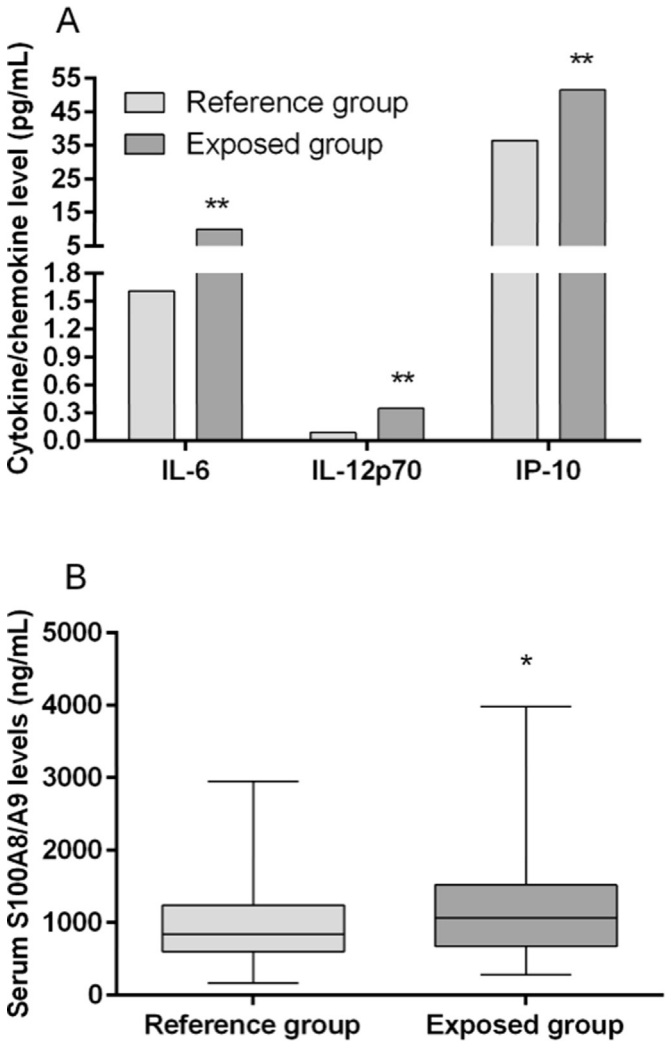

Fig. 2. Levels of pro-inflammatory cytokines and S100A8/A9 in child peripheral blood from e-waste-exposed and reference groups. A: exposed group, n = 80; reference group, n = 76; Data are presented by medians. B: exposed group, n = 105; reference group, n = 98. Results are presented as medians (minimum, maximum). A and B: *P*-values are the result of a Mann-Whitney *U* test. Values of $^{*}P < 0.05$ and $^{**}P < 0.01$ were considered statistically significant. IL, interleukin; IP-10, gamma interferon-inducible protein 10.

3.5. Correlations between inflammatory cells/cytokines and endothelial inflammatory biomarkers

The inflammatory cells (neutrophils, monocytes and $CD4^+$ T cells) and inflammatory cytokines (IL-6, IL-12p70) were further assessed to link with chemokine IP-10 and endothelial inflammatory biomarker S100A8/A9 (Fig. 3). IP-10 was positively associated with levels of IL-6, IL-12p70, monocyte counts and CD4+T cell percentages ($r_s = 0.673$, $r_s = 0.634$, $r_s = 0.276$, and $r_s = 0.249$, respectively; all $P < 0.01$). S100A8/A9 was positively associated with levels of IL-6, IL-12p70, monocyte and neutrophil counts ($r_s = 0.176$, $r_s = 0.162$, $r_s = 0.449$ and $r_s = 0.549$, respectively; all $P < 0.05$).

3.6. Multiple mediator model

To examine the effects of mediators, multiple mediator models were examined, as shown in Table 2. The direct effect and indirect relationships of blood Pb levels with S100A8/A9, blood Pb levels with IP-10, as well as urinary ΣOHFlu levels with IP-10, were employed. In the model of the relationship of blood Pb levels with S100A8/A9, bias corrected 95% CIs indicate a significant total indirect effect [B (95% CI) = 0.1388 (0.0065, 0.2865)], and an indirect effect through neutrophils was significant [0.0831 (0.0089, 0.1897)]. However, other parameters of the mediators (IL-6, IL-12p70 and monocytes), direct effect or contrasts were insignificant (all CIs crossed zero). These results indicate neutrophils are the exclusive mediator of the association between blood Pb levels and S100A8/A9. In the model of the relationship of blood Pb levels with IP-10, bias corrected 95% CIs indicate a significant total indirect effect [0.1227 (0.0800, 0.1751)] and an indirect effect through IL-6 was significant [0.0982 (0.0519, 0.1696)], indicating that IL-6 is the exclusive mediator of the association between blood Pb levels and IP-10. In the model of the relationship of urinary ΣOHFlu levels with IP-10, bias corrected 95% CIs indicate a significant total indirect effect [0.1291 (0.0438, 0.2069)] and indirect effect through IL-6 was significant [0.1077 (0.0474, 0.1922)], indicating that IL-6 is the exclusive mediator of the association between urinary ΣOHFlu levels and IP-10.

4. Discussion

In this study, our results demonstrate that higher levels of inflammatory cells, cytokines/chemokines and S100A8/A9 may be linked to elevated blood Pb levels and urinary PAH metabolite concentrations. Our study had noteworthy advantages and shortcomings. A considerable amount of other pollutants, such as organic pollutants and other heavy metals, also exist in this e-waste environment. Nevertheless, due to insufficient blood quantities, we only investigate the effect of blood Pb levels and urinary PAH metabolite concentrations on biomarkers of inflammatory processes and potential CVD risk. However, to our knowledge, this is the first study to provide evidence for a relationship between Pb and PAH co-exposure and cardiovascular endothelial inflammation in e-waste exposed populations, which may be a potential mechanism for CVD linked to environmental exposure.

4.1. Pb and PAH exposure and related factors

Our results show that children from an e-waste recycling area have elevated blood Pb levels compared with those from the reference area, which is consistent with that found by our previous studies in Guiyu (Zeng et al., 2017; Zhang et al., 2016, 2017). Urinary PAH metabolite concentrations in children are higher in the exposed group, which is similar to the findings of the previous study, in another e-waste area, showing elevated urinary ∑OH-PAH levels in participants from the e-waste recycling areas compared to both rural and urban reference areas (Lu et al., 2016). We evaluate the correlations between blood Pb levels and the investigated factors, and show that the child being born in the local area and child contact with e-waste have the highest correlation with child blood Pb levels. In addition, child contact with e-waste and use of the house as a family workshop may largely contribute to elevating the PAH concentrations in urine. We also analyzed the correlation between blood Pb levels and urinary PAH metabolites, and showed that the concentrations of blood Pb levels are positively associated with urinary 2-OHNap, 9-OHFlu, 2-OHFlu, ΣOHNap and ΣOHFlu levels. These results suggest that a common source of Pb in blood and PAH in urine of children may be linked to the informal e-waste recycling activities.

4.2. Pb exposure and inflammatory biomarkers

Absolute values of neutrophils and monocytes in the peripheral

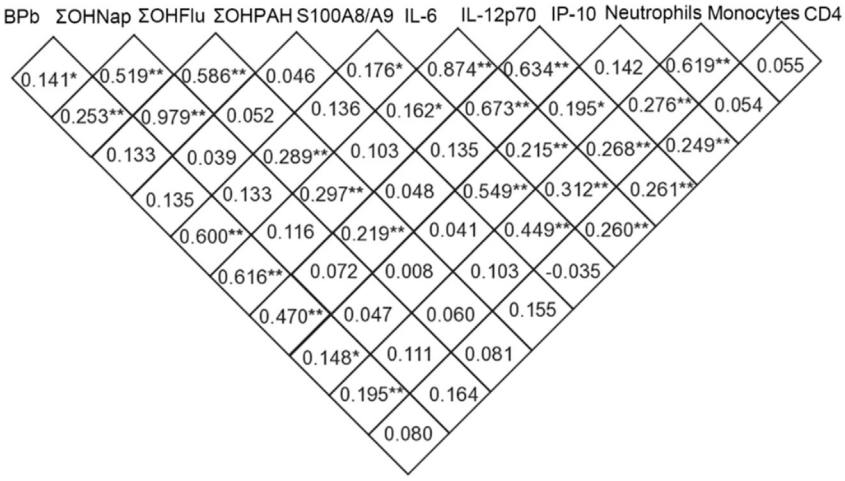

Fig. 3. Spearman correlation coefficients for blood Pb levels, urinary metabolites of PAHs, inflammatory cells and inflammatory biomarkers in children. BPb, blood Pb level; ΣOHNap, combined sum of the two examined naphthalene metabolites; ΣOHFlu, combined sum of the two examined fluorene metabolites; ΣOHPhe, combined sum of the three examined phenanthrene metabolites; ΣOHPAHs, combined sum of the eight examined PAH metabolites; IL, interleukin; IP-10, gamma interferon-inducible protein 10. $^{*}P < 0.05$; $^{**}P < 0.01$.

Table 2
Estimates of the mediating effects.

Model[a]	Product of coefficients		Bootstrapping Bias-corrected 95% CI		Product of coefficients		Bootstrapping Bias-corrected95% CI		Product of coefficients		Bootstrapping Bias-corrected 95% CI	
	B[b]	SE[b]	Lower[b]	Upper[b]	B[c]	SE[c]	Lower[c]	Upper[c]	B[d]	SE[d]	Lower[d]	Upper[d]
Direct effect	0.0616	0.0973	−0.1306	0.2539	0.0613	0.0392	−0.0162	0.1388	0.0310	0.0620	−0.0916	0.1536
Indirect effects												
Total	0.1388*	0.0707	0.0065	0.2865	0.1227*	0.0242	0.0800	0.1751	0.1291*	0.0419	0.0438	0.2069
IL-6	0.0111	0.0569	−0.1165	0.1114	0.0982*	0.0300	0.0519	0.1696	0.1077*	0.0360	0.0474	0.1922
IL-12	0.0099	0.0525	−0.0989	0.1132	0.0204	0.0260	−0.0414	0.0645	0.0221	0.0289	−0.0399	0.0795
Monocytes	0.0347	0.0267	−0.0035	0.1025	0.0051	0.0178	−0.0263	0.0467	−0.0009	0.0135	−0.0513	0.0132
Neutrophils	0.0831*	0.0457	0.0089	0.1897	−0.0009	0.0103	−0.0239	0.0178	0.0003	0.0119	−0.0214	0.0288
Contrasts												
Il-6 vs. IL-12p70	0.0012	0.0984	−0.1988	0.1890	0.0778	0.0512	−0.0044	0.2019	0.0856*	0.0541	0.0022	0.2261
IL-6 vs. monocytes	−0.0235	0.0651	−0.1658	0.0913	0.0931*	0.0361	0.0320	0.1734	0.1086*	0.0387	0.0446	0.2012
IL-6 vs. neutrophils	−0.0720	0.0745	−0.2324	0.0573	0.0991*	0.0315	0.0504	0.1711	0.1074*	0.0366	0.0447	0.1923
IL-12p70 vs. monocytes	−0.0248	0.0603	−0.1497	0.0888	0.0153	0.0322	−0.0521	0.0762	0.0230	0.0297	−0.0318	0.0880
IL-12p70 vs. neutrophils	−0.0732	0.0705	−0.2285	0.0547	0.0213	0.0271	−0.0442	0.0672	0.0218	0.0312	−0.0421	0.0836
Monocytes vs. neutrophils	−0.0484	0.0460	−0.1646	0.0213	0.0060	0.0266	−0.0407	0.0653	−0.0012	0.0205	−0.0722	0.0266

B, unstandardized coefficients; SE, standard error; CI, confidence interval; ΣOHFlu, Combined sum of the two examined fluorene metabolites; IL, interleukin; IP-10, gamma interferon-inducible protein 10. $^{*}P < 0.05$.

[a] All regression analyses are 5000 bootstrap samples.

[b] Model 1 (n = 152): mediating effect of inflammatory biomarkers on the relationship between blood Pb levels (LnBPb, ln-transformed blood Pb level) and serum S100A8/A9 level (LnS100A8/A9, ln-transformed S100A8/A9 level).

[c] Model 2 (n = 152): mediating effect of inflammatory biomarkers on the relationship between blood Pb levels (LnBPb, ln-transformed blood Pb level) and serum IP-10 level (LnIP-10, ln-transformed IP-10 level).

[d] Model 3 (n = 153): mediating effect of inflammatory biomarkers on the relationship between urinary ΣOHFlu levels (LnΣOHFlu, ln-transformed ΣOHFlu level) and serum IP-10 level (LnIP-10, ln-transformed IP-10 level).

blood of children from the e-waste-exposed group are increased compared with the reference area. Neutrophils and monocytes both exhibit a positive association with blood Pb levels (Fig. 3). These observations are consistent with our previous findings (Zhang et al., 2016, 2017). Inflammation is promoted by Pb as a result of ROS production, causing rises in endogenous waste demanding elimination by neutrophils and monocytes (Li et al., 2017; Zhang et al., 2017). Therefore, elevated exposure levels of Pb may increase levels of monocytes and neutrophils in peripheral blood.

Our data show that serum IL-6 and IL-12p70 concentrations are higher in e-waste-exposed children, and exhibit a positive correlation with blood Pb levels, which is similar to epidemiological data on Pb-exposed workers (Di Lorenzo et al., 2007). These observations indicate that higher serum levels of IL-6 and IL-12p70 may be linked to elevated blood Pb levels in e-waste-exposed children. In animals, Pb-pretreated mouse macrophages show enhanced production of proinflammatory cytokines in response to LPS (e.g. TNF-α, IL-6 and IL-12p70), likely by activation of protein kinase C (Flohe et al., 2002). In humans, Villanueva et al. exposed human peripheral blood mononuclear cells to Pb at 10, 50, and 100 μM for 3 d, and showed that production of TNF-α and IL-6 is enhanced by inorganic Pb exposure at concentrations greater than 10 μM (Villanueva et al., 2000). Therefore, these results suggest that elevated exposure levels of Pb exposure may increase levels of IL-6 and IL-12p70 secretion from peripheral blood cells.

Linear regression analysis between blood Pb and IP-10 levels showed that serum IP-10 concentrations are positively associated with blood Pb levels (Table S6), which suggests Pb may increase levels of IP-10. Serum levels of IP-10 are higher in e-waste-exposed

children and are positively correlated with IL-6 and IL-12p70 levels. These observations suggest that the increase in IP-10 level may be related to the increase in IL-6 and IL-12p70 level. Previous studies reported that macrophages/peripheral blood mononuclear cells, exposed to IL-6 and IL-12, secrete increased levels of IP-10 (Lee et al., 2000; Xu et al., 2012). Furthermore, IL-12 can induce T and NK cells to further secrete IFN-γ (Chan et al., 1992; Gately et al., 1994), which plays an important role in IP-10 expression (Cassatella et al., 1997; Torvinen et al., 2007). Moreover, our study found elevated blood Pb levels may increase levels of IL-6 and IL-12p70 secretion from peripheral blood cells, and IL-6 exerts a significant mediation effect on the relationship between blood Pb levels and IP-10. In all, our results can be explained by higher levels of IP-10 being related to elevated exposure levels of Pb.

Our data show serum S100A8/A9 levels, and median absolute values of neutrophils and monocytes are higher in exposed children. Serum S100A8/A9 levels exhibit a positive correlation with absolute values of neutrophils and monocytes. These observations indicate that the increase in S100A8/A9 levels is related to the increase in absolute values of neutrophils and monocytes, which is similar to the findings of a previous epidemiological study, on middle-aged healthy individuals, that showed neutrophils are the only cell population that strongly and independently correlate with plasma S100A8/A9 levels (Cotoi et al., 2014). Extracellular S100A8/A9 is primarily released from activated or necrotic neutrophils and monocytes/macrophages, and the protein can be also released during the interaction of activated phagocytes with activated endothelium (Ehrchen et al., 2009; Frosch et al., 2000; Nacken et al., 2003; Pruenster et al., 2015). Moreover, we found elevated exposure levels of Pb may increase levels of monocytes and neutrophils, and neutrophils exert the significant mediation effect on the relationship between blood Pb levels and S100A8/A9. Taken collectively, this suggests that elevated exposure levels of Pb may enhance S100A8/A9 release from peripheral blood cells.

4.3. PAH exposure and inflammatory biomarkers

Urinary 2-OHNap levels and $CD4^+$ T cell percentages of children are higher in the exposed group, and urinary 2-OHNap levels show a positive correlation with CD4+T cell percentages (data not shown), which is similar to the findings of a previous epidemiological study on asphalt workers showing the percentage of $CD4^+$ cells and the CD4+/CD8+ ratio are higher in PAH-exposed workers (Karakaya et al., 1999). A previous finding found significant associations between PAHs in ambient air during early gestation and increases in $CD3^+$ and $CD4^+$ lymphocyte percentages in cord blood (Herr et al., 2010). In all, elevated exposure levels of PAHs may increase levels of $CD4^+$ T cell percentages.

Our study shows that higher serum IL-6 and IL-12p70 concentrations exhibit a positive correlation with elevated urinary ΣOHFlu levels. In vitro PAH-pretreated human macrophages show enhanced production of proinflammatory cytokines (e.g. IL-6, IL-12) (Goulaouic et al., 2008; Wang et al., 2017). DiNatale et al. showed PAH can bind to the AhR, and activated AhR is involved in priming the IL-6 promoter through binding to dioxin response elements located upstream of the IL-6 start site, promoting IL-1β-induced binding of NF-κB components and synergistically leading to IL-6 expression (DiNatale et al., 2010). Therefore, these results suggest that elevated exposure levels of PAHs may increase levels of IL-6 and IL-12p70 secretion from peripheral blood cells.

Linear regression analysis between urinary ΣOHFlu and IP-10 levels showed that serum IP-10 concentration is positively associated with urinary ΣOHFlu levels (Table S6), which suggests PAHs may increase levels of IP-10. In this study, elevated exposure levels of PAHs may increase levels of IL-6 and IL-12p70 secretion from peripheral blood cells. Moreover, we found the increase in IP-10 level is related to the increase in IL-6 and IL-12p70 levels, and IL-6 exerts a significant mediation effect on the relationship between urinary ΣOHFlu levels and IP-10. In all, our results suggest that higher levels of IP-10 are related to the increase in urinary ΣOHFlu levels.

4.4. Pb and PAH coexposure and inflammatory biomarkers

In the current study, multiple linear regression analyses were performed to identify the contributions of Pb, PAHs, and the mixture of Pb and PAHs to inflammatory biomarkers in children (Table S6). The joint effects of blood Pb and urinary ΣOHFlu have a weaker correlation with serum IL-6, IL-12p70 and IP-10 concentrations compared to individual effects of either blood Pb or urinary ΣOHFlu levels. PAHs can bind to the AhR, which interacts with the xenobiotic response element (XRE) regions located at the two sides of NF-κB binding sites in the NLRP3 promoter, and the association may attenuate NF-kB transcriptional activity, leading to a decrease of NLRP3 transcription and subsequent inflammasome activation (Huai et al., 2014). In contrast, Pb and excessive Pb-induced ROS can activate numerous intracellular signaling pathways (such as the mitogen-activated protein kinase, and protein kinase C pathways), which may converge on NF-κB and ultimately lead to increased cytokine production (Cheng et al., 2006; Liu et al., 2012; Vaziri, 2008). We speculate the probable interaction effects derived from Pb and PAHs would have some implications on their resultant toxicity by competing for NF-kB transcription.

4.5. The role of inflammatory biomarkers in endothelial inflammation

Leukocytes are recruited to the vascular endothelium by a combination of chemokines and adhesion molecules, which is a key event in the inflammatory response. Extracellular S100A8/A9 is able to affect different stages of the leukocyte recruitment process and adhesion by binding to pattern recognition receptors including the Toll-like receptor-4 (TLR4) and the receptor of advanced glycation endproducts (RAGE) (Croce et al., 2009; Ehlermann et al., 2006; Pruenster et al., 2016), which is supported by our epidemiological data showing that increased levels of neutrophils and monocytes correlate with higher levels of serum S100A8/A9. Pruenster et al. found S100A8/A9 is released by neutrophils interacting with E-selectin- P-selectin glycoprotein-1 (PSGL1), and binds to TLR4 to activate the MyD88-induced small GTPase Rap1 and β2 integrin, leading to slow neutrophil rolling and promoting adhesion of the neutrophils to inflamed endothelium (Pruenster et al., 2015). Our results suggest that the levels of IP-10 are elevated by Pb and PAH co-exposure (Table S6), and IP-10 is positively associated with monocytes and $CD4^+$ T lymphocyte cells. IP-10, an important chemokine of the CXC chemokine family, has been found to be the main chemoattractant for T lymphocytes and monocytes as well as potentiates T cell adhesion to the endothelium (Taub et al., 1993, 1996). The previous study also showed that IP-10 and S100A8/A9 can induce migration and proliferation of vascular smooth muscle cells (Croce et al., 2009; Inaba et al., 2009; Wang et al., 1996), which may play a role in the pathogenesis of arterial intimal thickening. Therefore, these results suggest increased S100A8/A9 and IP-10 levels accompanied by elevated Pb and PAH co-exposure may prompt more neutrophil, monocyte and $CD4^+$ T cell chemotaxis to the endothelium, as well as contribute to the proliferation and migration of smooth muscle cells from the media to the intima.

Our present and recent research demonstrate the absolute counts of monocytes, as well as the percentages of $CD4^+$ T cells and ratios of total cholesterol to HDL (Tc/HDL) and low-density

lipoprotein to HDL (LDL/HDL), are significantly higher in exposed children compared with those in reference children (Lu et al., 2018). S100A8/A9 can impair endothelial cell integrity and trigger endothelial cell apoptosis (Viemann et al., 2007). Endothelial injury causes increased vascular permeability and leukocyte adhesion and migration to the vessel wall intima, where monocytes transform into macrophages, followed by accumulation of lipoproteins in the vessel wall (Khan et al., 2010). This suggests that increased levels of lipoproteins, monocytes and T cells may enter the vessel wall intima in e-waste-exposed children, which may cause T cell and macrophage infiltration. The pathological feature of atherosclerosis is characteristically noted as an initial event of endothelial fibrosis, which includes macrophage infiltration to the endothelium and the proliferation and migration of smooth muscle cells from the media to the intima (Hansson and Hermansson, 2011; Khan et al., 2010; Lan et al., 2013). Taken collectively, our results support that elevated Pb and PAH co-exposure damage the endothelium by modulation of inflammatory processes, indicating future CVD risk.

5. Conclusions

Overall, increased blood Pb levels and urinary PAH metabolite concentrations are accompanied by elevated absolute counts of inflammatory cells (monocytes, neutrophils, and $CD4^+$ T cells), and serum S100A8/A9 endothelial inflammation biomarker, and other inflammatory biomarkers (IL-6, IL-12p70, IP-10) in e-waste-exposed children. Available data support the conclusion that children with elevated exposure levels of Pb and PAHs exacerbate vascular endothelial inflammation, which may indicate future CVD risk in the e-waste recycling area. Our study provides the basis for further research on the effect of heavy metal and organic pollutant co-exposure on development of the cardiovascular system in children, and indicates that it is necessary to implement suitable measures to further help reduce sources of heavy metal and organic pollutant exposure and pay more attention to protect child cardiovascular health from e-waste contamination.

Declarations of interest

None.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant numbers 21577084 and 21876065). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We would like to thank Dr. Stanley Lin for his constructive comments and English language editing. Finally, the authors are grateful to all the recruited children and their guardians for participating in this project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2018.12.055.

References

Alabi, O.A., Bakare, A.A., Xu, X., Li, B., Zhang, Y., Huo, X., 2012. Comparative evaluation of environmental contamination and DNA damage induced by electronic-waste in Nigeria and China. Sci. Total Environ. 423, 62–72. https://doi.org/10.1016/j.scitotenv.2012.01.056.

Betts, K.S., 2012. CDC Updates Guidelines for Children's Lead Exposure. Environ. Health Perspect. 120 a268–a268. https://doi.org/10.1289/ehp.120-a268.

Bhattacharjee, R., Kim, J., Alotaibi, W.H., Kheirandish-Gozal, L., Capdevila, O.S., Gozal, D., 2012. Endothelial dysfunction in children without hypertension: potential contributions of obesity and obstructive sleep apnea. Chest 141, 682–691. https://doi.org/10.1378/chest.11-1777.

Burstyn, I., Kromhout, H., Partanen, T., Svane, O., Langard, S., Ahrens, W., Kauppinen, T., Stucker, I., Shaham, J., Heederik, D., Ferro, G., Heikkila, P., Hooiveld, M., Johansen, C., Randem, B.G., Boffetta, P., 2005. Polycyclic aromatic hydrocarbons and fatal ischemic heart disease. Epidemiology 16, 744–750. https://doi.org/10.1097/01.ede.0000181310.65043.2f.

Cao, J., Xu, X., Zhang, Y., Zeng, Z., Hylkema, M.N., Huo, X., 2018. Increased memory T cell populations in Pb-exposed children from an e-waste-recycling area. Sci. Total Environ. 988–995, 616–617. https://doi.org/10.1016/j.scitotenv.2017.10.220.

Cassatella, M.A., Gasperini, S., Calzetti, F., Bertagnin, A., Luster, A.D., McDonald, P.P., 1997. Regulated production of the interferon-gamma-inducible protein-10 (IP-10) chemokine by human neutrophils. Eur. J. Immunol. 27, 111–115. https://doi.org/10.1002/eji.1830270117.

Chan, S.H., Kobayashi, M., Santoli, D., Perussia, B., Trinchieri, G., 1992. Mechanisms of IFN-gamma induction by natural killer cell stimulatory factor (NKSF/IL-12). Role of transcription and mRNA stability in the synergistic interaction between NKSF and IL-2. J. Immunol. 148, 92–98.

Cheng, Y.J., Yang, B.C., Liu, M.Y., 2006. Lead increases lipopolysaccharide-induced liver injury through tumor necrosis factor-α overexpression by monocytes/macrophages: role of protein kinase C and p42/44 mitogen-activated protein kinase. Environ. Health Perspect. 114, 507–513. https://doi.org/10.1289/ehp.8550.

Cong, X., Xu, X., Xu, L., Li, M., Xu, C., Qin, Q., Huo, X., 2018. Elevated biomarkers of sympatho-adrenomedullary activity linked to e-waste air pollutant exposure in preschool children. Environ. Int. 115, 117–126. https://doi.org/10.1016/j.envint.2018.03.011.

Cotoi, O.S., Dunér, P., Ko, N., Hedblad, B., Nilsson, J., Björkbacka, H., Schiopu, A., 2014. Plasma S100A8/A9 correlates with blood neutrophil counts, traditional risk factors, and cardiovascular disease in middle-aged healthy individuals. Arterioscler. Thromb. Vasc. Biol. 34, 202–210. https://doi.org/10.1161/ATVBAHA.113.302432.

Croce, K., Gao, H., Wang, Y., Mooroka, T., Sakuma, M., Shi, C., Sukhova, G.K., Packard, R.R.S., Hogg, N., Libby, P., Simon, D.I., 2009. Myeloid-related protein-8/14 is critical for the biological response to vascular injury. Circulation 120, 427–436. https://doi.org/10.1161/CIRCULATIONAHA.108.814582.

Deng, W.J., Louie, P.K.K., Liu, W.K., Bi, X.H., Fu, J.M., Wong, M.H., 2006. Atmospheric levels and cytotoxicity of PAHs and heavy metals in TSP and $PM_{2.5}$ at an electronic waste recycling site in southeast China. Atmos. Environ. 40, 6945–6955. https://doi.org/10.1016/j.atmosenv.2006.06.032.

Di Lorenzo, L., Vacca, A., Corfiati, M., Lovreglio, P., Soleo, L., 2007. Evaluation of tumor necrosis factor-alpha and granulocyte colony-stimulating factor serum levels in lead-exposed smoker workers. Int. J. Immunopathol. Pharmacol. 20, 239–247. https://doi.org/10.1177/039463200702000204.

DiNatale, B.C., Schroeder, J.C., Francey, L.J., Kusnadi, A., Perdew, G.H., 2010. Mechanistic insights into the events that lead to synergistic induction of interleukin 6 transcription upon activation of the aryl hydrocarbon receptor and inflammatory signaling. J. Biol. Chem. 285, 24388–24397. https://doi.org/10.1074/jbc.M110.118570.

Ding, N., Wang, X., Weisskopf, M.G., Sparrow, D., Schwartz, J., Hu, H., Park, S.K., 2016. Lead-related genetic loci, cumulative lead exposure and incident coronary heart disease: the normative aging study. PLoS One 11, e0161472. https://doi.org/10.1371/journal.pone.0161472.

Ehlermann, P., Eggers, K., Bierhaus, A., Most, P., Weichenhan, D., Greten, J., Nawroth, P.P., Katus, H.A., Remppis, A., 2006. Increased proinflammatory endothelial response to S100A8/A9 after preactivation through advanced glycation end products. Cardiovasc. Diabetol. 5, 6. https://doi.org/10.1186/1475-2840-5-6.

Ehrchen, J.M., Sunderkotter, C., Foell, D., Vogl, T., Roth, J., 2009. The endogenous Toll-like receptor 4 agonist S100A8/S100A9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer. J. Leukoc. Biol. 86, 557–566. https://doi.org/10.1189/jlb.1008647.

Eue, I., Pietz, B., Storck, J., Klempt, M., Sorg, C., 2000. Transendothelial migration of 27E10+ human monocytes. Int. Immunol. 12, 1593–1604.

Flohé, S.B., Brüggemann, J., Herder, C., Goebel, C., Kolb, H., 2002. Enhanced proinflammatory response to endotoxin after priming of macrophages with lead ions. J. Leukoc. Biol. 71, 417–424.

Frosch, M., Strey, A., Vogl, T., Wulffraat, N.M., Kuis, W., Sunderkötter, C., Harms, E., Sorg, C., Roth, J., 2000. Myeloid-related proteins 8 and 14 are specifically secreted during interaction of phagocytes and activated endothelium and are useful markers for monitoring disease activity in pauciarticular-onset juvenile rheumatoid arthritis. Arthritis Rheum. 43, 628–637. https://doi.org/10.1002/1529-0131(200003)43:3<628::AID-ANR20>3.0.CO;2-X.

García-Lestón, J., Roma-Torres, J., Vilares, M., Pinto, R., Prista, J., Teixeira, J.P., Mayan, O., Conde, J., Pingarilho, M., Gaspar, J.F., Pásaro, E., Méndez, J., Laffon, B., 2012. Genotoxic effects of occupational exposure to lead and influence of polymorphisms in genes involved in lead toxicokinetics and in DNA repair. Environ. Int. 43, 29–36. https://doi.org/10.1016/j.envint.2012.03.001.

Gately, M.K., Warrier, R.R., Honasoge, S., Carvajal, D.M., Faherty, D.A., Connaughton, S.E., Anderson, T.D., Sarmiento, U., Hubbard, B.R., Murphy, M., 1994. Administration of recombinant IL-12 to normal mice enhances cytolytic lymphocyte activity and induces production of IFN-γ in vivo. Int. Immunol. 6, 157–167. https://doi.org/10.1093/intimm/6.1.157.

Goulaouic, S., Foucaud, L., Bennasroune, A., Laval-Gilly, P., Falla, J., 2008. Effect of polycyclic aromatic hydrocarbons and carbon black particles on pro-inflammatory cytokine secretion: impact of PAH coating onto particles. J. Immunot. 5, 337–345. https://doi.org/10.1080/15476910802371016.

Guo, T.L., Mudzinski, S.P., Lawrence, D.A., 1996. The heavy metal lead modulates the expression of both TNF-alpha and TNF-alpha receptors in lipopolysaccharide-activated human peripheral blood mononuclear cells. J. Leukoc. Biol. 59, 932–939.

Guo, Y., Huo, X., Wu, K., Liu, J., Zhang, Y., Xu, X., 2012. Carcinogenic polycyclic aromatic hydrocarbons in umbilical cord blood of human neonates from Guiyu, China. Sci. Total Environ. 427–428, 35–40. https://doi.org/10.1016/j.scitotenv.2012.04.007.

Hansson, G.K., Hermansson, A., 2011. The immune system in atherosclerosis. Nat. Immunol. 12, 204–212. https://doi.org/10.1038/ni.2001.

Hayes, A.F., Preacher, K.J., Myers, T.A., 2011. Mediation and the estimation of indirect effects in political communication research. Sourcebook for political communication research: Methods, measures, and analytical techniques 23, 434–465.

Herr, C.E.W., Dostal, M., Ghosh, R., Ashwood, P., Lipsett, M., Pinkerton, K.E., Sram, R., Hertz-Picciotto, I., 2010. Air pollution exposure during critical time periods in gestation and alterations in cord blood lymphocyte distribution: a cohort of livebirths. Environ. Health 9, 46. https://doi.org/10.1186/1476-069X-9-46.

Hokamura, K., Inaba, H., Nakano, K., Nomura, R., Yoshioka, H., Taniguchi, K., Ooshima, T., Wada, K., Amano, A., Umemura, K., 2010. Molecular analysis of aortic intimal hyperplasia caused by Porphyromonas gingivalis infection in mice with endothelial damage. J. Periodontal. Res. 45, 337–344. https://doi.org/10.1111/j.1600-0765.2009.01242.x.

Hu, C., Hou, J., Zhou, Y., Sun, H., Yin, W., Zhang, Y., Wang, X., Wang, G., Chen, W., Yuan, J., 2018. Association of polycyclic aromatic hydrocarbons exposure with atherosclerotic cardiovascular disease risk: A role of mean platelet volume or club cell secretory protein. Environ. Pollut. 233, 45–53. https://doi.org/10.1016/j.envpol.2017.10.042.

Huai, W., Zhao, R., Song, H., Zhao, J., Zhang, L., Zhang, L., Gao, C., Han, L., Zhao, W., 2014. Aryl hydrocarbon receptor negatively regulates NLRP3 inflammasome activity by inhibiting NLRP3 transcription. Nat. Commun. 5, 4738. https://doi.org/10.1038/ncomms5738.

Huo, X., Peng, L., Qiu, B., Zheng, L.K., Yekeen, T.A., Xu, X.J., 2014. ALAD genotypes and blood lead levels of neonates and children from e-waste exposure in Guiyu, China. Environ. Sci. Pollut. Res. Int. 21, 6744–6750. https://doi.org/10.1007/s11356-014-2596-2.

Inaba, H., Hokamura, K., Nakano, K., Nomura, R., Katayama, K., Nakajima, A., Yoshioka, H., Taniguchi, K., Kamisaki, Y., Ooshima, T., Umemura, K., Murad, F., Wada, K., Amano, A., 2009. Upregulation of S100 calcium-binding protein A9 is required for induction of smooth muscle cell proliferation by a periodontal pathogen. FEBS Lett. 583, 128–134. https://doi.org/10.1016/j.febslet.2008.11.036.

Karakaya, A., Yücesoy, B., Turhan, A., Erdem, O., Burgaz, S., Karakaya, A.E., 1999. Investigation of some immunological functions in a group of asphalt workers exposed to polycyclic aromatic hydrocarbons. Toxicology 135, 43–47. https://doi.org/10.1016/S0300-483X(99)00048-7.

Khan, F., Galarraga, B., Belch, J.J.F., 2010. The role of endothelial function and its assessment in rheumatoid arthritis. Nat. Rev. Rheumatol. 6, 253–261. https://doi.org/10.1038/nrrheum.2010.44.

Lan, T.H., Huang, X.Q., Tan, H.M., 2013. Vascular fibrosis in atherosclerosis. Cardiovasc. Pathol. 22, 401–407. https://doi.org/10.1016/j.carpath.2013.01.003.

Lee, E.Y., Lee, Z.H., Song, Y.W., 2009. CXCL10 and autoimmune diseases. Autoimmun. Rev. 8, 379–383. https://doi.org/10.1016/j.autrev.2008.12.002.

Lee, T.V., Kim, D.K., Peoples, G.E., Castilleja, A., Murray, J.L., Gershenson, D.M., Ioannides, C.G., 2000. Secretion of CXC chemokine IP-10 by peripheral blood mononuclear cells from healthy donors and breast cancer patients stimulated with HER-2 peptides. J. Interferon Cytokine Res. 20, 391–401. https://doi.org/10.1089/107999000312333.

Li, M., Huo, X., Pan, Y., Cai, H., Dai, Y., Xu, X., 2018. Proteomic evaluation of human umbilical cord tissue exposed to polybrominated diphenyl ethers in an e-waste recycling area. Environ. Int. 111, 362–371. https://doi.org/10.1016/j.envint.2017.09.016.

Li, Q., Zhang, P., Yu, X., Zhao, Y., Li, Q., Zhang, Y., Yang, Z., Xie, Y., Xue, P., Sun, S., Jia, X., Zhou, Z., He, M., Zhang, Y., 2017. Lead transiently promotes granulocyte-macrophage progenitor differentiation and subsequently suppresses common myeloid progenitor differentiation. Toxicol. Sci. 160, 268–283. https://doi.org/10.1093/toxsci/kfx176.

Liu, C.M., Sun, Y.Z., Sun, J.M., Ma, J.Q., Cheng, C., 2012. Protective role of quercetin against lead-induced inflammatory response in rat kidney through the ROS-mediated MAPKs and NF-κB pathway. Biochim. Biophys. Acta 1820, 1693–1703. https://doi.org/10.1016/j.bbagen.2012.06.011.

Liu, M., Guo, S., Hibbert, J.M., Jain, V., Singh, N., Wilson, N.O., Stiles, J.K., 2011. CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications. Cytokine Growth Factor Rev. 22, 121–130. https://doi.org/10.1016/j.cytogfr.2011.06.001.

Liu, Y., Huo, X., Xu, L., Wei, X., Wu, W., Wu, X., Xu, X., 2018. Hearing loss in children with e-waste lead and cadmium exposure. Sci. Total Environ. 624, 621–627. https://doi.org/10.1016/j.scitotenv.2017.12.091.

Lu, S., Li, Y., Zhang, J., Zhang, T., Liu, G., Huang, M., Li, X., Ruan, J., Kannan, K., Qiu, R., 2016. Associations between polycyclic aromatic hydrocarbon (PAH) exposure and oxidative stress in people living near e-waste recycling facilities in China. Environ. Int. 94, 161–169. https://doi.org/10.1016/j.envint.2016.05.021.

Lu, X., Xu, X., Zhang, Y., Zhang, Y., Wang, C., Huo, X., 2018. Elevated inflammatory Lp-PLA2 and IL-6 link e-waste Pb toxicity to cardiovascular risk factors in preschool children. Environ. Pollut. 234, 601–609. https://doi.org/10.1016/j.envpol.2017.11.094.

Moser, B., Willimann, K., 2004. Chemokines: role in inflammation and immune surveillance. Ann. Rheum. Dis. 63, ii84–ii89. https://doi.org/10.1136/ard.2004.028316.

Nacken, W., Roth, J., Sorg, C., Kerkhoff, C., 2003. S100A9/S100A8: Myeloid representatives of the S100 protein family as prominent players in innate immunity. Microsc. Res. Tech. 60, 569–580. https://doi.org/10.1002/jemt.10299.

Navas-Acien, A., Guallar, E., Silbergeld, E.K., Rothenberg, S.J., 2007. Lead exposure and cardiovascular disease - a systematic review. Environ. Health Perspect. 115, 472–482. https://doi.org/10.1289/ehp.9785.

Nicaise, C., Weichselbaum, L., Schandene, L., Gangji, V., Dehavay, F., Bouchat, J., Balau, B., Vogl, T., Soyfoo, M.S., 2017. Phagocyte-specific S100A8/A9 is upregulated in primary Sjögren's syndrome and triggers the secretion of pro-inflammatory cytokines in vitro. Clin. Exp. Rheumatol. 35, 129–136.

Peng, H., Han, S.H., Liu, H.Y., Chandni, V., Cai, X.Q., Zhang, Y.H., 2013. Relationship of inflammation and endothelial dysfunction with risks to cardiovascular disease among people in Inner Mongolia of China. Biomed. Environ. Sci. 26, 792–800. https://doi.org/10.3967/bes2013.002.

Plé, C., Fan, Y., Ait Yahia, S., Vorng, H., Everaere, L., Chenivesse, C., Balsamelli, J., Azzaoui, I., de Nadai, P., Wallaert, B., Lazennec, G., Tsicopoulos, A., 2015. Polycyclic aromatic hydrocarbons reciprocally regulate IL-22 and IL-17 cytokines in peripheral blood mononuclear cells from both healthy and asthmatic subjects. PLoS One 10, e0122372. https://doi.org/10.1371/journal.pone.0122372.

Preacher, K.J., Hayes, A.F., 2008. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav. Res. Methods 40, 879–891. https://doi.org/10.3758/BRM.40.3.879.

Prokopowicz, A., Sobczak, A., Szuła-Chraplewska, M., Zaciera, M., Kurek, J., Szołtysek-Bołdys, I., 2017. Effect of occupational exposure to lead on new risk factors for cardiovascular diseases. Occup. Environ. Med. 74, 366–373. https://doi.org/10.1136/oemed-2016-103996.

Pruenster, M., Kurz, A.R.M., Chung, K., Cao-Ehlker, X., Bieber, S., Nussbaum, C.F., Bierschenk, S., Eggersmann, T.K., Rohwedder, I., Heinig, K., Immler, R., Moser, M., Koedel, U., Gran, S., McEver, R.P., Vestweber, D., Verschoor, A., Leanderson, T., Chavakis, T., Roth, J., Vogl, T., Sperandio, M., 2015. Extracellular MRP8/14 is a regulator of β2 integrin-dependent neutrophil slow rolling and adhesion. Nat. Commun. 6, 6915. https://doi.org/10.1038/ncomms7915.

Pruenster, M., Vogl, T., Roth, J., Sperandio, M., 2016. S100A8/A9: From basic science to clinical application. Pharmacol. Ther. 167, 120–131. https://doi.org/10.1016/j.pharmthera.2016.07.015.

Rammes, A., Roth, J., Goebeler, M., Klempt, M., Hartmann, M., Sorg, C., 1997. Myeloid-related protein (MRP) 8 and MRP14, calcium-binding proteins of the S100 family, are secreted by activated monocytes via a novel, tubulin-dependent pathway. J. Biol. Chem. 272, 9496–9502. https://doi.org/10.1074/jbc.272.14.9496.

Ryckman, C., Vandal, K., Rouleau, P., Talbot, M., Tessier, P.A., 2003. Proinflammatory activities of S100: proteins S100A8, S100A9, and S100A8/A9 induce neutrophil chemotaxis and adhesion. J. Immunol. 170, 3233–3242. https://doi.org/10.4049/jimmunol.170.6.3233.

Schmidt, J.V., Bradfield, C.A., 1996. Ah receptor signaling pathways. Annu. Rev. Cell Dev. Biol. 12, 55–89. https://doi.org/10.1146/annurev.cellbio.12.1.55.

Suryono, Kido, J., Hayashi, N., Kataoka, M., Nagata, T., 2003. Effect of Porphyromonas gingivalis lipopolysaccharide, tumor necrosis factor-alpha, and interleukin-1beta on calprotectin release in human monocytes. J. Periodontol. 74, 1719–1724. https://doi.org/10.1902/jop.2003.74.12.1719.

Tardif, M.R., Chapeton-Montes, J.A., Posvandzic, A., Pagé, N., Gilbert, C., Tessier, P.A., 2015. Secretion of S100A8, S100A9, and S100A12 by neutrophils involves reactive oxygen species and potassium efflux. J. Immunol. Res. 296149. https://doi.org/10.1155/2015/296149.

Taub, D.D., Lloyd, A.R., Conlon, K., Wang, J.M., Ortaldo, J.R., Harada, A., Matsushima, K., Kelvin, D.J., Oppenheim, J.J., 1993. Recombinant human interferon-inducible protein 10 is a chemoattractant for human monocytes and T lymphocytes and promotes T cell adhesion to endothelial cells. J. Exp. Med. 177, 1809–1814. https://doi.org/10.1084/jem.177.6.1809.

Taub, D.D., Longo, D.L., Murphy, W.J., 1996. Human interferon-inducible protein-10 induces mononuclear cell infiltration in mice and promotes the migration of human T lymphocytes into the peripheral tissues and human peripheral blood lymphocytes-SCID mice. Blood 87, 1423–1431.

Torvinen, M., Campwala, H., Kilty, I., 2007. The role of IFN-gamma in regulation of IFN-gamma-inducible protein 10 (IP-10) expression in lung epithelial cell and peripheral blood mononuclear cell co-cultures. Respir. Res. 8, 80. https://doi.org/10.1186/1465-9921-8-80.

Vaziri, N.D., 2008. Mechanisms of lead-induced hypertension and cardiovascular disease. Am. J. Physiol. Heart Circ. Physiol. 295, H454–H465. https://doi.org/10.1152/ajpheart.00158.2008.

Veldhoen, M., Hirota, K., Westendorf, A.M., Buer, J., Dumoutier, L., Renauld, J.C., Stockinger, B., 2008. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature 453, 106–109. https://doi.org/10.1038/nature06881.

Viemann, D., Barczyk, K., Vogl, T., Fischer, U., Sunderkötter, C., Schulze-Osthoff, K., Roth, J., 2007. MRP8/MRP14 impairs endothelial integrity and induces a caspase-dependent and -independent cell death program. Blood 109, 2453–2460. https://doi.org/10.1182/blood-2006-08-040444.

Viemann, D., Strey, A., Janning, A., Jurk, K., Klimmek, K., Vogl, T., Hirono, K., Ichida, F.,

Foell, D., Kehrel, B., Gerke, V., Sorg, C., Roth, J., 2005. Myeloid-related proteins 8 and 14 induce a specific inflammatory response in human microvascular endothelial cells. Blood 105, 2955–2962. https://doi.org/10.1182/blood-2004-07-2520.

Villanueva, M., Koizumi, S., Jonai, H., 2000. Cytokine production by human peripheral blood mononuclear cells after exposure to heavy metals. J. Health Sci. 46, 358–362.

Wang, C., Yang, J., Zhu, L., Yan, L., Lu, D., Zhang, Q., Zhao, M., Li, Z., 2017. Never deem lightly the "less harmful" low-molecular-weight PAH, NPAH, and OPAH — Disturbance of the immune response at real environmental levels. Chemosphere 168, 568–577. https://doi.org/10.1016/j.chemosphere.2016.11.024.

Wang, L., Luo, H., Chen, X., Jiang, Y., Huang, Q., 2014. Functional characterization of S100A8 and S100A9 in altering monolayer permeability of human umbilical endothelial cells. PLoS One 9, e90472. https://doi.org/10.1371/journal.pone.0090472.

Wang, X., Yue, T.L., Ohlstein, E.H., Sung, C.P., Feuerstein, G.Z., 1996. Interferon-inducible protein-10 involves vascular smooth muscle cell migration, proliferation, and inflammatory response. J. Biol. Chem. 271, 24286–24293. https://doi.org/10.1074/JBC.271.39.24286.

Wong, M.H., Wu, S.C., Deng, W.J., Yu, X.Z., Luo, Q., Leung, A.O., Wong, C.S., Luksemburg, W.J., Wong, A.S., 2007. Export of toxic chemicals - a review of the case of uncontrolled electronic-waste recycling. Environ. Pollut. 149, 131–140. https://doi.org/10.1016/j.envpol.2007.01.044.

Wu, K., Xu, X., Liu, J., Guo, Y., Li, Y., Huo, X., 2010. Polybrominated diphenyl ethers in umbilical cord blood and relevant factors in neonates from Guiyu, China. Environ. Sci. Technol. 44, 813–819. https://doi.org/10.1021/es9024518.

Wu, K., Xu, X., Peng, L., Liu, J., Guo, Y., Huo, X., 2012. Association between maternal exposure to perfluorooctanoic acid (PFOA) from electronic waste recycling and neonatal health outcomes. Environ. Int. 48, 1–8. https://doi.org/10.1016/j.envint.2012.06.018.

Xu, L., Ge, J., Huo, X., Zhang, Y., Lau, A.T.Y., Xu, X., 2016. Differential proteomic expression of human placenta and fetal development following e-waste lead and cadmium exposure in utero. Sci. Total Environ. 550, 1163–1170. https://doi.org/10.1016/j.scitotenv.2015.11.084.

Xu, W., Joo, H., Clayton, S., Dullaers, M., Herve, M.C., Blankenship, D., De La Morena, M.T., Balderas, R., Picard, C., Casanova, J.L., Pascual, V., Oh, S., Banchereau, J., 2012. Macrophages induce differentiation of plasma cells through CXCL10/IP-10. J. Exp. Med. 209, 1813–1823. https://doi.org/10.1084/jem.20112142.

Xu, X., Hu, H., Kearney, G.D., Kan, H., Sheps, D.S., 2013a. Studying the effects of polycyclic aromatic hydrocarbons on peripheral arterial disease in the United States. Sci. Total Environ. 461–462, 341–347. https://doi.org/10.1016/j.scitotenv.2013.04.089.

Xu, X., Liu, J., Huang, C., Lu, F., Chiung, Y.M., Huo, X., 2015. Association of polycyclic aromatic hydrocarbons (PAHs) and lead co-exposure with child physical growth and development in an e-waste recycling town. Chemosphere 139, 295–302. https://doi.org/10.1016/j.chemosphere.2015.05.080.

Xu, X., Yekeen, T.A., Xiao, Q., Wang, Y., Lu, F., Huo, X., 2013b. Placental IGF-1 and IGFBP-3 expression correlate with umbilical cord blood PAH and PBDE levels from prenatal exposure to electronic waste. Environ. Pollut. 182, 63–69. https://doi.org/10.1016/j.envpol.2013.07.005.

Yang, H., Huo, X., Yekeen, T.A., Zheng, Q., Zheng, M., Xu, X., 2013. Effects of lead and cadmium exposure from electronic waste on child physical growth. Environ. Sci. Pollut. Res. Int. 20, 4441–4447. https://doi.org/10.1007/s11356-012-1366-2.

Zeller, I., Knoflach, M., Seubert, A., Kreutmayer, S.B., Stelzmüller, M.E., Wallnoefer, E., Blunder, S., Frotschnig, S., Messner, B., Willeit, J., Debbage, P., Wick, G., Kiechl, S., Laufer, G., Bernhard, D., 2010. Lead contributes to arterial intimal hyperplasia through nuclear factor erythroid 2-related factor-mediated endothelial interleukin 8 synthesis and subsequent invasion of smooth muscle cells. Arterioscler. Thromb. Vasc. Biol. 30, 1733–1740. https://doi.org/10.1161/ATVBAHA.110.211011.

Zeng, X., Xu, X., Boezen, H.M., Huo, X., 2016a. Children with health impairments by heavy metals in an e-waste recycling area. Chemosphere 148, 408–415. https://doi.org/10.1016/j.chemosphere.2015.10.078.

Zeng, X., Xu, X., Boezen, H.M., Vonk, J.M., Wu, W., Huo, X., 2017. Decreased lung function with mediation of blood parameters linked to e-waste lead and cadmium exposure in preschool children. Environ. Pollut. 230, 838–848. https://doi.org/10.1016/j.envpol.2017.07.014.

Zeng, X., Xu, X., Zheng, X., Reponen, T., Chen, A., Huo, X., 2016b. Heavy metals in $PM_{2.5}$ and in blood, and children's respiratory symptoms and asthma from an e-waste recycling area. Environ. Pollut. 210, 346–353. https://doi.org/10.1016/j.envpol.2016.01.025.

Zhang, Y., Huo, X., Cao, J., Yang, T., Xu, L., Xu, X., 2016. Elevated lead levels and adverse effects on natural killer cells in children from an electronic waste recycling area. Environ. Pollut. 213, 143–150. https://doi.org/10.1016/j.envpol.2016.02.004.

Zhang, Y., Xu, X., Chen, A., Davuljigari, C.B., Zheng, X., Kim, S.S., Dietrich, K.N., Ho, S.M., Reponen, T., Huo, X., 2018. Maternal urinary cadmium levels during pregnancy associated with risk of sex-dependent birth outcomes from an e-waste pollution site in China. Reprod. Toxicol. 75, 49–55. https://doi.org/10.1016/j.reprotox.2017.11.003.

Zhang, Y., Xu, X., Sun, D., Cao, J., Zhang, Y., Huo, X., 2017. Alteration of the number and percentage of innate immune cells in preschool children from an e-waste recycling area. Ecotoxicol. Environ. Saf. 145, 615–622. https://doi.org/10.1016/j.ecoenv.2017.07.059.

Zheng, X., Xu, X., Yekeen, T.A., Zhang, Y., Chen, A., Kim, S.S., Dietrich, K.N., Ho, S.M., Lee, S.A., Reponen, T., Huo, X., 2016. Ambient air heavy metals in $PM_{2.5}$ and potential human health risk assessment in an informal electronic-waste recycling site of China. Aerosol Air Qual. Res. 16, 388–397. https://doi.org/10.4209/aaqr.2014.11.0292.