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Mercury exposures and symptoms in smelting workers of artisanal mercury mines in Wuchuan, Guizhou, China

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Abstract

Mercury exposures to smelting workers of artisanal mercury mines in Wuchuan, Guizhou, China were evaluated by urine and hair mercury survey. The mean urinary mercury (U-Hg), hair total mercury (T-Hg), and hair methyl mercury (Me-Hg) for smelting workers was 1060 μ g/g creatinine (μ g/g Cr), 69.3 and 2.32 μ g/g, respectively. The results were significantly higher than that of control group, which is 1.30 μ g/g Cr, 0.78 and 0.65 μ g/g, correspondingly. The average urinary β 2-microglobulin (β 2-MG) was 248 μ g/g Cr for the exposed group contrasting to $73.5 \mu g/g$ Cr for the control group and the data showed a serious adverse effect on renal system for the smelting workers. The workers were exposed to mercury vapor through inhalation, and the exposure route of Me-Hg may be through intake of polluted diet. The results indicate that age, alcohol drinking, and smoking are not crucial factors controlling the urine and hair mercury levels for the exposed and the control group. Clinical symptoms including finger and eyelid tremor, gingivitis, and typical darkline on gums were observed in six workers. This study indicated that the smelting workers in Wuchuan were seriously exposed to mercury vapor.

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Keywords: Mercury exposures; Smelting workers; Artisanal mercury mines; Urine; Hair

1. Introduction

Mercury (Hg) and its compounds are recognized as potentially hazardous materials and are rated in the top category of environmental pollutants. Mercury can cause significantly adverse effects on human health. The toxicity of mercury depends on its chemical form. Inhalation of mercury vapor and ingestion of Me-Hg via the diet are the most important routes of human exposure to mercury [\(WHO, 1990, 1991\)](#page-6-0). For occupational exposure (such as workers in chlor-alkali plants, mercury mines, mercurybased gold extraction, mercury processing and sales, thermometer factories, dental clinics), inhalation of mercury vapor is the most important route of human exposure

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to mercury, while the dental amalgam filling is the predominant source of human exposure to elemental mercury in the general population ([Clarkson, 2002\)](#page-6-0).

About 80% of the inhaled mercury vapor is retained in the bloodstream and from there is distributed to the tissues. Urine and feces are the principal routes of mercury elimination and the urinary route dominates when exposure is high ([WHO, 1991\)](#page-6-0). The half-time for Hg in urine is about 2 months. U-Hg measurements are widely used for assessment of inorganic mercury (mainly mercury vapor) exposure in humans because U-Hg is thought to be indicating most closely the mercury levels present in the kidneys [\(Clarkson et al., 1988;](#page-6-0) [Barregard,](#page-6-0) [1993\)](#page-6-0).

Elemental mercury may cause a variety of adverse effects. Neurological effects, renal effects, cancer, respiratory effects, cardiovascular effects, gastrointestinal and

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hepatic effects, effects on the thyroid gland, effects on the immune system, effects on the skin, reproductive and developmental effects, and genotoxicity have been observed following exposure to mercury vapor [\(WHO, 1991;](#page-6-0) [USEPA, 1997;](#page-6-0) [ATSDR, 1999](#page-6-0); [UNEP, 2002](#page-6-0)). The specific symptoms are found in the central nervous system and the kidney.

China is rich in Hg and the reserve of Hg ranks the third in the world. The most important Hg production center in China is Guizhou Province. From the perspective of the global plate tectonics, Guizhou Province is situated in the circum-Pacific mercuriferous belt [\(Qiu et al., 2006](#page-6-0)), and so far 12 large or super-large Hg deposits have been discovered there. The reserve of cinnabar deposits in Guizhou, approximately 80,000 tons of metal Hg, represents approximately 70% of the total in China ([Qu, 2004\)](#page-6-0). Wuchuan mercury mine is one of the largest mercury mines in China with mercury reserve up to 23,320 metric tons. It has a long history of mercury production for approximately 400 years and more than 4000 tons of metal mercury has been produced. The large-scale mercury mining activities began in 1949 and ceased in 2003 mainly due to insufficient profits. However, the market for mercury demand has been increasing since China started to restrict importing mercury from Europe and other regions a few years ago. Consequently, mercury prices in the market went up sharply recently, which stimulated the revival of small-scale or artisanal mercury smelting activities in Guizhou. The illegal artisanal mining activities are extensively existed in mercury mining areas especially in Wuchuan area. The mercury ore (cinnabar) was crushed and then heated to $700-800\degree C$ to produce mercury vapor that condensed in cooling wooden barrel, which contained water. Because the simple smelting processes are without any environmental protection measures, the mercury emission factors (the proportion of mercury in ore is released to the ambient air) ranged from 6.9% to 32.1% and the annual mercury emission from artisanal mercury smelting activities was up to 3.7–9.6 tons in Wuchuan area ([Li et al., 2006](#page-6-0)). The artisanal mining activities have resulted in serious mercury pollution to the local environment [\(Qu, 2004;](#page-6-0) [Qiu et al., 2006\)](#page-6-0). Therefore, the health of the workers may be negatively affected through inhalation of mercury-polluted air.

Up to date, only a few studies regarding mercury contamination to the local environments have been carried out in Wuchuan mercury mining areas ([Qu, 2004](#page-6-0); [Li et al.,](#page-6-0) [2006](#page-6-0); [Qiu et al., 2006](#page-6-0)). To the best of our knowledge, human mercury exposure survey has not been reported in the study areas. The present study was designed to investigate mercury exposure to the smelting workers in Wuchuan areas by measuring their urine and hair mercury levels. Efforts were also made to identify clinical symptom of mercury poisoning for the smelting workers. In a companion paper, the effects of mercury vapor exposure on the neuromotor function of the artisanal smelting workers were examined [\(Iwata et al., 2007](#page-6-0)).

2. Materials and methods

2.1. Study area and selected population

The Wuchuan county (E: $107^{\circ}31' - 108^{\circ}31'$, N: $28^{\circ}11' - 29^{\circ}05'$) is located at the northeast of Guizhou Province (Fig. 1) with an area of approximately 2778 km². The Wuchuan district is hilly, karstic and its average altitude is 1034 m (with a range of $325-1743 \text{ m}$) above the sea level. Smelting workers in the artisanal mercury mine in Wuchuan area were chosen to the mercury exposure survey. For comparison, residents in Changshun County were selected as the control group. Changshun County is located in the south of Guizhou Province (Fig. 1) and is about 90 km away from Guiyang City, the capital of the province. A large proportion of residents is ethnic minority groups both in Wuchuan and in Changshun County. The ethnic groups live on farming and have own traditional custom. Basically, the two selected groups have similar living habits so that it is favorable for comparison. The smelting workers in Wuchuan are mostly male, so that the control group also consists of all male residents. The present study obtained the ethics approval by the Institute of Geochemistry. All participants were required to sign a consent form.

2.2. Sample collection

In June 2005, 22 smelting workers in Wuchuan and 40 residents in Changshun joined in the investigation. Urine and scalp hair samples were collected for investigating mercury exposure levels. The urine samples were collected in pre-cleaned plastic centrifugal tubes, hermetically sealed and frozen at 4° C until analysis. Hair samples were cut with a stainless steel scissors from the occipital region of the scalp, bundled together with scrip, placed and sealed in polyethylene bags, properly identified and taken to the laboratory for analysis.

Fig. 1. Locality of the study areas and the control site.

A questionnaire was utilized to collect information on residential history, occupational history, dietary habits, life style (smoking habit and alcohol drinking), and health history. Clinical examinations were carried out by specialists from National Institute for Minamata Disease, Japan.

2.3. Analytical method

Hair samples were washed with nonionic detergent, distilled water, and acetone, and dried in an oven at 60° C overnight. Urine and hair samples were digested in a water bath (95 °C) with a fresh mixed acid of $HNO₃:H₂SO₄$ (4:1, v/v) for T-Hg analysis ([Horvat et al., 1991\)](#page-6-0). For Me-Hg analysis, prepared hair samples were digested using KOH–methanol/ solvent extraction technique ([Liang et al., 1994, 1996, 1996](#page-6-0)). T-Hg concentrations in hair and U-Hg were determined by cold vapor atomic fluorescence spectrometer (CVAFS) or cold vapor atomic absorption spectrometry (CVAAS). Me-Hg contents in hair samples were measured using gas chromatography–cold vapor atomic fluorescence spectrometer (GC–CVAFS) detection.

Urinary parameters (including urinary pH, protein, and hematuria) were assayed by test paper upon sample collection. Urinary creatinine (U-Cr) contents were analyzed with a HITACHI 7170A automatic analyzer. In order to take hydration and urinary flow rate into account, the assessment of U-Hg as a biomarker for exposure to mercury vapor was adjusted by creatinine excretion. The results of U-Hg were given in μ g/g creatinine (μ g/g Cr). Normal creatinine values are between 0.5 and 3.0 g/L ([ACGIH, 1997\)](#page-6-0) so that the extreme creatinine concentrations were excluded from the evaluations. Urinary β 2-MG concentrations were analyzed by radioimmunoassay method [\(Chen, 1985\)](#page-6-0). For the control group, we found some participants had kidney dysfunction from the urinary parameters tests. Since both mercury exposure and kidney dysfunction may cause the increase of β 2-MG concentration in urine, we only selected those urine samples collected from participants who had no kidney dysfunction problem for β 2-MG determination.

As part of a strict Quality Assessment/Quality Control program, method blanks, blank spikes, matrix spikes, certified reference material, and blind duplicates were analyzed along with field samples. The mean T-Hg in the certified reference material of hair samples (NIES-13) was $4.4\pm0.1 \,\mu$ g/g (n = 6) consistent with the certified value of $4.4\pm0.2 \,\mu$ g/g. The average Me-Hg was $3.5 \pm 0.1 \,\mu$ g/g (n = 5) in good agreement with the certified value of 3.8 ± 0.4 µg/g. The recoveries from spiked samples ranged from 83% to 120% for Me-Hg in hair sample, and ranged from 98% to 103% for T-Hg in urine samples. The relative percent difference was lower than 5% for T-Hg in hair and urine duplicate samples.

2.4. Statistical analyses

Statistical analyses were performed using SPSS 11.5 for windows. Mean values were compared between the exposed and control groups using independent-samples t test to evaluate the differences between the two groups. The correlation coefficients among U-Hg, hair T-Hg, and Me-Hg in each group were studied by Pearson correlation analysis. The results of a statistical test were considered statistically significant if $p < 0.05$.

3. Results and discussion

3.1. Urine, hair mercury levels

Urine, hair mercury levels, and other parameters were compared between the mercury exposed group and the control group, and the results are summarized in Table 1. The mean age was similar between the two selected groups. Furthermore, there was no significant difference in both U-Cr and U-pH in the two groups. However, there were significant differences in mean U-Hg $(p<0.001)$, mean urinary β 2-MG ($p < 0.05$), mean hair T-Hg ($p < 0.001$), mean hair Me-Hg ($p < 0.001$) and mean percentage of T-Hg as Me-Hg $(p<0.05)$ between the two groups, respectively. The maximum urinary mercury concentration for occupational workers recommended by the [WHO \(1991\)](#page-6-0) was $50 \,\mu g/g$ Cr. Urine mercury levels rarely exceed $5 \,\mu g/g$ Cr in people who are not occupationally exposed to mercury [\(UNIDO, 2003\)](#page-6-0). The geometric mean U-Hg for smelting workers was $463 \mu g/g$ Cr which was considerably higher than 1.30μ g/g Cr for the control group. The highest U-Hg in smelting workers reached $6150 \mu g/g$ Cr which is about 120 times higher than the occupational exposure limit level $(50 \,\mu\text{g/g Cr})$ recommended by [WHO \(1991\).](#page-6-0) Ninety-five percent (21/22) of U-Hg exceeded the limit value indicating that the smelting workers in the Wuchuan area were seriously exposed to mercury vapor.

 β 2-MG as a renal biomarkers can be used to study human nephrotoxicity at an early stage [\(Lauwerys and](#page-6-0) [Bernard, 1989;](#page-6-0) [Mueller and Jay, 1989\)](#page-6-0). It is useful to define the effects for assessing re-absorption function to indicate

Table 1

Comparison of urine mercury, hair mercury levels, and other parameters between the mercury exposed group and the control group

	The exposed group $(N = 22)$		The control group $(N = 40)$	
	$Mean + S.D.$	Range	$Mean + S.D.$	Range
Age	$42 + 9$	$30 - 63$	$41 + 13$	$23 - 67$
U -p H	$5.5 + 0.7$	$5.0 - 7.5$	$5.5 + 0.6$	$5.0 - 7.0$
U-Cr (g/L)	$1.39 + 0.47$	$0.56 - 2.01$	$1.03 + 0.40$	$0.57 - 1.97$
U-Hg $(\mu g/L)$	1080 ± 1260 ***	$41 - 4830$	$1.27 + 0.47$	$0.56 - 2.96$
U-Hg $(\mu g/g Cr)$	1060 ± 1510 ***	$28 - 6150$	$1.30 + 0.39$	$0.68 - 2.32$
Urinary β 2-MG (μ g/g Cr)	$248 + 295^*$	$26.3 - 1030$	$73.5 + 36.2$	$32.0 - 134$
Hair T-Hg $(\mu g/g)$	69.3 ± 44.4 ***	$9.91 - 143$	$0.78 + 0.28$	$0.32 - 1.72$
Hair Me-Hg $(\mu g/g)$	$2.32 + 1.26r$	$0.83 - 5.89$	$0.65 + 0.25$	$0.26 - 1.38$
$\%$ Hg as Me-Hg $(\%)$	$7.18 + 8.65^*$	$0.71 - 31.6$	$83.5 + 12.6$	$52.7 - 99.9$

*Significant difference was observed between the exposed group and the control group at $p < 0.001$.

 $*$ Significant difference was observed between the exposed group and the control group at $p < 0.05$.

tubular injury. The average urinary β 2-MG concentration for the exposed workers was $248 \mu g/g$ Cr, which was substantially higher than $73.5 \mu g/g$ Cr for the control group. In conclusion, the present study showed a serious adverse effect on renal system due to mercury vapor exposure for smelting workers. Significant correlation $(r = 0.85, p < 0.01)$ was found between U-Hg and urinary β 2-MG concentrations of smelting workers in Wuchuan area (Fig. 2). This also confirmed that the elevation of

Fig. 2. Relationship between urinary mercury and β 2-MG concentrations of workers in Wuchuan areas.

Table 2 Mercury levels in human urine from different exposed populations

urinary β 2-MG concentrations of smelting workers is indeed resulted from the exposure to mercury vapor.

The smelting workers in Wuchuan showed very high U-Hg levels of which the mean value reached 1060μ g/g Cr $(1080 \,\mu\text{g/L})$. The data are comparable to the results obtained from smelting workers in Abbadia San Salvatore mine, Italy which was 1111.6 µg/L [\(Bellander et al., 1998\)](#page-6-0). The comparison among mercury levels in human urine from different exposed populations is summarized in Table 2. Obviously, urinary mercury levels from smelting workers in the Wuchuan area were much significantly higher than those from other exposed populations around the world, indicating a high health risk to the smelting workers. The higher Me-Hg level in the hair of the exposed group also needed to be scrutinized though the percentage of Me-Hg as T-Hg was very low.

3.2. Correlation between hair Hg and U-Hg

The correlation coefficients between hair T-Hg, hair Me-Hg, and U-Hg for the two groups are listed in [Table 3](#page-4-0). For the control group, Me-Hg constitutes 83.5% of the T-Hg in the hair and shows significant correlation $(r = 0.90,$ $p<0.01$) with hair T-Hg which is consistent with most previous studies. Me-Hg usually constitutes at least 80% of the T-Hg analyzed in hair among fish consumers ([Mcdo](#page-6-0)[well et al., 2004](#page-6-0)). On the contrary, hair T-Hg concentrations had no significant correlation with hair Me-Hg concentrations, but had a significant correlation with U-Hg $(p<0.05)$ for the exposed group. Moreover, hair Me-Hg in

AM, arithmetic mean and GM, geometric mean.

Table 3 The correlation coefficients among hair T-Hg, hair Me-Hg, and U-Hg concentrations for the exposed group and the control group

		Hair Me-Hg	U-Hg $(\mu g/L)$	U-Hg $(\mu$ g/ g Cr)
The exposed group	Hair T-Hg Hair Me-Hg U-Hg $(\mu g/L)$	0.11	$0.55*$ 0.28	$0.55*$ 0.33 $0.84*$
The control group	Hair T-Hg Hair Me-Hg U-Hg $(\mu g/L)$	$0.90*$	-0.03 0.01	-0.18 -0.11 $0.36***$

*Significant correlation at $p < 0.01$.

*Significant correlation at $p < 0.01$.
**Significant correlation at $p < 0.05$.

the exposed group just accounted for 7.2% of T-Hg, which is very low compared with the control group. Research showed that hair T-Hg analysis is difficult in differentiating between exogenous metal contamination and the metal deposited endogenously [\(Hat and Krechniak, 1993\)](#page-6-0). Therefore, we measured both hair T-Hg and Me-Hg to distinguish the Me-Hg intake and external exposure to mercury vapor. Low percents Hg as Me-Hg and no correlation between hair T-Hg and hair Me-Hg explained the different exposure route of inorganic mercury and Me-Hg. The workers were exposed to mercury vapor through inhalation, and the exposure route of Me-Hg may be through intake of polluted diet. Previous studies demonstrated that rice cultivated in mercury mining areas generally contained high level of Me-Hg ([Horvat et al.,](#page-6-0) [2003;](#page-6-0) [Qiu et al., 2006\)](#page-6-0). The local residents in the study area seldom eat fish and rice is the staple food. Thus, the intake of mercury contaminated rice as a route of Me-Hg exposure to the local inhabitants in mercury mining areas in Guizhou Province should be taken into account.

3.3. The impacts of smoking and alcohol drinking on U-Hg and hair mercury

The correlation coefficients between age and hair T-Hg, Me-Hg, and U-Hg for the exposed group and the control group were analyzed and listed in Table 4. The comparison of hair Hg and U-Hg levels between smokers and nonsmokers for the exposed group and the control group is given in [Table 5.](#page-5-0) As tobacco leaves contain mercury, smoking may also contribute to inhalation exposure. However, no significant difference in U-Hg was observed between smokers and nonsmokers both for the exposed group and for the control group. It is obvious that smoking may have very less contribution to mercury exposure for smelting workers compared with inhalation of highly Hg contaminated air.

The comparison of hair Hg and U-Hg levels between alcohol drinkers and nondrinkers for the exposed group and the control group is listed in [Table 6](#page-5-0). For the control group, alcohol drinkers have significant lower mean hair T-Hg concentrations ($p = 0.003$) and hair Me-Hg concenTable 4

The correlation coefficients between age and hair T-Hg, hair Me-Hg, and U-Hg in the exposed and the control group

	Hair T-Hg		Hair Me-Hg		$U-Hg$	
	EG.	CG	EG	CG	EG	CG
Age	$0.452*$	0.276	-0.042	0.209	0.399	-0.209

EG, the exposed group and CG, the control group.

*Significant correlation was observed at a level of $p < 0.05$.

trations ($p = 0.002$) than the nondrinkers. A number of interactions have been identified for chemicals that affect the pharmacokinetics and/or toxicity of mercury compounds. Generally, ethanol inhibits the enzyme catalase, which is the main enzyme responsible for the oxidation of mercury vapor into ionic mercury in blood. Ethanol consumption can decrease mercury vapor absorption [\(USEPA, 1997](#page-6-0); [UNEP, 2002](#page-6-0)). However, for the control group, the source of hair T-Hg and Me-Hg is through diet intake, but not from inhalation of mercury vapor. Generally, the alcohol drinkers eat less rice, which may be responsible for the lower hair T-Hg and Me-Hg concentrations. For the exposed groups, on the other hand, drinking alcohol is not the decisive factor of hair Hg and U-Hg levels.

The results indicate that alcohol drinking and smoking are not crucial factors controlling the urine and hair mercury levels for the exposed and the control group.

3.4. Mercury in the air and its relationship with U-Hg

Total gaseous mercury (TGM) concentrations in the ambient air in Wuchuan mining areas were highly elevated. The mean TGM concentration near the smelting furnace was up to $40 \mu g/m^3$ (n = 10), greatly exceeding $20 \mu g/m^3$ according to the Chinese occupational criterion (GB 16227-1996). In 1980, the World Health Organization (WHO) recommended an 8-h time-weighted average (TWA) mercury exposure standard of $25 \mu g/m^3$ [\(WHO,](#page-6-0) [1980\)](#page-6-0). The serious mercury contamination in ambient air was certainly attributed to the artisanal mercury smelting activities. The mercury emission factors (the proportion of mercury in ore is released to the ambient air) during the smelting processes ranged from 6.9% to 32.1% [\(Li et al.,](#page-6-0) [2006\)](#page-6-0); as a result, a large amount of mercury vapor was released to the ambient air.

Many studies have showed a strong correlation between the level of mercury in urine and the level of elemental mercury in air in occupational settings. Values for air concentration (in μ g/m³) are approximately the same as those for urine mercury concentration (expressed in μ g/g Cr) ([WHO, 1991](#page-6-0)). A significant correlation $(r^2 = 0.599)$; $p<0.001$) was found between mercury in air versus urine from various studies, including at lower air concentrations ranging from approximately 10 to $50 \mu g/m^3$ ([Tsuji et al.,](#page-6-0) P. Li et al. / Environmental Research 107 (2008) 108–114 113

Table 6

Comparison of hair Hg and U-Hg levels between drinkers and nondrinkers for the exposed group and the control group

**Significant difference was observed between drinkers and nondrinkers at $p < 0.01$.

Table 7 Clinical symptoms and correlative parameters of mercury poisoning workers in Wuchuan areas

	Clinical symptom	Urinary T-Hg $(\mu g/g Cr)$	Urinary parameters	Urinary β 2-MG (μ g/g Cr)
Worker 1	Lightly tremor (finger and eyelid)	3680	Hematuria $(+ +)$	$\overline{}$
Worker 2	Lightly tremor (eyelid)	202	Hematuria (\pm)	72.7
Worker 3	Lightly tremor (eyelid)	567	Normal	105
Worker 4	Gingivitis and typical dark-line on gums	1370	Normal	52.7
Worker 5	Gingivitis and typical dark-line on gums	586	Normal	326
Worker 6	Lightly tremor (finger and eyelid), gingivitis	229	Proteinuria $(+)$	204

[2003](#page-6-0)). The regression equation fitting to all studies is

$$
urine = 3.24 \times air^{0.833}.
$$
\n⁽¹⁾

Using Eq. (1), however, the corresponding urine mercury should just be about $70 \mu g/L$, if the average mercury concentration in air is $40 \mu g/m^3$. According to the [WHO](#page-6-0) [\(1991\)](#page-6-0), air mercury concentration of $40 \mu g/m^3$ is approximately corresponding to $40 \mu g/g$ Cr urinary mercury. However, the geometric mean of urinary mercury for the smelting workers in YQG in Wuchuan mining areas was $602 \mu g/L$ (463 $\mu g/g$ Cr) corresponding to the air mercury concentration of $40 \mu g/m^3$. During our investigation, TGM concentrations in ambient air around the smelters were only measured at one site 2–3 m away from one of the smelter. The sampling time of TGM measurement only lasted for 5 min. It is obviously that the average TGM concentrations of $40 \mu g/m^3$ is not representative of the average personal exposure since air mercury levels are highly variable (i.e. [Symanski et al., 2000](#page-6-0)). In order to better evaluate Hg vapor exposure for the smelting workers, systematical air Hg measurements are needed ([Roels et al., 1987\)](#page-6-0).

3.5. Clinical symptoms

Most studies focused on the effects on the central nervous system following occupational exposure to mercury vapor, and the occupational exposure has resulted in erethism, irritability, excitability, excessive shyness, and insomnia as the principal features of a broad-ranging functional disturbance. With continuing exposure, a fine tremor develops, initially involving the hands and later spreading to the eyelids, lips, and tongue, causing violent muscular spasms in the most severe cases ([WHO, 1990\)](#page-6-0). Gingivitis and typical dark line on gums have been reported after high inhalation exposures [\(Bluhm et al.,](#page-6-0) [1992](#page-6-0); [Barregard et al., 1996\)](#page-6-0). Clinical symptoms were also found in the smelting workers by health examination. Clinical symptoms and correlative parameters of mercury poisoning workers in Wuchuan areas are listed in Table 7. Symptoms include finger and eyelid tremor, gingivitis and typical dark-line on gums were observed in six workers. These indicated that physical impairments were occurred and the workers were heavily exposed to mercury vapor during the process of cinnabar roasting.

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