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Thyroid disruption and reduced mental development in children from an informal e-waste recycling area: A mediation analysis

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HIGHLIGHTS

- Median values of Pb, Cd, FT_4 and TSH in Guiyu were higher than that of the reference area.
- Guiyu children had lower cognitive Scores than those in the reference area.
- Pb was negatively correlated with both cognitive and language scores.
- Thyroid disruption isn't involved in the neurotoxicity induced by Pb-Cd co-exposure.

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ABSTRACT

This paper aims to evaluate the effects of thyroid disruption on the mental development of children. A total of 258 three-year-old children in Guiyu (e-waste-exposed group) and Nanao (reference group), China were examined. FT_3, FT_4, TSH, lead (BPb) and cadmium (BCd) in blood were determined, and cognitive and language scores of children were assessed based on the Bayley Scales of Infant Development III. Stepwise multiple regression was used to estimate the relationship between heavy metals and cognitive and language scores; mediation analysis was performed to determine whether thyroid disruption was mechanistically involved. Medians of BPb and BCd in Guiyu were higher than of Nanao (11.30 ± 5.38 vs. 5.77 ± 2.51 μg/dL BPb; 1.22 ± 0.55 vs. 0.72 ± 0.37 μg/L BCd, both $p < 0.001$). Means of FT_4 and TSH in Guiyu were also higher than those in Nanao (16.65 ± 1.83 vs.16.06 ± 1.66 pmol/L FT_4, $p = 0.007$; 2.79 ± 1.30 vs. 2.21 ± 1.43 mIU/L TSH, $p = 0.001$). Guiyu children had lower cognitive scores (100.00 ± 25.00 vs. 120.00 ± 20.00, $p < 0.001$) and lower language scores (99.87 ± 7.52 vs. 111.39 ± 7.02, $p < 0.001$). Mediation analysis showed that Pb negatively correlated with both cognitive and language scores (both $p < 0.001$). However, FT_3, FT_4 and TSH did not significantly mediate the relationship between Pb and mental development of children (all $p > 0.05$). In contrast, Cd correlated with neither cognitive nor language scores (both $p > 0.05$). Results suggest exposure to heavy metal (Pb) reduces cognitive and language skills, and affects thyroid function, but fail to confirm that thyroid disruption is involved in the neurotoxicity induced by Pb–Cd co-exposure.

1. Introduction

The influence of thyroid hormone (TH) on brain development in children has been extensively studied (Chan and Kilby, 2000). The hypothalamus-pituitary-thyroid axis regulates thyroid function through thyrotropin-releasing hormone, also known as thyroid-stimulating hormone (TSH), and TH. It is clear that the fetus and neonate are quite sensitive to TH, and neurological growth and maturation can be compromised when disruptions in TH occur during fetal development (Zoeller et al., 2002). Studies have also revealed that relatively subtle deficits in circulating levels of TH, in pregnant women, could affect the neurological outcome of children (Ghassabian et al., 2011).

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Relevant abbreviations

BCd	blood cadmium
BMI	body mass index
BPb	blood lead
Cd	cadmium
FT_3	free triiodothyronine
FT_4	free thyroxine;
Pb	lead
PCBS	polychlorinated biphenyls
TH	thyroid hormone
TSH	thyroid stimulating hormone

Disposal of electronic waste, or e-waste, is an emerging global environmental issue. People living near hazardous waste sites can be exposed to soaring levels of toxic heavy metals and organic contaminants by breathing air, drinking water, eating food, or swallowing dust or dirt (Alabi et al., 2012; Heacock et al., 2016; Yekeen et al., 2016; Zhang et al., 2014; Zheng et al., 2016). Studies conducted in children have shown that developmental exposure to high levels of lead (Pb) and cadmium (Cd) can result in adverse neurocognitive and behavioral consequences in children, including mental retardation, lower neuropsychological test performance, lower child intelligence scores, and also decreasing child olfactory memory, which may extend into adulthood (Skerfving et al., 2015; Evens, et., 2015; Huang et al., 2012; Kippler et al., 2012; Zhang et al., 2017). Other studies show not only heavy metals, but also polybrominated flame retardants may lead to children suffering from lower scores of mental, or worse motor, cognitive and behavioral performance (Sagiv et al., 2015; Herbstman et al., 2010).

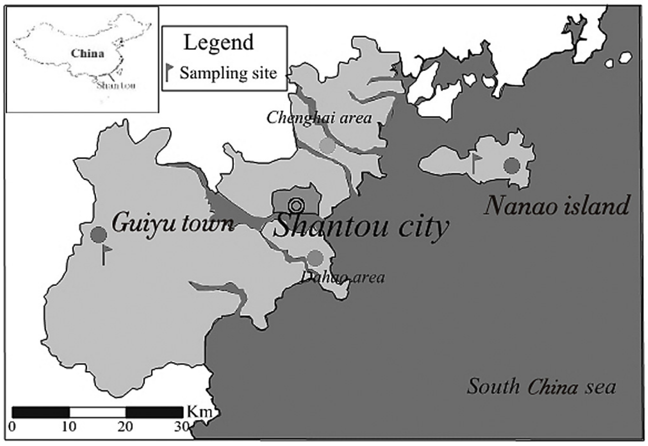
There is emerging evidence that children in informal recycling areas may have changes in TH and TSH concentrations. Especially relevant, concentrations of bisphenol A, polybrominated diphenyl ethers, polychlorinated biphenyls (PCBs) and hydroxylated PCBs correlate with TH and TSH levels (Lee et al., 2017; Eguchi et al., 2015; Xu et al., 2014). In recent years, a greater number of studies have focused on the relationship between levels of heavy metals and TH concentrations, although no consensus has been reached. Long-term, low-level Pb exposure resulting in low blood lead (BPb) levels (lower than 10 μg/dL), may lead to reduced free thyroxine (FT_4) levels without significant changes in TSH in adolescents (Dundar et al., 2006), and urinary Cd, but not associated with BPb, may be positively associated with free triiodothyronine (FT_3)(Chen et al., 2013). However, other studies report no statistically significant relationship between Pb concentrations and thyroid function (Siegel et al., 1989; Mendy et al., 2013).

Guiyu, is a crude and informal e-waste recycling town in Guangdong province. We have shown that concentrations of heavy metals, including Pb and Cd, are significantly higher and have adverse effects on the growth and development of the local neonates and children (Guo et al., 2010, 2012; Huo et al., 2007; Li et al., 2011, 2008a, 2008b; Wu et al., 2010, 2012; Xu et al., 2012, 2013, 2015, 2017; Zeng et al., 2016; Zhang et al., 2016, 2017; Zheng et al., 2013; Zheng et al., 2008). We recently showed child BPb and/or blood cadmium (BCd) correlate with certain behavioral abnormalities, such as alterations in temperament, conduct problems, antisocial behavior and decreased olfactory memory (Liu et al., 2011, 2014., Zhang et al., 2017). Studies are lacking on whether early childhood co-exposure to Pb and Cd directly affects the levels of TH and mental development of children in e-waste-contaminated areas, and what roles thyroid disruption plays in affecting child neurological outcomes. Therefore, we investigate whether thyroid disruption is involved in the mechanism of neurotoxicity induced by co-exposure to heavy metals. We measured BPb, BCd, FT_3, FT_4, and TSH in 258 three-year-old children in Guiyu and a reference location (Nanao), and assessed child cognitive and language scores based on the Bayley Scales of Infant Development III. In addition, we used mediation analysis, normally used to quantify and test the pathways of influence from causal variables to outcome variables (Hayes, 2013), to evaluate the effect of thyroid disruption on mental development in children co-exposed to Pb and Cd.

2. Material and methods

2.1. Study population

Volunteers from a selected kindergarten, in either the e-waste-exposed or reference area, were recruited, and screened based on the inclusion and exclusion criteria for this study. E-waste-exposed children were recruited from Guiyu in Shantou, China. Guiyu is a town with a more than 30-year history of informal e-waste recycling, which is often performed by family-run workshops. The soaring levels of toxic heavy metals and organic contaminants in the air, dust, soil, river sediment, surface water, and ground water of Guiyu have been previously reported (Zhang et al., 2014; Yekeen et al., 2016; Zheng et al., 2016). We recruited non-exposed subjects from Nanao Island, in Shantou, to serve as the reference group. This island is 104 km away from Guiyu, has a climate, cultural background, and socioeconomic status similar to the exposed area, and is populated with people of similar ethnicity and lifestyle. Because of its geographical isolation and lack of industrial workshops, Pb and Cd exposure is expected to be minimal among people living on this island.



Inclusion criteria were: (1) children approximately 3 years old (more than 30 months, but less than 42 months), (2) residence in Guiyu (or Nanao) for more than 2 years after birth, and (3) enrollment in a local regular kindergarten, which was a public daycare center, but with optional attendance. Exclusion criteria were: (1) mixed receptive-expressive language disorder, and (2) having been diagnosed with a severe neurologic or psychiatric disorder (cerebral palsy, seizure disorders, schizophrenia, obsessive-compulsive disorder). In total, two hundred eighty-four 3-year-old kindergarteners were recruited for our survey (135 in Guiyu and 149 in Nanao) from December 2011 to March 2012. After informed consent was obtained from the children's parents or guardians, blood samples were collected, and questionnaires were administered to the caretakers. Because of the shortage in volume of blood samples collected at Guiyu, total triiodothyronine and total thyroxine measurements were not included in this study. For this reason, only 120 Guiyu children were measured for BCd, FT_3, FT_4 and TSH, and 115 for BPb analysis. Because of some uncooperative children, only 117 were assessed for cognitive and language ability. For the same reasons, only 136 of 149 children in Nanao Island were assessed for cognitive ability, and 135 for language ability, while 138 of their blood samples were included in all analyses. This study was approved by the Human Ethics Committee of Shantou University Medical College.

2.2. Blood sample collection and metal analyses

Samples of venous blood were obtained from each volunteer by trained nurses at the kindergartens. Two ml whole blood was collected, in an EDTA anticoagulant-containing Vacutainer® tube, for heavy metal analysis. Serum, from another Vacutainer® tube, without anticoagulants, was used for determination of FT_3, FT_4 and TSH. Immediately upon returning to the laboratory, serum was isolated following separation of blood cells after clotting. All samples were stored at −20 °C until further measurement.

The levels of BPb and BCd were determined by graphite furnace atomic absorption spectroscopy consisting of an MPE60 auto sampler with an injection volume set at 10 μL (Yang et al., 2013). The main parameters used for BPb determination were a wavelength of 283.3 nm, a lamp current of 4.0 mA, a slit width of 0.8 nm, drying at 90 °C, 105 °C, and 120 °C, ashing at 600 °C, and atomization at 1500 °C. The accuracy of the method was confirmed by recoveries of between 94% and 107% from spiked blood samples. The detection limit for BPb was 0.043 μg/dL. The parameters for BCd analysis were a wavelength of 228.8 nm, current of 2.0 mA, slit width of 0.8 nm, drying at 90 °C, 105 °C and 300 °C, ashing at 900 °C, and atomization at 1500 °C. The recoveries of this method were 100–104%, which were also determined from spiked blood samples. The detection limit for BCd was 0.018 μg/L.

2.3. Thyroid function determination

All serum samples were sent to the Central Hospital of Chaozhou for immunoassay determination of FT_3, FT_4, and TSH levels by using an automatic chemiluminescence instrument (ADVIA-CentautXP). All reagents used were analytical grade (Siemens AG).

2.4. Mental development testing

We administered cognitive and language subscales, from the Bayley Scales of Infant Development III, because we focused on the mental development of children (Moore et al., 2012). The cognitive and language performances were assessed according to their cognitive and language scores, respectively. The standardization of mental performance assessment and scoring was based on the examiner's hands-on training at the University of Cincinnati Medical College in 2011.

2.5. Questionnaire survey

An interview-based structured questionnaire was used to attain basic information, including birth status, dietary supplementation, duration of residency at the address, child e-waste contact (the frequency of child e-waste contact), history of preventive inoculation, lifestyle, main source of drinking water, parental occupation and education, and passive smoking. The questionnaire included 29 factors that could influence heavy metal levels in child blood, such as diet, housing conditions, parent educational background, and socio-economic status. Parental occupation was classified by the type of job in e-waste recycling, such as transporting, selecting, and splitting of e-waste, printed circuit board grilling, acid bathing, and burning to recover metals.

2.6. Statistical analyses

Statistical analysis was carried out using SPSS 20.0 (SPSS, Inc., Chicago, IL, USA) and PROCESS (Hayes) software packages. All data were analyzed and modeled according to the data type. Considering differences between the two groups, such as the child age, economic status and parent educational background, multiple regression analysis was performed to adjust for these covariates. The demographic profile of the two groups was described. Independent-sample t-tests or covariance analyses were performed to determine the differences between the two groups. Nonparametric analyses were used for data with skewed distributions, while the χ^2 test was performed for categorical data. The concentrations of FT_3, FT_4 and TSH, levels of BPb and BCd, and cognitive and language scores between the two groups were compared. Independent-sample t-tests or covariance analyses were used to determine the mean level differences of these variables between the two groups. After adjusting the covariates for sex, age, BMI at birth, BMI at examination, passive smoking, parent educational background, economic income, eating canned food, and biting pencil stubs, stepwise multiple regression analysis was performed to respectively explore the factors related to cognitive and language scores, while considering co-exposure to Pb and Cd. Stepwise multiple regression analysis also was constructed to sequentially determine the relationship between the dependent variable (cognitive scores, language scores, FT_4 and TH) and independent variables (recycling industry), and control for relevant confounders (sex, age, BMI at birth, BMI at examination, passive smoking, parent educational background, economic income).

A parallel multiple mediator model was conducted to evaluate the effects of thyroid disruption on the mental development of children co-exposed to Pb and Cd. Antecedent variables (Pb and Cd) were modeled as influencing consequent variables (cognitive and language scores) directly, as well as indirectly through multiple mediators (FT_3, FT_4 and TSH). The direct and indirect effects were combined to represent total effect. Baseline levels of the dependent variables, age, sex, BMI at birth, BMI at examination, passive smoking, parent educational background, and economic income were included as covariates in the models. The significance levels of the total, direct and indirect effects were set at 0.05 using a two-tailed test (Hayes, 2013).

3. Results

3.1. General characteristics of children

The characteristics of the children and their parents in this study are listed in Table 1. All children were approximately 36 months of age, with the average age of the exposed children being one month younger than that of the reference group. All children in the exposed group had different levels of e-waste contact, while there was no e-waste-derived contaminant contact for any of the children from the reference group. Although there was no significant difference in the body mass index (BMI) at birth between the two groups, the BMI of the exposed group was 3.4% higher at the time of examination ($p < 0.01$). The duration of residence at the current address was significantly different between the two groups for parents, but not for children, and a 19.4% higher percentage of passive exposure to smoking was found in the exposed group compared to the reference group ($p < 0.05$). Guiyu parents had a much lower degree of education compared with the reference

Table 1
Characteristics of children and their parents in e-waste-exposed and reference groups.

Variables		Exposed group		Reference group		Statistics	P
		N	M±SD/%	N	M±SD/%		
Age (months)		120	37.49 ± 3.40	138	38.80 ± 2.37	t'=*3.526*	0.001
Sex	Male	57	47.50%	75	54.30%	x^2 = 1.205	0.272
	Female	63	52.50%	63	45.70%		
BMI at birth		120	12.27 ± 0.98	138	12.03 ± 1.31	t = 1.647	0.101
BMI at examination		120	15.47 ± 1.33	138	14.96 ± 1.30	t = 3.127	0.002
Passive smoking		119	2.15 ± 1.35	137	1.80 ± 1.30	t = 2.148	0.033
Parent education		118	4.99 ± 2.03	136	7.70 ± 2.10	t = 10.415	0.000
Income (ten thousand yuan)						Z = 1.829	0.067
<0.1		4	3.40%	3	2.20%		
0.1–0.15		8	6.80%	7	5.10%		
0.15–0.2		29	24.80%	24	17.50%		
>0.2		76	65.00%	103	75.20%		
Child e-waste contact		120		138		Z = 10.073	0.000
None		53		138			
Seldom		43		0			
Most time		6		0			
Every day		18		0			
Duration of child residence (years)						Z = 0.940	0.347
<1		4	3.50%	1	0.70%		
1–3		104	91.20%	136	99.30%		
>3		6	5.30%	0	0.00%		
Duration of parent residence (years)						Z = 2.883	0.004
<1		0	0.0%	1	0.70%		
1–3		2	1.70%	1	0.70%		
3–5		4	3.40%	2	1.50%		
5–8		5	4.20%	3	2.20%		
8–10		10	8.50%	1	0.70%		
>10		97	82.20%	129	94.20%		

group ($p < 0.001$). No significant differences were found with respect to child gender distribution or economic conditions of the families.

3.2. Comparison of Pb, Cd, FT_3, FT_4, TSH, and cognitive and language scores

The mean values of BPb and BCd were significantly higher in the exposed group than those in the reference group (BPb: 11.30 ± 5.38 μg/dL vs. 5.77 ± 2.51 μg/dL; BCd: 1.22 ± 0.55 μg/L vs. 0.72 ± 0.37 μg/L; both $p < 0.001$) (Table 2). Although the concentrations of FT_3 in children from the exposed group tended to be lower than in children from the reference group, no statistical significance was achieved ($p = 0.056$). However, both FT_4 (16.65 ± 1.830 pmol/L vs. 16.06 ± 1.66 pmol/L, $p = 0.007$) and TSH (2.79 ± 1.30 mIU/L vs. 2.21 ± 1.43 mIU/L, $p = 0.001$) levels were significantly higher in the exposed children. The average levels of FT_4 and TSH in the exposed children were 3.7% and 26.2% higher respectively, than those in the reference group. Compared with reference children, children in the exposed group had lower cognitive scale (100.00 ± 25.00 vs. 120.00 ± 20.00, $p < 0.001$) and language scale (99.87 ± 7.52 vs. 111.39 ± 7.02, $p < 0.001$) scores.

3.3. Cognitive score, language scores, FT_4, TSH and the recycling industry

After adjusting the covariates for sex, age, BMI at birth, BMI at examination, passive smoking, parent educational background, and economic income, multiple stepwise regression analysis was used to evaluate the factors related to cognitive scores and language scores, the relationship between cognitive/language scores and recycling industry, and also the association between FT_4/TSH and the recycling industry. As shown in Table 3, the cognitive and language test scores were negatively correlated to the recycling industry, whereas elevated levels of both FT_4 and TSH were positively related to the recycling industry. On the other hand, multiple

Table 2
Lead, cadmium, free triiodothyronine, free thyroxine, thyrotropic hormone, cognitive scales, and language scales in e-waste-exposed and reference groups.

	Exposed group		Reference group		Statistics	P
	N	M±SD	N	M±SD		
Pb (μg/dL)	115	11.30 ± 5.38[a]	138	5.77 ± 2.51[a]	$t' = 11.574$	0.000
Cd (μg/L)	120	1.22 ± 0.55[a]	138	0.72 ± 0.37[a]	$t' = 9.942$	0.000
FT_3 (pmol/L)	120	5.94 ± 0.71	138	6.11 ± 0.69	$t = 1.917$	0.056
FT_4 (pmol/L)	120	16.65 ± 1.83	138	16.06 ± 1.66	$t = 2.710$	0.007
TSH (mIU/L)	120	2.79 ± 1.30	138	2.21 ± 1.43	$t = 3.401$	0.001
Cognitive scales	117	100.00 ± 25.00[a]	136	120.00 ± 20.00[a]	$t' = 9.157$	0.000
Language scales	117	99.87 ± 7.52	135	111.39 ± 7.02	$t = 12.574$	0.000

[a] Median ± interquartile range for data with skewed distribution.

Table 3
The relationships between cognitive scores/language scores/FT$_4$/TSH and recycling industry.[a]

	Regression coefficient (SE)	Standard regression coefficient	*t*	*P*
Related factors (X$_i$) to cognitive scores (Y$_1$)				
Recycling industry	−18.767 (1.827)	−0.555	10.274	0.000
Age (months)	−1.514 (0.341)	−0.240	4.442	0.000
Constant	138.009 (2.866)	–	9.207	0.000
Related factors (X$_i$) to language scores (Y$_2$)				
Recycling industry	−11.488 (1.003)	−0.619	11.448	0.000
Age (months)	−1.210 (0.157)	−0.350	7.710	0.000
Parent educational background	0.519 (0.200)	0.138	2.593	0.010
Constant	165.974 (6.736)	–	24.639	0.000
Related factors (X$_i$) to FT$_4$ (Y$_3$)				
Recycling industry	0.606 (0.219)	0.171	2.774	0.006
Constant	15.434 (0.339)	–	45.532	0.000
Related factors (X$_i$) to TSH (Y$_4$)				
Recycling industry	0.504 (0.175)	0.180	2.886	0.004
Age (months)	−0.064 (0.029)	−0.135	2.171	0.031
Sex	−0.350 (0.170)	−0.125	2.059	0.040
Constant	4.712 (1.244)	–	3.787	0.000

Model 1: Adj R ^{2} = 0.303, F = 55.439, $p = 0.000$; Model 2: Adj R ^{2} = 0.510, F = 87.496, $p = 0.000$.
Model 3: Adj R ^{2} = 0.026, F = 7.695, $p = 0.006$; Model 4: Adj R ^{2} = 0.062, F = 6.594, $p = 0.000$.

[a] All models were generated after the stepwise regression analysis (forward method, entry: $p = 0.05$, removal: $p = 0.10$).

Table 4
Regression coefficients, standard errors, and model summary information for presumed media influence parallel multiple mediator model (n = 246).

Antecedent	Consequent											
	M$_1$(FT$_3$)			M$_2$(FT$_4$)			M$_3$(TSH)			Y(Cognitive)		
	Coeffi.	SE	*p*	Coeffi.	SE	*p*	Coeffi.	SE	*p*	Coeffi.	SE	*p*
X(Pb)	a_1 −0.005	0.009	0.617	a_2 0.045	0.024	0.059	a_3 0.029	0.018	0.115	c' −1.571	0.181	0.000
M$_1$(FT$_3$)	–	–	–	–	–	–	–	–	–	b_1 0.864	1.360	0.526
M$_2$(FT$_4$)	–	–	–	–	–	–	–	–	–	b_2 −0.774	0.520	0.138
M$_3$(TSH)	–	–	–	–	–	–	–	–	–	b_3 0.808	0.639	0.207
Constant	6.162	1.036	0.000	15.852	2.687	0.000	7.036	2.091	0.001	199.296	22.813	0.000
	$R^2 = 0.035$			$R^2 = 0.042$			$R^2 = 0.065$			$R^2 = 0.406$		
	F(9,236) = 0.956, $p = 0.477$			F(9,236) = 1.160, $p = 0.322$			F(9,236) = 1.813, $p = 0.067$			F(12,233) = 13.262, $p = 0.000$		

Direct effect: Pb (X) → cognitive scores (Y).
Indirect effect: Pb (X) → M$_1$/M$_2$/M$_3$ → cognitive scores (Y).
Total effect: combined direct effect and indirect effect.
Cd and 7 confounding factors were analyzed.
Normal theory tests for specific indirect effects, M$_1$(FT$_3$), Z = −0.248, $p = 0.805$; M$_2$(FT$_4$), Z = −1.082, $p = 0.280$; M$_3$(TSH), Z = 0.886, $p = 0.376$.

Table 5
Regression coefficients, standard errors, and model summary information for presumed media influence parallel multiple mediator model (n = 245).

Antecedent	Consequent											
	M1(FT$_3$)			M2(FT$_4$)			M3(TSH)			Y(Language)		
	Coeffi.	SE	*p*	Coeffi.	SE	*p*	Coeffi.	SE	*p*	Coeffi.	SE	*p*
X(Pb)	a_1 −0.004	0.009	0.639	a_2 0.044	0.024	0.061	a_3 0.029	0.018	0.117	c' −0.803	0.094	0.000
M1(FT$_3$)	–	–	–	–	–	–	–	–	–	b_1 0.667	0.706	0.346
M2(FT$_4$)	–	–	–	–	–	–	–	–	–	b_2 −0.082	0.270	0.761
M3(TSH)	–	–	–	–	–	–	–	–	–	b_3 0.222	0.331	0.503
Constant	6.155	1.037	0.000	15.859	2.692	0.000	7.038	2.095	0.001	154.726	11.822	0.000
	$R^2 = 0.036$			$R^2 = 0.042$			$R^2 = 0.064$			$R^2 = 0.475$		
	F(9,235) = 0.972, $p = 0.464$			F(9,235) = 1.149, $p = 0.329$			F(9,235) = 1.773, $p = 0.074$			F(12,232) = 17.502, $p = 0.000$		

Direct effect: Pb (X) → language scores (Y).
Indirect effect: Pb (X) → M$_1$/M$_2$/M$_3$ → language scores (Y).
Total effect: combined direct effect and indirect effect.
Cd and 7 confounding factors were analyzed.
Normal theory tests for specific indirect effects, M$_1$(FT$_3$), Z = −0.305, $p = 0.760$; M$_2$(FT$_4$), Z = −0.267, $p = 0.790$; M$_3$(TSH), Z = −0.533, $p = 0.594$.

stepwise regression analysis was constructed to evaluate the factors related to cognitive and language scores of children co-exposed to Pb and Cd. The models were estimated as: cognitive score = 6.135 + 1.067 language score−0.783 BPb+3.219 eating canned food, AdjR^2 = 0.546, $p = 0.000$; language score = 121.985 + 0.226 cognitive score−6.300 recycling industry−0.867 age + 0.420 parent educational background, AdjR^2 = 0.653, $p = 0.000$. These results showed Pb was negatively correlated with cognitive scores ($p < 0.001$), but uncorrelated with language scores ($p > 0.05$). On the other hand, Cd correlated with neither cognitive nor language scores ($p > 0.05$).

Table 6
Regression coefficients, standard errors, and model summary information for presumed media influence parallel multiple mediator model (n = 246).

Antecedent	Consequent											
	M1(FT_3)			M2(FT_4)			M3(TSH)			Y(Cognitive)		
	Coeffi.	SE	p	Coeffi.	SE	p	Coeffi.	SE	p	Coeffi.	SE	p
X(Cd)	a_1 −0.173	0.128	0.177	a_2 0.174	0.332	0.560	a_3 -0.262	0.258	0.310	c' −3.182	2.526	0.209
M1(FT_3)	–	–	–	–	–	–	–	–	–	b_1 0.864	1.360	0.526
M2(FT_4)	–	–	–	–	–	–	–	–	–	b_2 0.774	0.520	0.138
M3(TSH)	–	–	–	–	–	–	–	–	–	b_3 0.808	0.639	0.207
Constant	6.162	1.036	0.000	15.852	2.687	0.000	7.036	2.091	0.001	199.296	22.813	0.000
	$R^2 = 0.035$			$R^2 = 0.042$			$R^2 = 0.065$			$R^2 = 0.406$		
	F(9,236) = 0.956, $p = 0.477$			F(9,236) = 1.160, $p = 0.322$			F(9,236) = 1.813, $p = 0.067$			F(12,233) = 13.262, $p = 0.000$		

Direct effect: Cd (X) → cognitive scores (Y).
Indirect effect: Cd (X) → $M_1/M_2/M_3$ → cognitive scores (Y).
Total effect: combined direct effect and indirect effect.
Pb and 7 confounding factors were analyzed.
Normal theory tests for specific indirect effects, $M_1(FT_3)$, Z = −0.478, $p = 0.633$; $M_2(FT_4)$, Z = −0.419, $p = 0.676$; M_3(TSH), Z = −0.675, $p = 0.500$.

Table 7
Regression coefficients, standard errors, and model summary information for presumed media influence parallel multiple mediator model (n = 245).

Antecedent	Consequent											
	M1(FT_3)			M2(FT_4)			M3(TSH)			Y(Language)		
	Coeffi.	SE	p	Coeffi.	SE	p	Coeffi.	SE	p	Coeffi.	SE	p
X(Cd)	a_1 −0.177	0.128	0.168	a_2 0.178	0.333	0.593	a_3 -0.261	0.259	0.314	c' −2.030	1.310	0.123
M1(FT_3)	–	–	–	–	–	–	–	–	–	b_1 0.667	0.706	0.346
M2(FT_4)	–	–	–	–	–	–	–	–	–	b_2 −0.082	0.270	0.761
M3(TSH)	–	–	–	–	–	–	–	–	–	b_3 −0.222	0.331	0.503
Constant	6.155	1.037	0.000	15.859	2.692	0.001	7.038	2.095	0.001	154.726	11.822	0.000
	$R^2 = 0.036$			$R^2 = 0.042$			$R^2 = 0.064$			$R^2 = 0.475$		
	F(9,235) = 0.972, $p = 0.464$			F(9,235) = 1.149, $p = 0.329$			F(9,235) = 1.773, $p = 0.074$			F(12,232) = 17.502, $p = 0.000$		

Direct effect: Cd (X) → language scores (Y).
Indirect effect: Cd (X) → $M_1/M_2/M_3$ → language scores (Y).
Total effect: combined direct effect and indirect effect.
Pb and 7 confounding factors were analyzed.
Normal theory tests for specific indirect effects, $M_1(FT_3)$, Z = −0.670, $p = 0.503$; $M_2(FT_4)$, Z = −0.139, $p = 0.890$; M_3(TSH), Z = 0.431, $p = 0.667$.

3.4. Mediation analysis of the effects

As can be seen from Table 4, in a parallel multiple mediator model, using the mediator variables (M) included FT_3 (M_1), FT_4 (M_2) and TSH (M_3), the direct effect of Pb was measured as c' = −1.571, the negative coefficient indicating that the cognitive scores of children was estimated to be 1.571 points lower with each 1 μg/dL increase in BPb ($p < 0.001$). The total indirect effects, were estimated as $a_1b_1+a_2b_2+a_3b_3=$ (−0.005) 0.864 + 0.045(-0.774) + 0.029(0.808). However, as shown in Table 4, a_1, a_2, a_3, b_1, b_2 and b_3 each were not statistically different from zero (all $p > 0.05$). All the specific indirect effects were tested again, using normal theory tests, and similarly showed that all indirect effects in this model were not statistically different from zero (all $p > 0.05$). The total effect of Pb on cognitive scores was derived by summing the direct and indirect effects ($p < 0.001$) (Table 4).

As shown in Table 5, the direct effect, c' = −0.803, quantified the effect of Pb on language scores, meaning that the language scores of children was estimated to be 0.803 units lower with each 1 μg/dL increase in BPb ($p < 0.001$). Likewise, a_1, a_2, a_3, b_1, b_2 and b_3, individually, were not statistically different from zero (all $p > 0.05$). Normal theory tests for specific indirect effects also showed all of them were not statistically different from zero (all $p > 0.05$). The total effect of Pb on language scores was derived by summing the direct and indirect effects ($p < 0.001$).

Even though the direct effects of Cd on both cognitive and language scores were negative, meaning that Cd also may be negatively related to both cognitive and language scores, there was no statistical significance (both $p > 0.05$). The indirect effect and total effect were not statistically different from zero (Tables 6 and 7).

4. Discussion

Significantly higher blood levels of heavy metals (Pb and Cd), changing FT_4 and TSH concentrations, as well as reduced cognitive and language scores, are observed in Guiyu 3-year-olds compared to reference children. In this study, we found that children living in an e-waste recycling town, Guiyu, had higher BPb and BCd levels compared with the reference area. Though is the levels are much lower than the BPb levels of children residing in a smelting craft village in Vietnam (median, 21.5 μg/dL) (Sanders et al., 2014), and also lower than the BCd levels of residents living near abandoned metal mining areas in Korea (median, 1.60 μg/L) (Park et al., 2014), this result is consistent with our previous investigations conducted in Guiyu (Huo et al., 2007; Zheng et al., 2008). The reference area, Nanao, has a specific advantage as a reference site because of its lack of industrial workshops. Aquaculture and fishing are the two principle occupations of the inhabitants on this island. However, in the current study, the median BPb level in the reference group is unexpectedly high, at 5.77 μg/dL, possibly due to exposure to leaded fuel combustion from fishing boats, because tetraethyl lead until recently has long been used as an anti-knock gasoline additive at the reference site.

We also show that cognitive and language scores are lower in the exposed group than in the reference group (Table 2), and both cognitive and language test scores are negatively related to blood levels of e-waste contaminants (Table 3), suggesting that cognitive or language scores are lower due to e-waste exposure, consistent with previous studies in e-waste areas (Kippler et al., 2012).

Other studies have shown that residents of informal recycling areas have changes in TH and TSH concentrations (Dundar et al., 2006; Eguchi et al., 2015; Wang et al., 2010; Zhang et al., 2010). Our present study also shows that the mean values for FT_4 and TSH in children living in an e-waste recycling site are elevated, and FT_4 and TSH levels are correlated to blood levels of e-waste contaminants (Table 3), further suggesting that changes in FT_4 and TSH concentrations may occur because of exposure to e-waste. The hypothalamus-pituitary-thyroid axis regulates thyroid function through thyrotropin-releasing hormone, and negative feedback may be involved whenever the levels of TH are changed. So, there usually is a negative correlation between TH and TSH. However, our study shows both FT_4 and TSH levels of children are elevated simultaneously in the e-waste recycling site. Previous studies show significant age-related declines in TH and TSH during childhood (Zurakowski et al., 1999), which rapidly decrease within the first 2 years of birth, then stabilize after about age 4 years (Kapelari et al., 2008). All children in our study are approximately 36 months of age, still within the unstable stage. The changes of FT_4 serum levels in this study may be too weak to create a negative feedback, possibly due to a not yet fully developed cerebrum.

Our results are consistent with expectations prior to experimentation. However, the primary goal of this study was to examine the relationship between heavy metals (Pb and Cd) and mental development of children (cognitive and language scores), and also evaluate whether thyroid disruption mediates this relationship. Using two different statistical approaches, multiple stepwise regression analysis and mediation analysis, we respectively evaluate the relationship between heavy metals (Pb and Cd) and mental development of children (cognitive and language scores). Both approaches showed Cd does not correlate with either cognitive or language scores ($p > 0.05$). However, the relationship between Pb and mental development of children differs in different aspects of mental development. Multiple stepwise regression analysis shows Pb is negatively correlated with cognitive scores ($p < 0.001$), but is uncorrelated with language scores ($p > 0.05$). However, mediation analysis reveals Pb negatively predicts both cognitive and language scores of children (both $p < 0.001$). Mediation analysis may be more objective because it comprehensively considers both direct and indirect effects on child mental development. Our findings about the neurotoxic impact of Pb are consistent with the literature (Min et al., 2007). Other findings from prospective studies also show the neurotoxic impact of very low levels of prenatal lead exposure in infants and very young children (Jedrychowski et al., 2009), and postnatal BPb levels in children 2–5 years of age may also cause lagging effects on neurodevelopment observed in children 5–8 years of age (Huang et al., 2012). On the other hand, the present study shows Cd correlates with neither cognitive scores nor language scores ($p > 0.05$) (Tables 5 and 6), which is inconsistent with a previous study by Thatcher et al. (1982).

Studies on mechanisms of lead neurotoxicity involve neuronal apoptosis (He et al., 2000), disrupted neurotransmission (Guilarte and McGlothan, 2003), and effects on glial cell function (Deng and Poretz, 2002). In this study, correlation with FT_3, FT_4 and TSH indicate they do not mediate the effect of heavy metals (Pb and Cd) on child mental development (Tables 4–7).

We do not find any statistically significant dependence relationships between heavy metals (Pb and Cd) and TH and TSH, though we demonstrate higher levels of FT_4 and TSH in e-waste-exposed children. Other studies show the action of some chemicals (dioxins and/or PCB) on TH in the fetus and neonate may contribute to neurodevelopmental impairment (Jacobson and Jacobson, 1996; Koopman-Esseboom et al., 1994). Therefore, the higher levels of FT_4 and TSH in our study may result from other kinds of environmental pollutants in the e-waste recycling area, such as organic pollutants.

Our study evaluates the associations among heavy metals, child neurodevelopment and TH based on their correlativity. We only recruited exposed subjects from a local regular kindergarten, which may restrict representation. On the other hand, we excluded children with developmental deficits that may also be related to exposure. Moreover, because of occasional missing data in the questionnaire, we mainly assess cognitive and language scales. Furthermore, we lack measures of adaptive behavior in children. Moreover, we also lack simultaneous measurements of organic pollutants, which may also correlate with thyroid disruption, as revealed by other studies. Finally, as a typical e-waste recycling area, where many metals and persistent organic pollutants co-exist in the environment, multiple pollutants may act as confounding factors. Further confirmation based on prospective studies with a large sample size will be utilized in our future studies.

5. Conclusions

Significantly higher blood levels of assessed heavy metals (Pb and Cd), changing FT_4 and TSH concentrations, as well as reduced cognitive and language scores, are observed in e-waste-exposed 3-year-olds from Guiyu. Pb is negatively correlated with both cognitive and language scores. However, Cd shows no correlation with either cognitive scores or language scores. The results of this study fail to confirm the hypothesis that thyroid disruption is a mechanism of neurotoxicity induced by co-exposure to Pb and Cd.

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