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Methylmercury Accumulation in Rice (*Oryza sativa* L.) Grown at Abandoned Mercury Mines in Guizhou, China

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Mercury is a global pollutant that can transform into methylmercury, a highly toxic and bioaccumulative organic form. Previous surveys have shown that fish is the main source of human methylmercury exposure, whereas most other food products have an average value below 20 μg/kg and primarily in the inorganic form. This paper reports that methylmercury in rice (*Oryza sativa* L.) grown at abandoned mercury mining areas contained levels >100 μg/kg in its edible portion and proved to be 10−100 times higher than other crop plants. The daily adult intake of methylmercury through rice consumption causes abnormally high methylmercury exposure to humans. The results demonstrate that rice is a methylmercury bioaccumulative plant and the main methylmercury source for human exposure in the areas studied.

KEYWORDS: Methylmercury; bioaccumulative; rice (*Oryza sativa* L.); human exposure; mercury mines

INTRODUCTION

Mercury (Hg) is a global and extremely toxic pollutant (*1*). Its mobility and toxicity are highly dependent on the speciation in environments (*2*). Both inorganic and methylated Hg species cause severe health problems in humans. However, methylmercury (MeHg), which is usually produced by anaerobic bacteria acting on inorganic Hg, is the main bioaccumulative species in the food web (*3*). The biogeochemistry of Hg in the food chain has received considerable attention because of the accumulation of MeHg in biota and its biomagnification.

Generally, Hg concentrations in most foodstuffs are below 20 μg/kg, and mainly in inorganic forms (*4*). Due to its lipophilic and protein-binding properties (*5*), MeHg can readily bioaccumulate in the organisms at the top of aqueous food webs, thus leading to elevated concentrations of MeHg in fish. Hence, human exposure to MeHg is predominantly derived from fish consumption, posing a worldwide human health threat (*3, 6*).

Mercury mining is one of the leading sources of environmental Hg contamination. Guizhou, in southwestern China, is in the largest circum-Pacific mercuriferous belt and produces numerous large cinnabar deposits, such as Wanshan, Danzhai, and Wuchuan Hg mines. The Wanshan Hg mines are in the eastern part of the province and are ranked as the largest Hg-producing center in China. Mining at Wanshan began in 221 B.C. and lasted until 2001. Significant quantities of gangues and mine-waste calcines, containing as much as 4400 mg/kg of total mercury (THg) (*7*) are scattered near old abandoned mine processing sites and retorts upstream of aqueous systems.

The mine-waste calcines produced by the inefficient smelting of Hg ores contain abundant elemental Hg and Hg salts (*8, 9*). Such toxic Hg compounds can be readily mobilized into streams and methylated in a variety of different habitats within an ecosystem and then bioaccumulate through complex food webs (*10, 11*). Therefore, the abandoned Hg mines continue to affect surrounding environments, in which one of most significant concerns is the conversion from inorganic Hg to MeHg and, in turn, its bioaccumulation.

Characterization of Hg in the Wanshan Hg mines has shown that active transformation of inorganic Hg to MeHg takes place. Elevated MeHg levels of up to 23 μg/kg (*12*) were reported in soil samples. Our recent study showed that MeHg in rice paddies was usually higher than that in cornfields (*7*). Because MeHg can be translocated within the plants more easily than inorganic Hg forms (*13, 14*), abundant MeHg in paddy soils might serve as a source of MeHg to rice plants. If so, then the rice could potentially contribute to critical human health issues because it is a traditional food staple for the residents in the study region.

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Our purpose here was to determine MeHg levels in rice harvested from the paddies in Hg mines in Wanshan. For comparison, MeHg in most other representative crop plants was analyzed as well. We also conducted human risk assessments of daily consumption of MeHg-contaminated rice.

MATERIALS AND METHODS

We sampled rice (*Oryza sativa* L.) in the vicinity of five Hg mines, including Laowu, Gouxi, Dashui, Sikeng, and Gaolou. Recently, Laowu and Gouxi have experienced some small-scale artisanal Hg mining activity; Gaolou is a region that is about 10 km downstream from abandoned Hg mines; Dashui and Sikeng are old Hg mining districts, which are piled with large quantities of calcines.

The rice plants collected were cropped on a regular basis for human consumption. The irrigating water in the paddies of the sample rice was heavily Hg-contaminated. Total Hg concentrations in water samples varied from 60 to 4460 ng/L with pH values ranging from 7.0 to 11. The rice plants can be harvested after 3 months of growth. During our sampling campaign, a final rice sample (rice seeds) was composed of three to five subsamples from several localities at each paddy. Similar to the rice sampling, four other typical crop species that grew in the Hg mining areas, including corn (*Zea mays* L.), rape (*Brassica campestris*), tobacco (*Nicotiana tabacum*), and cabbage (*Brassica oleracea*), were sampled for comparison. Those crops were grown in cornfields with no irrigation. All collected samples were stored in sealed polyethylene bags to avoid cross contamination and then shipped to the laboratory as soon after collection as possible.

In the laboratory, the hull from the rice seeds was initially removed using a scalpel. The remaining parts (edible portions) were thoroughly rinsed using deionized Milli-Q water and then air-dried at room temperature. Afterward, portions of dried samples were selected, crushed, and ground prior to digestion processes. The procedures for preparing other plant samples were similar to the process for the edible portions of rice.

Appropriate aliquot samples were prepared and then used for digestion at 95 °C in a water bath with a fresh mixture acid of HNO_3/H_2SO_4 for THg analysis (*15*). Other aliquot samples were digested using a KOH−methanol/solvent extraction technique for MeHg analysis. In this process, samples were first digested with KOH−methanol and then acidified. After that, MeHg in samples was extracted with methylene chloride and back-extracted from the solvent phase into water and then aqueous phase ethylated for MeHg analysis (*16, 17*). Total Hg was determined using dual-stage gold amalgamation method and cold vapor atomic fluorescence spectrometry (CVAFS) detection following method 1631 (*18*). Methylmercury was measured using gas chromatography (GC)−CVAFS detection according to method 1630 (*19*).

Quality assurance and quality control were determined using duplicates, method blanks, matrix spikes, and certified reference materials (TORT-2). Limits of determination were 0.01 μg/kg for THg and 0.003 μg/kg for MeHg in samples. An average MeHg concentration of 154 ± 3.3 μg/kg ($n = 5$) was obtained from TORT-2 with a certified value of 152 ± 13 μg/kg. Recoveries on matrix spikes of MeHg in samples were in the range of 83−110%. The relative percentage difference was <8.5% in duplicate samples.

RESULTS AND DISCUSSION

THg concentrations in rice and other crops were in the ranges of 10.3−1120 and 8.92−1160 μg/kg of dry weight, respectively. The peak value was presented in cabbage samples, which probably reflects a direct uptake of Hg^0 via foliage (*20*). The rice samples were found to exhibit slightly lower concentrations of THg than those of other samples; however, there was no significant variation of THg between rice and other crops (**Figure 1a**). The concentrations of THg in all samples averaged 300 μg/kg and greatly exceeded the National Permitted Limit of 20 μg/kg in foods in China. This demonstrated that entire crop plants in Wanshan were heavily affected by Hg mining activities.

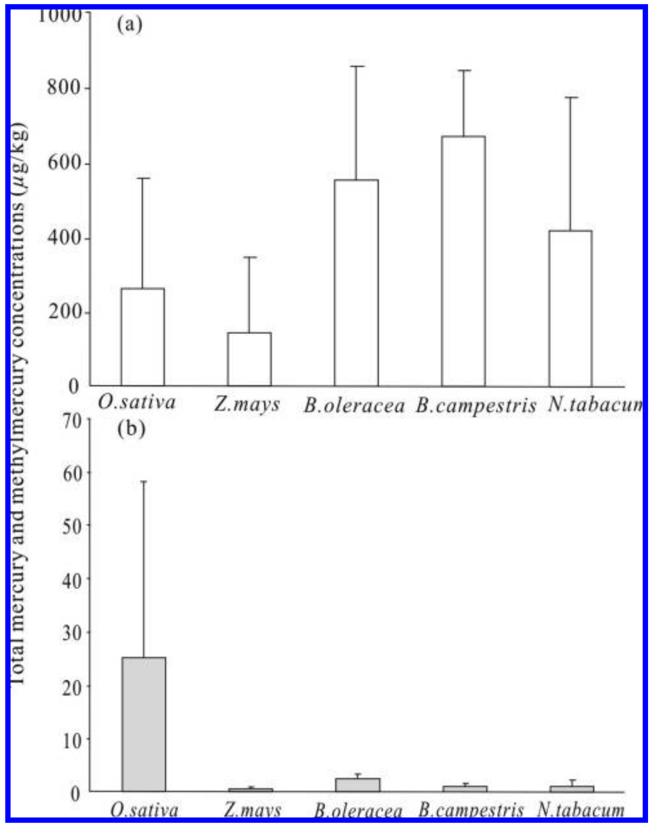

Figure 1. Average concentrations of total mercury (**a**) and methylmercury (**b**) in crops (with standard deviations; dry weight).

MeHg concentrations in rice and other crops were in the ranges of 1.61−174 and 0.278−4.21 μg/kg of dry weight, respectively. The rice samples displayed extremely high MeHg levels compared to other crops (**Figure 1b**). A mean value as high as 25.3 μg/kg MeHg in rice was observed.

Table 1 lists the MeHg concentrations in rice harvested from the five sites in Wanshan. The highest MeHg levels were present in Gouxi, ranging from 22.3 to 174 μg/kg with an average value as high as 62.3 μg/kg. Similar results for MeHg ranging from 10.1 to 43.1 μg/kg were observed in Laowu, with an average of 26.3 μg/kg. The concentrations of MeHg in rice yielded from Dashui, Sikeng, and Gaolou areas were slightly lower, ranging from 1.61 to 27.3 μg/kg. The maxima of MeHg levels in Gouxi and Laowu might reveal episodes heavily affected by Hg emissions from quantities of artisanal Hg mining activities.

No significant positive correlation between MeHg and THg in rice was observed (**Figure 2**). This probably reflects the fact that inorganic Hg and MeHg uptake in rice has complex mechanisms. The poor correlation might also suggest that MeHg in rice could not be a biotransformation of inorganic Hg in the plant. Interestingly, high percentages of MeHg to THg concentrations were found in rice, ranging from 1.4 to 93% (**Table 1**). The highest percentage was presented in a sample from Gouxi. An average value as high as 45% of MeHg to THg was found in samples yielded from Gaolou that are downstream from Hg mines. This showed that MeHg contamination of rice pervades even large areas in the region.

Previous surveys have documented that Hg in most foodstuffs is usually in inorganic forms except for fish (*4*). Our MeHg data in corn, rape, tobacco, and cabbage samples were comparable to the results published in the literature (*21–23*). However, MeHg data in rice were totally different and revealed that rice can accumulate MeHg to high levels. In our results, MeHg concentrations in rice usually were 10−100 times higher than

Table 1. Total Mercury and Methylmercury Concentrations in Rice Grown at Wanshan Hg Mines

site	mercury concn, μg/kg of dry wt, mean ± SD (*n*, range)		% of methylmercury
	total mercury	methylmercury	
Laowu	353 ± 240 (8, 246–928)	26.3 ± 13 (8, 10.1–43.1)	2.2−22
Gouxi	608 ± 290 (19, 49.4–1120)	62.3 ± 42 (19, 22.3–174)	2.3−93
Dashui	113 ± 120 (30, 31.0–565)	8.76 ± 7.3 (30, 1.61–27.3)	1.4−36
Sikeng	46.7 ± 31 (9, 19.3–100)	9.42 ± 8.2 (9, 1.84–21.2)	6.4−58
Gaolou	17.5 ± 9.5 (4, 10.3–31.3)	7.31 ± 2.4 (4, 4.29–10)	32−58

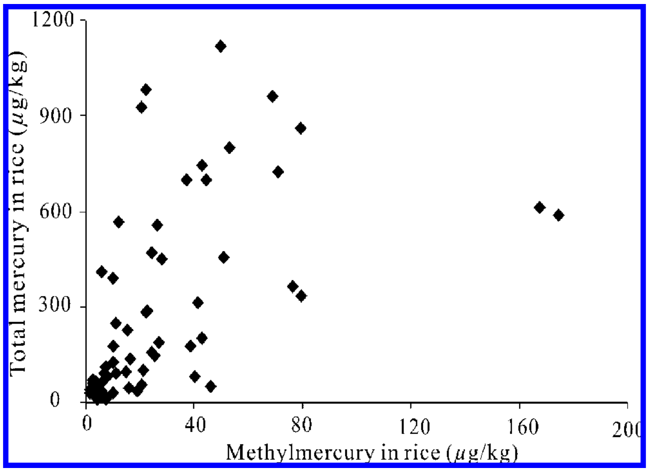

Figure 2. Association between total mercury and methylmercury in rice (linear regression: $y = 133 + 5.06x$; $R^2 = 0.32$).

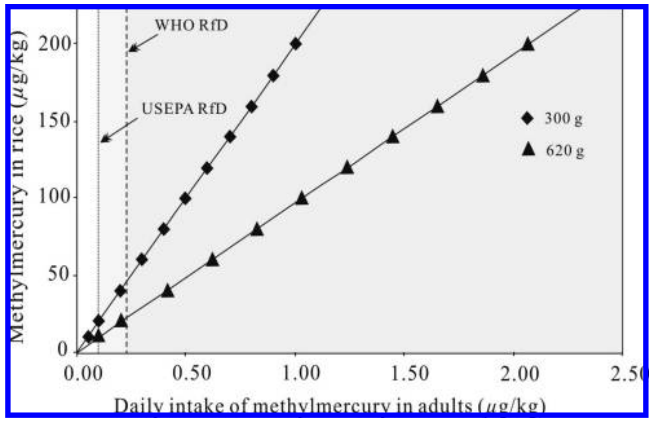

Figure 3. Assessment of human exposure to methylmercury via rice consumption: estimated daily intakes of methylmercury in an adult's rice consumption of 300 g (diamonds) and 620 g (triangles) in Wanshan. Intake values are calculated as micrograms per kilogram of body weight of a 60 kg person.

in other crops. Horvat et al. (*12*) also reported a MeHg value as high as 144 μg/kg in the rice harvested from the Dashui district in Wanshan. Although MeHg in rice varied widely in our survey, it might be the highest value record of MeHg on most foodstuffs. Those findings may confirm that MeHg can be accumulated in rice plants.

Plant uptake of Hg from the soil may be limited by the roots (*24*), but as mentioned above, MeHg can be translocated within plants more easily than inorganic Hg forms. The poor correlation between THg and MeHg concentrations in rice indicated that MeHg in rice could not be biotransformation of inorganic Hg in the plant and that the high MeHg levels in paddies in Wanshan (*7*) might be one of the most important MeHg sources to rice.

Rice is a traditional staple food for the local people in Wanshan. The daily per capita consumption of rice in 2005 in Dashui, for instance, was up to 620 g of dry weight per day for adults with average weight of 60 kg, that is, equivalent to MeHg ingestion of 10.3 g/kg of body weight per day.

Assuming the absorption of MeHg in bodies was 100%, we roughly calculated adult daily intake of MeHg via rice consumption. For an adult, routine consumption of 620 g per day of rice containing the average MeHg value of 25.3 μg/kg obtained in our study would provide a MeHg intake of 0.26 μg/kg of body weight per day. This value is slightly higher than the new dietary reference dose (RfD) of 0.23 μg/kg of body weight per day for MeHg recommended by United Nations Committee (*25*), but much higher than the RfD of 0.1 μg/kg of body weight per day for high MeHg exposure recommended by the U.S. EPA (*26*) (**Figure 3**). For the peak value of 174 μg/kg, however, we could get an extremely high MeHg intake of 1.8 μg/kg of body weight per day. This indicates that rice is a major source of human MeHg exposure in Wanshan.

Apparently, the population living in Gouxi was heavily exposed to MeHg, which resulted from the abnormally high MeHg levels in rice. Owing to the biomagnifications of MeHg, however, the present low MeHg levels in rice harvested from other areas might also result in an undesirable MeHg exposure to residents, especially the susceptible populations who live near those Hg mines and daily consume the rice. Therefore, the rice yielded from the Hg mines in Wanshan contained concentrations of this neurotoxin high enough to cause exposure risks to local peoples.

Recent experiments of rats' 20 day exposure to rice from Wanshan revealed an inducible lipid peroxidation in rat brain, liver, and kidney tissue, exhibiting an increase of c-jun mRNA (*27, 28*). Moreover, a study of 98 persons from the Wanshan Hg mining area showed a significant positive relationship between estimated rice MeHg intake and hair MeHg levels ($r = 0.65$, $p < 0.001$) (*29*). This investigation confirmed that rice in Wanshan indeed was an important route of MeHg exposure for the local residents. Those studies might also suggest that residents living in Hg mining areas in Wanshan are now suffering from heavy rice dietary MeHg exposure. In many Hg mining areas similar to Wanshan in Guizhou, therefore, MeHg contamination of rice would negatively affect the health of humans and wildlife.

Although fish is the primary source of MeHg exposure in human diets (*3*), our finding provides evidence of significant MeHg uptake by plants and also provides insight into a new pathway of human MeHg exposure (*29*). However, the mechanism of MeHg uptake and translocation by rice is unknown. Further study is needed for mechanisms on rice plants exposed in situ to high levels of MeHg in order to assess roles in plant uptake of MeHg. Consequently, the systematic assessment of human exposure and biomonitoring measurements remains to be further investigated in this region.

ABBREVIATIONS USED

MeHg, methylmercury; THg, total mercury; CVAFS, cold vapor atomic fluorescence spectrometry; GC, gas chromatography; RfD, reference dose; SD, standard deviation.

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