Mercury Exposure in Children of the Wanshan Mercury Mining Area, Guizhou, China

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Abstract: To evaluate the mercury (Hg) exposure level of children located in a Hg mining area, total Hg concentrations and speciation were determined in hair and urine samples of children in the Wanshan Hg mining area, Guizhou Province, China. Rice samples consumed by these same children were also collected for total mercury (THg) and methyl-mercury (MeHg) analysis. The geometric mean concentrations of THg and MeHg in the hair samples were 1.4 (range 0.50–6.0) µg/g and 1.1 (range 0.35–4.2) µg/g, respectively, while the geometric mean concentration of urine Hg (UHg) was 1.4 (range 0.09–26) µg/g Creatinine (Cr). The average of the probable daily intake (PDI) of MeHg via rice consumption was 0.052 (0.0033–0.39) µg/kg/day, which significantly correlated with the hair MeHg concentrations (r = 0.55, p < 0.01), indicating that ingestion of rice is the main pathway of MeHg exposure for children in this area. Furthermore, 18% (26/141) of the PDIs of MeHg exceeded the USEPA Reference Dose (RfD) of 0.10 µg/kg/day, indicating that children in this area are at a high MeHg exposure level. This paper for the first time evaluates the co-exposure levels of IHg and MeHg of children living in Wanshan mining area, and revealed the difference in exposure patterns between children and adults in this area.

Keywords: mercury exposure; Methyl Hg; total Hg; probable daily intake (PDI); children; mercury mining; Wanshan; China

1. Introduction

Mercury (Hg) is a toxic metal that has harmful effects on human health. The toxicity depends on its chemical forms, among which inorganic Hg (IHg) and methyl Hg (MeHg) are the most important chemical forms. IHg may cause a variety of adverse effects, with damage to the central nervous system and kidneys being the most prominent [1,2]. MeHg may pose a threat to the sensory, visual, and auditory functions, cerebellum, as well as to the cardiovascular system [3,4].

The most important routes of human exposure to IHg are inhalation of Hg vapor and accidental ingestion of Hg^{2+} [1]. Hg in bloodstream is then distributed to the tissues around the body including the central nervous system [5,6]. The major elimination routes of IHg are urinary and fecal excretion [7]. The half-life of inorganic Hg (IHg) in urine is about 30–70 days [1]. Urinary Hg measurements are widely used for the exposure assessment of IHg to humans [8]. As for MeHg, up to 95% of MeHg in diet is absorbed into the blood stream, then distributed to the tissues—especially in brain [9]. The primary route of excretion of MeHg is mainly as Hg^{2+} in feces (90%) and urine (10%) [10]. The half-life of MeHg...
in body is about 50 days [3,9]. Both hair and blood Hg levels are widely used as biomarkers of MeHg exposure [8].

Compared with adults, children are more susceptible to the health effects of Hg. Hg exposure may damage the developing central nervous system of children due to the permeable blood–brain barrier [11–13]. Additionally, even low or moderate levels of Hg exposure may also cause subtle neurodevelopmental abnormalities in the children [14–17]. Axelrad et al. [18] estimated that in low level MeHg exposure populations, a child could lose 0.18 Intelligence Quotient (IQ) points for each part per million increase of Hg in the mother’s hair. Another studies, researchers found that MeHg can interfere with the normal functioning of the cardiovascular system. Exposed to low doses of MeHg during the prenatal period can cause the coefficient of variance of the heart rate in boys to decrease by 47% seven years old [19]. More importantly, neurotoxicity associated with MeHg exposure in young children has been found to be irreversible [17,20,21], and the impairment of the intellect with a probable adverse impact on general mental ability may last throughout life [17].

Children are much more vulnerable to Hg exposure (both IHg and MeHg forms) than adults. Hg vapor inhalation, dental amalgamation as well as the consumption of Hg-contaminated food (e.g., fish or rice) are main Hg exposure pathways for both adults and children. On the other hand, there are Hg exposure pathways that are specific to children. For instance, the placental barriers do not protect the fetus from the MeHg transfer and bioaccumulation from the mother’s blood. Breast milk can also be a relevant MeHg exposure source [22]. Further, certain types of medical agents, such as thimerosal-containing vaccines, can lead to IHg exposure [22]. Meanwhile, certain behaviors associated with childhood (e.g., playing outside in the sand or soil, putting their hands in their mouths, etc.) tend to make children more vulnerable to exposure to Hg. In addition, working or living close to mercury-mining areas may also result in high Hg (both MeHg and IHg) exposure in children [23]. Children are much more likely to be exposed to Hg compared with adults.

In China, Wanshan is a historical Hg-mining area; this activity began during the Qin Dynasty (221 B.C.) and ended recently, in 2003. The long history of Hg-mining activities has seriously polluted the local environment [24,25] and has also posed serious threats to the health of local residents [26–30]. Mine wastes and the simple artisanal mercury smelting processes caused a serious gaseous Hg contamination in ambient air [28,29,31]. On the other hand, MeHg accumulated in the rice cultivated in the mining area [28,32], and its consumption is the main route of human MeHg exposure in Wanshan [33].

However, studies on Hg exposure in children and related health risks have not been conducted in the study area. The purpose of this study was to evaluate the implications of co-exposure of IHg and MeHg to the children (ages six to thirteen) in this area and to reveal and understand the difference in exposure patterns between children and adults.

2. Materials and Methods

2.1. Study Area

Wanshan features a hilly, karstic terrain covering an area of approximately 338 km\(^2\) and with an elevation varying from 270 to 1149 m above sea level. Karstic terrain is a landscape formed from the dissolution of soluble rocks such as limestone, dolomite, and gypsum. It has a subtropical, humid climate characterized by abundant precipitation and mild temperatures. Accordingly, the area is divided into four regions associated with its corresponding watershed: Xiaxi, Aozhai, Huangdao and Gaolouping (regions A, B, C and D, respectively). Most of the Hg mines and mine wastes are scattered in the valleys of the mid-western region, situated upstream of the rivers (Figure 1). Surface waters around the Wanshan Hg mines and retorts were significantly Hg-contaminated by the mine waste, but the impacted areas are limited to 6–8 km downstream from Hg mine wastes [34].
The local economy of Wanshan is undeveloped, as the per capita gross domestic product (14,914 RMB, 2400 USD) was about half of the national average in China in 2011 [35]. The population in 2012 was 64,000, with the rural population constituting about 80% of the total. There are 4202 primary school students (five to fourteen years old) living in this area, among which 45% are female and 55% are male. These data were obtained from the local government [35].

2.2. Sample Collection and Preparation

A total of 29 primary schools were located in this area. In the study, two primary schools within each region were selected. Inside regions A, B, and C, the selected two schools were: (1) distributed along the respective rivers of Hg catchment, which is easier to be compared with the results of adults in formal studies; and (2) within varying distances from the sources. A1 was located on the upper stream of A2 in region A, along the Xiaxi River; B1 was located upper stream of B2 in region B, along the Aozhai River; and C1 was located on the upper stream of C2 in region C, along the Huangdao River. In region D, only one primary school was located on the downstream. As a result, D1 was located in the center of the Wanshan town, upstream of all of the mining sites, while D2 was located downstream of the Gaolouping River. Spatial distribution of the sampling sites within the Wanshan mining area is shown in Figure 1.

Sampling was conducted in March 2013. All of the students at school in A1 and C1 (100%) were invited to participate in the experiments. For the other six sampling sites, a class in grade three was chosen randomly in each school, and all of the students in the class were invited to participate in the experiment. In total, 237 primary school children were sampled. After eliminating the samples from children who did not complete hair and urine samples and questionnaires, 217 children were analyzed in this study. All of the participants lived in villages near their schools, and none of them had left the area in the last three months. Hair samples were cut with stainless steel scissors from the occipital region of the scalp, bundled together with strips of scrip, placed and sealed in polyethylene bags, properly identified and brought to the laboratory. Hair samples were washed with nonionic...
detergent, distilled water and acetone and then dried in an oven at 60 °C overnight prior to analysis. Urine samples were collected by cups, then transferred into 50 mL pre-cleaned plastic centrifugal tubes, preserved by adding trace-metal grade HNO₃ (to 10% of the total volume), hermetically sealed, transported to the laboratory and kept at 4 °C until analysis.

It is assumed that there are no losses of MeHg during cooking processes for rice [36], so, in our study, only raw rice (white rice) was sampled. One raw rice sample from each student’s home and three raw rice samples from school cafeterias at each site were collected. Rice from the children’s home was brought to us by the children themselves, while rice from school cafeterias was collected directly from schools. According to the information in the questionnaires, rice from the children’s home was cultivated by themselves, while rice from the school cafeterias was purchase from the local market. The numbers of samples vary at the different sites (range from 7 to 25). A total of 146 rice samples were collected from the students’ home eventually (12 samples in A1, 25 samples in A2, 15 samples in B1, 21 samples in B2, 7 samples in C1, 22 samples in C2, 19 samples in D1, and 25 samples in D2), while a total of 24 rice samples were collected from school cafeterias. Rice samples were air-dried, crushed, poured through a 150-µm sieve and stored in polyethene bags.

Each child was required to fill out a questionnaire which included information such as age, gender, home location, amalgam use by his family, illness, food consumption habits and frequency of eating at the school cafeterias. The teachers explained the questionnaire and helped the children to fill the questionnaires. All children and their guardian gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol obtained ethics approval from the Institute of Geochemistry, Chinese Academy of Sciences (20111201).

2.3. Analytical Methods

The first 3 cm hair sample from the scalp, which reflected the recent exposure in the study area, was selected for THg and MeHg analysis. For the THg analysis, hair samples were measured using Lumex RA915+ Hg analyzer coupled with PYRO 915+ pyrolysis attachment by thermal decomposition. Urine and rice samples were digested in a water bath (95 °C) using a fresh mixture of acid composed of HNO₃/H₂SO₄ (v/v 4:1). A suitable aliquot of the digest was taken for THg analysis by cold vapor atomic fluorescence spectrometry (CVAFS, Tekran 2500, Tekran Inc., Toronto, ON, Canada) preceded by BrCl oxidation, SnCl₂ reduction and purge and thermo-reduction of Hg following USEPA Method 1631 [37].

For the MeHg analysis, hair samples were digested with 25% KOH, while rice samples were digested using the KOH-methanol/solvent extraction technique [38]. The digested samples were then measured using aqueous ethylation, purge, trap and GC-CVAFS (Brooks Rand Model III, Brooks Rand Laboratories, Seattle, WA, USA) following USEPA Method 1630 [39].

2.4. Quality Control

Method blanks, certified reference materials (CRM) and duplicates were used for conducting quality control. The corresponding analytical results of CRMs are listed in Table 1.

| Producer          | CRM       | Matrix           | n  | Hg Speciation | Obtained Value | Certified Value | Recovery (%) |
|-------------------|-----------|------------------|----|---------------|----------------|----------------|--------------|
| IGGE, CAGS        | GBW10020  | Citrus Leaves    | 6  | THg (µg/kg)   | 149 ± 2        | 150 ± 20       | 99 ± 5       |
| China CDC         | ZK200-2   | Human Urine      | 13 | THg (µg/L)    | 45 ± 4.0       | 49 ± 4.2       | 92 ± 7       |
| IGGE, CAGS        | GBW07001  | Human Hair       | 28 | THg (µg/kg)   | 363 ± 9        | 360 ± 80       | 101 ± 5      |
| Japan, NIES       | NIES-13   | Human Hair       | 17 | MeHg (mg/kg)  | 3.4 ± 0.4      | 3.8 ± 0.4      | 89 ± 10      |
| Canada, NRC       | TORT-2    | Lobster          | 15 | MeHg (µg/kg)  | 132 ± 11       | 152 ± 13       | 87 ± 14      |

IGGE, CAGS: Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences, China; CDC: China Center for Disease Control and Prevention; NIES: National Institute for Environmental Studies; NRC: National Research Council.
The Detection Limits for THg were 0.5 ng/g and 0.1 ng/g for hair and rice, respectively, and 0.2 ng/L for UHg. The CRMs GBW07601, GBW10020 and ZK020-2 were used for the THg analysis of hair, rice and urine, respectively. The recoveries were 101%, 99%, and 92%, respectively. The THg analysis accuracy, obtained from ten duplicate measurements of samples, were 7.8% for hair, 7.6% for rice and 4.0% for urine.

The CRM NIES-13 was used for the MeHg analysis of hair while TORT-2 was used for the MeHg analysis of rice. The recoveries were 89% and 87%, respectively, while the relative percentage difference was lower than 10% for MeHg in hair and rice duplicate samples.

To take hydration and urinary flow rate into account, Hg concentrations in urine were adjusted by Creatinine (Cr) excretion. Urine Cr concentrations were determined by an HITACHI 7170A automatic biochemical analyzer (HITACHI, Tokyo, Japan) in Guizhou Provincial People's Hospital within 24 h after sampling. UHg results were given in the unit of µg/g Cr.

2.5. Calculation of the Probable Daily Intake (PDI) of MeHg

The probable daily intakes of MeHg via rice consumption were calculated according to the following equation:

\[ \text{PDI} = \left( \text{Ch} \times f + \text{Cs} \times (1 - f) \right) \times \text{DI/W} \]  

where PDI refers to the probable daily intake of MeHg (µg/kg/day); Ch and Cs refer to the MeHg concentrations (µg/g) in rice collected from the students’ homes and schools, respectively; f refers to the frequency at which the children ate rice at home, which was obtained in the questionnaires; W refers to the students’ body weights; and DI refers to the daily intake of rice: 250 g/day in this study according to results obtained from the questionnaires, which is comparable to the daily rice intake of the Guizhou rural population (371 g/day) from the official statistical yearbook in 2012 [35].

2.6. Statistical Analysis

Statistical analyses were performed with SPSS 19 for Windows. The data are tested for normal distribution by the Kolmogorov–Smirnov test. If they are not normally distributed, the data are log transformed for further statistical analysis. The characteristics of the data were described in Mean ± Standard Deviation (SD) and Geomean for descriptive statistics. Mean values of the data at different sites were compared using independent-sample t tests and analysis of variance (ANOVA). The correlation coefficients among UHg, hair THg, MeHg and PDIs in each site were studied by the Pearson correlation analysis. Results of statistical tests were considered statistically significant if \( p < 0.05 \).

3. Results

3.1. Basic Information

Details of the basic information of the participants are listed in Table 2. A total of 18 children in A1 and 29 children in C1 (100% of all students at the schools), from grade one to grade three were investigated in this study. For the other six sampling sites, 88% of all students at the school in B1, and 6.3%–14% in A2, B2, C2, D1 and D2 were investigated in this study. The pupils were distributed within 51% girls and 49% boys. The average age of the children was 9.8 ± 1.3 (6–13) years old, and the average body weight (bw) and height were 28 ± 7.3 (17–70) kg and 133 ± 9.0 (110–160) cm, respectively.
3.2. Hg in Rice

THg and MeHg concentrations are shown in Figure 2. THg concentrations in rice from the children’s homes ranged from 1.3 to 166 ng/g, with a mean of 14 ng/g, and, 37% exceeded the tolerance limit of Hg in human food (20 ng/g) as recommended by the Chinese National Standard Agency (CNSA) [40]. The averages of rice THg concentrations at each site ranged from 5.2 to 57 ng/g. MeHg concentrations in rice from the children’s homes ranged from 0.29 to 65 ng/g, with a mean of 6.1 ng/g. Among the eight sampling sites, average of rice MeHg concentrations ranged from 2.6 to 17 ng/g.

![Figure 2. Concentrations of THg and MeHg in rice from: school cafeterias (a); and students’ homes (b) at different sites in the Wanshan Province (March 2013).](image-url)

THg concentrations in rice samples collected from school cafeterias ranged from 4.7 to 42 ng/g, with a mean of 9.1 ng/g. These results were significantly lower than those of the THg concentrations in rice from the students’ homes but were still higher than concentrations in rice measured from the Guiyang market (2.7 ng/g) [41]. The averages of THg concentrations in the rice from school cafeterias in A1 and A2 (39 and 40 ng/g) exceeded the maximum permissible limit of 20 ng/g in foods set by CNSA [40]. The MeHg concentrations in the rice from school cafeterias (5.9 ng/g, ranging from 0.85 to 21 ng/g) were significantly lower than those in rice from the children’s homes.
3.3. Hg in Hair

THg and MeHg concentrations in the hair samples of the children at different sites are shown in Figure 3. The mean THg concentration in all hair samples was 1.4 µg/g (with a range of 0.50 to 6.0 µg/g), but significant differences were shown among the eight sites. Site A1 showed significantly higher (p < 0.05, AVOVA) hair THg concentrations (with a mean of 3.3 µg/g) than seven other sites (0.96–1.9 µg/g). In addition, 75% (163/217) of hair THg concentrations exceeded the reference value of 1 µg/g as suggested by the United States Environmental Protection Agency (USEPA) [42], while 18% (40/217) exceeded the reference value of 2.3 µg/g as suggested by the Joint Expert Committee on Food Additives (JECFA) [43]. Remarkably, in A1, 100% of the hair THg samples surpassed the USEPA reference value, and 80% surpassed that of the JECFA.

The mean hair MeHg concentration in all of the children was 1.1 µg/g (with a range of 0.35 to 4.2 µg/g). The average value across the eight sites ranged from 0.75 to 2.6 µg/g, with the highest in A1 and the lowest in C2. MeHg concentrations in hair accounted for 78% ± 15% of THg for all of the children (ranging from 39% to 99%) with no obvious difference found between the different sites (p > 0.05, AVOVA). Significant correlations were found between hair THg and MeHg concentrations at the eight sites (R² values were between 0.74 and 0.94, p < 0.01 for all pairs). No differences of hair Hg concentrations between different genders or different ages have been observed (p > 0.05 ANOVA).

3.4. Hg in Urine

The mean UHg concentration was 1.4 µg/g Cr for all eight sites (Figure 3). Similar to hair THg, the mean UHg concentration was highest at site A1 (8.6 µg/g Cr). Nine percent of the children at
all eight sites exceeded the reference value of 5 \( \mu g/g \) Cr suggested by the United Nations Industrial Development Organization (UNIDO) [44]. However, at site A1, about 73% of the children exceeded this reference value. No differences of UHg concentrations between different genders or different ages have been observed (\( p > 0.05 \) ANOVA).

### 3.5. PDIs of MeHg

The PDIs of MeHg in children across the eight sites ranged from 0.0033 to 0.34 \( \mu g/kg/day \), with a mean of 0.052 \( \mu g/kg/day \) (Figure 4). Eighteen present (26/141) of the PDIs exceeded the reference dose (RfD) of 0.10 \( \mu g/kg/day \) suggested by the USEPA [39], while, in site A1, 100% (11/11) of the PDIs exceeded this RfD. The PDIs of MeHg was significantly correlated with the hair MeHg concentrations (\( r = 0.55, p < 0.01 \)), indicating that children in this area are at high level of MeHg exposure.

![Figure 4. PDIs of MeHg in the children at different sites. Each box represents the interquartile range (25th and 75th percentile), the band near the middle of the box is the 50th percentile (the median), and the whisker represents 5th and 95th percentile.](image)

### 4. Discussion

#### 4.1. Geographical Differences

Compared with the sampling sites downstream, hair Hg concentration upstream was higher in regions A, B and C (\( p < 0.001 \) for A1 and A2; \( p < 0.001 \) for B1 and B2; \( p < 0.01 \) for C1 and C2, independent \( t \)-test) (Figure 3). The same tendency was also observed in soil, river sediment and rice cultivated in this area [24,35,45,46]. The THg concentrations of the samples were inversely proportional to their distance from the mine waste. Indeed, Hg levels in the samples matched their background concentrations in this area, even at a distance of several kilometers [24,45]. The distance-related concentrations of Hg in rice finally resulted in distance-related concentrations of hair Hg. For UHg, distance-related concentration variations were found only in region A, while no obvious concentration variations were observed in the other three areas (\( p < 0.05 \) for A1 and A2, \( p > 0.05 \) for the other pairs, independent \( t \)-test). Hg contained in urine primarily originated from: (1) inhalation of contaminated air; (2) ingestion of contaminated vegetables with a high ratio of IHg [47–49]; or, (3) ingestion of soil accidentally and deliberately by children during playing.
4.2. Comparison with Adults

Four sampling sites were identified that matched the location previously undertaken on adults in the same geographic area by Li [49] (Table 3). No significant differences have been observed between Hg concentrations in rice from students’ home and adults’ home (p > 0.05, independent t-test).

Hg concentrations in children’s hair were slightly less than in adults within A1 and A2 zones (p < 0.05 for THg in A1, while p < 0.01 for THg in A2 and MeHg in both A1 and A2, independent t-test). This may be explained by two reasons. First, the ingestion of the lower Hg-contaminated rice at school cafeterias than at home (Table 3) may lower the exposure levels of children compared to adults. Second, the specific metabolic reactions of children may affect the behavior of Hg in children’s bodies. For instance, the mean breathing rate over the first 12 years of life is almost twice as fast compared to adult breathing rates (452 vs. 232 L/kg/day, respectively) [50], showing that the metabolic reactions of children is much more active than adults, which can affect the Hg transfer and speciation in children’s bodies. Additionally, the enzymatic activity of children may modify different reactions kinetics such as absorption, methylation/demethylation, distribution, and excretion compared with adults’ activity. More studies are needed to understand the details of Hg metabolic reactions of children’s body [51,52].

Conversely, UHg was higher in the children’s urines than in adults ones in A1 and A2 areas (p < 0.01, independent t-test), which suggests a higher IHg exposure for children than for adults in these areas. Except for the more active MeHg demethylation reactions inside children’s bodies [50,52], which may result in a higher UHg level [53–56], other processes may also be important. For instance, the higher breathing rate of children may cause a higher respiring amount of Hg vapor; smaller airways of children would tend to increase particle deposition compared with adults [57]. Additionally, the breathing zone height for children is much lower than for adults, where there is more Hg vapor as well as particle Hg. More importantly, the unique behaviors of children, such as regularly placing their hands in their mouths, causing the accidental or deliberate ingestion of contaminated soil during play [58–60], may also cause a higher IHg exposure level.

4.3. Worldwide Comparison

Hair Hg concentrations in children in this study were compared with other research studies conducted the past 10 years in mining sites throughout the world (Table 4). Brazilian children living in a gold-mining region showed the highest hair THg concentrations, with average values of 2.3 to 17 µg/g [61–67]. The results in this study were comparable with THg concentrations in children’s hair from Ghana and Portugal [68,69]. Children located in the Philippines, Thailand and Bolivia showed relatively low hair THg concentrations (with average values ranging from 0.49 to 0.99 µg/g) [70–72].
### Table 3. Comparison of Hair THg, MeHg and UHg between children and adults in the Wanshan mining area.

| Sampling Site | Hair THg (µg/g) | Hair MeHg (µg/g) | Hair MeHg/THg (%) | Urine THg (µg/g Cr) | Urine MeHg (µg/g) | Rice (Home) THg (µg/g) | Rice (School) THg (µg/g) | Rice (Home) MeHg (µg/g) | Rice (School) MeHg (µg/g) | Location and Description |
|---------------|-----------------|------------------|-------------------|---------------------|-----------------|------------------------|-------------------------|------------------------|-------------------------|--------------------------|
| D             | 5.1 ± 3.2       | 3.7 ± 2.2        | 75 ± 16           | 9.1 ± 13            | 81 ± 71         | -                      | -                       | -                      | -                       | Near A1 [49], adults     |
| F             | 2.7 ± 1.2       | 1.9 ± 0.93       | 74 ± 21           | 1.3 ± 0.82          | 21 ± 5.5        | -                      | -                       | -                      | -                       | Near A2 [49], adults     |
| B             | 1.5 ± 0.59      | 0.79 ± 0.35      | 55 ± 14           | 1.1 ± 0.45          | 15 ± 10         | -                      | -                       | -                      | -                       | Near B1 [49], adults     |
| C             | 1.3 ± 0.89      | 0.80 ± 0.36      | 66 ± 18           | 2.6 ± 5.8           | 83 ± 3.3        | -                      | -                       | -                      | -                       | Near B2 [49], adults     |
| A1            | 3.3 ± 1.4 **    | 2.5 ± 0.98 **    | 71 ± 14           | 8.6 ± 8.3 **        | 59 ± 41         | 17 ± 7.3               | 17 ± 7.3                | 40 ± 3.9               | 9.1 ± 1.4               | This research, children  |
| A2            | 1.5 ± 0.68 **   | 1.0 ± 0.47 **    | 69 ± 17           | 3.1 ± 3.5 **        | 25 ± 34         | 9.5 ± 12               | 39 ± 3.0                | 8.9 ± 1.2              | 1.2 ± 0.4               | This research, children  |
| B1            | 1.3 ± 0.63      | 1.2 ± 0.40 **    | 80 ± 17 **        | 1.4 ± 1.5           | 11 ± 8.7        | 6.4 ± 8.7              | 1.4 ± 1.2               | 1.2 ± 0.4              | 1.2 ± 0.4               | This research, children  |
| B2            | 0.9 ± 0.26      | 0.75 ± 0.26      | 83 ± 14 **        | 0.76 ± 0.91         | 5.9 ± 3.5       | 2.6 ± 2.5              | 37 ± 1.2                | 1.6 ± 1.5              | 1.3 ± 1.5               | This research, children  |

**p < 0.01 compared with adults in the comparable sampling site; *p < 0.05 compared with adults in the comparable sampling site.**

### Table 4. Comparison of hair THg concentrations in children around the world.

| Location                  | n   | Mean ± SD (µg/g) | Range (µg/g) | Remarks                                      | References                  |
|----------------------------|-----|------------------|--------------|----------------------------------------------|-----------------------------|
| Wanshan area, China       | 227 | 1.40 (GM)        | 0.09–5.98    | 5–12 years old, living in mine sites         | This research               |
| Kayabi, Amazonia, Brazil  | 40  | 16.55 ± 11.44    |              | Children, fish consumers in gold-mining area | [64]                        |
| Cururu, Amazonia, Brazil  | 86  | 4.76 ± 2.09      |              | Children, fish consumers in gold-mining area | [64]                        |
| Kaburua, Amazonia, Brazil | 77  | 2.87 ± 2.13      |              | Children, fish consumers in gold-mining area | [64]                        |
| Negro river basin, Brazil |     |                  |              | <15 years old, fish consumers in gold-mining area |                             |
| Sao Luiz do Tapajos, Brazil| 40  | 11.41 ± 7.16     | 0.8–28.17    | 0–12 years old, fish consumers in gold-mining area | [66]                        |
| Barreiras, Brazil         | 37  | 5.64 ± 5.55      | 0.4–27.82    | 0–12 years old, fish consumers in gold-mining area | [66]                        |
| Maranhao, Brazil          | 118 | 2.27 ± 1.84      | 0.13–9.54    | Far from prospecting areas, in the city of Abacetuba | [66]                        |
| RioTapajo’s, Amazonia, Brazil | 51  | 10.2             | 0.5–41.4     | A traditional riverine village, children eating fish | [65]                        |
| Rondonia, Brazil          | 11  | 6.24 ± 5.89      |              | 3–9 years old, in Gleba do Rio Preto riverine | [67]                        |
| Rondonia, Amazonia, Brazil| 31  | 3.57 ± 1.86      |              | 3–9 years old, Demarcacao area riverine      | [67]                        |
| Anwiaso, Ghana            | 7   | 4.27             | 0.06–28.3    | Children in gold-mining area                 | [68]                        |
| Sahuma, Ghana             | 21  | 1.61             | 0.15–5.86    | Children in gold-mining area                 | [68]                        |
| Tanoso, Ghana             | 11  | 1.21             | 0.07–3.19    | Children in gold-mining area                 | [68]                        |
| Elulbo, Ghana             | 15  | 0.62             | 0.32–2.19    | Children in gold-mining area                 | [68]                        |
| Madeira Island, Portugal  |     | 4.09             | 0.38–25.95   | 7 years old                                  | [63]                        |
| Island of Madeira, Portugal| 40  | 4.08 ± 7.07      |              | Children working with Hg                     | [69]                        |
| Island of Madeira, Portugal| 3.82 (GM)| 0.4–26         |              | 6.4–7.4 years old, fish consumers             | [69]                        |
| Island of Madeira, Portugal| 2.27 ± 0.83 |              |              | Children living in Hg-exposed area           | [69]                        |
| Faro Islands              | 917 | 2.99             | 1.7–6.1      | Children, 7-year-old, eating seafood         | [61]                        |
| Tagum, Davao del Norte, Philippines | 59  | 0.99 ± 1.16      | 0.28–20.39   | Schoolchildren, near a gold processing and refining plant | [70]                        |
| Phanom Pha, Phichit, Thailand | 59  | 0.93 ± 0.01      |              | Children living near the gold mining area    | [71]                        |
| Bolivian Altiplano, Bolivian | 242 | 0.49 (GM)        | 0.09–8.44    | 7–10 years old, live in polymetallic mining communities | [72]                        |

SD: Standard deviation; GM: Geometric mean value.
4.4. Relationship between Hair MeHg and PDI

A single-compartment model calculated the steady-state Hg concentration in blood from the average daily dietary intake as shown in the following equation [42]:

$$C = d \times A \times \frac{bw}{V} \times f$$ (2)

where:

- $C$ = MeHg concentration in blood (μg/L);
- $d$ = daily dietary intake (μg/kg/day);
- $A$ = absorption factor (unitless, 0.95);
- $b$ = elimination constant (0.014 per days for adults);
- $bw$ = body weight (kg);
- $V$ = volume of blood in the body (L); and
- $f$ = fraction of daily intake taken up by blood (unitless, 0.05 for adults).

The third equation was about ratios between hair and blood:

$$\frac{Ch}{Cb} = \frac{250}{1}$$ (3)

where Ch refers to the Hg concentration in hair (μg/g), Cb refers to the Hg concentration in blood (μg/L).

In this study, a significant correlation ($r = 0.55$, $p < 0.01$) was found between hair MeHg concentrations and the PDI of MeHg from rice in the children (Figure 5). The regression coefficient was 15, which was lower than that for adults reported by Li et al. ($y = 23x$, $R^2 = 0.74$, $p < 0.001$; [49]). Since both children and adults in this region have similar dietary habits, while the Hg inputs by less contaminated rice from school cafeterias was higher in children, the different regression coefficients can also be due to differences in metabolic processes between children and adults.

![Figure 5. Relationship between hair MeHg and PDIs of MeHg via rice consumption in the children at different sites.](image)

Firstly, the elimination constant ($b$) of children is higher than that of adults. Mammals are able to demethylate MeHg into IHg via intestinal bacteria, tissue macrophages and the liver [62,63]. Demethylation of MeHg in young children is much more effective than in adults [51,52], which results in a higher elimination constant of MeHg in children than in adults. Secondly, the proportion of blood to body weight ($\frac{V}{bw}$) is higher for children than for adults (8%–10% for children, 6%–8% for adults) [73].
4.5. Implication for Health of Children

Hair THg concentration is considered as a good biomarker for human MeHg exposure. The hair THg levels of 1.0 and 2.3 μg/g recommended by USEPA and JECFA, respectively, were adopted for risk assessment of MeHg exposure on developing fetuses. The cumulative frequencies of hair THg concentration levels in this survey are shown in Figure 6a. It was supposed that the distribution of different levels of hair Hg and UHg concentrations for all of the primary school students in each region were consistent with the results obtained in this study. The total numbers of children exceeding the limit levels in each region were obtained by multiplying the total number of primary school children in each region by exceeding rate (Figure 6b). In total, 61% (2556/4202) of the THg concentrations in primary school children’s hair exceeded 1.0 μg/g, while 8% (327/4202) exceeded 2.3 μg/g. These proportions were 75% (348/464) and 18% (84/464) in region A, and, 74% (1740/2348) and 10% (243/2348) in region D.

![Figure 6](image1.png)

*Figure 6.* The cumulative frequencies of hair THg concentration levels in each region (a); and the number of children at each level in each region (b).

UHg is considered a good biomarker for human IHg exposure, and UNIDO set a limit of 5 μg/g Cr for the general population. The cumulative frequencies of UHg concentrations in this survey are shown in Figure 7a. After multiplying the total number of primary school children in each region by the frequency of each UHg concentration level, the total number of children for each concentration level in each region was obtained (Figure 7b). Approximately 9.7% (272) of UHg concentrations in the children exceeded 5 μg/g Cr. Most of these children lived in region A (172) and region D (80), which indicates a high level of IHg exposure close to the Hg mines of the Wanshan region.

![Figure 7](image2.png)

*Figure 7.* The cumulative frequencies of UHg concentration levels in each region (a); and the number of children at each level in each region (b).
5. Conclusions

Hg exposure levels in the children were the highest in region A, two kilometers downstream of the Hg mine sites, which were seriously contaminated by mining activities. THg and MeHg concentrations in the children’s hair were high in the sites located upstream, especially in mining site A1. UHg concentrations were higher in children than in adults in the same region, which proved that children are more vulnerable to IHg exposure. Hair THg concentrations were lower in the children than the adults in the same region, which resulted from lower contaminated rice in school cafeterias. In the future, more research should be done to understand the metabolic processes the influence Hg bioaccumulation and excretion in young children. Moreover, pertinent policies should be taken to reduce the Hg exposure level of the children living in this area.

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