Science of the Total Environment 624 (2018) 621–627

Contents lists available at ScienceDirect

Science of the Total Environment

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

Hearing loss in children with e-waste lead and cadmium exposure

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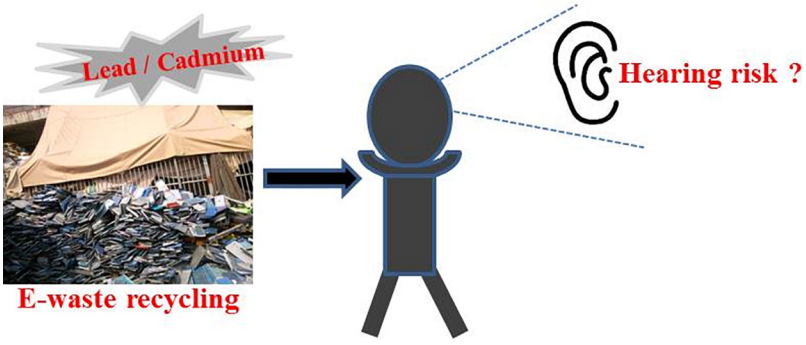
HIGHLIGHTS

- Higher blood Pb but not urinary Cd was found in the e-waste-exposed children.
- Elevated hearing thresholds and hearing loss prevalence in the exposed children were observed.
- Child hand-to-mouth behavior was an important factor for Pb and Cd exposure.
- Pb showed a significant OR for hearing loss in children by adjusting confounding factors.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 November 2017
Received in revised form 7 December 2017
Accepted 7 December 2017
Available online 27 December 2017

Editor: Jay Gan

Keywords:
Lead
Cadmium
E-waste
Hearing loss
Preschool children

ABSTRACT

Environmental chemical exposure can cause neurotoxicity and has been recently linked to hearing loss in general population, but data are limited in early life exposure to lead (Pb) and cadmium (Cd) especially for children. We aimed to evaluate the association of their exposure with pediatric hearing ability. Blood Pb and urinary Cd were collected form 234 preschool children in 3–7 years of age from an electronic waste (e-waste) recycling area and a reference area matched in Shantou of southern China. Pure-tone air conduction (PTA) was used to test child hearing thresholds at frequencies of 0.25, 0.5, 1, 2, 4 and 8 kHz. A PTA ≥ 25 dB was defined as hearing loss. A higher median blood Pb level was found in the exposed group (4.94 ± 0.20 *vs* 3.85 ± 1.81 μg/dL, $p < 0.001$), while no significance was found for creatinine-adjusted Cd. Compared with the reference group, the exposed group had a higher prevalence of hearing loss (28.8% *vs* 13.6%, $p < 0.001$). The PTA in the left, right and both ears, and hearing thresholds at average low and high frequency, and single frequency of 0.5, 1 and 2 kHz were all increased in the exposed group. Positive correlations of child age and nail biting habit with Pb, and negative correlations of parent education level and child washing hands before dinner with Pb and Cd exposure were observed. Logistic regression analyses showed the adjusted OR of hearing loss for Pb exposure was 1.24 (95% CI: 1.029, 1.486). Our data suggest that early childhood exposure to Pb may be an important risk factor for hearing loss, and the developmental auditory system might be affected in e-waste polluted areas.

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https://doi.org/10.1016/j.scitotenv.2017.12.091
0048-9697/

1. Introduction

Hearing loss is one of the most common chronic disabling conditions. Roughly 360 million people around the world suffer from moderate to profound hearing loss due to various causes, and a recent systematic review reports that the incidence of neonatal hearing loss in the U.S. is near 1.1 per 1000 infants and the average prevalence of mild or worse unilateral or bilateral hearing impairment in children and adolescents exceed 3% (Davis et al., 2016; Roland et al., 2016). Many factors are attributable for hearing loss. Studies have shown that modern lifestyles may cause hearing impairment, including smoking, acoustic over-stimulation, drinking alcohol and emotional stress (Canlon et al., 2013; Durusoy et al., 2017; Sumit et al., 2015). For decades, most efforts have been implemented to link the occupational noise or ototoxic drug (*e.g.*, antibiotics) with hearing impairment, and their exposure can initiate a series of pathological changes resulting in damages of spiral ganglion neurons within the cochlea, some of which occur even when the hair cells are not sufficiently injured to undergo degeneration (Caciari et al., 2013; Francis and Cunningham, 2017). Recent studies have shown that heavy metal exposure may also be considered as the risk factors for hearing loss in humans (Hwang et al., 2009; Schaal et al., 2017).

Lead (Pb) is a ubiquitous environmental toxicant that associates with many potential health problems, and nervous system is especially affected with decreasing intelligence quotient and delayed cognitive function during early development in children even at a low-level exposure (Huang et al., 2012; Lee et al., 2017). An early comparison study has been performed to investigate the changes in Pb-induced neurobehavior, finding that the brainstem auditory evoked potentials both in animals and school-age children are deterred (Lilienthal et al., 1990). Then the association of environmental Pb exposure and impaired hearing is also observed among general adult population and workers (Castellanos and Fuente, 2016). Cd is a typical nephrotoxicant that may produce a series of physiological disorders and even cancer potential (Antila et al., 1996; Rango et al., 2015; Zeng et al., 2017). A recent *in vivo* study finds that high Cd exposure may cause an increase in auditory threshold and a decrease in response latency (Low and Higgs, 2015). Upon human epidemiological data of Cd and hearing loss, to date, only 2 studies in a general population have been conducted (Choi et al., 2012; Shargorodsky et al., 2011). Although research on the ototoxic effects of Pb and Cd are not clearly understood, experimental data demonstrate that they can damage cochlea or vestibular function through ROS generation and apoptosis, leading to disorders in auditory nerve conduction and finally significant hearing loss (Kim et al., 2008; Klimpel et al., 2017).

Guiyu, as a typical electrical waste (e-waste) recycling area, has attracted much attention among researchers due to informal dismantling without proper environmental protection measures over the last few decades (Song and Li, 2014; Zeng et al., 2016). In our previous studies, the levels of heavy metals in children and neonates of Guiyu are much higher compared to other areas (Liu et al., 2016; Xu et al., 2015). In perspective of the ototoxic effect of heavy metals and limited epidemiologic studies available for the prevalence of hearing loss in children, especially in an e-waste-polluted area, we therefore attempted to investigate whether environmental chemical pollutant exposure was able to affect hearing abilities of preschool children living in Guiyu, to find out the association of blood Pb and urinary Cd exposure with hearing loss in early childhood.

2. Material and methods

2.1. Study population and questionnaires

A cross-sectional study was performed, with a total of 234 preschool children 3–7 years of age were voluntarily enrolled to participate in a hearing test in 2014. In this, 146 children were from Guiyu town, an e-waste recycling area, and the remaining 88 children were from Haojing area. The reference area is about 31.6 km to the east of Guiyu, Shantou city, in the southeastern coast of Guangdong province in China. Haojiang was selected as the reference area because it lacks of e-waste processing as well as its similarities to Guiyu in population, traffic density, residential lifestyle and socioeconomic status. A questionnaire on lifestyle and residential environment was administrated to parents or guardians of all children participating in the study. Children with otitis media or other hearing impairment problems in medication, family hearing genetic history and chronic diseases such as asthma, or acute cough or cold were excluded. The questionnaire addressed factors that might influence hearing status, including questions related to residence, physical activity, dietary habits, nutrition, behavior habits, whether the parent's occupation was related to e-waste processing, and family member smoking status, education status, and income. Some characteristics for relevant factors were given (Supplemental Table 1). This study protocol was approved by the Human Ethics Committee of Shantou University Medical College, China.

2.2. Sample collection and exposure measurement

A total of 2 mL venous blood from each participant was collected between 7:00 AM and 10:00 AM by trained nurses and stored in a Vacutainer with EDTA-2K as an anticoagulant. Whole blood samples were then transported to lab and stored at −20 °C until measurement of Pb concentration. First morning urine was collected, between 7:00 AM and 8:00 AM, into a polypropylene conical centrifuge tube, divided into two tubes, and frozen at −80 °C until analysis. Blood Pb and urinary Cd levels were both determined by graphite furnace atomic absorption spectrometry (GFAAS, Jena Zeenit 650, Germany). Details for the pretreatment of samples and measurement procedures of instrument were as described previously, which was quite experienced in our laboratory (Huo et al., 2014; Zheng et al., 2008). Another part of urine was diluted tenfold in ultrapure water to detect urinary creatinine. The concentrations of creatinine were determined by a modified Jaffé reaction (Husdan and Rapoport, 1968) and optical density at 490 nm was recorded using a microplate reader (Infinite M200PRO, Tecan, Switzerland).

2.3. Hearing test

In this study, pure-tone air conduction (PTA) has been applied to evaluate the hearing function in children. Audiometric examination was conducted in a quite silent and sound-proofed room in local kindergartens by two audiologists from the affiliated hospitals in Shantou University Medical College and the detailed procedure was based on a clinical protocol (GB/T 16403-1996, China). Before the test, the audiologists did an otoscopic examination on all children to ensure that the ear was free of infection and obstructions, and hearing test procedure was performed using the clinical standard protocol of the hospitals. PTA hearing thresholds were examined in both ears, at frequencies of 0.25, 0.5, 1, 2, 4 and 8 kHz, by a clinical audiometer (model SA203, Entomed AB, Sweden) equipped with headphones (model EF120075, Entomed AB, Sweden). In order to guarantee the quality of participants' responses, we first tested twice at the frequency of 1 kHz in each ear (Choi and Kim, 2014). Hearing loss was defined as a PTA ≥ 25 dB 25 dB (dB) in one or both ears (Austeng et al., 2013).

2.4. Statistical analysis

Normally distributed data was reported as the mean ± standard deviation (SD). The differences between the compared groups were tested using an independent sample *t*-test or analysis of variance (ANOVA). The chi-square test was used for categorical data, such as the ratio of hearing loss. Spearman correlations were used to find the potential risk factors in child exposure to Pb and Cd. Bivariate logistic models

were performed to examine odds ratios (ORs) of environmental exposure to Pb and Cd for hearing loss as well as their interaction effect after adjusting confounders especially for environmental noise. Statistical analyses were conducted by SPSS (Statistical Product and Service Solutions) 19.0 software, while figures were produced by GraphPad Prism 5.0. The level of significance was set at $p < 0.05$ or <0.01.

3. Results

3.1. General characteristics of study participants

Table 1 shows general characteristics of the study subjects. The average age of the exposed group was higher than that of the reference group (mean ± SD: 5.22 ± 0.59 *vs* 4.73 ± 0.74). Gender showed no difference. Significant differences were observed in parent education level, family monthly income and family member smoking between two groups. Residence environment such as residence distance to the road, residence nearby noise, residence renovation noise within a year differed between the two groups. There were no statistical significance for child often listening music with earphones within a year and often watching television programs in loud noise, while the exposed children had often play (*i.e.*, toys or music, *etc.*) in loud noise compared to the reference children (83.6% *vs* 16.4%, $p < 0.01$).

Median blood Pb levels of children from the exposed group were higher than that from the reference group (4.94 ± 0.20 *vs* 3.85 ± 1.81 μg/dL, $p < 0.001$). The percentage of Pb level exceeding 5 μg/dL in the exposed group was higher than the reference group (43.8% *vs* 14.8%, $p < 0.001$). No significance was observed between two groups for the ratio of a Pb level over 10 μg/dL. No significance was found for Cd levels between the two groups.

Subjects of the exposed group had a higher prevalence of hearing loss in one or both ears compared to that of the reference group (28.8% *vs* 13.6%, $p < 0.01$). The pure-tone average (PTA) in the left (20.24 ± 4.73 *vs* 18.21 ± 5.26 dB, $p < 0.01$), right (20.69 ± 4.53 *vs*

Table 1
Characteristics of the participants.

Characteristics	Reference (n = 88)	Exposed (n = 146)	Statistics	p
Age (years, mean ± SD)	4.73 ± 0.74	5.22 ± 0.59	$t = 5.232$	<**0.001**
Gender, n (%)			$\chi^2 = 0.005$	0.945
Male	46 (52.3%)	77 (52.7%)		
Female	42 (47.7%)	69 (47.3%)		
Parent education level			$\chi^2 = 28.777$	<**0.001**
Primary school or lower	2 (2.3%)	18 (12.7%)		
Middle/high school	57 (65.5%)	114 (80.3%)		
College or above	28 (32.2%)	10 (7.0%)		
Family monthly income, RMB			$\chi^2 = 15.587$	**0.004**
<1500	1 (1.1%)	9 (7.1%)		
1500~	9 (10.3%)	22 (17.3%)		
3000~	16 (18.4%)	32 (25.2%)		
4500~	20 (23.0%)	34 (26.8%)		
>6000	41 (47.1%)	30 (23.6%)		
Family member smoking			$\chi^2 = 16.356$	**0.003**
No	36 (41.4%)	31 (22.5%)		
Around two cigarette	12 (13.8%)	26 (18.8%)		
Half bag	24 (27.6%)	28 (20.3%)		
One bag	10 (11.5%)	35 (25.4%)		
Over one bag	5 (5.7%)	18 (13.0%)		
Residence distance to the road, n (%)			$\chi^2 = 36.520$	<**0.001**
<10 m	7 (8.0%)	51 (36.2%)		
10–50 m	23 (26.4%)	30 (21.3%)		
50–100 m	9 (10.3%)	28 (19.9%)		
>100 m	48 (55.2%)	32 (22.7%)		
Residence nearby noise, n (%)			$\chi^2 = 32.215$	<**0.001**
No	83 (95.4%)	87 (61.7%)		
Yes	4 (4.6%)	54 (38.3%)		
Residence renovation noise within a year, n (%)			$\chi^2 = 6.139$	**0.013**
No	6 (6.9%)	113 (81.3%)		
Yes	81 (93.1%)	26 (18.7%)		
Often listening music with earphones within a year, n (%)			$\chi^2 = 0.124$	0.725
No	1 (1.1%)	1 (0.7%)		
Yes	86 (98.9%)	141 (99.3%)		
Often watching television programs in loud noise, n (%)			$\chi^2 = 3.484$	0.062
No	56 (65.1%)	74 (52.5%)		
Yes	30 (34.9%)	67 (47.5%)		
Often play (*i.e.*, toys or music, *etc.*) in loud noise, n (%)			$\chi^2 = 7.025$	**0.008**
No	82 (95.3%)	117 (83.6%)		
Yes	4 (4.7%)	23 (16.4%)		
Blood Pb (μg/dL), median ± SE	3.85 ± 1.81	4.94 ± 0.20	$Z = -5.057$	<**0.001**
>5 μg/dL (%)	13 (14.8%)	64 (43.8%)	$\chi^2 = 21.006$	<**0.001**
>10 μg/dL (%)	2 (2.3%)	11 (7.5%)	$\chi^2 = 2.897$	0.089
Urinary Cd, μg/g creatinine, median ± SE	1.80 ± 2.11	2.49 ± 2.54	$Z = -1.500$	0.134
Pure-tone average[a], mean ± SD				
Left ear (dB)	18.21 ± 5.26	20.24 ± 4.73	$t = 3.046$	**0.003**
Right ear (dB)	17.49 ± 4.85	20.69 ± 4.53	$t = 5.105$	<**0.001**
Both ears (dB)	17.85 ± 4.65	20.47 ± 4.17	$t = 4.449$	<**0.001**
Hearing loss[b], n (%)	12 (13.6%)	42 (28.8%)	$\chi^2 = 7.081$	**0.008**

[a] Pure-tone average (PTA) in either ear and both ears derives from the average of hearing thresholds of 0.5, 1, 2 and 4 kHz.
[b] Hearing loss indicates the PTA value over 25 dB (≥25 dB) in either ear. The bold number indicates the statistical significance.

17.49 ± 4.85 dB, $p < 0.001$) and both ears (20.47 ± 4.17 *vs* 17.85 ± 4.65 dB, $p < 0.001$) was found to be significantly higher in the exposed group.

3.2. Comparisons of PTA and average hearing at each single frequency in both ears

To examine whether there was impact of child gender and age on PTA, we compared the PTA in both ears between the two groups (Table 2). We found higher PTA in the exposed children for 4~ (19.60 ± 3.21 *vs* 17.53 ± 4.08 dB, $p < 0.05$) and 5~ year-old age groups (20.83 ± 4.66 *vs* 18.58 ± 5.13 dB, $p < 0.05$). Gender also showed a difference between the two groups (male: 20.10 ± 4.16 *vs* 16.85 ± 3.70 dB, $p < 0.001$; female: 20.87 ± 4.19 *vs* 18.94 ± 5.35 dB, $p < 0.05$).

The both ear hearing thresholds at low frequencies of 0.25, 0.5, 1, and 2 kHz, and high frequencies of 4 and 8 kHz were analyzed (Table 3). The total hearing thresholds at both low (24.03 ± 4.39 *vs* 21.69 ± 4.69, $p < 0.001$) and high frequencies (13.90 ± 5.68 *vs* 12.19 ± 6.40, $p < 0.05$) were higher in the exposed group compared to that in the reference group. To each low frequency, the hearing thresholds of the exposed group were significantly higher at 0.5 (29.04 ± 5.35 *vs* 26.79 ± 6.13, $p < 0.01$), 1 (22.77 ± 4.97 *vs* 19.14 ± 5.27, $p < 0.001$) and 2 kHz (16.51 ± 5.14 *vs* 13.47 ± 5.69, $p < 0.001$). However, there was no statistical significance for single high frequency at 4 kHz and 8 kHz between the two groups.

3.3. Potential factors related to Pb and Cd exposure

Spearman correlation was used to analyze potential factors related to Pb and Cd exposure (Table 4). We found positive correlations of child age ($r_s = 0.152$, $p = 0.021$) and nail biting habit ($r_s = 0.151$, $p = 0.023$), and negative correlations of parent education level ($r_s = -0.238$, $p < 0.001$) and child washing hands before dinner ($r_s = -0.210$, $p = 0.001$) with Pb exposure. Negative correlations of parent education level ($r_s = -0.141$, $p = 0.033$) and child washing hands before dinner ($r_s = -0.144$, $p = 0.030$) was found with Cd exposure.

3.4. Hearing loss risk assessment

Bivariate logistic regression was conducted to analyze the odds ratio (OR) of environmental exposures for child hearing loss among all subjects (Table 5). We found the OR for hearing loss was 1.24 (95% CI: 1.029, 1.486) in Pb exposure after adjusting potential confounders such as age, gender, weight, height, BMI, parent education level, family member smoking, family monthly income, residence distance to the road, residence nearby noise, residence renovation noise within a year, often listening music with earphones within a year, often watching television programs in loud noise, and often play (*i.e.*, toys or music, *etc.*) in loud noise. The ORs of Cd and joint exposure of Pb × Cd for hearing loss were 1.06 (95% CI: 0.970, 1.152) and 0.98 (95% CI: 0.962, 1.007), respectively, but without statistical significance. We also did not find statistical significance of Pb and Cd exposure for hearing loss at both low and high frequency. To examine whether there was difference for ORs of hearing loss between the exposed and the reference group due to different exposure levels, we found no statistical significance for the exposed subjects and results cannot be shown for the reference subjects due to the little sample size of hearing loss children resulting in the absence of 95% confidence interval (Data not shown).

Table 2
Comparisons of PTA in both ears of children (Mean ± SD, dB).

Categories	Reference (n = 88)	Exposed (n = 146)	t-value	*p*
Gender				
Male	16.85 ± 3.70	20.10 ± 4.16	4.379	<**0.001**
Female	18.94 ± 5.35	20.87 ± 4.19	2.112	0.037
Age (y)				
3~	17.40 ± 4.89	18.96 ± 7.81	0.475	0.640
4~	17.53 ± 4.08	19.60 ± 3.21	2.645	**0.010**
5~	18.58 ± 5.13	20.83 ± 4.66	2.081	**0.040**
6–7	18.28 ± 7.02	21.48 ± 3.09	0.891	0.433

Note: The bold number indicates the statistical significance.

Table 3
Comparisons of hearing thresholds in both ears at each single frequency (Mean ± SD) between the reference and the exposed group.

Frequencies	Reference (n = 88)	Exposed (n = 146)	t-value	*p*
Low frequency				
0.25 kHz	27.36 ± 5.73	27.80 ± 5.40	0.572	0.568
0.5 kHz	26.79 ± 6.13	29.04 ± 5.35	2.849	**0.005**
1 kHz	19.14 ± 5.27	22.77 ± 4.97	5.201	<**0.001**
2 kHz	13.47 ± 5.69	16.51 ± 5.14	4.104	<**0.001**
Total	21.69 ± 4.69	24.03 ± 4.39	3.780	<**0.001**
High frequency				
4 kHz	11.98 ± 7.14	13.54 ± 5.63	1.847	0.066
8 kHz	12.38 ± 7.14	14.25 ± 7.32	1.912	0.057
Total	12.19 ± 6.40	13.90 ± 5.68	2.062	**0.041**

Note: The total is a result of an average for all low or high individual frequencies. The bold number indicates the statistical significance.

4. Discussion

In this cross-sectional study, we have measured blood Pb and urinary Cd levels in an e-waste area and estimate the association of Pb and Cd exposure with child hearing abilities. Our data show reduced hearing ability in children living in the e-waste area compared to the reference area, and Pb exerts a significant risk for child hearing loss. These suggest that individuals living in e-waste recycling areas, especially for the susceptible population may get higher risk in hearing loss due to the long-term environmental chemical insults.

Pb and Cd are ubiquitously environmental contaminants that are associated with many health hazards. Pb as one of the main neurotoxins is a mainly accumulated in body bone and has been estimated with a half-life of over 10 years in adults (Berglund et al., 2000; Chisolm Jr, 1974). Nearly 45 and 70% of blood Pb is released from long-term stores and the half-life of blood-Pb ranges from 9.96 ± 3.92 day in children (Gulson et al., 1995; Specht et al., 2016). Thus blood Pb level can be assessed for human short-term exposure. In the present study, we found that children in the e-waste-exposed area had higher average Pb levels at 4.94 μg/dL, and 43.8% of them had a level over 5 μg/dL

Table 4
Potential influencing factors related to child body burden of Pb and Cd exposure.

Relevant factors	Urinary Cd		Blood Pb	
	r_s	*p*	r_s	*p*
Age	−0.082	0.217	0.152	**0.021**
Parent education level	−0.141	**0.033**	−0.238	<**0.001**
Family average income	−0.098	0.154	−0.045	0.516
Time for outdoor play	−0.030	0.658	−0.030	0.652
Washing hands before dinner	−0.144	**0.030**	−0.210	**0.001**
Nail biting habit	0.080	0.226	0.012	0.860
Chewing pencil habit	0.017	0.795	0.151	**0.023**
Toy biting habit	−0.001	0.983	−0.015	0.817
Preserved eggs taken within a year	0.060	0.364	0.047	0.483
Milk product frequency within a year	−0.113	0.088	−0.129	0.051
Eating soy products within a year	−0.026	0.695	−0.013	0.847
Canned food taken within a year	0.028	0.673	0.106	0.111
Ca-Fe-Zn supplement within a year	−0.027	0.684	0.107	0.107
Iron rich food taken within a year	0.022	0.741	−0.064	0.338
Family member smoking	0.027	0.690	0.097	0.147
Residence distance to the road	−0.071	0.283	0.035	0.604

Note: The bold number indicates the statistical significance.

Table 5
Logistic regression analyses to predict the odds ratio (OR, 95% CI) for hearing loss among all subjects.

Independent variables	Hearing loss		Low frequency		High frequency	
	OR	95% CI	OR	95% CI	OR	95% CI
Pb	1.24	(1.029, 1.486)*	1.02	(0.869, 1.190)	1.08	(0.839, 1.379)
Cd	1.06	(0.970, 1.152)	0.97	(0.910, 1.023)	1.04	(0.937, 1.151)
Pb × Cd	0.98	(0.962, 1.007)	1.01	(0.994, 1.024)	0.99	(0.964, 1.019)

Note: Models were selected by way of "enter method" and adjusted for child age, gender, weight, height, BMI, parent education level, family member smoking, family monthly income, residence distance to the road, residence nearby noise, residence renovation noise within a year, often listening music with earphones within a year, often watching television programs in loud noise, and often play (*i.e.*, toys or music, *etc.*) in loud noise; * $p < 0.05$; ** $p < 0.01$.

adopted by US CDC in 2012, and the ratio was over 3 times higher than the reference area. This is consistent with those studies that populations living in or neighboring high polluted areas always tend to have a high Pb level, such as discharge sites, mining or industrial areas (Cabral et al., 2015; Lin et al., 2017; Yu et al., 2016). Our data suggest that quite a few children are still under a high risk of Pb exposure in e-waste-polluted areas, and long-term accumulation may lead to adverse health effects. Cd is known as a renal toxicant with an estimated biological half-time of ten years to decades, but even a low-level exposure in early life can result in adverse neurodevelopment (Wang et al., 2017; Wang et al., 2016). In our study, we did not find significant difference for Cd level between two groups. There are many sources for human Cd exposure, and most studies have reported that dietary intake is regarded as the main environmental source of general population (He et al., 2013; Satarug et al., 2010). The reference children in this study are from an area nearby inland sea and seafood is the major source of food intake, which may add to the increase of Cd exposure. On the other hand, the individual difference of Cd exposure may also contribute as we find that concentrations in some subjects are high when we analyze the results. Further studies should be paid attention on Cd exposure to verify this finding.

Studies have demonstrated that the internal exposure of Pb and Cd primarily comes from contaminated food, water and house dust as well as through industrial activities such as metal recycling and the battery manufacturing (Barbosa et al., 2005; Cheng and Hu, 2010; Yoshinaga et al., 2014). Our results show that Pb exposure correlates to child age and nail biting habit, while decreased Pb and Cd levels are related to higher parent education level and child washing hands before dinner. This suggests that long-term exposure along with age increasing and child poor habit may contribute to the accumulation of Pb in body. Washing hands and family education are protective factors reducing the opportunity of exposure. It is recognized by other epidemiological studies that the high susceptibility of child exposure is related to behavioral factors such as hand to mouth habits, physical activity, nutrition, and education (Acosta-Saavedra et al., 2011).

Recent advance has shown the evidence of ototoxic chemicals in increasing the danger to pathologic changes in both peripheral and central parts of the auditory pathway, resulting in hearing loss (Godfrey et al., 2017; Sliwinska-Kowalska, 2015). In our study, children in the exposed area had a higher prevalence of hearing loss in one or both ears compared to that in the reference group, and the average hearing thresholds in one or both ears in the exposed children were higher, including gender stratification. This indicates that children in the e-waste-polluted area suffer more hearing impairment. It has a similar trend with prior findings of occupational environment that individual hearing impairment may be at high risk due to chronic exposure to mixed solvents (Kim et al., 2005). Further we observed that PTA in both ears was higher at the 4~ and 5~ year-old age in the exposed group, implying an effect of age sensitivity on hearing during development. Hearing ability is sensitive to age and most of the hearing loss is age related (Lie et al., 2016; Park et al., 2016). Developmental children may also be impacted due to varying factors especially for the potentially environmental toxicants. Results in this study also showed the hearing level of the exposed group was significantly higher, especially at the frequency of 0.5, 1 and 2 kHz. It appears that child hearing of the low frequency is affected in e-waste-polluted areas. A previous epidemiological study reports that children living near a burned coal plant with a high concentration of arsenic present significantly higher pure-tone thresholds than non-exposed children for 0.125, 0.25, 0.5, and 1 kHz (Bencko and Symon, 1977). Children are at higher risk of having elevated hearing threshold at low frequencies in the right ear due to the gestational exposure to organic toxicants (Li et al., 2015). These results suggest that low frequency of hearing is more likely deterred by environmental toxicants.

At present, increasing studies has investigated about the adverse auditory effects of heavy metal exposure. For instance, Co, Mn, Cd, Pb, and Hg has the potential to affect hearing in humans and experimental animals, but sometimes the results are contradictory and few has shown a linkage of hearing loss with environmental Pb and Cd exposure in children, especially in an e-waste polluted area (Roth and Salvi, 2016; Sliwinska-Kowalska, 2015). We examined the odds risk of hearing loss for all subjects by logistic regression models with adjustment for several covariates, especially for child noise exposure environment such as residence nearby noise, residence renovation noise within a year, often listening music with earphones within a year, often watching television programs in loud noise, and often play (*i.e.*, toys or music, *etc.*) in loud noise. We only found that the adjusted OR of Pb exposure for hearing loss was 1.24 (95% CI: 1.029, 1.486) among all subjects. Pb-induced hearing dysfunction has been proved by animal models and the relevant mechanism has also proportionally elucidated (Lasky et al., 2001; Yamamura et al., 1989). An *in vivo* study shows that high Pb exposure during development can result in alterations of axonal structure and function within brainstem auditory nuclei (Jones et al., 2008). Besides, abnormal shapes and loss of the outer hair cells are found in the cochlear basilar membrane following the prolonged Pb exposure (Liu et al., 2013). It has been identified in another study that after adjusting for sociodemographic and clinical risk factors and exposure to noise, high Pb level is associated with 18.6% (95% CI: 7.4%, 31.1%) increases in PTA. Therefore, given the current Pb level in the e-waste area, individuals living or around there may be at a high risk of hearing loss. Experimental studies show that Cd exposure can induce cell death, reactive oxygen species generation, mitochondrial membrane depolarization, cytochrome *c* release, caspase-3 and caspase-9 activation, and an increase of extracellular signal-regulated kinase activation in auditory cells (Chen et al., 2011; Kim et al., 2008). A previous epidemiological study demonstrates that a low-level exposure to cadmium is associated with hearing loss in the general adult population when they use blood samples (Choi et al., 2012). However, we did not find statistical association of urinary Cd level with child hearing loss in this study, and a further study should be continued to verify this finding because the specimen source, study size and body burden of exposure may partially influence the final results.

Again several limitations in this study have to be considered. First, it is restricted to compare the ORs in the e-waste-polluted area and reference area because of the quite low prevalence of hearing loss in the reference area. Secondly, we find that individual difference exists in level of creatinine-adjusted Cd in the urine with a large standard deviation, which may cause some statistical bias especially when we compare it between two groups, as well as in the subsequent regression models.

Thirdly, we have only measured Pb and Cd elements and other chemicals may synergistically affect the child hearing due to the complex e-waste pollution. The ongoing study should be continued to explore various risk factors of hearing loss and determine possible mechanisms. Despite these probable limitations, to our knowledge, this is the first study on the relationship between Pb and Cd exposure and hearing loss of children in an e-waste recycling area. This study extends limited evidence of heavy metals on hearing impairment from adults to preschool children (Rooney and Dorea, 2012; Shargorodsky et al., 2011; Tak and Calvert, 2008).

5. Conclusion

On the whole, this study is the first attempt to determine hearing loss in 3- to 7-year-old children who live in an informal e-waste recycling area. We find a high proportion of hearing loss and increased average hearing thresholds among children living in the e-waste area compared to the reference area, and low-frequency hearing is affected and associated with blood Pb level but not Cd exposure. Thus, the present study suggests that early childhood exposure to Pb from e-waste recycling areas may affect hearing development. It is necessary to pay more attention to the developmental auditory system of children and reduce environmental chemical pollutant exposure in e-waste recycling areas.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.12.091.

Conflicts of interest

All authors declare no competing financial interests.

Acknowledgement

This work was supported by National Natural Science Foundation of China (21577084) and the Project of International Collaboration and Innovation Platform in Guangdong Universities (2013gjhz0007). We wish to thank Dr. Stanley Lin for his constructive comments and language editing. We are grateful to all the recruited children and their guardians for participating in this study.

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