ABSTRACT: The health effects of exposure to pollutants from electronic waste (e-waste) pose an important issue. In this study, we explored the association between oxidative stress and blood levels of e-waste-related pollutants. Blood samples were collected from individuals living in the proximity of an e-waste recycling site located in northern China, and pollutants, as well as reactive oxygen species (ROS), were measured in comparison to a reference population. The geometric mean concentrations of PCBs, dechlorane plus, and 2,2′,4,4′,5,5′-hexabromobiphenyl in plasma from the exposure group were 60.4, 9.0, and 0.55 ng g⁻¹ lipid, respectively, which were 2.2, 3.2, and 2.2 times higher than the corresponding measurement in the reference group. Correspondingly, ROS levels in white blood cells, including in neutrophil granulocytes, from the exposure group were significantly higher than in those from the reference group, suggesting potential ROS related health effects for residents at the e-waste site. In contrast, fewer ROS were generated in the respiratory burst of neutrophil granulocytes for the exposure group, indicating a depressed innate immune function for the individuals living at the e-waste site. These findings suggest a potential linkage between exposure to pollutants from e-waste recycling and both elevated oxidative stress and altered immune function.

INTRODUCTION

With the constant rapid updating of electronic products, waste electrical and electronic equipment or electronic waste (e-waste) has received a great deal of attention in recent decades.¹⁻³ The global annual output of e-waste is estimated to be 20⁻50 million tons, and most of this output is transported to developing countries for disposal.²,³ Among these countries, China has received nearly 70% of global e-waste.² This cross-border transfer has resulted in severe pollution at the recycling sites because pollutants in e-waste are readily released into the environment during the primitive disposal processes. These pollutants include persistent organic pollutants (POPs) such as polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/PCDFs), among others.²⁻⁴

The adverse health effects of exposure to POPs have attracted attention for many decades due to their persistence in the environment, bioaccumulation in biota, and particularly their toxicity.⁵ For example, several studies have demonstrated an association between exposure to PCBs and toxic effects such as alteration of neuropsychological,⁶⁻⁷ immune,⁸⁻⁹ and thyroid functions.¹⁰⁻¹¹ Although to date the underlying mechanism is not fully understood, these negative health effects are supposed to be related to the generation of reactive oxygen species (ROS) following pollutant exposure.¹²⁻¹³

ROS, including superoxide anions, hydrogen peroxide, and the hydroxyl radical, comprise a group of chemically reactive species formed during the incomplete reduction of oxygen.¹⁴ In physiological systems, ROS are generated through the electron transport chain in mitochondria, oxidative protein folding in the endoplasmic reticulum, and nicotinamide dinucleotide phosphate (NADPH) oxidases in cell membranes.¹⁵ Acting as important signaling molecules, ROS have a critical role in cell function,¹⁶ cell differentiation,¹⁷ and wound healing.¹⁸ Particularly, ROS generated in the respiratory burst of certain immune cells (mainly neutrophil granulocytes) are essential for microbe elimination after infection.¹⁹⁻²⁰ However, excessive production of ROS (i.e., oxidative stress) is harmful because they can cause damage to biomolecules including nucleic acid, lipids, and proteins,²¹ which can severely compromise cell viability and function or induce a variety of cellular responses, ultimately leading to cell death by necrosis or apoptosis.¹⁶

Oxidative stress is usually evaluated indirectly through biomarkers in human body fluids in epidemiological studies.²² Such biomarkers include malondialdehyde (MDA), a product of
ROS-induced lipid peroxidation, and 8-hydroxydeoxyguanosine (8-OHdG), an oxidized nucleoside. However, both 8-OHdG and MDA are secondary products of oxidative stress, and they could be influenced by many factors. In this regard, intracellular ROS level is a more direct marker of the oxidative stress in human bodies.

In this study, we explored the association between pollutant exposure and oxidative stress based on the measurement of ROS in immune cells for a population living in an e-waste recycling region located in northern China. In addition, the respiratory burst of neutrophil granulocyte was evaluated to explore the alteration of this important nonspecific immune function. This study could be valuable for revealing the etiology of some prevalent diseases such as cancers and some infectious diseases that we preliminarily investigated in the region of this highly polluted e-waste recycling site, particularly from the viewpoint of oxidative stress and immune function.

MATERIALS AND METHODS

Subjects and Sample Collection. A cross-sectional rural population was enrolled in this study in October 2011. One group (n = 23) living in an e-waste recycling region (38.825° N, 116.777° E) with many family dismantling workshops was defined as the exposure group, and a second group (n = 28) living in a region without any e-waste dismantling activity (38.636° N, 117.135° E) was considered to be the reference group. The e-waste recycling region has a history of more than twenty years in dismantling imported e-waste, particularly cables, electric motors, and transformers to recycle copper. Participants of each group have been living in these areas for nearly 20 years averagely, and none of the participants from the reference group worked in or lived near a polluted area before. The distance between the two sites, which were both located in the same county of Tianjin, was approximately 40 km. Both groups shared similar environmental conditions and personal lifestyles, except that no e-waste dismantling occurred in the reference region.

Fasting peripheral blood was collected from all subjects in EDTA-coated vacuum blood tubes for hematology and ROS analysis within 6 h of sampling. An aliquot of blood sample was store at 4 °C for blood lead analysis within a week. Another aliquot of the blood sample was centrifuged at 2000 rpm for 10 min to separate the plasma fraction and then frozen at −25 °C for pollutant analysis.

The demographic information of the subjects, including age, sex, body mass index (BMI), occupation, and personal lifestyle, was obtained through a face to face questionnaire survey. Hematological parameters were determined in anticoagulated peripheral blood using routine blood procedures. These parameters included the red blood cell count (RBC), hemoglobin level (Hb), white blood cell count (WBC), lymphocyte count (LY), and neutrocyte count (NEUT). This study was approved by the institutional review board of Tianjin Medical University, and informed consent was obtained from each participant.

ROS in Immune Cells. ROS in peripheral white blood cells and the respiratory burst of neutrophil granulocytes were measured according to a previous method with a slight modification. In brief, three aliquots of 100 μL anticoagulated blood from each participant were placed into three polypropylene tubes. The three samples were used as a reagent blank and for determining the ROS generated in resting cells and the respiratory burst of neutrophil granulocytes, respectively. Phosphate buffer saline (PBS; 25 μL) was spiked into tubes of the reagent blank, while dihydrodihydroxide123 solution (25 μL, 100 nM; Sigma-Aldrich, St. Louis, MO, USA) was added to the other two sets of tubes. After incubating in a shaking air bath at 37 °C for 15 min, 25 μL of PBS was spiked into tubes of the reagent blank and resting ROS tests, while 25 μL of phorbolmyristate acetate (PMA) solution (5 nM; Sigma-Aldrich) was spiked into tubes for the respiratory burst test as a stimulator. All samples were incubated at 37 °C for a further 15 min, and then 2 mL of BD FACS lysing solution (Becton Dickinson, San Jose, CA, USA) was added to each tube. After incubation in darkness at room temperature for 8–10 min, samples were centrifuged at 1000 rpm for 5 min. The supernatant was discarded, and the residue cells were washed twice with 2 mL of PBS. Finally, cells were resuspended in 1 mL of PBS that contained 1% paraformaldehyde.

Sample analyses were carried out on a BD FACs Calibur flow cytometer (Becton Dickinson). The cells were excited under 488 nm blue laser, and the fluorescence of the FL1 green channel (530 nm) was measured. For each sample 10,000 cells were acquired. Forward scatter (FSC) and side scatter (SSC) were applied to determine the subgroups of white blood cells.

Pollutants Analysis. Organohalogen pollutants including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), dechlorane plus (DP), hexachlorobenzene (HCB), β-hexachlorocyclohexane(β-HCH), and p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) in plasma were measured using a modification of a previous method. Briefly, after spiking with 13C-labeled PCBs as recovery surrogate standards, plasma was desaturated with hydrochloric acid (0.5 mL, 6 M) and 2-propanol (3 mL). Samples were extracted three times with hexane/methyl tertiary-butyl ether (MTBE) (1:1, by volume). The extract was blown down to 1.5 mL with nitrogen, and interference was removed with gel permeation chromatography (GPC), followed by alumina (3% water-deactivated) column chromatography. Finally, samples were blown down and spiked with 13C-labeled PCB-138 as an internal standard. All samples were analyzed by gas chromatography–mass spectrometry (GC-MS; 7890A-5975C; Agilent, Santa Clara, CA, USA) with an electron-impact (EI) ion source for p,p'-DDE and an electron-capture negative ionization (ECNI) ion source for all other pollutants. Blood lead was measured using atomic fluorescence spectroscopy (AFS, model 610A; Rayleigh Analytical Instrument Co. Ltd., Beijing, P. R. China) with 50 μL of peripheral blood.

Quality Control. The following quality control criteria were applied: a) the difference in retention time of the target substance between plasma samples and the standard was less than ±0.1 min, b) the signal-to-noise ratio was greater than 5:1, and c) the isotope ratios for selected ion couples were less than 10% of the theoretical values. The recoveries (mean ± standard deviation) of surrogate standards were 81.1 ± 13.4, 77.6 ± 12.7, 73.2 ± 12.2, 72.8 ± 12.2, 65.3 ± 16.6, and 79.7 ± 14.6% for 13C12−PCB118, 13C12−PCB133, 13C12−PCB137, 13C12−PCB153, 13C12−PCB180, 13C12−PCB194, 13C12−PCB208, and 13C12−PCB209, respectively. Five blank samples were prepared with water as blank matrix. For pollutants with no significant difference (i.e., p > 0.05) between blank and plasma samples were rejected from further analysis. All measurements were neither blank nor recovery corrected.

Statistics. The Shapiro–Wilk’s test was performed to check the normality of all the continuous or log-transformed data. For normally distributed data, mean (with one standard deviation) was reported; while for logarithmic normally distributed data, geometric mean (with 95% confidence interval, CI) was
presented in the text. A Student’s $t$ test was used to compare the normally or log-normally distributed parameters of the two groups. For the categorical parameters, a chi-square test was applied. For correlation analysis, the Pearson correlation coefficient was obtained. A two-tailed $p$-value <0.05 was considered to be statistically significant. Since some analytes were not detected in all samples, the 1/2 method detection limit (MDL) was applied as a substitute for statistical analysis when necessary. All statistical analyses were conducted using the SPSS software package 16.0 (SPSS Inc., Chicago, IL, CA).

**RESULTS**

Demographic and Hematological Characteristics. Fifty-one subjects, including 23 from the e-waste recycling region and the other 28 from the reference site, were enrolled in this study. For the exposure group, two participants were e-waste dismantling workers, and the others were residents at the e-waste site. Since no significant difference in serum levels of PCBs and most other e-waste related pollutants were observed between e-waste dismantling workers and local residents,26 we reported the combined data of these two subgroups in the following section. The demographic information of the participants was summarized in Table 1. No statistically significant difference was observed in age, BMI, gender, or the number of smokers between the two groups.

<table>
<thead>
<tr>
<th>Table 1. Demographic Characteristics and Blood Routine Parameters for the Exposure and Reference Groups</th>
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<td>exposure group</td>
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</tr>
<tr>
<td>number of participants</td>
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<tr>
<td>age (years, mean ± SD)</td>
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<tr>
<td>BMI (kg/m², mean ± SD)</td>
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<tr>
<td>no. of females/males</td>
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<tr>
<td>no. of smokers/nonsmokers</td>
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<tr>
<td>RBC (1×10¹²/L, mean ± SD)</td>
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<td>Hb (g/L, mean ± SD)</td>
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<td>WBC (1×10⁹/L, mean ± SD)</td>
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<td>LY (1×10⁹/L, mean ± SD)</td>
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<tr>
<td>NEUT (1×10⁹/L, mean ± SD)</td>
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<td>*Student’s $t$ test. *chi-square test.</td>
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The hematological parameters of all participants were within the medical reference range (Table 1).27 No difference in red blood count (RBC) or hemoglobin levels (Hb) existed between the two groups, whereas the white blood cell count (WBC), lymphocyte count (LY), and neutrocyte count (NEUT) were significantly higher for the exposure group compared to the reference group.

Pollutants in Plasma or Blood. Among the 51 participants, 48 plasma samples were measured for pollutants, while not enough plasma was left for this analysis for the other 3 participants. In total 21 PCB congeners (including PCB congeners −105, −118, −138, −153/−132, −156, −157, −167, −170, −171, −177, −180, −183, −187, −194, −195, −199, −202, −206, −208, and −209), 10 PBDE congeners (including PBDE congeners −28, −47, −153, −171, −183, −197, −201, −207, −208, and −209), some other PBDE congeners were either not detected or rejected for their blank values), DP (including syn- and anti-isomers), 2,2′,4,4′,5,5′-hexabromobiphenyl (PBB153), HCB, β-HCH, and p,p’-DDE were measured, and the results were given in Table 2. Plasma levels of almost all PCB congeners, DP, and PBB153 of the exposure group were significantly higher than those of the reference group. In contrast, no significant difference was observed between the two groups in the plasma levels of HCB, β-HCH, or p,p’-DDE, which were pesticide related pollutants rather than e-waste-originated pollutants.

As for blood lead, the geometric mean concentration was 77.1 μg L⁻¹ for the exposure group and 66.7 μg L⁻¹ for the reference group, with no statistically significant difference between the two groups. However, 5 participants from the exposure region had their blood lead concentration higher than the international blood lead diagnostic criteria (i.e., 100 μg L⁻¹),28 while there was only one such participant in the reference group.

ROS in Immune Cells. In this study, ROS levels for each subject were expressed as the mean channel fluorescence (MCF) of acquired cells after deducting the fluorescence of the corresponding reagent blank. Figure 1 shows the flow cytometric scatter plots of resting and stimulated white cells and fluorescence histograms of resting and stimulated neutrophils. In both neutroph granulocytes and white blood cells, the exposure group had higher ROS levels than the reference group ($p < 0.001$), but lower levels of ROS were generated in the respiratory burst in neutroph granulocytes for the exposure group ($p < 0.001$; Figure 2, panel A). To quantify the capacity of ROS generation in neutroph granulocytes, we introduced a stimulation index (SI) for neutroph granulocytes by calculating the ratio of the MCF of the respiratory burst cells to that of resting cells.29 The geometric mean of stimulation index was 8.3 (95% CI: 6.3–10.8) for the residents at the e-waste recycling site, which was significantly lower than that of 28.4 recorded for the reference group (95% CI: 21.8–37.0) ($p < 0.001$; Figure 2, panel B).

**DISCUSSION**

Organic pollutants, particularly organohalogen pollutants released directly from mechanical disposal processes or formed during the burning of e-waste, are important pollutants in regions where waste dismantling occurs. Such pollutants include PCBs, PBDEs, and PCDDs/PCDFs, among others.3,4 Taken PCBs for instance, commercial PCBs have been widely used as dielectric and coolant fluids in transformers, capacitors, and electric motors.5 During the dismantling process of e-waste, a potential exists for large quantities of such pollutants to be released into the environment.3,4 In this study, we found significantly higher plasma levels of PCBs, DP, and PBB153 in the residents living in the e-waste recycling region. The geometric mean concentration of 21 PCBs in plasma from the reference group was 60.4 ng g⁻¹ lipid, which was 2.2 times [95% confidence interval (CI): 1.4–3.3] higher than the concentration in the reference group. In particular, the geometric mean concentration of five dioxin-like PCBs (ΣdI-PCBs, including PCB congeners −105, −118, −156, −157, and −167) was 2.7 times (95% CI: 1.7–4.3) higher in plasma samples from the exposure group than in those from the reference group. It was not surprising given that the main e-waste dismantling in the exposure area were electric motors and transformers as well as cables. This finding was in accordance with other studies of exposure to PCBs in Guiyu, southern China,22 and Taizhou, eastern China,30,31 which both were large scale e-waste recycling sites. Similar to a study in Guiyu,32 plasma concentrations of DP, an unregulated highly chlorinated flame retardant, in the exposure group were 3.2-fold higher (95% CI: 1.7–6.1) than those in the reference group ($p < 0.001$) in our study. As for PBB153, it was never produced or consumed in
China and was prohibited in the United States in 1976 after a contamination incident in Michigan. This pollutant was detected in 65% of the plasma samples from the exposure group while in 18% of the samples from the reference group. Although the concentrations observed in this study were lower than those observed in the United States, the relatively higher levels of PBB153 in plasma from the exposure group further confirmed that recycling of historically produced electrical appliances from abroad could cause elevated body burdens of related pollutants in the exposure region.

As for PBDEs, although there was significant difference in plasma concentrations of some congeners such as PBDE153 and 183 between the two groups (Table 2), no significant difference was observed for the overall PBDE levels. This might be because of the existence of other PBDE sources such as household electrical appliances containing PBDEs. In fact, commercial Deca-BDE (almost pure PBDE209) is being produced and consumed in China intensively nowadays. It could be the source of PBDE209 or even congeners with less bromines through photodegradation or through debromination in humans. Likewise, interference from other sources, such as coal combustion and traffic emission, could be the cause of no statistically significant difference of blood lead levels between the two groups.
Presumably as a result of exposure to PCBs and other e-waste relevant pollutants, certain hematological parameters were significantly altered in participants living in the e-waste recycling region. The mean counts of white blood cells, lymphocytes, and neutrophils of the exposure group were 20.9%, 18.6%, and 24.0% higher, respectively, than those of the reference group. Furthermore, a significantly positive correlation \((R = 0.36, p < 0.05)\) between white blood cell counts and total PCB concentration was observed.

**Figure 1.** Flow cytometric plots of one blood sample. Panels A and B show scatter plots of white blood cells in resting and stimulated conditions, respectively. Subpopulations of white blood cells can be isolated by forward scatter (relative size) and side scatter (relative complexity). The gated a, b, and c represent neutrophils, lymphocytes, and monocytes, respectively. Panels C and D are corresponding fluorescence histograms of panels A and B, respectively, which show fluorescence in resting and stimulated neutrophils.

**Figure 2.** Geometric mean levels of ROS in resting white blood cells (WBC), resting neutrophil granulocytes (NG), and the respiratory burst in neutrophil granulocytes after stimulation (panel A) and the stimulation index between the two groups (panel B). These parameters were logarithmic normal distributed and Student’s t test was performed after log-transformation. *** indicates \(p < 0.001\).
alteration of the production of ROS has been proposed as a mechanism to the impaired immune function. This kind of alteration of immune cell counts has been found in many studies focusing on the immunotoxicity of PCBs and other halogenated pollutants. The number of lymphocytes in 3-month-old infants was positively correlated with prenatal PCB exposure, and goat kids exposed to PCB153 had significantly higher numbers of white blood cells, neutrophils, and lymphocytes than controls. These findings suggested that immune cell counts could be altered by exposure to halogenated contaminants.

In addition to the alteration of immune cell counts, exposure to PCBs has been shown to result in a series of adverse health effects such as dysfunction of the nervous system. Although the underlying mechanism was not fully understood, an alteration of the production of ROS has been proposed as a potential mechanism. In this study, we found levels of PCBs in plasma from the exposure group were 2.2 times higher than in plasma from the reference group; correspondingly, ROS levels in the white blood cells (including neutrophil granulocytes) from the exposure group were higher than in those from the reference group. Actually, a significant positive correlation was found between concentrations of PCBs in plasma and ROS levels in both white blood cells and neutrophil granulocytes (both \( p < 0.05 \); Figure 3), while no significant relation was observed between ROS in immune cell and plasma levels of PBDEs, DP, or PBB153. Regression analysis suggested a 2.0% (95% CI: 0.1%–3.9%) incremental increase of ROS in neutrophil granulocytes when plasma PCBs increased by 10%. Significant positive correlation still existed when the regression analysis was performed on either dioxin-like PCBs (dl-PCBs) (\( p = 0.02 \), \( p < 0.05 \) for both white blood cells and lymphocytes), while it was marginally significant on nondioxin-like PCBs (nondl-PCBs; \( p = 0.07 \) for white blood cells and \( p = 0.052 \) for neutrophils). This finding was in accordance with many in vitro toxicological studies, which have demonstrated that halogenated organic chemicals, including PCBs, could induce ROS formation in human neutrophil granulocytes by activating the NADPH oxidase complex through mitogen-activated protein kinase (MAPK) and protein kinase C (PKC) pathways. In addition, some studies showed that the production of ROS in neutrophil granulocytes after exposure to PCBs was impacted by the congener size, ortho-chlorine substitution, and absolute hardness. The elevated ROS levels in immune cells indicated a higher oxidative stress in human bodies, which could bring about a series of adverse effects such as lipid peroxidation and oxidative damage to DNA. This has been supported by many studies; for example, Wen et al. reported the incremental increase of urinary 8-OHdG in workers dismantling e-waste and exposed to organohalogen pollutants including PCBs and PBDEs, with the 8-OHdG level being about four times higher in postworkshift urine samples than in the preworkshift urine samples. Elevated ROS levels can influence the onset of atherosclerosis, Alzheimer’s disease, and even cancers. The higher ROS levels in blood immune cells indicated a higher oxidative stress for the residents living in the e-waste recycling region, which might further suggest an increased risk of ROS-related diseases for this population.

As the most abundant subtype of white blood cells, neutrophil granulocytes play an important role in the innate immune system. During infection, neutrophil granulocytes migrate to the inflammatory site and kill pathogens by generating high levels of ROS through NADPH oxidase, a physiological response termed respiratory burst. Since the respiratory burst is the central function of neutrophil granulocytes, defects in this function may result in immune diseases such as chronic granulomatous disease (CGD). Patients with CGD have mutations in NADPH oxidase subunits, which lead to defective ROS generation in their neutrophil granulocytes after stimulation. Consequently, they suffer recurrently from life-threatening microbial infections. In the present study, although the exposure group showed higher ROS levels in resting neutrophil granulocytes than the reference group (\( p < 0.001 \)), less ROS were generated in the neutrophil granulocytes from the exposure group than in those from the reference group after stimulating with PMA (\( p < 0.001 \); Figure 2, panel A).

Accordingly, the geometric mean of the stimulation index, a parameter to evaluate the capacity of ROS generation in neutrophil granulocytes, was significantly lower in the exposure group than in the reference group (\( p < 0.001 \); Figure 2, panel B). In addition, a significant inverse correlation between ROS generated in the respiratory burst and plasma PCBs was found (\( p < 0.01 \); Figure 3). Significantly negative correlation was still existed when correlation analysis was performed on either dl-

Figure 3. ROS levels in immune cells versus the total concentration of PCBs in plasma.
PCBs (p < 0.01) or non-d-PCBs (p < 0.01). Similar results have been observed in many other studies, particularly toxicological tests. An in vitro study showed that in isolated human leukocytes, the respiratory burst of monocytes, which have similar function in ROS generation with neutrophics, was depressed by around 20% - 40% after exposure to PCBs. In vivo experiments using Japanese medaka (Oryzias latipes) also demonstrated that ROS production in the respiratory burst was depressed at 3 days post-PCB exposure, indicating an association between exposure to PCBs and innate immune dysfunction. Combined with the increased immune cell counts as discussed above, we conclude that exposure to e-waste-related pollutants could lead to innate immune dysfunction by weakening the respiratory burst of neutrophil granulocytes.

Note that pollutants such as PCBs are ubiquitous in the environment. These pollutants can be detected in human blood from the general population around the world. Several epidemiological studies have proposed an association between blood PCB concentrations and adverse health effects such as impaired mental development in children, although the underlying mechanism is not currently fully understood. Dysfunction of the immune system and particularly oxidative stress might be an explanation of adverse health effects following exposure to these pollutants, even for the general populations not living in the e-waste dismantling region.

In conclusion, together with the high levels of plasma PCBs and other e-waste related pollutants in individuals living near an e-waste recycling site located in northern China, we found increased ROS levels in resting white blood cells (including neutrophil granulocytes) and a weakened respiratory burst of neutrophil granulocytes. Although further studies with a prospective design were warranted, this preliminary finding suggested a possible linkage between pollutant exposure and subsequent health effects in terms of ROS and immune dysfunction, for example infectious diseases or even cancers after long-term exposure of people. In consideration of the increased health risk, substantial measures should be taken to protect the residents at the e-waste dismantling site and particularly occupational workers. For instance, implementing centralized and unified management, adopting environmentally friendly dismantling technologies, and providing workers with personal protection equipment are all effective measures.\(^{51}\)

**REFERENCES**


article:


