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Traditional Tibetan Medicine Induced High Methylmercury Exposure Level and Environmental Mercury Burden in Tibet, China

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Abstract

Highly elevated concentrations of total mercury (THg) and methylmercury (MeHg) were found in the municipal sewage in Tibet. Material flow analysis supports the hypothesis that these elevated concentrations are related to regular ingestion of Hg-containing Traditional Tibetan Medicine (TTM). In Tibet in 2015, a total of 3,600 kg of THg was released from human body into the terrestrial environment as a result of TTM ingestion, amounting to 45% of the total THg release into the terrestrial environment in Tibet, hence substantially enhancing the environmental Hg burden. Regular ingestion of TTM leads to chronic exposure of Tibetans to inorganic Hg (iHg) and MeHg, which is 34 to 3,000-fold and 0 to 12-fold higher than from any other known dietary

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ASSOCIATED CONTENT

Supporting Information

Additional information on the TTM (Table S1) and parameters of the PBPK model (Table S2).

sources, respectively. Application of a human physiology model demonstrated that ingestion of TTM can induce high blood IHg and MeHg levels in the human body. Moreover, 180 days would be required for the MeHg to be cleared out of the human body and return to the initial concentration i.e. prior to the ingestion of 1 TTM pill. Our analysis suggests that high Hg level contained in TTM could be harmful to human health and elevate the environmental Hg burden in Tibet.

Abstract Art

1. INTRODUCTION

Human activities have fundamentally altered the global biogeochemical cycle of the toxic element mercury (Hg) in the environment, which has been considered as one of the chemicals to cause serious public health problems worldwide.^{1–5} Chemical form, dose and exposure rate of Hg represent the most significant factors that are known to trigger toxic effects in the human body.^{6, 7} Methylmercury (MeHg) is one of the most toxic forms of Hg. It is a potent neurotoxin that biomagnifies in food-webs,⁸ and can cause long-term developmental delays in children and impaired cardiovascular health in adults.^{6, 7, 9} Chronic exposure to MeHg is of major concern for human health, and consumption of marine fish has been claimed the dominant exposure route to people globally.^{10–12} Nevertheless, in some regions of Asia, MeHg exposure routes remain highly debated. Recent studies have concluded that rice consumption in some Asian regions could be a significant dietary source of MeHg to people.^{10, 13, 14}

The Tibetan Plateau, generally labeled as “the third pole of the world” due to its very high altitude (average 4,500 meters), is recognized as the cleanest (i.e. not impacted by chemical pollution) region in China.¹⁵ However, in contrast to that notion, a recently published article showed that per capita releases of Total Hg (THg, refers to all forms of Hg) and MeHg from the municipal sewage in Tibet were higher than anywhere else in China.¹⁶ Traditional Tibetan Medicine (TTM, see the abstract art), is produced by using a complex herbal and mineral pharmacopeia, and it is commonly used by Tibetans. The very high THg concentrations in TTM have been shown in past publications and it is known that pharmacists intentionally add heavy metals, including Hg, into medicinal products with a belief of their therapeutic effects.^{17–19} For over a thousand years, Tibetan nobility have ingested daily Hg-containing pills, and currently common people of Tibet have also access to these formerly very expensive pills. Thus far, only THg concentration in TTM have been

reported, while there have been no reports on the levels of MeHg in the TTM. Therefore, toxicity risks associated with the human exposure to MeHg from ingested TTM have not been evaluated and remain unknown. Furthermore, the environmental fate of THg, including its inorganic and organic forms that are excreted from human bodies, have also remained unknown.

The aim of the present study was to understand the life cycle of Hg (including THg and MeHg) in TTM from ingestion by human body to its final release into the terrestrial environment of Tibet. Therefore in this study, we have measured THg and MeHg concentrations in seven commonly used TTMs that are available on the market. We have also determined human exposure levels to MeHg, THg and to eight other toxic heavy metal(loid)s i.e. lead (Pb), copper (Cu), zinc (Zn), chromium (Cr), Arsenic (As), nickel (Ni), cobalt (Co) and cadmium (Cd) to Tibetan inhabitants who regularly ingests any of the TTMs that are available on the market. A physiologically Based Pharmacokinetic (PBPK) model was used to quantify MeHg and inorganic Hg (IHg) cycles in the human body upon ingestion of the TTM. Finally, we have applied a material flow analysis of THg and MeHg to quantify the fate of THg and MeHg excreted from human body in the environment in Tibet.

2. MATERIALS AND METHODS

2.1. Sample Collection.

As was done previously, TTM products were sampled from local Tibetan pharmacies, and sampling was conducted during the summer of 2016^{17, 18}. We ensured that all TTMs evaluated in the present study represented local Tibetan products. Nowadays, there are 11 registered companies producing TTM in Tibet (*China Food and Drug Administration*, <http://www.sda.gov.cn/>). The details on production and consumption of TTM are however not publically available. Based on previous studies and our personal communication with the local Tibetan pharmacies during the sampling,^{17, 19–22} there are seven commonly used TTM types, which are produced by 8 major factories, hence we have focused on these products in the present study. These TTMs are: Renqing Mangjue, Qishiwei Zhenzhu Wan, Ershiwuwei Zhenzhu Wan, Zuozhu Daxi, Renqing Changjue, Zhenzhu Qishi Wan, and Shiliu Jianwei San. We also compared concentrations in the same medicine type i.e. Renqing Mangjue, Qishiwei Zhenzhu Wan and Ershiwuwei Zhenzhu, which were produced by two different companies. Three pills from each type of TTM were analyzed in triplicate (sample size n=3). More information on these medicinal products is provided in Table S1, Supporting Information (SI).

2.2. Analytical Methodology.

All samples were freeze-dried and homogenized into fine powder. THg and MeHg concentrations of TTM products were analyzed in the *Ministry of Education Laboratory of Earth Surface Process in Peking University* and *Institute of Tibetan Plateau Research of Chinese Academy of Sciences*, respectively. The method of the THg extraction in the medicine followed Sallon et al.¹⁷ Each sample was digested in a solution of aqua regia (HNO₃ (69%) : HCl (38%) = 1:3 vol./vol.) in Teflon vessels at 130 °C for 20 min in a

graphite oven. Analysis of THg followed the U.S. EPA Method 1631.^{16, 23} THg was oxidized to Hg(II) by BrCl overnight, neutralized by hydroxylamine hydrochloride [NH₂OH·HCl] for at least 5 minutes to keep it free from halogens after incubation, and then reduced to Hg⁰ by SnCl₂. Cold Vapor Atomic Fluorescence Spectrometry (CVAFS) was used to measure Hg⁰. The detection limit for THg was 0.1 ng/L and spike recoveries for ambient THg ranged from 90% to 106%.

MeHg was extracted by two methods and comparison was made between them to determine any artifact MeHg formation during the two distinct processes.²⁴ For Method 1, samples were leached with 4.5 M HNO₃ in a water bath at 60 °C for 12 h, following previous studies.^{16, 25} For Method 2, as previously reported,^{26, 27} samples were leached with H₂SO₄/KBr/CuSO₄ at room temperature, followed by CH₂Cl₂ extraction and back extraction into water. Extracting solutions from Method 1 were neutralized with KOH. All the extracting solutions were buffered with acetate, derivatized into methylethylmercury with sodium tetraethyl borate, and quantified using Cold Vapor Atomic Fluorescence Spectrometry (GC-AFS).^{16, 25, 28} Due to extremely high THg concentration, digests for MeHg were diluted in a range of 10⁴ - 10⁶-fold to avoid damaging the analytical equipment.¹⁷ The detection limit for MeHg was 0.01 ng/L and recoveries for ambient MeHg ranged from 70% to 120%.

Other metal(loid)s i.e. Pb, Cu, Zn, As, Cr, Ni, Co, Cd and Selenium (Se), were analyzed at the *Institute of Geographic Sciences and Natural Resources Research of Chinese Academy of Sciences*. Samples were digested by HNO₃/HClO₄/HF mixture inside Teflon vessels at 140 °C in the graphite oven for 24 to 48 h. All resulting solutions were dried and dissolved in dilute HNO₃ (5% vol./vol.).²⁹ Pb, Cu, Zn and As were measured by Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP–OES), and Cr, Ni, Co, Cd and Se were measured by Inductively Coupled Plasma–Mass Spectrometry (ICP–MS). The detection limits were 0.1 to 0.3 µg/L for Pb, Cu, Zn and As, and 0.002 to 0.017 µg/L for Cr, Ni, Co, Cd and Se, respectively. The recoveries of the 9 metal(loid)s spiked into samples ranged from 83% to 108%. Except Se, concentrations of all metals used in the present study were above the detection limits. All concentrations are provided on wet weight basis. Percentages of water contents of the TTM in this study ranged from 0 to 13%.

2.3. Dietary Intake.

Probable daily intake (PDI) values for Hg (including THg and MeHg) and the remaining 8 heavy metal(loid)s (Pb, Cu, Zn, As, Cr, Ni, Co and Cd) were applied to evaluate the exposures due to TTM intake by an individual Tibetan population. This method has been extensively used to estimate dietary intake of chemical contaminants. Daily human exposure to given contaminant was calculated based on known contaminant concentrations in various foods and on food intake rates.^{30, 31} This calculation is based on the assumption that contaminant exposures from other routes such as ambient atmosphere, dental amalgam fillings or dermal exposure are negligible.^{13, 31–33} The calculation method is described below:

$$PDI_i = \frac{I \times C_i}{bw} \quad (1)$$

where PDI_i is the PDI of contaminant i due to intakes of TTM in Tibet ($\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$); I is the ingestion rate of the medicine; C_i is the concentration of contaminant i ($\mu\text{g}/\text{g}$); and bw is body weight (kg, set as 60 kg).^{13, 34}

The detailed production and consumption information of each TTM is not well documented. Hence, to gain a realistic image of human exposure to THg and MeHg from ingestion of TTM, we set TTM ingestion rates as 0.2, 1 and 2 $\text{g}\cdot\text{day}^{-1}$, based on the recommended doses of different TTM products (Table S1, SI). Our best estimate of the average daily intake of the TTM was 0.76 g, however ranging from 0.1 to 2.0 $\text{g}\cdot\text{day}^{-1}$ as described in previous studies.^{35, 36} Since most of the medicinal products are consumed by the Tibetans,^{17, 36} population weights based on the number of the Tibetans and other nationalities in Tibet were used when calculating the PDIs of contaminants in this area. In Tibet, Tibetan nationals accounted for 91% of the population in 2010.³⁷ In order to verify the reliability of the intake estimates, we compared these estimates with the annual production of TTMs. The annual medicine intake was estimated at 0.88 Gg (ranged from 0.12 to 2.3 Gg) in Tibet, while the average annual production was 1.2 Gg from 2005 to 2012, hence the intake and production values are in agreement.

In the majority of cases, people around the globe are exposed to MeHg by consuming fish.^{11, 38} Therefore, when considering MeHg exposure in the Tibetan population intake of fish was considered along with the TTM intake. Values of THg and MeHg concentrations in muscle tissue of fishes most commonly consumed in Tibet were taken from our previous study.³⁹ Data on fish consumption in Tibet, i.e. 5.8 $\text{g}\cdot\text{day}^{-1}$ in 2015, was taken from the yearbook.⁴⁰ Differently from other regions in China, the staple crops used in Tibet are highland barley and wheat.^{41, 42} MeHg intake related to consumption of highland barley and wheat were not considered, as there is no evidence of detectable MeHg in these two crops. A previous study showed that Hg concentration in wheat grain was low, because most of the Hg taken up from soils accumulates in the roots.⁴³

2.4. Physiologically Based Pharmacokinetic (PBPK) Modeling.

The PBPK model was used to quantitatively describe both the uptake and clearance of MeHg in the human body upon ingesting various diets that contained MeHg. The human body was compartmentalized into red blood cells, blood plasma, brain, hair, adipose, gut, intestine, kidney, liver, richly perfused tissue (e.g., myocardium), and slowly perfused tissue (e.g., muscle).⁴⁴ The structure of the PBPK model of MeHg, including its absorption, degradation, metabolism, and elimination in the human body, has been described in previous studies.^{44, 45} The toxicokinetic model was coupled with the PBPK, to connect the kinetic behavior of MeHg and IHg, because MeHg can be converted to IHg in human body,^{44, 45} and to understand the fate of the major fraction of IHg upon its ingestion.⁴⁶

In the PBPK model, MeHg and THg intake rates ($\mu\text{g}\cdot\text{day}^{-1}$) were calculated based on PDIs and human body weights. Based on previous study, the plasma flow between different tissues and tissue volumes we considered to follow normal distributions, while the partition coefficients between different tissues and blood, and kinetic parameters in human body follow a lognormal distribution.⁴⁴ All the distributions were considered in the uncertainty analysis based on the Monte Carlo simulation method, following Clewell et al.⁴⁴ Based on previous publications, the present study assumed 95% as the fraction of MeHg absorbed by the human body.^{13, 47} Absorption efficiency of IHg from ingested TTMs remains unknown. Previous studies indicated that about 96% of IHg are in the form of cinnabar ($\alpha\text{-HgS}$), and the assimilation efficiency of Hg from cinnabar was lower than 0.2%.^{18, 20, 48} The bioavailability of Hg^{2+} to human body from different species (excluding HgS) that are present in TTM is still under question, while the bioavailability of Hg^{2+} from various food sources to human body is known to range from 0.2% to 94%.⁴⁹ Based on these values, we set the fraction of IHg assumed to be absorbed by human body in a range of 0 - 4%, and generated by the Monte Carlo simulation in the PBPK model.⁴⁴ Moreover, we set MeHg and IHg intake from consumed fish as the initial input for the oral dose to quantify the contribution of the TTM. All parameters of the models are included in Table S2, SI.

2.5. Material Flow Analysis.

Material flow analysis was used as an effective tool to provide a system-oriented view of the interlinked processes and fate of contaminants,^{50, 51} here specifically to understand the fate of TTM Hg in the environment in Tibet and assessing the impact of human physiology in the Hg cycle. Calculation of material flow for either THg or MeHg considered the influences of human physiology, including ingestion of TTM and other dietary intake, and excretion of THg and MeHg from the human body was incorporated with the treatment and transport (i.e., municipal sewage treatment plant and sewage sludge) term with a final step of release into the terrestrial ecosystem and landfill.¹⁶ The analysis was established based on the mass balance principle, to ensure that the amount of Hg in the sources was equal to the amount of Hg in the sinks as below:¹⁶

$$\sum_i \text{Source}_i = M_i + IW_i + A_i \quad (2)$$

where Source_i is the source of THg ($i=1$, $\text{kg}\cdot\text{yr}^{-1}$) or MeHg ($i=2$, $\text{kg}\cdot\text{yr}^{-1}$) in the environment in Tibet, including municipal sewage discharge (M_i , $\text{kg}\cdot\text{yr}^{-1}$), industrial wastewater discharge (IW_i , $\text{kg}\cdot\text{yr}^{-1}$) and atmospheric deposition (A_i , $\text{kg}\cdot\text{yr}^{-1}$). (M_i) is calculated by the mass of THg or MeHg ingested by the Tibetan population (I_i , $\text{kg}\cdot\text{yr}^{-1}$, equation (3)) and retention in human body (H_i , $\text{kg}\cdot\text{yr}^{-1}$), or estimated from the amount of THg or MeHg in treated municipal sewage discharge (TM_i , $\text{kg}\cdot\text{yr}^{-1}$), untreated municipal sewage discharge (UM_i , $\text{kg}\cdot\text{yr}^{-1}$) and retention in the sewage sludge (S_i , $\text{kg}\cdot\text{yr}^{-1}$) as below:¹⁶

$$M_i = I_i - H_i = TM_i + UM_i + S_i \quad (3)$$

We calculated the (I_i) based on the equation (4) as below:

$$I_i = PDI_{ij} \times bw \times P \times K \quad (4)$$

where P is the Tibetan population size and K is the unit conversion factor. In equation (5) shown below, $(Sink_i)$ is the fate of THg or MeHg, including inputs to the terrestrial ecosystem (T_i , kg·yr⁻¹), landfill (L_i , kg·yr⁻¹) and the human body (H_i , kg·yr⁻¹).

$$\sum_i Sink_i = T_i + L_i + H_i = \sum_i Source_i \quad (5)$$

We regarded the human body as a temporary sink of THg and MeHg with a residence time of one year, as discussed further below. We assumed that sewage sludge was the primary source of THg and MeHg to the landfill in Tibet. More details of the material flow analysis are included in previous studies.^{16, 52} Amounts of THg and MeHg excreted by human body in Tibet were generated by the PBPK model. According to the yearbook data in 2015, 19% of municipal sewage were received by municipal sewage treatment plants in Tibet.⁵³ Other data that we used in the material flow analysis have been derived from our previous studies.^{16, 54} These data include concentrations of THg and MeHg in untreated influent and treated effluent sewage output of the municipal sewage treatment plants, amount of THg in sewage sludge deposited in landfills or other terrestrial systems and THg released from industrial wastewater into the terrestrial environment in Tibet.^{16, 54} In our previous study,¹⁶ we have sampled treated and untreated sewage from the largest in Tibet municipal sewage treatment plant in Lhasa, and measured both the THg and MeHg. A value representing the annual deposition of atmospheric THg in Tibet was taken from a previous study.⁵⁵

2.6. Uncertainty Analysis.

Monte Carlo simulation was applied to analyze the robustness of PBPK of THg and MeHg, and we ran the models 10,000 times following previous studies.^{54, 56} Median values and 60% confidence intervals (range from 20% to 80%) of the results were calculated to quantify the uncertainties.^{54, 56}

3. RESULTS AND DISCUSSION

3.1. Toxic Heavy Metal Exposures Induced by Traditional Tibetan Medicine in Tibet.

THg and MeHg concentrations in TTMs are provided in Figure 1. In this study, we have found that the average THg concentration in seven commonly used Tibetan medicinal products was $5,600 \pm 3,900$ (standard deviation, range: 24 - 12,000, wet weight) µg/g (Figure 1). Most of the THg concentrations of the medicinal products that were measured in the present study were within the range of values that were previously published.^{17, 18, 21, 22} Tenzin et al. analyzed the THg concentration of five kinds of commonly used TTMs, i.e. Qishiwei Zhenzhu Wan, Ershiwuwei Zhenzhu Wan, Renqing Changjue, Renqing Mangjue, and Zuozhu Daxi. THg concentrations of these medicines ranged from 2,300 to 11,000 µg/g.^{21, 22} Chen et al. also analyzed the THg concentrations in four Zuozhu Daxi pills and found them ranging from 4,100 to 5,000 µg/g.⁵⁷ We found that THg levels in the same type of

TTM such as Renqing Mangjue and Qishiwei Zhenzhu Wan ($P < 0.05$, Figure 1) produced by different leading manufacturers were significantly different. Although as previously shown, 96% of THg in TTM is present as α -HgS,^{18, 20, 48} a fraction not likely to be available for absorption by the human body, a large amount of THg would be excreted. The average MeHg concentration in TTM (present study) was 16 ± 12 (range: 0.022 to 37) $\mu\text{g/g}$, and made up a very small percentage (average: 0.29%; range: 0.086% - 1.1%) of the THg, yet these concentrations were higher than in any other local and commonly consumed food products,^{11, 13, 14} or in rice from the Hg mining area reputable for the high MeHg concentration (e.g. MeHg = 0.15 $\mu\text{g/g}$, dry weight).⁵⁸

Other toxic heavy metals were also elevated in TTM (Figure 2). The amount of THg in TTM accounted for $0.56 \pm 0.39\%$ of the total TTM mass, followed by Pb (0.27%), Cu (0.13%), Zn (0.11%) and As (0.077%). Like Hg, Pb and As are also considered in the top ten of chemicals of public health concern according to the World Health Organization (WHO, website: www.who.int). Overall, wet weights of TTM are $\sim 1\text{g/pill}$ across the various types (Table S1) and the manufacturer recommended dosage of TTM is $\sim 1\text{ pill}\cdot\text{day}^{-1}$ (Table S1). If a person consumes one pill each day, the PDI values for Pb and As could reach 45 and 13 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$, being 13- and 6.2-fold, above the recommended intake, respectively, based on FAO/WHO.⁵⁹ It would be of interest that future research focused on chemical forms of these elements, assessing their bioavailability and possible toxicological implications. For example, organic As compounds, which are abundant in seafood, are less harmful to human health in comparison to inorganic As compounds e.g. arsenates found in drinking water in some Asian countries e.g. Bangladesh, Taiwan but also certain regions in the US and possibly elsewhere.^{60, 61}

In evaluating MeHg concentrations in TTM, the two extraction methods i.e. Method 1 and 2, yielded similar results i.e. 17 ± 12 and 15 ± 11 $\mu\text{g/g}$, ($P > 0.05$), respectively. Bloom et al. had found Method 2 as most suitable for extracting MeHg from solids because there was no artifactual formation of MeHg.²⁷ Other studies also supported this conclusion.^{62–64} The mechanism leading to the observed MeHg in the TTM is unknown. Abiotic formation of MeHg as a result of the interaction of IHg and organic matter has been previously postulated.⁶⁵ Most of TTMs are produced by adding various medical herbal and mineral pharmacopeia, and subject to processes which include heating, cooling, washing and mixing.⁶⁶ Although identification of MeHg origin in TTM is beyond the scope of the present study, we hypothesize that MeHg could be formed during production.

Tibetans regularly consuming TTM are chronically exposed to high THg and MeHg. PDIs for THg and MeHg were 65 and 0.18 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$, accounting for $\sim 100\%$ and 94% of the total intake, respectively. In fact, among all regions considered in the present study (Figure 3), people in Tibet had the highest average PDI of THg. We have found that PDI value for THg in this region was 34-fold higher than in other Hg-contaminated regions such as one rural area of China specializing in Hg mining, Wanshan County. In comparison to Japan, Norway and the U.S., PDI in Tibet was higher by $2 \times 10^2 - 3 \times 10^3$ times (Figure 3). In contrast to THg, differences in MeHg PDIs between Tibet and other countries were smaller. PDI for MeHg in Tibet was 1.9 times the value for the Hg mining area in rural China, 3.1 times the value for Norway, it was 14 times higher than in the U.S., but the MeHg PDI of

Tibet was slightly lower than in Japan, where consumption rate of marine fish is among the highest worldwide (Food and Agricultural Organization, www.fao.org/home/en/). It is therefore evident that among various Asian regions, especially considering China, Tibet is quite unique in terms of THg exposure, with lower differences in exposure to MeHg (Figure 3).^{13, 14, 67–69} It is noteworthy that in China exposure to THg and MeHg is driven by fish consumption,⁷⁰ while the contribution of fish consumption to the overall Hg exposure is not significant in Tibet (Figure 3). Due to cultural preferences, the fish consumption rate in Tibet is low ($5.8 \text{ g}\cdot\text{day}^{-1}$).⁴⁰

This study demonstrates that ingestion of the TTM contributes most of the MeHg exposure in Tibet. Considering a person taking one pill each day ($\sim 1 \text{ g}\cdot\text{day}^{-1}$), the MeHg PDI could be as high as $0.24 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$, while the U.S. EPA recommended intake of MeHg is equal to or less than $0.1 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ (Figure 3). Se is a nutrient that could be highly active in counteracting neurological disturbances caused by MeHg toxicity.^{71, 72} Nevertheless, Se concentrations in TTM products selected in the present study are undetectable. It is therefore possible that toxicity to the human body associated with TTM ingestion might be higher than upon ingestion of equal amount of MeHg in fish.⁷¹ While in the past, the high price of TTM prevented regular Tibetans from its use, nowadays, the prices are lower and an increasing number of people can afford it. For example, in 2015, TTM prices ranged from 0.60 to 3.1 \$/pill (Table S1). Whether people who regularly consume TTM experience any adverse effects is not clear. One previous study using clinical trials indicated that THg in the TTM products could exert beneficial effects on neurocognition.¹⁷ This is contrary to the general perception of the impact of THg on humans and thus the effects of human exposure to IHg and MeHg from TTM require future focus and study to further examine its potential impact.^{6, 7}

3.2. Fate of TTM Hg in the Human Body.

Results of PBPK modeling suggest that upon daily ingestion of TTM, MeHg concentration in blood increases rapidly during the first 202 days of exposure and reaches a steady state of $3.0 \mu\text{g/L}$ (range: $1.7 - 5.7 \mu\text{g/L}$ based on the 60% confidence interval – see Materials and Methods; Figure 4). High concentration of IHg in blood occurs instantaneously upon the ingestion of TTM, although a value of $6.0 \mu\text{g/L}$ (range: $2.8 - 14$) is reached after 360 days and continues to increase but very slowly. Blood concentrations of both the MeHg and IHg in people who regularly ingest TTM were determined as higher than for inhabitants of other regions in China, where the exposure sources were associated with consumption of fish and rice. Blood concentrations in people from regions other than Tibet ranged from 0.58 to 1.1 and from 0.60 to $1.9 \mu\text{g/L}$, for MeHg and IHg respectively.^{34, 73, 74}

Uncertainties of blood MeHg and IHg concentrations estimated through PBPK modeling were similar to the PBPK modeling results based on the Monte Carlo analysis in a previous study.⁴⁴ High uncertainty in the present study could be attributed partly to uncertainties associated with THg and MeHg intake levels used in the model, driven primarily by the analytical uncertainty of the THg and MeHg concentrations between different medicines. Values of some partition coefficients had higher coefficient of variation (i.e. 70%) therefore some critical parameters, e.g. hair / blood and kidney / blood, MeHg partition coefficients,

also contributed to the high uncertainty of the model output⁴⁴. Despite the uncertainty, the PBPK modeling is able to demonstrate the general trends in MeHg and IHg concentrations in blood during the yearlong exposure associated with daily ingestion of TTM.

Results of modeling the fate of MeHg and IHg in human organs, upon ingesting just one TTM pill showed a rapid increase and a slow (i.e. >60 days) release due to metabolism and excretion (Figure 5). Most MeHg is projected to accumulate in the slowly perfused tissue. Between 47 to 180 days are required for different compartments of human body to return to MeHg levels that existed prior to the ingestion of one TTM pill. Furthermore, kidney appears to be a significant organ in terms of IHg retention (Figure 5 - bottom panel). A previous study indicated that under continuous exposure, the time required to reach equilibrium in kidney IHg concentration was longer in comparison to other tissues/organs such as blood, liver and brain.⁴⁶ Prolonged lifetime of IHg in the various tissues and organs is of concern due to published evidence of its damage to these and other (e.g. nervous, immune, gastrointestinal systems) organs.⁷⁵ The result of our model suggests that ~2 years are required for kidney to reach IHg levels that existed pre-ingestion. However, IHg in other compartments declined by 99% in the first three days, yet we have determined that to fully purge the body out of this IHg ~0.8 year is needed and this is largely due to the slow release of IHg from kidneys.⁴⁶ Hence, we suggest that the impact of Hg from the TTMs should be carefully evaluated to minimize the health risks to the people who regularly ingest these products.

3.3. Influence of TTM on the Hg Budget in the Environment in Tibet.

In 2015, 4,500 kg of THg and 13 kg of MeHg were ingested by Tibetan inhabitants, respectively, primarily with TTM (Figure 6). Given the high amount of THg in TTM products, and its low assimilation by the human body (<4%), which then drive high concentrations in human excretions, it is necessary to understand its fate in the environment in Tibet. The material flow analysis of THg showed that 860 kg of THg excreted by Tibetan inhabitants entered into municipal sewage treatment plants in Tibet in 2015, while 3,600 kg were directly released into the terrestrial environment (Figure 6).¹⁶

Based on our updated budget calculations, the estimated amount of THg in the municipal sewage treatment plants in Tibet is 34% higher than in our previous estimates, which were based on environmental sample measurements.¹⁶ This discrepancy possibly originates from: (1) measurement errors of THg concentrations; (2) modeling errors from the PBPK model; and (3) statistical errors of the yearbook data and possibly for other reasons that we are not aware of. Despite the errors, the comparison of our previous and present studies suggested that the THg content in the influent sewage of municipal sewage treatment plants could be an index of regional population exposure to THg in Tibet, and potentially to other populations in underdeveloped regions.

In the case of MeHg, our current estimate of input into the municipal sewage was 9.3×10^{-2} kg in 2015, which is 79% lower than what we have determined in our previous study.¹⁶ This difference could be due to occurrence of substantial IHg methylation in the sewage during its delivery, or the heterogeneity in the sewage treatment plant releases itself. Hence sampling and analysis of the effluent would reflect the MeHg levels altered by other natural

processes, including methylation by members of abundant microflora of the sewage. Combination of our former analyses and current modeling preliminarily suggests that between human excretion and arrival to the municipal sewage treatment plant, MeHg levels could increase by a factor of 5. In addition to household contributions, MeHg in the municipal sewage might also originate from other sources such as industrial wastewater and road runoff,^{16, 76} although due to low level of industrialization in Tibet these sources are likely of low significance. Further studies are needed to evaluate MeHg sources from the municipal sewage treatment plants.

Our analysis has indicated that THg released from the human body into the terrestrial environment associated with the TTM was a major contributor of THg in Tibet (Figure 6). In total, 3,600 kg of THg was released from human body into the terrestrial environment in Tibet in 2015 and accounted for 45% of the total THg source, while atmospheric deposition and industrial wastewater discharge contributed 54% and < 0.2%, respectively. As the assimilation efficiency of IHg in people is low, most ingested TTM IHg will be excreted and pass through the sewage treatment plants, resulting in the highest per capita THg and MeHg releases from the Tibetan municipal sewage into the natural environment among all regions in China.¹⁶

3.4. Implications for Human Health and Environment.

In our present study we have found that Tibetans are chronically exposed to high IHg and elevated MeHg levels through regular ingestion of TTM. The calculated exposure levels upon ingestion of TTM are higher than previously reported for populations from other regions in the world including those that are known to be Hg polluted regions.^{13, 77} Mercurous chloride (HgCl_2), which has a relatively high assimilation ranging between 1 and 38%, was widely used in medical preparations in the past, especially as a laxative but also in infant teething powders.^{48, 75} Assimilation of Hg from cinnabar is lower (<0.2%) than for HgCl_2 .⁴⁸ In consideration of the extremely high IHg concentration in TTM, further research on the chemical form of Hg, as well as its bioavailability and possible human health impacts would be highly desirable. Future research should also evaluate the bioavailability of Hg from TTM in consideration of possible interactions with specific foods e.g. protein rich products.

Although the exposure levels for MeHg were substantially lower than for IHg, they were tens of times higher than from fish.^{11, 12} Therefore, a small quantity of the daily ingested products believed to be beneficial to human health could serve as a significant source to drive the high PDI of MeHg for the people of Tibet. Since the impacts of high MeHg, IHg and other toxic heavy metals in TTMs are still unclear, and the method used to manufacture these products is confidential, we argue for urgency of future research in this area. Focus on high THg use and effects of IHg and MeHg in the Tibetan medicinal products on human health, as well as the origin of MeHg in the medicine, would be of high societal benefit in Tibet and provide insights to other cultures, which may use similar products.⁷⁸

As described above, statistics for the consumption of TTM products in Tibet are not available, and the percentage of the Tibetan population not consuming the TTM products in Tibet is not known. Such knowledge gaps likely result in large uncertainties for the

population-wide estimates of the exposure level, and for the Hg budget when considering human physiology. Further detailed investigations on the ingestion of TTM products by local populations are needed. Due to the high THg level in the influent of municipal sewage in Tibet, which is a consequence of TTM ingestion, we also suggest that regulatory authorities in China should formulate relevant standards and contaminant-control policies that are mindful of protecting human and environmental health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

THg and MeHg concentrations in seven commonly used TTMs. Error bars represent standard deviations of measurement (n = 3).

Figure 2.

Total concentrations of eight selected toxic heavy metals (a) and their percentages of the all measured metals (b) in traditional Tibetan medicines. Error bars in panel “a” represent standard deviations of measurement (n = 3).

Figure 3.

Calculated PDIs of THg and MeHg due to ingesting TTM in Tibet. Error bars represent 95% of confidence intervals due to analytical uncertainty. Data on PDIs in Wanshan Hg mining area in China, Japan, Norway and the U.S. are from previous studies.^{13, 67–69} The black solid line represents the MeHg daily intake of 0.23 µg·kg⁻¹·day⁻¹ recommended by JECFA,⁵⁹ and the black dotted line is the MeHg daily intake of 0.10 µg·kg⁻¹·day⁻¹ recommended by U.S. EPA.³¹

Figure 4. Responses of blood MeHg and IHg levels to the chronic exposure resulted from the ingestion of TTM. Shaded area represents 60% of confidence intervals generated by the Monte Carlo simulation. The dotted line represents time of equilibrium reached by MeHg in blood.

Figure 5. Responses and distributions of MeHg and IHg levels in different compartments of human body induced by the ingestion of one pill (~1 gram) of TTM.

All fluxes in kg/yr

Figure 6.

Diagram describing the lifecycles of THg and MeHg from the time of ingestion to their release into the terrestrial environment in Tibet. THg and MeHg data representing sources from the municipal sewage, sewage sludge, industrial wastewater as well as from the atmospheric deposition are from previous studies.^{16, 54, 55}