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ORIGINAL PAPER

Detection and Analysis of 12 Heavy Metals in Blood and Hair Sample from a General Population of Pearl River Delta Area

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Abstract To detect the content of 12 heavy metals in blood and hair sample from a general population of Pearl River Delta area, and to analyze the influence of duration of residence, gender, age, smoking and drinking on the heavy metal content. Use inductively coupled plasma mass spectrometry to detect the content of 12 heavy metals lead (Pb), mercury (Hg), cadmium (Cd), aluminum (Al), arsenic (As), copper (Cu), chrome (Cr), manganese (Mn), nickel (Ni), zinc (Zn), tin (Sn) and antimony (Sb) in blood and hair samples of a total of 50 subjects from a general population, collected by multistage stratified cluster random sampling method. The geometric mean of heavy metal content in blood samples of general population (μg/L): blood aluminum 214.00; blood chrome 92.82; blood manganese 21.43; blood nickel 20.59; blood copper 0.67; blood zinc 11.50; blood arsenic 0.55; blood cadmium 2.45; blood tin 0.00; blood antimony 1.92; blood lead 158.84; and blood mercury 1.19. The geometric mean of heavy metal content in hair samples of general population (μg/g): hair aluminum is 84.65; hair chrome 0.00; hair manganese 2.44; hair nickel 0.61; hair copper 28.49; hair zinc 136.65; hair arsenic 0.75; hair cadmium 0.46; hair tin 1.04; hair antimony 0.05; hair lead 8.97; and hair mercury 0.69. Some heavy metals were correlated with duration of residence, gender, age, smoking and drinking. This was the first time that simultaneously detecting heavy metal content in blood and hair was used to analyze the internal heavy metal burden in resident population of Pearl River Delta area. These data can serve as reference for further research.

Keywords Heavy metal · Internal exposure · Lead · Cadmium · Mercury · Arsenic · Aluminum

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Introduction

Some heavy metals are essential for normal human function; however, overexposure to heavy metals will have adverse effects to human health. Heavy metals usually are referred to metals with relative density larger than 5 g/cm^3. The common heavy metals include lead (Pb), cadmium (Cd), mercury (Hg), chrome (Cr), copper (Cu), zinc (Zn), nickel (Ni), antimony (Sb), manganese (Mn). Although aluminum (Al) and arsenic (As) have relative density <5 g/cm^3, they often arise from similar sources and cause similar harm as the other heavy metals and therefore are put into the same category in medical field.

In recent years along with industrialization, China is becoming one of the countries facing most serious pollution in the world. Industrial waste containing a great amount of heavy metals was disposed into the soil and water bodies, causing damage to ecological environment. Heavy metals in the environment are not degradable and can enter into the plant and animal body mainly through the ways of air, water and soil. Eventually those substances can be accumulated in human body through food chain. Extremely low concentration can destroy the normal physiological activities and deteriorate human health [1, 2].

In the area of Pearl River Delta of China, with the rapid proliferation of township, heavy metal pollution tends to

spread and the threat of pollution is imminent. It was reported that pollution area of 8 elements, including Cd, Hg, As, Cu, Pb, Ni and Cr, has reached 5,500 km^2, in which Hg pollution itself is up to 1,257 km^2 in area and 40 cm in depth. The levels of these elements detected from vegetable, fruit and rice samples were found to exceed the limit of standards for food hygiene, indicating that heavy metal pollution had posed a threat to agricultural product quality, food security and human health [3, 4].

The purpose of this study is to evaluate levels of 12 heavy metals in human blood and hair in general population of Pearl River Delta area and investigated the relationship of factors such as duration of residence, gender, age, smoking and drinking, on the heavy metal content.

Methods

Subjects and Sampling Method

This study was conducted in Pearl River Delta area, Guangdong province, in southeast of China from Jan 1 to 31, 2014. The study protocol was approved by the Medical Ethics Committee of Southern Medical School of China, and written informed consent was obtained from all study participants.

This study used a multistage clustering sampling with proportional stratified method. Firstly, Guangzhou city and Qingyuan city in Pearl River Delta area were chosen as the first stage, and then Haizhu district of Guangzhou city and Qingcheng district of Qingyuan city were chosen as the second stage. A total of 50 subjects from a general population at the age of 18 or above were included. Inclusion criteria include: subject living in the area over 0.6 years; free of five chronic wasting diseases of liver disease, kidney disease, diabetes, hyperthyroidism and tumors; not taking calcium, iron, zinc and complex trace elements drugs and health care products within 3 months.

Blood Sample Collection

Venous blood sample was taken from each subject, mixed in anticoagulant tube, stored at 4 °C for transportation and finally put under −80 °C for frozen preservation. A quality control sample after every 50 collected samples was added following the same processing and preserving procedures.

Hair Sample Collection and Processing

Hair sample <10 cm in length, about 1.5–2.0 g was taken from occipital or from the front hairline. The sample was soaked in 1 % detergent and rinsed with distilled water. The same procedure was repeated twice. Then, the sample was rinsed with deionized water 3 times, dried in oven at 60 °C, cut into 0.5 cm piece and stored in plastic bags. For digesting the hair sample, one segment of 0.5 cm sample was weighed (accurate to 0.0001 g) and digested with nitric acid 2 mL and hydrogen peroxide 0.5 mL in a microwave high-pressure cooker. After several cycles, the sample was taken out and cooled to room temperature. Finally, 25 mL of 5 % nitric acid was added into the digested sample. Blank was conducted following the same procedures.

Data Collection

Under the informed consent, we carried out an epidemiological survey with questionnaire. Questionnaire content included: basic personal information, such as gender, age, date of birth, duration of residence, marital status, education level and occupation; lifestyle, including smoking, drinking and the frequency, whether to drink/eat local water and produce; family history; current health conditions including whether suffering from chronic diseases, such as cardiovascular disease, digestive system disease and diabetes. In accordance with the requirements of the questionnaire guidance, all questionnaires were filled item by item by the trained investigators after the inquiry with the subjects.

Detection of Heavy Metals in Blood and Hair Samples

Levels of 12 heavy metals in blood and hair samples were determined using Quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-MS, 7500ce, Agilent Technologies, Inc., USA).

Statistics

Experimental data were first input with Epidata 3.0 software for verification and eliminating the unqualified ones. Then, data were imported into SPSS 15.0 software for statistical analysis. $p < 0.05$ indicated statistically significant difference.

Results

Demographics

A total of 50 subjects were included in the investigation, with mean age of 37.92 ± 9.58 (mean ± standard deviation) ranging from 23 to 64. Among them, males were 26 (52.0 %) and females were 24 (48.0 %). Smokers were 10 (20.0 %), and non-smokers were 40 (80.0 %). Alcohol drinkers were 15 (30.0 %), and no-alcohol drinkers were

Table 1 Personal characteristics of inhabitant in Pearl River Delta of China, 2014

Personal characteristics	*N*	%
Gender		
Male	26	52.0
Female	24	18.0
Age (years)		
23–29	6	12.0
30–35	22	44.0
36–40	6	12.0
40+	16	32.0
Years lived in Pearl River Delta		
≤1	2	4.0
2–5	21	42.0
6–9	14	28.0
≥10	13	26.0
Current smoker		
Yes	6	12.0
No	44	88.0
Current drinkers		
Yes	14	28.0
No	36	72.0

35 (70.0 %). The residence time in Pearl River Delta area fluctuated between 0.6 and 33 years, with average of 8.20 ± 7.37 years. See Table 1.

Heavy Metal Content in Blood and Hair Samples

The geometric mean of heavy metal content in blood samples of investigation subjects (μg/L): blood aluminum is 214.00; blood chrome 92.82; blood manganese 21.43; blood nickel 20.59; blood copper 0.67; blood zinc 11.50; blood arsenic 0.55; blood cadmium 2.45; blood tin 0.00; blood antimony 1.92; blood lead 158.84; and blood mercury 1.19.

The geometric mean of heavy metal content in hair samples of investigation subjects (μg/g): hair aluminum is 84.65; hair chrome 0.00; hair manganese 2.44; hair nickel 0.61; hair copper 28.49; hair zinc 136.65; hair arsenic 0.75; hair cadmium 0.46; hair tin 1.04; hair antimony 0.05; hair lead 8.97; and hair mercury 0.69. See Table 2.

Comparison of Heavy Metal Content Between Genders

Heavy metal content in blood and hair samples in different gender groups is shown in Table 3. Except that blood zinc content in male was significantly higher than that in female, there was no statistical difference in blood content of other heavy metals between genders. Hair content of arsenic and tin in female was significantly higher than that in male. And there was no statistical difference in hair content of other heavy metals between genders.

Comparison of Heavy Metal Content Among Different Age Group

Heavy metal content in blood and hair samples in different age groups is shown in Table 4. In blood, there was statistical difference only in mercury content among age groups. In hair, there was statistical difference in content of aluminum, mercury, lead, tin, cadmium and zinc, with higher content all in groups aged 35 or above.

Relationship of Heavy Metal Content with Duration of Residence in Pearl River Delta Area

The influence of duration of residence in Pearl River Delta area on heavy metal content is shown in Table 5. The

Table 2 Levels of heavy metal in blood and hair sample of inhabitant in Pearl River Delta of China, 2014 (μg/L for blood, μg/g for hair)

Metals	Range		Mean ± SD	
	Blood	Hair	Blood	Hair
Aluminum	0.00–2,638.00	0.00–908.50	214.00 ± 508.07	84.65 ± 163.96
Chrome	34.40–153.80	0.00–0.00	92.83 ± 32.63	0.00 ± 0.00
Manganese	11.92–44.26	0.00–88.38	21.43 ± 7.35	2.44 ± 12.55
Nickel	0.40–141.00	0.00–5.71	20.59 ± 20.91	0.61 ± 1.09
Copper	0.14–0.85	0.00–309.90	0.67 ± 0.09	28.49 ± 66.15
Zinc	2.90–33.05	0.00–784.39	11.50 ± 8.03	136.65 ± 140.40
Arsenic	0.00–18.36	0.00–20.97	0.55 ± 2.73	0.75 ± 3.18
Cadmium	0.00–8.29	0.00–8.91	2.45 ± 2.06	0.46 ± 1.52
Tin	0.00–0.00	0.00–6.60	0.00 ± 0.00	1.04 ± 1.62
Antimony	0.14–4.43	0.00–0.61	1.92 ± 1.09	0.05 ± 0.09
Lead	0.00–652.50	0.00–141.73	158.84 ± 160.38	8.97 ± 25.77
Mercury	0.00–5.85	0.00–8.06	1.19 ± 1.40	0.69 ± 1.30

Table 3 Heavy metal content in different gender groups

Metals	Blood		Hair	
	Male	Female	Male	Female
Aluminum	271.89 ± 586.69	151.29 ± 409.78	94.02 ± 130.27	74.5 ± 196.53
Chrome	96.32 ± 32.52	89.04 ± 33.01	0.00–0.00	0.00–0.00
Manganese	22.64 ± 8.49	20.11 ± 5.76	1.03 ± 2.54	3.96 ± 17.99
Nickel	24.45 ± 27.22	16.42 ± 9.53	0.63 ± 1.38	0.59 ± 0.69
Copper	0.64 ± 0.09	0.7 ± 0.09	18.23 ± 44.04	39.61 ± 83.46
Zinc	13.31 ± 8.8$^{\Delta}$	9.55 ± 6.75	128.36 ± 121.66	145.64 ± 160.46
Arsenic	0.88 ± 3.68	0.19 ± 0.94	0.32 ± 1.02	1.22 ± 4.47$^{\Delta}$
Cadmium	2.82 ± 2.08	2.06 ± 2.01	0.45 ± 1.24	0.47 ± 1.8
Tin	0.00–0.00	0.00–0.00	0.6 ± 1.26	1.52 ± 1.85$^{\Delta}$
Antimony	2.03 ± 1.12	1.8 ± 1.08	0.06 ± 0.13	0.04 ± 0.04
Lead	193.56 ± 174.06	121.23 ± 137.93	9.7 ± 23.3	8.18 ± 28.7
Mercury	1.25 ± 1.53	1.12 ± 1.27	0.65 ± 0.92	0.73 ± 1.63

$^{\Delta}$ $p < 0.05$

Table 4 Heavy metal content in different age groups

Metals	Blood		Hair	
	<35 years	≥35 years	<35 years	≥35 years
Aluminum	255.74 ± 603.74	165 ± 373.52	42.28 ± 76	134.4 ± 219.69$^{\Delta}$
Chrome	92.02 ± 32.18	93.77 ± 33.84	0.00–0.00	0.00–0.00
Manganese	21.1 ± 8.36	21.81 ± 6.12	0.68 ± 2.38	4.51 ± 18.32
Nickel	21 ± 25.96	20.11 ± 13.3	0.44 ± 0.66	0.82 ± 1.44
Copper	0.69 ± 0.1	0.63 ± 0.08	22.04 ± 59.46	36.06 ± 73.87
Zinc	10.85 ± 7.21	12.26 ± 9	115.71 ± 84.87	161.24 ± 184.99$^{\Delta}$
Arsenic	0.34 ± 1.23	0.8 ± 3.83	0.3 ± 1.01	1.27 ± 4.56
Cadmium	2.2 ± 2.11	2.75 ± 2.02	0.12 ± 0.21	0.85 ± 2.19$^{\Delta}$
Tin	0.00–0.00	0.00–0.00	0.75 ± 1.12	1.38 ± 2.04$^{\Delta}$
Antimony	1.96 ± 1.24	1.87 ± 0.92	0.05 ± 0.12	0.05 ± 0.06
Lead	158.79 ± 164.57	158.9 ± 159	2.8 ± 5.29	16.21 ± 36.67$^{\Delta}$
Mercury	0.88 ± 0.8	1.55 ± 1.83$^{\Delta}$	0.49 ± 0.54	0.92 ± 1.82$^{\Delta}$

$^{\Delta}$ $p < 0.05$

Table 5 Heavy metal content in different groups by years lived in Pearl River Delta

Metals	Blood		Hair	
	<5 years	≥5 years	<5 years	≥5 years
Aluminum	276.35 ± 666.52	175.79 ± 388.73	133.5 ± 219.25	54.72 ± 112.39
Chrome	95.28 ± 32.56	91.32 ± 33.11	0.00 ± 0.00	0.00 ± 0.00
Manganese	23.04 ± 8.02	20.43 ± 6.85	5.91 ± 20.18$^{\Delta}$	0.31 ± 0.57
Nickel	26.34 ± 28.68	17.07 ± 13.66	0.88 ± 1.59$^{\Delta}$	0.45 ± 0.6
Copper	0.67 ± 0.11	0.66 ± 0.09	30.96 ± 73.82	26.97 ± 62.21
Zinc	13.89 ± 8.37	10.04 ± 7.58	145.01 ± 104.58	131.53 ± 159.89
Arsenic	1.21 ± 4.29	0.15 ± 0.83	0.54 ± 1.79	0.88 ± 3.82
Cadmium	2.79 ± 2.07	2.25 ± 2.06	0.55 ± 1.43	0.4 ± 1.59
Tin	0.00 ± 0.00	0.00 ± 0.00	1.39 ± 2.14$^{\Delta}$	0.83 ± 1.2
Antimony	2.41 ± 0.78$^{\Delta}$	1.62 ± 1.16	0.05 ± 0.04	0.05 ± 0.12
Lead	201.76 ± 191.69	132.53 ± 134.46	11.45 ± 26.48	7.45 ± 25.66
Mercury	0.91 ± 0.96	1.36 ± 1.6	0.21 ± 0.39$^{\Delta}$	0.99 ± 1.56

$^{\Delta}$ $p < 0.05$

Table 6 Heavy metal content in different groups by smoking

Metals	Blood		Hair	
	Smoking	No smoking	Smoking	No smoking
Aluminum	728.83 ± 1,058.35$^{\Delta}$	143.8 ± 349.13	16.78 ± 15.64	93.91 ± 172.85
Chrome	105.66 ± 33.47	91.08 ± 32.51	0.00 ± 0.00	0.00 ± 0.00
Manganese	19.55 ± 4.38	21.68 ± 7.66	0.10 ± 0.22	2.76 ± 13.36
Nickel	14.59 ± 10.37	21.41 ± 21.91	0.47 ± 0.58	0.63 ± 1.15
Copper	0.63 ± 0.1	0.67 ± 0.09	6.75 ± 5.97	31.45 ± 70.05
Zinc	8.34 ± 5.56	11.93 ± 8.26	74.73 ± 12.77	145.09 ± 147.77
Arsenic	0.77 ± 1.89	0.52 ± 2.84	0.07 ± 0.18	0.84 ± 3.38
Cadmium	2.29 ± 1.35	2.48 ± 2.15	0.14 ± 0.27	0.50 ± 1.62
Tin	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.19$^{\Delta}$	1.16 ± 1.70
Antimony	1.73 ± 1.23	1.95 ± 1.09	0.02 ± 0.01	0.05 ± 0.10
Lead	146.74 ± 147.31	160.49 ± 163.59	3.2 ± 5.38	9.76 ± 27.36
Mercury	1.54 ± 2.19	1.14 ± 1.28	1.01 ± 0.99	0.65 ± 1.34

$^{\Delta}$ $p < 0.05$

Table 7 Heavy metal content in different groups by drinking

Metals	Blood		Hair	
	Drinking	No drinking	Drinking	No drinking
Aluminum	95.53 ± 168.47$^{\Delta}$	260.08 ± 585.71	141.99 ± 152.63	62.36 ± 164.8
Chrome	96.2 ± 33.68	91.51 ± 32.6	0.00 ± 0.00	0.00 ± 0.00
Manganese	23.49 ± 7.9	20.62 ± 7.07	1.74 ± 3.33	2.71 ± 14.7
Nickel	22.23 ± 9.27	19.95 ± 24.05	0.91 ± 1.83$^{\Delta}$	0.50 ± 0.62
Copper	0.63 ± 0.11	0.68 ± 0.08	25.23 ± 59.39	29.76 ± 69.35
Zinc	14.53 ± 8.22	10.33 ± 7.75	142.9 ± 147.49	134.22 ± 139.63
Arsenic	1.64 ± 4.97$^{\Delta}$	0.13 ± 0.77	0.03 ± 0.11	1.03 ± 3.72
Cadmium	3.19 ± 1.89	2.17 ± 2.08	0.76 ± 1.64	0.34 ± 1.48
Tin	0.00 ± 0.00	0.00 ± 0.00	0.87 ± 1.67	1.11 ± 1.63
Antimony	2.5 ± 0.94	1.7 ± 1.08	0.10 ± 0.16$^{\Delta}$	0.03 ± 0.03
Lead	232.08 ± 185.26	130.36 ± 142.41	16.28 ± 30.5	6.13 ± 23.56
Mercury	1.22 ± 1.46	1.18 ± 1.39	0.37 ± 0.73	0.81 ± 1.45

$^{\Delta}$ $p < 0.05$

duration of residence in Pearl River Delta area only had influence on the difference in blood antimony content statistically. In hair, there was statistical difference in content of manganese, nickel, tin and mercury. It showed that except mercury, the longer the duration of residence in Pearl River Delta area, the lower the heavy metal content.

Effect of Smoking on Heavy Metal Content

Blood aluminum content and hair tin content were significantly higher in smoking subjects than in non-smoking subjects. The results are shown in Table 6.

Effect of Alcohol Drinking on Heavy Metal Content

Drinking can affect aluminum and arsenic content in blood. In alcohol drinking, subjects' blood aluminum content was lower, but blood arsenic content was higher than in no-alcohol drinking subjects in the area. Drinking can also affect nickel and antimony content in hair, which were both higher in no-alcohol drinking subjects. The results are shown in Table 7.

Discussion

Up to date, there is still lack of standard method to accurately assess heavy metal content in human body. For low-dose chronic exposure of heavy metals, blood sample is generally considered a better indicator as it can reflect the recent comprehensive information of various elements in human body [5, 6]. Hair sample, on the other hand, can reflect the long-term accumulative effect of heavy metals in human body and therefore is particularly useful for long-term study. However, the collection method of hair sample is essential for accurate detection of hair heavy metal

content. Based on the origins, heavy metal elements in human hair come from three sources: endogenous, exogenous and endoexogenous [7, 8]. Endogenous elements refer to elements contained in the hair before coming out from the scalp. Exogenous elements may have two combination forms with the hair: one form is that aerosols and large particles containing heavy metal elements are absorbed on the hair surface; another form is that heavy metal elements diffuse through the outermost layer of the hair into the internal structure. Endoexogenous elements include both endogenous elements and exogenous elements. Only endogenous heavy metal elements content in hair can reflect the human body load quantity of this element. Therefore, removing the exogenous elements in hair is the key to use of hair as the biological monitoring material. So far, an effective method to pretreat the exogenous elements in human hair has not been established. In our pilot study, we used isotope tracer technique to evaluate the effects of several hair pretreatment methods on the content of elements lead, cadmium, chrome and mercury in hair, and found that the method used in this study achieved the best result in reducing the exogenous elements.

It was reported that aluminum can damage human brain cells [9]. According to the evaluation by world health organization (WHO), daily intake of aluminum was set 0–0.6 mg/kg, but normal value of blood aluminum or hair aluminum was not set so far. Aluminum content in blood and hair of investigation population in this area was 214.00 μg/L and 84.65 μg/g, respectively, higher than results of other relevant reports [10]. Chrome is an essential trace element for human body. Trivalent chromium is beneficial to human, mainly distributed in the bone, skin, adrenal gland, brain and muscles. It can help insulin to promote the efficiency of glucose entering the cells and thus is an important blood sugar regulator. Chromium content in hair and blood still lacks standard value. This study failed to detect the chromium content in hair, which may be related to its water insolubility property. In the 50 cases of blood samples in this study, no tin content was detected, neither was in some cases of hair samples, which indicated that the human body content of this metal was relatively low in this area. This study showed that arsenic content in blood and hair did not exceed the standard value of general population. According to American guidelines for the diagnosis and management of lead exposure in adults, normal value is $<$100 μg/L and average value is 30 μg/L. In this experiment, blood lead content was abnormal in 25 cases (50 %), which showed that lead content exceeded the standard very much, as needed to pay attention to. About the detection of mercury content in human body, it should be noted that mercury element in human includes inorganic, organic and elemental mercury. Determination of urinary mercury is mainly determination of inorganic and elemental mercury, which is usually seen in population having occupational contact and overexposure of mercury. Determination of blood mercury is mainly determination of organic mercury, which is usually seen in population eating more food containing organic mercury. Determination of mercury content in hair is rare. For general population, determination of blood mercury can be considered to use as measure of mercury load in human body. Blood mercury did not exceed the European standard in this investigation population.

Heavy metal content in blood had no direct correlation with gender. In hair, female had higher arsenic and tin content than male, which may relate to women doing more hair curling and coloring. Previous study had shown that heavy metals in hair dye, such as arsenic, can increase the heavy metal content in hair [11]. The frequent use of hair dye had correlation with heavy metals in human body, such as arsenic, and bladder cancer incidence rate, which should be paid attention to [12, 13]. Our study showed that heavy metal internal exposure level had very strong correlation with exposure time. Therefore, the older the age, the longer the exposure time, and the greater the body heavy metal load, which was especially evident in mercury in blood and in aluminum, mercury, lead, tin, cadmium and zinc in hair. It indicated that the above heavy metals had prominent accumulation effect, and probably also reflected that the exposures of different age groups were influenced by multiple factors, such as gender, exposure time, exposure extent, dietary habits and their physiological conditions. The influence of duration of residence in Pearl River Delta area to heavy metal content in blood was reflected in antimony content in blood and in manganese, nickel, tin and mercury in hair. The longer the time of the stay in Pearl River Delta area was, the lower the content of the above heavy metals, indicating that the content of the above heavy metals was probably higher in other areas in China, which agreed with some research results [14, 15].

About the influence of smoking to heavy metal content in human body, there were more reports. Ashraf and Han [16, 17] both reported that content of cadmium and lead was higher in smokers than in non-smokers. In this study, it was found that smoking had limited influence on heavy metal content in blood, while blood aluminum and hair tin content were significantly higher in smoking population than in non-smoking population. It was different from results of other research, probably related to the smaller sampling population in this study. Studies on the relation between drinking and heavy metal content were rare. It was found in our study that in the blood of alcohol drinking population aluminum content was lower, but arsenic content was higher than that in no-alcohol drinking population in this area. And drinking can influence heavy metal content of nickel and antimony in hair, which were lower than that in no-alcohol drinking population.

In summary, 12 heavy metals were detected in both blood and hair samples of a general population in Pearl River Delta area of southern China. Relationship of factors such as duration of residence, gender, age, smoking and drinking, with the heavy metal content was investigated. These data can serve as reference for further research.

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